1st International Conference

IPSAPHARM

November 18-19, 2017

Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences

Scientific Proceeding

Motivators

Motivators

Publication Associate

Journal of Drug Delivery and Therapeutics (JDDT)
(UGC approved - 45744)
An official publication of
Society of Pharmaceutical Technocrats (SoPhTech)

Organized by

IPS Academy College of Pharmacy

Knowledge Village, Rajendra Nagar, A.B. Road, Indore-452012 (M.P.) India
Website: www.ipsapharm.com E-mail: ipsapharmcop@gmail.com

1st International Conference

IPSAPHARM

18th - 19th November 2017

Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences

About Conference

Pharmaceutical innovation is a complex creative process that harnesses the application of knowledge and creativity for discovering, developing and utilizing clinically, new medicinal products that extend or improve the lives of patients. Pharmaceutical sciences is a vibrant and interdisciplinary field that combines a broad range of scientific disciplines which are critical to the discovery and development of new drugs and therapies. Over the years, pharmaceutical scientists are influential in discovering and developing innovative drugs that save people's lives and improve the quality of life.

This International conference on **Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences** will provide an open forum for the academicians, researchers, budding pharmacists, and professionals from pharmaceutical industry to enrich their knowledge in the area of drug discovery, development, and regulatory requirements. It will help them to share their ideas and research work which may culminate with new concepts, innovations and novel strategies for various challenges in the pharmaceutical field.

The conference features plenary sessions which will be delivered by eminent international speakers from different disciplines of pharmaceutical field. The conference will also provide the opportunity to scientists and research scholars from various organizations to put forth their innovative ideas and research findings by means of deliberations, discussions and poster presentations.

This conference shall enhance the cornucopia of knowledge of all participants in the field of pharmacy and will be a blend of eminent speakers, industry professionals, research scholars etc. Discussing on various imperative topics will add an insight to confer over today's scientific and technological perk up. It will facilitate better understanding about the technological developments and scientific advancements across the world by simultaneously equating with the velocity of science and technology.

IPS Academy College of Pharmacy, Indore

Dr. Neelam Balekar
Organizing Secretary
IPSAPHARM, 1st International Conference

Message

It gives me immense pleasure to thank every one participating IPSAPHARM, the first International Conference, organized by IPS Academy, College of Pharmacy, and Indore. The scientific theme of the International Conference 2017 is ***Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences***.

With the technical advancement, the entire world has come closer. We have to break our boundaries and should move together for the service to mankind. Our little efforts in this direction resulted in the form of today's theme, "Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences." A galaxy of internationally renowned keynote speakers and pharmaceutical professionals would enlighten and enrich us with their ideas and expertise. The Conference will witness keynote speakers from 5 different countries from USA, Prof. Timothy Stemmler, Thailand, Prof. Teerapol Srichana and Prof. Pornsak Sriamornsak, Mauritius, Prof. Dhanjay Jhurry, Bulgaria, Prof. Milen Georgiev, Malaysia, Prof. Zoriah Aziz.

Array of topics will include research in pharmaceuticals, newer drug delivery, and application of biotechnology, biochemistry, clinical pharmacology and need of nanotechnology. Orators will cover very intricate topics in a way that will help academicians, researchers, industry professionals and students to have better understanding of the problems and challenges associated with drug discovery in 21st century. A key feature of the conference is the chance for budding researchers and research students to present their work in the form of poster presentation. The proceeding of the conference is in digital format. Collaboration with Journal of Drug Delivery and Therapeutics (a UGC approved journal) has been made where research papers presented in the conference shall be published. The program of plenary lectures and poster presentation will be scientifically stimulating and lead to new collaborations and research opportunities.

This conference will no doubt, provide an excellent opportunity for all to exchange information and views and I am sure you would agree that effective international collaboration will help us to achieve above said goals. On behalf of the Organizing Committee, I appreciate your interest and welcome you to Indore.
It is my privilege to put scientific proceeding of the conference before you.

Neelam Balekar

INDEX

Keynote Speakers

Research Papers

PA: Pharmaceutics and Pharmaceutical Technology

PB: Medicinal Chemistry, Pharmaceutical Analysis, Quality Assurance and Drug Regulatory Affairs

PC: Pharmacognosy, Phytochemistry and Pharmaceutical Biotechnology

PD: Pharmacology, Toxicology, Clinical Pharmacy, Pharmacoepidemiology and Pharmacovigilance

Review Articles

Biography of Keynote Speakers

Prof. Timothy Louis Stemmler

Timothy Louis Stemmler, Ph.D. in Biophysical Chemistry, is Professor and Director of Research; Associate Dean of Postdoctoral Studies in The Graduate School Wayne State University, Detroit USA. He has 25+ years of experience in Physical, Biophysical and Pharmaceutical Chemistry research and has published in excess of 80 papers in reputed International Journals.

Dr. Stemmler did his postdoctoral work in University of Utah, Salt Lake City in 2000 as NIH Fellow. He has one US patent to his credit for in vitro assay for inhibitors of HIV capsid formational changes. Dr. Stemmler serves as reviewer with many international journals and several grants like NIH special emphasis section, German Research Foundation and French National Research Agency to name a few. He has published 3 book chapters and served as resource person to more than 53 invited talks in various conferences.

Dr. Stemmler received outstanding Graduate Student Research Award at University of Michigan in 1996. He received American Heart Association Scientist Development Award (2001-2004); School of Medicine College Teaching Award in 2005 & 2010; School of Medicine Faculty Research Excellence Award in 2006 & 2011.

His area of expertise includes Structural Biology, Protein Spectroscopy, Molecular Biology, Chemistry, and Biophysics. His current research focuses on characterizing the functional role proteins play in regulating cellular iron homeostasis, with special interest regarding iron-sulfur cluster (ISC) bioassembly.

The topic of his presentation in the conference was ***Molecular factor that drives mitochondrial Fe-S cluster Biosynthesis.***

Molecular Factor that Drives Motochondrial Fe-S cluster Biosynthesis

Timothy L. Stemmler

ABSTRACT

The chemical versatility inherent in Fe-S clusters allow these cofactors, when bound to their partner proteins, to play a direct role in several diverse biochemical processes including respiration, photosynthesis and RNA/DNA production/modification/repair. During regulation, these Fe-cofactors can modulate the activity of their regulatory protein partner by serving in a structural, catalytic or electron transferrole. Activation of the Fe-S cluster containing regulatory protein is initiated by the bioassembly of this essential Fe cofactor, and the bulk of the eukaryotic Fe-S clusters are produced within the mitochondria. The highly coordinated activity of the mitochondrial Fe-S cluster (ISC) assembly apparatus of proteins, which in yeast includes the assembly scaffold protein (Isu1), the cysteine desulfurase (Nfs1) the NFS accessory protein (Isd11), the ferrodoxin (Yah1) and the assembly/Fe delivery regulatory protein frataxin (Yfh1), work together to meet the bulk of the cellular Fe-S cluster demands, and also trigger the export of the mitochondrially produced S-molecule that triggers Fe-S cluster assembly in the cytosol. This coordinated pathway for Fe homeostasis in under investigation by our laboratory. Using the yeast model system, our laboratory has prepared and characterized all protein partners in the ISC cluster assembly pathway. Anaerobic spectroscopic and thermodynamic analysis (CD, ITC, DSC, XAS) has been used to characterize the structure and function of iron during assembly and the molecular activities of the protein partners during coordinated Fe-S cluster bioproduction events. Our model for assembly and the early stages for delivery of clusters for eventual incorporation into gene regulation proteins will be discussed.

Prof. Dhanjay Jhurry

Dhanjay JHURRY, Ph.D. in Polymer Chemistry from Bordeaux University France, is the Head of African Network for Drugs and Diagnostics Innovation (ANDI) Centre of Excellence for Biomedical and Biomaterials Research (CBBR), and National Research Chair in Biomaterials and Drug Delivery at University of Mauritius.

He has 20+ years of experience in teaching, management and research level and has published in excess of 70 papers in reputed International Journals. He holds international patent on 'A method of preparing an amphiphilic graft copolymer'. Dr. Dhanjay has worked as R&D Scientist at Flamel Technologies, Lyon, France in 1992 and since 1995 he is affiliated to University of Mauritius at different levels.

Dr. Dhanjay was conferred Bruce Hartmann Award for a Young Investigator at International Conference on Advanced Materials held in Singapore in 2005. He received the First Best Mauritian Scientist Award from the Ministry of Tertiary Education, Science, Research and Technology, Mauritius in 2011.

Dr. Dhanjay was conferred with 'Chevalier dans l'ordre des Palmes Académiques' {Knighthood/ Membership in the Order of Academic Palms} by French Republic in 2007. Dr. Dhanjay serves as Member of the Advisory Board of Polymer International, Wiley-Blackwell. He was the founder and President, Chemical Society of Mauritius (2004 to 2013). He was instrumental in starting a Postgraduate Course 'Pharmaceutical Technology, Drug delivery and Regulatory Aspects' at University of Mauritius in 2010. He has organized several regional and international conferences.

His mainstream research is currently at the frontier of materials science/polymer science and emerging fields such as nanotechnology/nanomedicine including biomaterials and nano-drug delivery carriers in treatment of cancer, tuberculosis, and malaria.

The topic of his presentation in the conference was ***Nanostrategies to fight cancer.***

Nanostrategies to Fight Cancer

Anisha Veeren, Archana Bhaw-Luximon & Dhanjay Jhurry

ABSTRACT

Due to the complexity of cancer, multiple chemotherapy regimens are required to combat the disease causing at the same time enhanced toxicity. The advent of nanotherapeutics has undeniably led to better chemotherapy efficacy, lesser toxicity as opposed to conventional drug delivery and improved overall patient survival rates [1]. Recently nanotherapeutics has been applied to exploiting the synergistic action of multiple-drug combination. For instance, nab-PTX and gemcitabine (GEM) combination has already been approved for metastatic breast cancer, small lung cancer and pancreatic cancer. Combination chemotherapy indeed makes it possible to disrupt different stages of the cell reproduction cycle. Polymeric nanomicellar systems offer the possibility of simultaneous use of two or more drugs for a single treatment cycle and concomitantly provide enhanced efficacy with reduced toxicity [2]. The challenge of multiple drug loading in a nanoparticle system remains drug compatibility and effective loading and release. The condition for mixed drug-loaded micelles to be effective is that the combined concentration of released drug is within permissible toxicity.

Two strategies to enhance drug cytotoxicity on cancer cells are discussed: (i) a physical mixture of individually loaded GEM and doxorubicin (DOX) PVP-b-PCL micelles gives rise to enhanced toxicity on cell lines compared to the individually loaded micelles (Fig. 1) [3]. (ii) Combination of anti-cancer drugs and biomolecules into nanoparticle formulations can enhance synergistic effect and cytotoxicity.

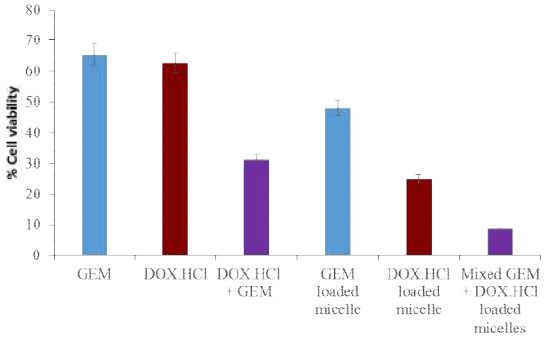


Figure1: Enhanced cytotoxicity of mixed micelles (PVP_{50}-*b*-PCL_{75}) on BxPC-3 cell line compared to free drugs (GEM and DOX) or single-loaded micelles

References:

[1] A. Bhaw-Luximon, and D. Jhurry, Cancer Lett., (2015): 369(2);266-273.

[2] H. Wang, Y. Zhao, Y. Wu, Y.L. Hu, K. Nan, G. Nie, H. Chen, Biomaterials (2011): 32(32); 8281-8290.

[3] A. Veeren, A. Bhaw-Luximon, D. Mukhopadhyay, D. Jhurry. EJPS (2017):102 (2017); 250–260

Prof. Pornsak Sriamornsak

Pornsak Sriamornsak, Ph. D. in Pharmaceutics, is Vice-President (Academic Affairs and Research) at Silpakorn University, Thialand. He completed B. Sc. and M. Sc. in Pharmacy from Mahidol University, Bangkok. In 1999, he received the International and CSU Postgraduate Research Scholarships from Federal Government of Australia and Charles Sturt University, respectively, to study in Australia for his PhD in 2002.

At present, Prof. Sriamornsak is Secretary General and Executive Board Member, Thai Industrial Pharmacist Association; Board member of Silpakorn University Computer Center. He is an editorial board member of several journals such as Asian Journal of Pharmaceutical Sciences, The Scientific World Journal, Journal of Pharmaceutics, Songklanakarin Journal of Science and Technology, etc.

He was awarded 2012 FAPA Ishidate Award for Pharmaceutical Research from Federation of Asian Pharmaceutical Associations (FAPA), 2012-2013 Thailand Distinguished Polymer Scientist from Polymer Society of Thailand. In 2009, he was awarded the (1) Silpakorn University Outstanding Research Award, (2) TRF-CHE-Scopus Researcher Award 2009 in Health Sciences, and (3) Research Council Award: Research Award 2009 in Chemistry and Pharmaceutical Sciences, for his research on mucoadhesive mechanism of pectin and its application to drug delivery system.

Prof. Sriamornsak's current researches focus on drug delivery system and dosage form design, application of biopolymer in pharmaceutics, and pharmaceutical nanotechnology. He is the author or co-author of more than 140 research papers published in peer-reviewed international journals, 17 books/book chapters, and 9 Thai patent applications. He has given over 320 presentations, invited lectures and seminars, in national and international conferences.

The topic of his presentation in the conference was ***Biopolymer-based nano-sytems for effective drug delivery.***

Biopolymer-Based Nano Systems for Effective Drug Delivery

Pronsak Sriamornsak

ABSTRACT

Biopolymers, including pectin and dextrin, have been used extensively in pharmaceutical fields according to their unique properties that enable it to be used as carrier for oral drug delivery system. We have identified the benefits of biopolymer-based dlivery systems for oral drug administration. Few examples of biopolymer-based nano systems were given here, i.e., pectin-liposome nanocomplexes, pectin-based nanoparticles prepared from nanoemulsion templates, pH-responsive dextrin nanogels. The pectin-liposome nanocomplexes were prepared by simply mixing cationic liposomes with pectin solution and tested ex vivo and in vivo. The mucoadhesive performance of pectin in the GI mucosa and its mechanisms were also investigated. The selection of suitable pectin as a mucoadhesive material helps to retain the dosage form at its site of application. The pectin-based nanoparticles were used for improving the dissolution of poorly water-soluble drug. The nanoparticles were prepared via nanoemulsion templates. In vivo drug absorption from the pectin-based nanoparticles showed an excellence result, which was better than the commercial product, suggesting a promising for drug absorption enhancement. Anticancer drug-loaded pH-responsive dextrin nanogels were fabricated by emulsion cross-linking technique using formaldehyde or glyoxal as a cross-linking agent to form pH-sensitive bond. An in vitro anti-tumor activity and in vivo anti-tumor efficacy demonstrated an enhanced cytotoxicity and anti-tumor efficacy. The success in anticancer drug delivery suggests that dextrin nanogels may be a promising targeted drug delivery system for colorectal cancer treatment.

Keywords: Nano system, nanocomplexes, nanoparticles, nanogels, biopolymer, pectin, dextrin

Prof. Teerapol Srichana

Teerapol Srichana, Ph.D. in Pharmaceutics from King's College London, UK is Dean Graduate School and Director of Drug Delivery System Excellence Center, and NANOTEC-PSU Center of Excellence on Drug Delivery, at Prince of Songkla University (PSU), Thailand. Earlier, he was also Associate Dean for Planning & Development and Research & Graduate Studies at Prince of Songkla University (PSU), Thailand.

He has 20+ years of experience in Pharmaceutical Drug Delivery Research and has published in excess of 100 papers in reputed International Journals. He has supervised nine doctoral candidates; 20+ postgraduate students. He had completed his post doctoral studies from US & Austria.

Dr. Srichana has received outstanding researcher award in 2008 and PSU Grand Slam Researcher Award in 2012 at PSU. He has received best poster presentation award in National TB symposium, Penang, Universiti Sains, Malaysia and Best Presenter Award at 3rd National Research University Meeting, Bangkok in 2014.

His current research focuses on Liquid Crystal Drug Delivery System, Pelletization technology and topical sprays focusing on pulmonary and wound healing medicines.

*The topic of his presentation in the conference was –**Nanotechnology and health.***

Nanotechnology and Health

Teerapol Srichana

ABSTRACT

Nanoparticles are used for site specific drug delivery. This technique required drug dose is lowered therefore side-effects are lowered significantly as the active agent is deposited in that region only. This highly selective approach can reduce costs and pain to the patients. Various nanoparticles such as liposomes, liquidcrystals, micelles find an application. Liquid crystal and micelles synthesizedin house are used for drug encapsulation. Nanodelivery system together with drug targeting to the organ candeliver drug molecules to the desired location. A targeted medicine reduces the drug dose and side effects.

Nanomedicines may improve drug bioavailability both at specific places in the body and over a specified period of time. The molecules are targeted and deliveredto precised cells. Thus nanoparticles are promising tools for drug delivery advancement. The biodistribution of nanoparticles is still under investigated due to the difficulty in targeting specific organs. Efforts are made to optimize and understand the potential and limitations of nanoparticulate systems. It is expected that the benefits will be gained from nanotechnology including lower drug toxicity, improved bioavailability, reduced cost of treatment and extended economic life of proprietary drugs.

Some examples of drug delivery systems are examples of research work in the NANOTEC-PSU on amphotericin B and rifampicin in liquid crystals systems were demonstrated the successful stories of nanotechnology. We can use the liquid crystal as nanocubic and nanovesicle to encapsulate the drugs into the system to give more effectiveness with less toxicity to the cells

Key words: drug delivery system, cells, bioimaging

Prof. Zoriah Aziz

Prof. Datin Zoriah Aziz, Ph.D. in Evidence-based Pharmacotherapy, is Professor and Head of Department of Pharmacy in University of Malaya, Kuala Lumpur, Malaysia. Dr. Zoriah completed her Ph.D. at Aston University, UK and her undergraduate studies at Universiti Sains Malaysia (USM). She has completed M.Sc. in Pharmacy and Applied Statistics.

She started her career as a Pharmacist with National Pharmaceutical Control Bureau, Malaysia; and then was Head of the Pharmacovigilance Department for six years, before getting associated with University of Malaya as lecturer in 1998. Since then her area of expertise has been Evidence-based herbal medicine and pharmacotherapy and Pharmacy Education. She has published in excess of 30 papersin Journals of International repute.

She has received the Thomas C. Chalmers award in 2000 from Conchrane Collaboration. In 2017 she was awarded with best lecturer for Pharmacy Award by University of Malaya.

Dr. Zoriah Aziz has served as Head and member of several Committees in University of Malaya. She is a member of several national and International Societies including Cochrane Consumer Network and Adverse Effects Methods group, International Society of Pharmacovigilance; Pharmacy Board of Malaysia etc.

The topic of his presentation in the conference was ***Understanding systematic review and meta-analysis.***

Understanding Systematic Review and Meta-Analysis

Zoriah Aziz

ABSTRACT

Pharmacists are increasingly required to provide advice to patients and other healthcare professionals on matters related to pharmacotherapy based on the best available evidence.Thus, the importance of pharmacists in understanding and applying the principles of Evidence-based Pharmacotherapy (EBP) has never been greater.We aim to cover the principles of systematic reviews and meta-analysesin EBP and introduce the steps in conducting the review with a demonstration of the steps using a real life scenario. The problems associated with traditional narrative reviews are discussed. We also provide steps taken in limiting bias associated with the conduct of systematic review and meta-analysis. Several terms used in the reporting of systematic reviews and meta-analyses such as outcomes measures, risk of bias assessment tool, heterogeneity, subgroup analysis, and the forest plot are introduced.

Prof. Milen Georgiev

Milen Georgiev, Ph.D. in Biotechnology is heading a group of Plant Cell Biotechnology and Metabolomics Research in Plovdiv, Bulgaria. He has 13+ years of experience in natural products research and has published in excess of 90 papers in reputed international journals. He has delivered invited lectures in 14 different countries. Dr. Georgiev holds several grants from the National Science Fund of Bulgaria and framework programs of European Union.

He did his postdoctoral work in Germany (2005-2007) and in The Netherlands (2010-2012), both supported by the Marie Curie postdoctoral program of European Union. In 2011 and 2015 he was awarded with Pythagoras award by the Bulgarian Government for outstanding scientist. At present he is the only scientist in Bulgaria to win this prestigious award twice.

Dr. Georgiev serves as an Associate Editor of Phytomedicine (Elsevier) and he is a member of *the Editorial board of Biotechnology Letters (Springer). He was a chairman of the International Conference on Natural Products Utilization: from Plants to Pharmacy Shelf (2013) and its second edition (2015). Recently he has organized 3rd International Conference on Natural Product Utilization: From Plants to Pharmacy Shelf (ICNPU-2017), 18-21 Oct. 2017 Bonsta, Bulgaria and it is noteworthy that delegates from more than 350 countries participated in the conference.*

His current research focuses on the biosynthesis of fine molecules and the development of biotechnological tools for their sustainable mass production. In addition his work is focused on the application of emerging modern platforms for comprehensive metabolite profiling (i.e. NMR-based metabolomics) and biochemometrics.

The topic of his presentation in the conference was ***NMR based metabolomics: Just another 'OMICS' or an ideal platform for accelerated lead finding?***

NMR Based Metabolomics: Just another 'OMICS' or an Ideal Platform for Accelerated Lead Finding?

Milen I. Georgiev

ABSTRACT

Per definition metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine, and in particular in cancer therapy [1].

Since the term metabolome has been introduced several platforms for high throughput analyses have been developed accordingly. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, because it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of proton NMR-spectrometry over the other analytical platforms is the possibility for quantification, hence direct comparison of concentrations of all compounds present in the sample, as the signal intensity is only dependent on the molar concentration of the solutes [2-4].

Some case studies of the application of NMR-based metabolomics concept in natural products research, plant biotechnology and lead finding [3-5] will be presented and discussed.

Acknowledgements: Financial support from National Science Fund of Bulgaria, under project DFNI-B02/14, is greatly acknowledged.

References:

[1] Nicholson JK (2010) Nature 463:32.

[2] Kim HK, Choi YH, Verpoorte R (2010) Nature Protocols 5:536.

[3]Georgiev MI, Ali K, Alipieva K, Verpoorte R, Choi YH (2011) Phytochemistry 72:2045.

[4] Zahmanov G, Alipieva K, Simova S, Georgiev MI (2015) Phytochemistry Letters 11:404.

[5] Alipieva K, Korkina L, Erdogan Orhan I, Georgiev MI (2014) Biotechnology Advances 32:1065.

Research Papers

PA-03

A Novel Class of Phototriggerable Liposomes Containing Paclitaxel for the Treatment of Skin Cancer

Neeraj Sharma[1], Vimal Kumar[2]
[1]IPS Academy College of Pharmacy, Indore, (M.P.)
[2]ITM School of Pharmacy, ITM Universe, Vadodara, Gujarat
E-mail address: neerajsharma236@gmail.com

Abstract: Success of nanocarriers-mediated drug delivery solely depends on delivery of therapeutics to a specified target. Secondly therapeutically active amount of drug should be released within defined space and time (triggered release). Recently, we formulated a novel class of photo-triggerable liposomes prepared from soy lecithin (SPC), cholesterol (CHOL) and photosensitive agent ketoprofen that can efficiently released entrapped paclitaxel upon UV light treatment. To explore these formulations for *in vivo* applications, we have examined the effect of released anticancer drugs on cellular toxicity. Liposomes were loaded with paclitaxel and biophysical properties (including liposome size and stability) and paclitaxel encapsulation efficiency of the liposomes were determined. Subsequently, the effect of UV light treatment on paclitaxel release, and cellular toxicity by released paclitaxel were examined. Since liposomes using the 5:1 molar ratio of SPC and CHOL, showed highest encapsulation of paclitaxel, these formulations were investigated further. UV light treatment of co-cultures containing paclitaxel loaded liposomes and cells (SK-MEL-2) resulted in improved cell killing as compared to untreated samples. These phototriggerable liposomes described here may provide a platform for future drug delivery applications.

Introduction:

The development of a drug delivery system is one of the most important technological challenges in the present time. Liposomes are bilayered vesicles made from fatty materials, predominantly phospholipids and ranging from 50 nm to several micrometers in diameter. It is highly explored drug delivery system and can be safely and effectively used in various fields like protein /drug delivery, controlled delivery, antiviral therapy, tumour therapy, gene delivery, vaccine delivery, cosmetics and dermatology and others [1]. However, to achieve therapeutic efficacy of the liposomal dosage form the encapsulated associated drug should become available to the target cells. A main reason for this is that accumulation of liposomes in the tumor area does not guarantee that the encapsulated drug becomes bioavailable to the tumor cells [2, 3]. Local administration of drugs has always been attractive because of the avoidance of systemic distribution of the drug and the need to use excessively high doses to enable effective concentrations at target sites. One of the crucial aspects of liposome use is to achieve the release of components at the target site. In local drug delivery, there are many methods to trigger the controlled release of drugs at the target site; such as the release of contents from liposomes in response to external stimuli of temperature, pH and light [4]. The use of

light to stimulate the release of encapsulated compounds from liposomes is attractive, because spatial and temporal delivery of the radiation can be possible to control. The aim of present study was to achieve release of model drug encapsulated in SPC based liposomes upon photoactivation of ketoprofen in bilayer by UV light exposure. The objective of the present study is to develop safe and effective phototriggerable liposome carrier system for effective delivery of anticancer drugs [5].

Materials and Methods:

Materials

Soya phosphatidylcholine (SPC) were purchased from HiMedia, India. CHOL was purchased from Sisco Research Laboratories Mumbai, India. Ketoprofen was purchased from Msd Laboratories Ltd., Delhi. Paclitaxel was obtained as a gift sample from Dabur India. All other chemicals and solvents used were purchased from local suppliers and were of analytical grade unless mentioned.

Formulation and optimization of novel phototriggerable liposomes

Liposomes were prepared by REV method. Both conventional and photosensitive liposomes were prepared (PC:CHOL 5:1 to 5:5 molar ratio) for study. Ketoprofen (drug-to-lipid ratio 0.25 wt/wt) was incorporated to membrane by dissolving in a mixture of chloroform and methanol (2:1v/v). Paclitaxel was incorporated in the liposomal bilayer. The shape and morphology of these liposomes were observed with the help of transmission electron microscope (Morgagni, 268, FEI, Electron microscope, Netherlands) after negative staining.

Light treatment

Effect of light-triggered release of entrapped contents from liposomes was evaluated after exposure to UV. Effect of exposure to UV was studied in a Kompakt UV cabinet, India fitted with a UV lamp.

Cellular toxicity assay

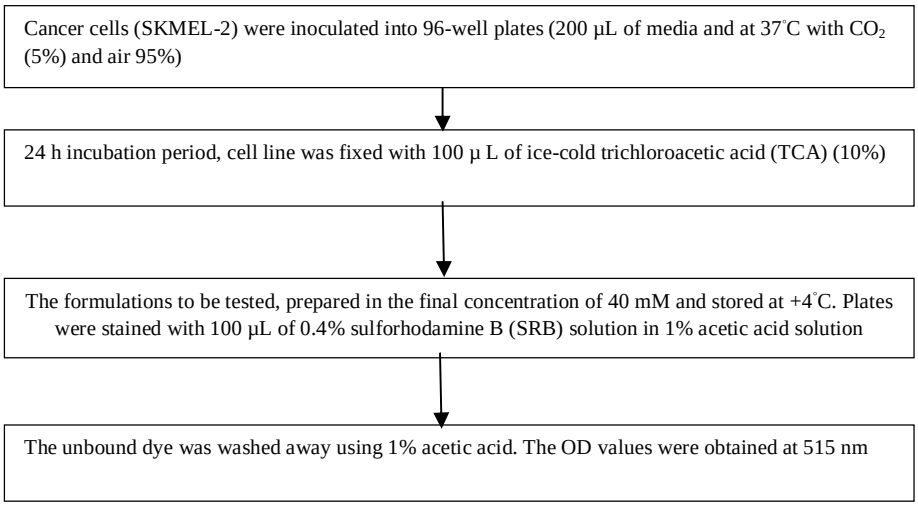


Figure 1: Cellular toxicity assay

Results and Discussion:

Formulation of photosensitive liposomes Several formulations of liposomes were prepared to study the effect of SPC:CHOL ratio. Five different batches of both conventional and photosensitive liposomes containing various SPC/CHOL molar ratios from 5:1 to 5:5 were prepared Table 1. Separation of liposomes was achieved by centrifugation at 16500 rpm for 90 min at –5 °C. The liposomal concentrate was washed twice with PBS pH 7.4. TEM micrograph was taken and clearly showed the formation of liposomes (Figures 1). Most of the liposomes formed appeared spherical and symmetrical in shape and were mainly unilamellar in nature. Sizes of formulated liposomes were found between 200-400 nm.

Table 1: % EE of formulations of photosensitive and conventional liposomes

S. No	Formulation Code	PC/CHOL ratio	Encapsulation efficiency (%)	
			With ketoprofen	Without ketoprofen
1.	LP-1	5:1	30.36 ± 1.49	26.55 ± 2.04
2.	LP-2	5:2	27.55 ± 0.50	17.40 ± 0.40
3.	LP-3	5:3	24.72 ± 0.78	14.62 ± 1.01
4.	LP-4	5:4	15.87 ± 0.56	11.75 ± 1.55
5.	LP-5	5:5	10.25 ± 0.55	07.82 ± 0.205

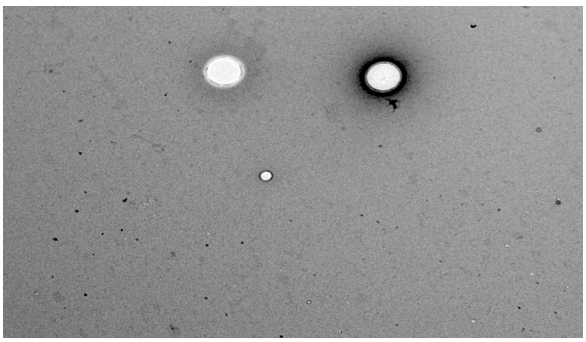


Figure 2: Transmission electron microscopic photograph of prepared liposomes

Light treatment

Shape was observed before and after exposure for photosentive liposomes using phase contrast microscope. It was observed visibly that size increased after exposure.

SRB assay Co-cultures containing paclitaxel-loaded phototriggerable liposomes and cells (SK-MEL-2), resulted in improved cell-killing as compared to untreated samples Table 2.

Table 2: GI_{50} values of different formulations

Samples	GI_{50} values
Free drug solution	55.6
Paclitaxel loaded liposomes	37.4
Paclitaxel loaded phototriggerable liposomes	24.0

Conclusion:

UV light treatment of co-cultures containing paclitaxel-loaded liposomes and cells (SK-MEL-2) resulted in improved cell-killing as compared to untreated samples. These phototriggerable liposomes described here may provide a platform for future drug delivery applications.

References:

[1] Thompson DH, Gerasimov OV & Wheeler JJ (1996) Biochim Biophys Acta 1279:25-34.

[2] Bisby RH, Mead C & Morgan CG (1999) FEBS Lett 463:165-168.

[3] Kono K, Yoshino K & Takagishi T (2002) J Control Release 80:321-332.

[4] Gerasimov OV, Boomer JA & Qualls MM (1999) Adv Drug Deliv Rev 38:317-338.

[5] Gerasimov OV, Schwan A & Thompson DH (1997) Biochim Biophys Acta 1324:200-214.

PA-05

Formulation and Evaluation of Tablets Containing Artemether Microspheres and Lumefantrine

Kuldeep Vinchurkar, Sheetal Mane, Neelam Balekar, D. K. Jain
IPS Academy College of Pharmacy, Indore - 452012 (M.P.)
E-mail address: kuldeepvinchurkar@gmail.com

Abstract: This study was aimed to fabricate and evaluate a combination of Artemether and Lumefantrine as tablets and to make Artemether in sustained form so as to prolong its elimination time. Artemether was formulated in form of microspheres by solvent evaporation technique and was then formed into the tablet along with the Lumefantrine. Artemether microspheres were prepared and compressed into compressible tablet by direct compression process using the compressible excipients along with Lumefantrine, and was further evaluated for various parameters such as hardness, thickness, weight variation, friability, drug content, in vitro drug release and stability.

Introduction:

Malaria is one of the most important and scourge infectious diseases in developing areas of the world. It is estimated that 300-500 million cases of malaria occur each year resulting in 750,000- 2 million deaths (World malaria situation, 1994). It is transmitted by the bite of female mosquito (Anopheles). Mainly four major species of *Plasmodium* are found in humans (*P.falciparum, P.vivax, P.ovale* and *P.malariae*) [1]. Effective treatment is dependent on the use of an efficacious anti malarial that is taken according to an optimized regimen. Artemisinin-based combination treatments (ACTs) have the most potent and rapid onset of anti-parasitic activity of any anti-malarial drug available today and are active against all *Plasmodium* species that infect humans [2]. Efficacy is determined by the drug partnering the Artemisinin derivatives and Artemether-Lumefantrine is one such drug combination. The rationale behind this combination is that artemether initially provides rapid symptomatic relief by reducing the number of parasites present before lumefantrine eliminates any residual parasites.

Materials and Methods:

Materials

Artemether (ART), Lumefantrine (LUM), Ethyl Cellulose (EC), Polyvinyl alcohol (PVA), Dichloromethane (DCM), Distilled water, Microcrystalline cellulose (MCC), Sodium Starch Glycolate (SSG), Croscarmellose Sodium (CCS), Magnesium Stearate, Colloidal Silicon Dioxide.

Preparation of Artemether Microspheres

Artemether microspheres were obtained by solvent evaporation technique Polymer was dissolved in dichloromethane and then the drug was added to the above solution. This solution was injected into the PVA solution maintained at variable speed using mechanical stirrer. Stirring was continued until

all the dichloromethane evaporated. The formed microspheres were collected by filtration and washed with n-Hexane and dried [3, 4].

Table 1: Formulation codes of Artemether-EC Microspheres

Formulation Codes	Drug (Artemether) (mg)	Polymer (Ethyl Cellulose) (mg)	Solvent (DCM) (ml)	Medium (PVA) (%)	Stirring rate (rpm)
M1	500	1000	20	0.5	200
M2	500	1000	20	0.5	400
M3	500	1000	20	0.5	600
M4	500	1250	20	0.5	200
M5	500	1250	20	0.5	400
M6	500	1250	20	0.5	600
M7	500	1000	20	0.3	200
M8	500	1000	20	0.3	400
M9	500	1000	20	0.3	600
M10	500	1250	20	0.3	200
M11	500	1250	20	0.3	400
M12	500	1250	20	0.3	600
M13	500	1000	20	0.1	200
M14	500	1000	20	0.1	400
M15	500	1000	20	0.1	600
M16	500	1250	20	0.1	200
M17	500	1250	20	0.1	400
M18	500	1250	20	0.1	600

Characterization of microspheres

Prepared microsphere should be characterized by following tests such as, Particle size analysis, surface Morphology**,** determination of Percentage yield of microspheres**,** determination of flow properties of microspheres**,** drug entrapment efficiency**,** *In vitro* release studies of microspheres and effect of different formulation variables on various evaluation parameters.

Formulation of Tablets containing microspheres

Tablets of ART microspheres and LUM were prepared by direct compression technique. The corresponding amount of ART microspheres equivalent to 20 mg drug, lumefantrine, MCC and superdisintegrants were accurately weighed and blended. Thereafter the corresponding amount of magnesium stearate and colloidal silicon dioxide were added to the mixture. The mixture was allowed for direct compression into tablets weighing 300mg using a tablet punching machine with 8 mm flat faced punches.

Characterization of tablets

Formulated tablet should be evaluated by following test such as, thickness, diameter, hardness, friability, weight variation, drug content, microscopic evaluation of tableted microspheres and In vitro release of Lumefantrine & Artemether from tablets

Table 2: Formulation codes of tablets containing ART Microspheres and LUM

FC	ART microsphere	LUM	MCC	SSG	CCS	Mag. Stearate	Colloidal SiO_2
MT1	20	120	107	3	-	7	3
MT2	20	120	105.5	4.5	-	7	3
MT3	20	120	104	6	-	7	3
MT4	20	120	102.5	7.5	-	7	3
MT5	20	120	101	9	-	7	3
MT6	20	120	99.5	10.5	-	7	3
MT7	20	120	98	12	-	7	3
MT8	20	120	96.5	13.5	-	7	3
MT9	20	120	95	15	-	7	3
MT10	20	120	97	-	3	7	3
MT11	20	120	95.5	-	4.5	7	3
MT12	20	120	94	-	6	7	3
MT13	20	120	92.5	-	7.5	7	3
MT14	20	120	91	-	9	7	3
MT15	20	120	89.5	-	10.5	7	3
MT16	20	120	88	-	12	7	3
MT17	20	120	86.5	-	13.5	7	3
MT18	20	120	85	-	15	7	3

Results and Discussions:

Table 3: Evaluation parameters of microspheres

Formulation Codes	Percentage Yield (%)	Entrapment Efficiency (%)	Particle Size (µm)	Cumulative Release (%)
M1	84.87	66.20	18.45	63.03
M2	79.40	60.49	18.30	68.38
M3	94.00	54.06	15.00	71.65
M4	90.28	70.57	23.12	57.85
M5	86.00	67.42	19.10	61.95
M6	70.34	63.23	16.92	69.19
M7	88.33	67.81	21.00	69.01
M8	92.27	61.15	21.00	71.79
M9	91.73	52.81	14.80	76.72
M10	86.68	73.31	22.77	63.38
M11	86.91	69.98	21.87	70.14
M12	66.63	66.98	19.35	74.48
M13	86.00	70.15	26.42	72.51
M14	84.06	68.39	23.80	76.45
M15	76.67	63.86	20.00	84.87
M16	71.88	74.42	26.10	71.02
M17	88.91	71.26	22.45	75.11
M18	90.11	67.07	18.95	79.48

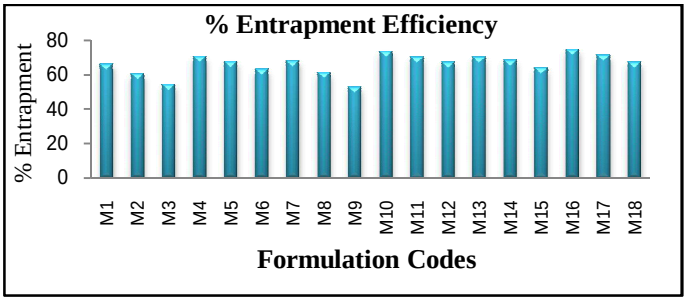


Figure 1: Entrapment efficiency (%) of microspheres

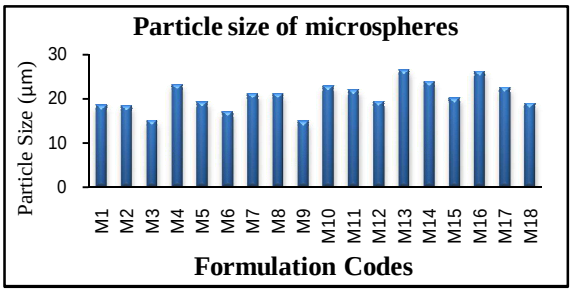


Figure 2: Particle size of microspheres

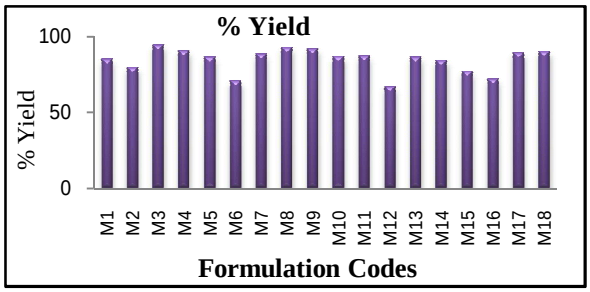


Figure 3: Percentage yield (%) of microspheres

The morphology of the prepared batches of Artemether microspheres was evaluated by Scanning Electron Microscopy (SEM). Scanning Electron micrographs of the microspheres are shown in figure at different magnifications of 70x, 100x and 10000x revealing the spherical and smooth surface.

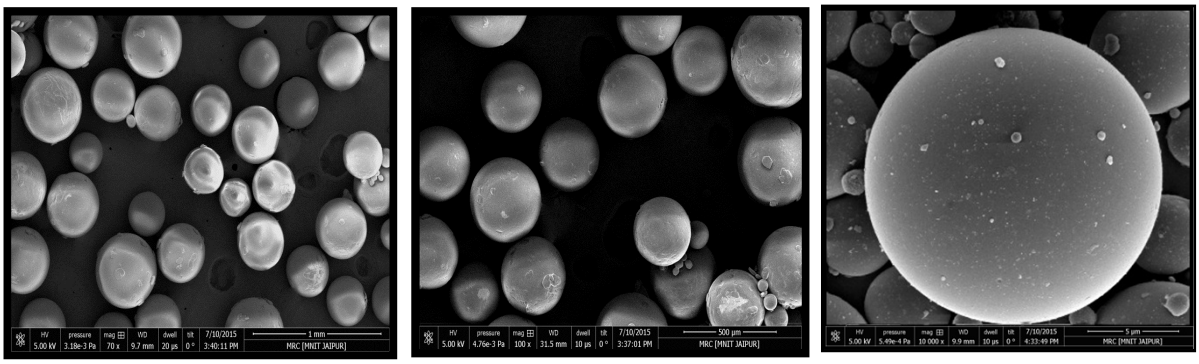


Figure 4: Scanning Electron Micrograph at 70, 100 and 10000 magnification

In vitro dissolution studies of art

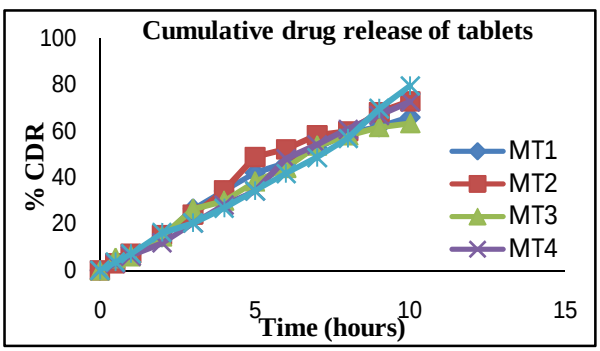


Figure 5: %CDR (ART) of MT1-MT5

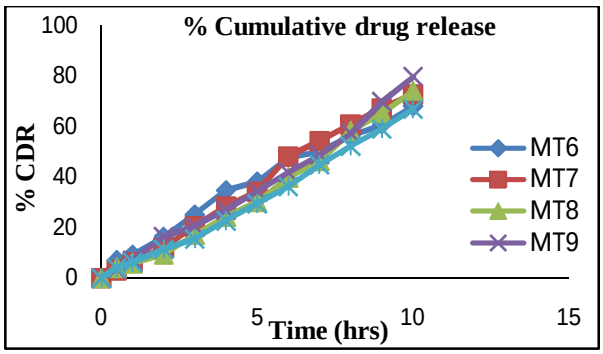


Figure 6: % CDR (ART) of MT6 - MT10

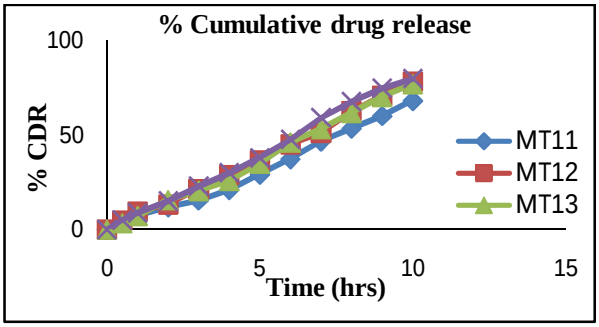


Figure 7: %CDR (ART) of MT11-MT14

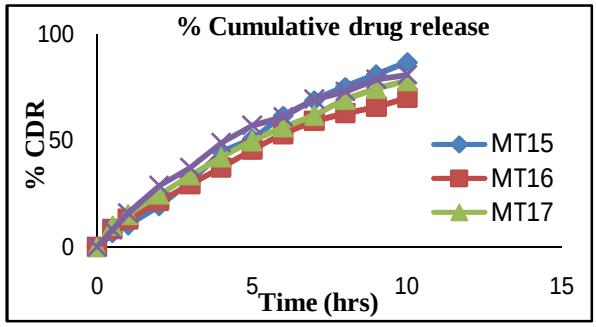


Figure 8: % CDR (ART) of MT15- MT18

Figure depicts the release rate of ART from the tableted microspheres for all the formulations. At 10th hours release rate of drug was between 61.24% - 86.92%. M15 formulation shows the maximum release and M4 shows the minimum release. It depicts that the release of ART either from microspheres or from the tableted microspheres was found to be relatively near about same. A slight increase is observed in the release from tableted microspheres.

Conclusion:

Sustained release microspheres of ART were successfully prepared using EC by solvent evaporation technique and after successfully incorporating ART into microspheres, this study aimed to obtain tablets as a final oral dosage form. ART microspheres along with LUM were formulated into tablets by direct compression technique using the excipients. Hence the present work suggest that, ART which has the lower half life and eliminates quickly from the body, when loaded with ethyl cellulose in form of microspheres and tableted along with LUM results in sustained release of drug in malaria. Therefore, ART and LUM in combination minimizes development of resistance as the malaria parasites are never exposed to artemether alone, so are considered as the best combination for treatment of malaria.

References:

[1] Aboghanem A, Alburyhi M & Alwan (2013) J Chem Pharm Res 5:617-625.

[2] Alagusundaram M, Chetty M, Umashankar K, Badarinath A & Ramkanth S (2009) Int J Chem Tech Res 1:526-534.

[3] Aulton, ME (2002) Pharmaceutics: The Science of Dosage Form Design, in: Wells, J., Second edition, Pharmaceutical preformulation: the physicochemical properties of drug substances. Churhill Livingstone, Leicester 114-136.

[4] Balaji A, Kumari H & Kumar U (2014) Int J Pharn Pharm Sci 4:180-185.

[5] Bannister LH & Sherman IW (2009) Plasmodium. Encyclopedia of Life Sciences John Wiley & Sons 26:1-10.

PA-06

Formulation and Evaluation of Taste Masked Oral Dispersible Tablet of Ciprofloxacin With Ion Exchange Resin

Vishwas Pathak[1], Govinda Bhatt[2], Sapna Malviya[2], Anil Kharia[2]
[1]IPS Academy, College of Pharmacy (Indore, MP)
[2]Modern Institute of Pharmaceutical Sciences (Indore, MP)
E-mail address: vishwas.pathak@gmail.com

Abstract: Bitter taste is one of the important formulation problems that are encountered with many drugs, like Ciprofloxacin. In modern times, the most improved & easy technique is to formulate tasteless complexes of ciprofloxacin with ion exchange resin (Indion 234). After acid activation and swelling in water, resin & ciprofloxacin (proper ratio) was stirred for definite time & unbound drug in filtrate was estimated spectrophotometrically and drug-loading efficiency was calculated. The molecular properties of drug complexes by FT-IR study confirm the complexation of ciprofloxacin with Indion 234. The influence of superdisintegrants, crospovidone and sodium starch glycolate on disintegration time, wetting time and water absorption ratio was studied.

Introduction:

Taste is the ability to detect the flavour of substances like food, drugs etc. Taste is now considered an important factor governing the patient compliance [1] .The purpose of the research was to formulate tasteless complexes of ciprofloxacin with Indion 234 and to evaluate molecular properties of drug complexes [2]. Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic. It has a narrow absorption window and is mainly absorbed in the proximal areas of GIT. The present study is an attempt to select best possible combination of drug and disintegrating agent to formulate rapidly disintegrating tablet of ciprofloxacin which disintegrates faster thereby reducing the time of onset of action[3] .

Materials and Methods:

Materials

Ciprofloxacin, Croscarmellose Sodium, Crosspovidone, MCC, Mannitol, SLS, Saccharin Sodium, Orange Flavor, Talc, Magnesium Stearate. Drug-excipent interactions were studied by IR spectroscopy at Modern Laboratories, Pvt. Ltd., Indore. All other chemicals used were of analytical reagent grade. Double distilled water was used in entire study.

Preparation of Oral Dispersible Tablet Drug-Resin Complex

Formation of drug-resin complex

The batch process was used for complexation. Drug-resin complex was prepared by placing 10 g of activated resin in a beaker containing 300 ml deionized water and allowed to swell for 30 minutes. Accurately weighed drug (as per 1:1, 1:1.2, and 1:1.3, drug-resin ratio) was added and stirred for 30

minutes. On filtration, the residue was washed with 700 ml of deionised water. Unbound drug in filtrate was estimated at 278 nm and drug-loading efficiency was calculated.

Method of Complexation

Selection of resins

Resins were selected on the basis of the nature of drug and requirement of formulation. Depending on the basis of acidic and basic nature of the drug, cation and anion exchange resins can be used. In the present work, weak cation exchange resin Indion 234 was selected based on their Ion exchange capacity and used for the taste masking of model drug

Table 1: Different Compositions of Oral Disintegrating Tablets

S.N.	Ingredients	Quantity of Ingredients (mg)							
		F1	F2	F3	F4	F5	F6	F7	F8
1	DRC Eq. to 100 mg of drug	235.1	235.1	235.1	235.1	235.1	235.1	235.1	235.1
2	Croscarmellose Sodium	10.0	16.5	23.0	29.5	-	-	-	-
3	Crosspovidone	-	-	-	-	10.0	16.5	23.0	29.5
4	MCC	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
5	Mannitol	40.85	34.35	27.85	21.35	40.85	34.35	27.85	21.35
6	SLS	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
7	Saccharin Sodium	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
8	Orange Flavour	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
9	Talc	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	Magnesium Stearate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total weight of tablet		330	330	330	330	330	330	330	330

Batch process

Indion 234 resin in three different drug- resin ratios (1:1, 1:1.2, and 1:1.3) was studied. Activated resins (Indion 234) (10 g) were taken in a 1000 ml beaker; 300 ml of deionized water was added. The resins were allowed to swell in water for 30 min. Model drug (Ciprofloxacin) (10 gm) was added. The contents of beaker were stirred on a magnetic stirrer for 30 min. The complex was filtered using a whatman's filter paper. The residue was further washed with 700 ml of deionized water to remove any unbound drug. The filtrate was then analyzed at 278 nm. By subtracting the quantity of unbound drug from the concentration of the drug solution added, the amount of bound drug was calculated.

Results and Discussion:

Determination of threshold bitterness concentration of model drug (Ciprofloxacin)

It was found that all the volunteers felt bitterness, after 30 sec of time, for the concentration of 150µg/ml and above.

Table 2: Determination of threshold bitterness concentration

No. of Volunteer	Concentration of Drug (µg/ml)					Interpretation
	50	75	100	150	200	
01	1	1	1	2	3	0= Good 1=Tasteless 2=Slightly Bitter 3=Bitter
02	1	1	1	2	3	
03	1	1	1	2	3	
04	1	1	1	2	3	
05	1	1	2	2	3	
06	1	1	1	2	3	

Process optimization

The process to prepare drug-resin complex was optimized. Model drug (Ciprofloxacin) was loaded on ion exchange resin by batch process to optimize the drug-resin complex.

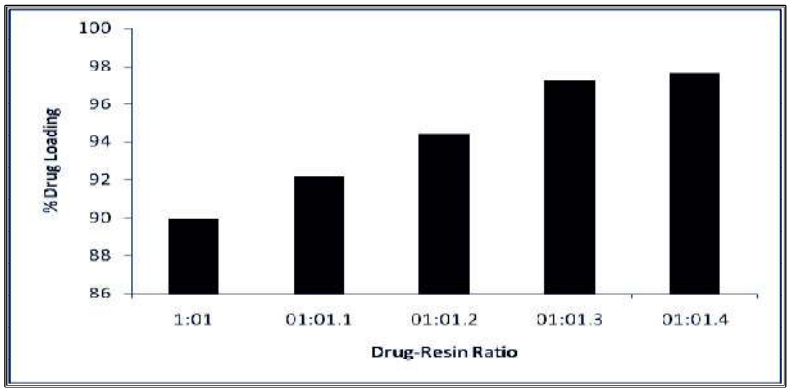


Figure 1: Optimization of drug-resin ratio

***In Vitro* Taste Masking Evaluation:** Results of *In Vitro* taste masking evaluation of drug-resin complex are tabulated, it was revealed that the drug released in phosphate buffer pH 6.8 from drug-resin complex at the end of 120 sec was less than the threshold bitterness concentration of model drug (Ciprofloxacin) i.e.150 µg/ml.

Table 3: *In Vitro* Taste Masking Evaluation of Indion 234

DRCs With Indion 234	Time (in sec)	Concentration (µg/ml)			
		1	2	3	Average Concentration
	60	99 .36	100.17	100.04	99.85 ± 0.435
	120	114.77	114.98	114.26	114.67 0.370

a. Sensory Evaluation of Taste Masked drug-resin complex: When the drug-resin complexes were subjected to sensory evaluation by human volunteers, the volunteers did not feel any bitter taste after keeping the drug resin complex in mouth for 30 sec, which confirmed that bitter taste of model drug (Ciprofloxacin) was masked successfully.

b. Determination of drug content

Table 4: Determination of drug content from drug-resin complex

S. N.	Drug-resin complex	% Drug content (in 30 min)
1	Drug-Indion 234 complex (1:1.3)	97.84 0.48

c. Drug releases from drug-resin complex: The release of model drug (Ciprofloxacin) from the drug-resin complex was observed in deionizer water , salivary pH of 6.8 and at gastric pH of 1.2 . The results are given in the following Tables and figure. The results indicated that insignificant amount of drug (less than 0.3%) was released in demonized water in 30 min, indicating the stability of complexes. In vitro drug release in salivary pH 6.8 was less than 5% within 60 sec. The drug-resin complex is stable in salivary pH for a period of administration. The amount released is insufficient to impart bitter taste while the formulation passes through the mouth to further parts of gastrointestinal (GI) tract. At gastric pH of 1.2, model drug (Ciprofloxacin) was completely released in 30 min.

Table 5: Drug release from drug-resin complex in deionised water

S. N.	Time (min)	Percent drug release
1	5	0.010±0.48
2	10	0.058±0.74
3	20	0.077±0.18
4	30	0.091±0.18

Table 6: Drug release from drug resin complex at salivary pH 6.8

S. N.	Time (sec)	Percent drug release
1	15	2.14±0.72
2	30	3.79±0.49
3	60	4.46 ±0.86
4	120	4.77±0.37

d. Evaluation of oral disintegrating tablet

Table 7: Post compression parameters of oral disintegrating tablets

Formulation code	Hardness (kg/cm^2)	Friability (%)	Wetting Time (sec)	*In vitro* Disintegration Time (sec)
F_1	2.97±0.45	0.512	88 ±1.21	65 ±1.26
F_2	2.99±0.41	0.447	72±1.41	53 ±1.34
F_3	2.94±0.38	0.411	62 ±1.35	39 ±1.67
F_4	3.01±0.37	0.276	46±1.02	32 ±1.01
F_5	2.55±0.49	0.534	76 ±1.61	59 ±1.23
F_6	2.67±0.61	0.476	60 ±0.98	40 ±1.64
F_7	2.79±0.17	0.379	40 ±1.11	28 ±1.89
F_8	2.68±0.45	0.345	34 ±1.94	22±1.91

e. Dissolution test of ODT F7 in Phosphate Buffer pH 6.8 (at salivary pH):

Dissolution testing of ODT F7 at salivary pH showed that at the end of 120 sec, less than 5% of drug was released which indicated adequate taste masking.

Table 8: *In Vitro* Drug Release of Batch F7 in Phosphate Buffer pH 6.8

S.N.	Time (sec)	% Drug Release	Standard deviation
1	0	0	± 0.00
2	5	0.66	± 0.21
3	10	1.33	± 0.21
4	20	1.83	± 0.15
5	30	2.64	± 0.14
6	60	3.76	± 0.24
7	120	4.37	± 0.26

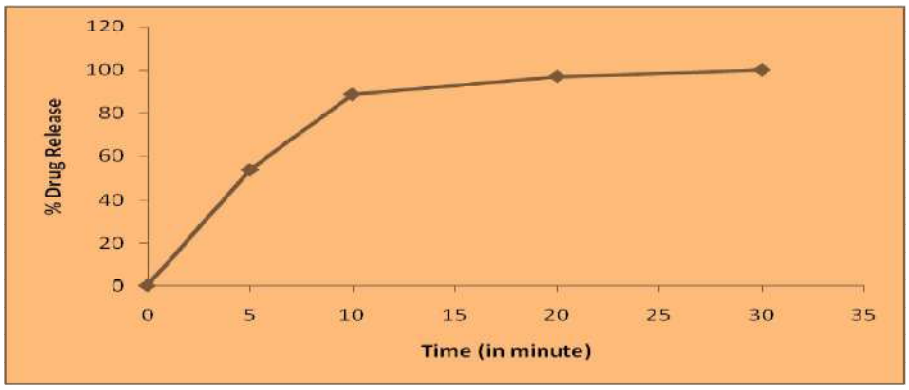


Figure 2: Drug release from drug resin complex at pH 1.2

Conclusion:

Drug release from drug resin complex was less than 5% at salivary pH 6.8. Bitterness evaluation results made by the consensus of trained persons confirmed that the bitter taste of Model drug (Ciprofloxacin) was masked by complexation with Resin. Micromeritics properties of drug-resin complex were evaluated. Amongst the various batches studied, Drug: Resin complex was successfully formulated into oral disintegrating tablet by direct compression method. Amongst the different superdisintegrants, combination of two superdisintegrants indicated improved disintegration time. Various drug formulations were compared with respect to *In Vitro* disintegration time and *in vitro* drug release profile. Formulation F7 was found to be palatable with *In Vitro* disintegration time of 28 sec. Dissolution studies showed complete release of F7 within 30 minutes. *In Vivo* taste masking evaluation revealed that the oral disintegrating tablet was adequately taste masked and had a pleasant mouth feel. In conclusion, an effective and pleasant tasting oral disintegrating tablet, of model drug (Ciprofloxacin) exhibiting satisfactory disintegration time and dissolution profile, was formulated using Indion 234 as a taste masking agent and crospovidone as a superdisintegrant by direct compression method.

References:

[1] Vummaneni V (2012) Int J of Res Pharm and Biomed Sci 3:510-518.

[2] Mahore G J & Wadher J K (2010) Int J of Pharm Sci Rev and Res 1:8-13.

[3] Harish G & Bhargavi C (2013) Ind J Res Pharm and Biotech 1:281-287.

PA-07

Formulation and Optimization of Fast Dissolving Tablets of Promethazine Theoclate using 3^2 Factorial Design

Shiv Hardenia[1], Gajanan Darwhekar[2]
[1]IPS Academy College of Pharmacy, Indore
[2]Acropolis Institute of Pharmaceutical Education and Research, Indore
E-mail address: shivsharma280485@gmail.com

Abstract: The fast dissolving tablets of Promethazine Theoclate were prepared by sublimation technique**,** using 3^2 full factorial design. This research work aimed to study and to develop a unique drug delivery system for immediate release of drugs which can dissolve readily when placed in the oral cavity. The different subliming agents (Camphor, Urea and Menthol) in varying concentration (5-15% w/w) were used to develop the tablets. Total 12 formulations were prepared and evaluated for pre-compression and post compression characteristics. The optimization of the batches was carried out using 3^2 full factorial design and results of polynomial equation were analyzed using ANOVA and regression analysis. By the use of desirability approach final optimized formulation was prepared.

Introduction:

Oral route of drug administration is one of the most appealing routes for the delivery of drugs. The task of developing rapidly disintegrating tablets is accomplished by using a suitable diluents and Superdisintegrants. The Promethazine Theoclate is classified under anti-emetic drug category which undergoes extensive gastric and first pass metabolism via oral administration causing low bioavailability, thereby reducing the efficacy of controlling vomiting. Fast dissolving tablets of Promethazine Theoclate were designed for rapid and complete oral absorption for achieving a therapeutic success. Thus the objective of the work was to formulate and optimize mouth dissolving tablets of Promethazine Theoclate, having adequate mechanical strength, rapid disintegration and fast action. A3^2 full factorial design is applied for the optimization of the fast dissolving tablets.

Materials and Methods:

Promethazine Theoclate was obtained from Cipla (Baddi, India), Menthol was obtained from S.D. Fine (Mumbai, India). Camphor and urea were obtained from Loba Chem Pvt. Ltd., (Mumbai, India).

Preparation of Promethazine Theoclate Tablets by Sublimation Technique

For preparation of fast dissolving tablets subliming agents (camphor, urea and menthol) were incorporated in varying concentration (5-15% w/w). All ingredients were co-grounded in glass pestle mortar. The mixed blends of excipients were compressed using a single punch machine to produce flat faced tablets weighing 100 mg. Tablets were subjected for drying for 6 h under vacuum (30 kpa) at 50º for sublimation to make tablets porous.

Evaluation

Precompression characterization: Bulk Density, Tapped Density, Compressibility index, Angle of repose and Hausners Ratio.

Post compression characterization

Friability, Wetting Time, *In Vitro* Dispersion time and Disintegration time

Table 1: Formulation by varying Subliming Agents

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10 *	F11 #	F12 $
PMT	6	6	6	6	6	6	6	6	6	6	6	6
Camphor	5	10	15							5	10	15
Menthol				5	10	15						
Urea							5	10	15			
Crospovidone										2	2	2
Avicel PH102	45	40	35	45	40	35	45	40	35	43	38	33
Dextrose	20	20	20	20	20	20	20	20	20	20	20	20
Lactose monohydrate	20	20	20	20	20	20	20	20	20	20	20	20
Talc	2	2	2	2	2	2	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2	2	2	2	2	2	2

Preparation of Factorial Design Batches

The raw materials were passed through a no. 100 screen prior to mixing. Promethazine Theoclate, Crospovidone, Camphor, urea, Avicel and lactose were mixed using a glass mortar and pestle. The blends were lubricated with 2% w/w Talc and 2% w/w Magnesium Stearate. The blends ready for compression were converted into tablets using a single-punch tablet machine. The composition of the factorial design batches is shown in Table 2 respectively.

Results and Discussion:

The fast dissolving tablets of Promethazine Theoclate were successfully prepared by sublimation technology. Total twelve formulations were prepared and optimized using $\mathbf{3^2}$ full factorial design; that after the ANOVA was applied and the formulations showed the significant model. Using the desirability approach the final optimized formulation was prepared with the targeted results.

Table 2: Optimized Formulation (PMT)

Formulation	**OPT**
Promethazine Theoclate	6
Camphor	10.62
Crospovidone	2.59
Lacotse Monohydrate	20
Avicel PH 102	36.79
Dextrose	20
Talc	2.00
Magnesium Stearate	2.00
Evaluation	
Weight (mg)	98.014±2.225
Hardness (kg/ cm^2)	3.8±0.135
Friability (%)	0.480±0.028
Disintegration time (s)	28±2.01
Wetting time (s)	22±1.98
Drug Content (%)	97.35±2.325

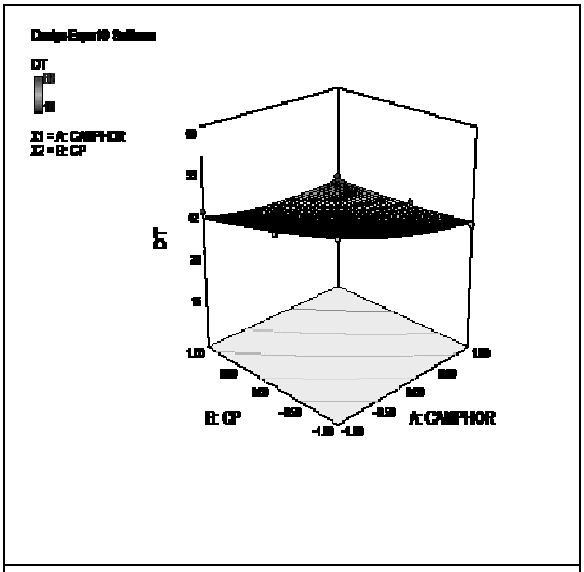


Figure 1. Response Surface for Disintegration Time

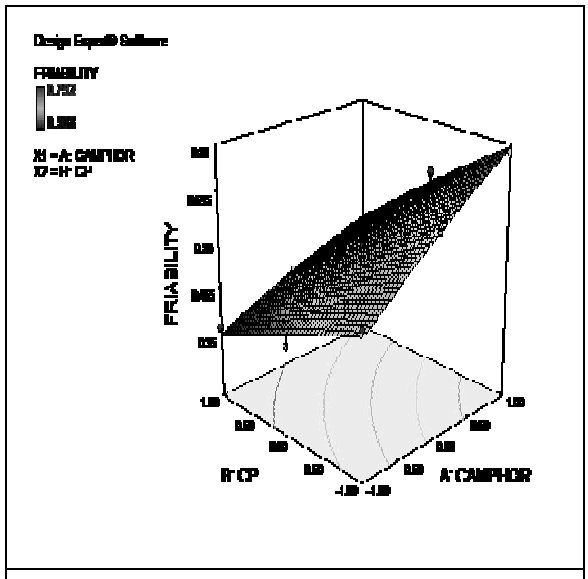


Figure 2. Response Surface for Friability

Conclusion:

The results of a 3^2 full factorial design revealed that the amount of Camphor and Crospovidone significantly affect the dependent variables, disintegration time, and percentage friability. It is thus concluded that by adopting a systematic formulation approach, an optimum point can be reached in the shortest time with minimum efforts.

References:

[1] Mishra DN & Vijaya KSG (2006) Indian Drugs 43:117-121.

[2] Zhao N & Augsburger LL (2005) AAPS Pharm Sci Tech 6:634-640.

[3] Khan S, Kataria P, Nakhat P & Yeole P (2007) AAPS Pharm Sci Tech 8:E1-E7.

[4] Sreenivas SA, Gadad AP, Dandagi PM, Mastiholimath VS & Patil MB (2006) Indian Drugs 43:35-38.

PA-15

Formulation and Evaluation of Ivermectin Solid Dispersion

Somya Verma, Urmi Patel, Rakesh P. Patel
S.K.Patel College of Pharmaceutical Education & Research, Ganpat University, Mehsana, Gujarat- 384012
E-mail address- somyav942@gmail.com

Abstract: This study was aimed to formulate and evaluate solid dispersion containing ivermectin to improve solubility. Ivermectin is BCS (Biopharmaceutical classification system) class-II drug, which has high permeability and low water solubility (0.005mg/ml) which is responsible for its poor dissolution rate and ultimately leads to variable absorption. Solid dispersion with Gelucire (44/14) has the ability to improve dissolution of poor water soluble drugs. So, solid dispersion ivermectin with Gelucire 44/14 was prepared to enhance the solubility and was further evaluated for different parameters such as assay, wettability, DSC, FT-IR, dissolution study.

Introduction:

Helminthiasis is the type of intestinal tract infection. Sometimes they may involve with other organs. The human body is with a parasitic worm such as roundworms and pinworms. The worms are usually present only in the intestinal tract. The severity of symptoms is classified by the type of worm and the part of the body infected. Ivermectin is the medication that is effective against many types of parasites. It is used to treat- Helminthiasis, Head lice, Scabies, Lymphatic filariasis, River blindness and Strongyloidiasis. Ivermectins are macro cyclic lactones derivatives [1]. It is derived from the bacterium Streptomycin avermitilis. Ivermectin kills by interfering with nervous system and muscle function, in particular by enhancing inhibitory neurotransmission. 3.0 mg and 6.0 mg tablets of ivermectin are available as marketed preparations [2]. Solid dispersion is defined as one or more active ingredients in an inert carrier or matrix at solid state prepared by the fusion-solvent or melting-solvent method. A product formed by converting a fluid drug-carrier combination to the solid state. Solid dispersion defines the texture of two different parts. One is the water soluble carrier and another is water in-soluble (polar) compounds. A carrier it can be either crystalline or amorphous in nature. They are based according to their drug molecular structure [3].

Materials and Methods:

Materials

Ivermectin, Gelucire 44/14, Distilled Water, Aspartame, Methanol, Hydrochloric acid, Lactose, HPMC (hydroxypropyl methylcellulose), SSG (sodium starch glycolate), MCC (microcrystalline cellulose), Magnesium Stearate, Talc.

Methods

For preparation of solid dispersion: Different methods involves physical mixture, melting, kneading

method, lyophilization, solvent evaporation, melt agglomeration process, extruding method, spray drying technology, use of surfactant, electro static spinning method and super critical fluid technology [4].

Physical Mixing

Physical mixing method of Gelucire 44/14 with drug is prepared by geometric mixing of drug & carrier respectively without applying pressure. An excess quantity of drug & carrier is taken in a glass mortar and mix for 20 minutes.

Melting Method

In Melting method the carrier Gelucire 44/14 is dissolved at 40^0c. After the melting process, add drug. They are proper miscible of mixture. Immediately the mixture is cooled in freezing point. or at room temperature. After cooling the samples they were scrapped out. And dried powder is passes through sieve no 100#**.**

Kneading Method

Solid dispersion by Kneading method is prepared by symmetrical mixing of drug with Gelucire 44/14. After mixing they were kneaded with appropriate ratio of solvent's fusion to get and extent with a pasty consistency, which is dried in hot air oven at 45˚ to 50˚C. The dried powder is passes through sieve no 100#**.**

Lyophilization Technique

In lyophilization process, the matrix & drug are dissolved in suitable solvent. After the geometric mixing of solution they are cooled& freeze dried in Vir-Tis Benchtop K lyophilizer (freeze drier) to 24 hrs. After lyophilization, get % yield amorphous powder. The dried solid dispersions were sieved through 100#.

Characterization of solid dispersion

Prepared solid dispersion can be characterized by differential scanning calorimetry (DSC) analysis, infrared (IR) analysis, wettability study, in-vitro dissolution study and effect of different formulation variables on various evaluation parameters [5].

Formulation of tablets using solid dispersion

Tablets were formulated using solid dispersion containing equivalent quantity of 3 mg of Ivermectin by different techniques like (I) Physical mixing, (II) Melting method, (III) Kneading method, and (IV) Lyophilization. Plain tablet of Ivermectin (3 mg) without Gelucire 44/14 was also prepared for

comparison in the dissolution behaviour. All these tablet formulations were prepared using direct compression techniques with necessary excipients shown in table 1.

Table 1: Excipients used in tablets containing plain Ivermectin, and with solid dispersion

Excipients	Plain Ivermectin Tablet (mg)	Tablet with Ivermectin & solid dispersion(mg)
Drug	3	33
SSG	10	10
MCC	20	20
Magnesium stearate	2	2
Talc	2	2
Aspartame	5	5
Menthol	2	2
Lactose	Upto q.s.	Upto q.s.
Total	300	300

Characterization of tablets

All these tablet formulations should be evaluated by various parameters such as size, shape, weight variation, hardness, friability, thickness, drug content, disintegration and in-vitro dissolution study.

Result and Discussion:

Table 2: Evaluation parameters of MDT tablet prepared using solid dispersion by melting method

Parameter	Value
Size-Diameter	7.2 mm
Shape	Round
Color	White
Thickness	4 mm
Hardness	4.5 Kg/cm^2
Weight variation	275.6 mg
Drug content	96.25 %
Disintegration time	3-4 min

***In-vitro* dissolution study of tablet formulation**

In-vitro dissolution study of tablet formulation was carried out in 0.1 N HCl and presented in Figure 1.

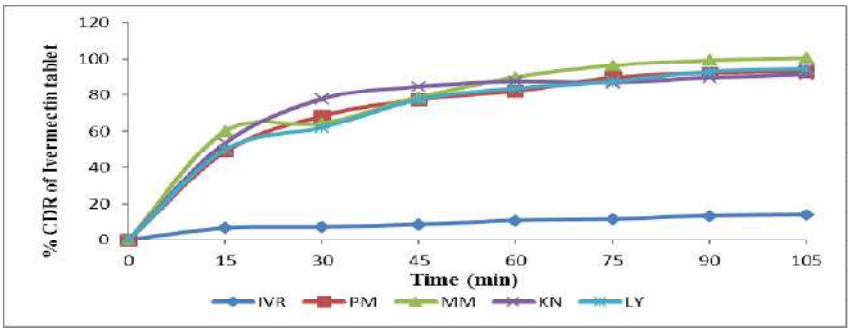


Figure 1: Dissolution profile MDT tablet of plain Ivermectin and PM, MM, KN & LY in 0.1 N HCL.

From this dissolution studies, it can be seen that the tablets prepared using solid dispersion by melting method showed highest cumulative % drug release (100% in both 0.1 N HCL) in comparison of plain Ivermectin. The tablets prepared using solid dispersion by other methods like physical mixing, kneading method, and lyophilization also showed in improvement of drug release in comparison to tablets prepared with plain Ivermectin. All these tablet formulations showed the maximum drug release in 90 minutes. Hence, melting method formulation was found the best formulation which gave highest % drug release at the end of 105 mins.

Table 3: % CDR of Tablet formulations in 0.1 N HCL in dissolution

Time (min)	**% CDR of Tablet formulations**				
	Ivermectin	**PM**	**MM**	**KN**	**LY**
15	6.45	48.92	60.22	53.76	50.00
30	7.12	68.72	64.65	77.26	62.29
45	8.33	77.60	78.82	84.87	77.49
60	10.65	82.33	90.02	87.69	83.89
75	11.39	89.82	96.58	87.11	87.59
90	13.22	92.58	99.47	89.81	93.00
105	14.00	93.22	100.77	91.45	94.72

Conclusion:

After optimizing the ratio of drug and carrier (1:10 %w/w) by phase solubility studies, Ivermectin solid dispersion was successfully prepared by different methods like physical mixture. These solid dispersions confirm the entrapment of Ivermectin in the Gelucire 44/14 by DSC and FTIR studies. All prepared solid dispersion of Ivermectin showed improvement in the Wettability and dissolution profile of Ivermectin. Highest improvement in the in-vitro dissolution profile was obtained in the solid dispersion prepared by melting method. Tablet formulation prepared using solid dispersions by melting method showed highest improvement in the dissolution profile in comparison to other solid dispersions as well as plain Ivermectin. Finally it can be concluded that poor dissolution profile of water insoluble Ivermectin can be overcome by preparing its solid dispersion with Gelucire 44/14.

References:

[1] Brig HK, Jain K, & Jain RC (2014) Med J Armed Forces India 70:144–148.

[2] Dourmishev AL, Dourmishev LA & Schwartz RA. (2005) J Dermatol 44:981–988.

[3] Abbas IM & Mahmoud ED (2014) Saudi Pharm J 22:149–156.

[4] Fox L & Anne M (2006) Int J Pharm Sci 19:588-593.

[5] Gupta MM, Patel MG, Patel NS, Kedawat M (2011) Int J Pharm Sci 3:204-206.

PA-17

Preparation and Evaluation of Irbesartan-Cyclodextrin Inclusion Complexes

Rajashree Hirlekar[1], V. J. Kadam[2]
[1]Vivekanand Education Society's College of Pharmacy, Chembur, Mumbai-400074
[2] BharatiVidyapeeth's College of Pharmacy, Navi Mumbai-400614
E-mail address: rajashree.hirlekar@ves.ac.in

Abstract: In the present work effect of methyl-β-cyclodextrin (MβCD) on physical properties and dissolution rate of Irbesartan (IRB) was studied.Based on A_L-type ofphase solubility diagram obtained, solid binary systems of the drug with MβCD were prepared in 1:1 molar ratio by various methods. Complexes were characterized using Differential Scanning Calorimetry and powder X-Ray Diffractometry. It could be concluded that IRB can form inclusion complex with MβCD. The dissolution profiles of inclusion complexes were compared with those of IRB alone and the physical mixture. The dissolution rate of IRB was increased remarkably by MβCD inclusion complexation.

Introduction:

Irbesartan (IRB) is used in the treatment of hypertension. Its poor aqueous solubility leads to limited bioavailability after oral administration [1,2]. Cyclodextrins (CDs) are macrocyclic oligosaccharides with six to eight D-glucose units called α- cyclodextrin, β- cyclodextrin and γ-cyclodextrin and have been widely used to improve the solubility and dissolution rate of poorly soluble drugs [3]. Significant improvement in the solubility resulting in increased rate of dissolution and absorbability is obtained by use of methylated βCD.

Co-evaporation, co-grinding andkneading methods were used to prepareinclusion complexes. Instrumental techniques like X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) were used for characterization of complexes. Dissolution patterns of plain drug,physical mixtures and inclusion complexes were evaluated.

Materials and Methods:

Materials: IRB was kindly supplied byAarati Drugs Ltd., India. MβCD (D.S. = 1.8 and M. W. = 1310) was gifted by Wacker fine chemicals.

Methods: Method described by Higuchi and Connors was used to conduct phase solubility studies [4]. An excess amount of IRB was added to MβCD aqueous solution (0-10mM) and subjected to shaking. After equilibrium attainment, concentration of IRB was determined. The apparent stability constant Ks was calculated from the phase solubility diagram according to the following equation:

$$Ks = \frac{Slope}{S_o(1 - Slope)}$$

S_0 is the solubility of IRB in absence of MβCD.

Preparation and evaluation of Binary Systems of IRB–MβCD:

Various methods such as co-grinding (CG), kneading (KN) and co-evaporation (CE) were used to prepare binary systems of IRB-MβCD with 1:1 molar ratio. The physical mixture (PM) was also prepared for the purpose of comparison. Intense trituration of IRB and MβCD was carried out to obtain CG product. Trituration of IRB with MβCD in glass mortar followed by kneading with 66% alcohol for 45 minresulted in KN product. The pasty mass obtained was dried at 60°C. The dried mass was passed through sieve no.80 and stored overnight in desiccator. For preparation of CE product, equimolar amounts of MβCD and IRB were dissolved in minimum volume of 1:1 mixture of 66% ethanol and water. The final solution was stirred with the help of magnetic stirrer at 60°C till pasty mass was obtained. The pasty mass was treated as above to get the dry sample.

Binary systems were characterized by DSC, XRD alongwith dissolution studies. Dissolution rate studies of IRB alone and from various IRB-MβCD systems were conducted using USP XXIII dissolution apparatus type-II (6 stations, VDA-6DR, Veego Scientific, India) at 37 $\pm$ 0.5°C stirring at 50 rpm. Seventy five mg of IRB or its equivalent amount of IRB-MβCD binary system was added to 1000 ml of distilled water. At predetermined time intervals, the samples were withdrawn and analyzed spectrophotometrically.

Results and Discussion:

The phase solubility profile of IRB-MβCD as presented in Figure1 is classified as A_L type. The curve showed a linear increase in IRB solubility as a function of MβCD concentration with a slope of 0.0904 (R^2 = 0.999) in the concentration range (0-10 mM) investigated. The apparent stability constant $K_{1:1}$ was 177.8 M^{-1}. Slope value was lower than one indicating inclusion complex in the molar ratio of 1:1.

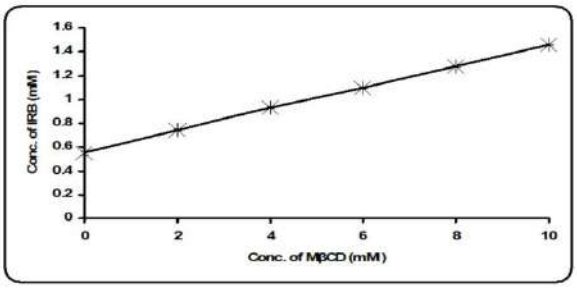


Figure 1: Phase solubility profile of IRB-MβCD in water

Thermograms of IRB and the binary systems are shown in Fig. 2. The thermogram of IRB was typical of a highly crystalline compound, characterized by a sharp endothermic peak at 181°C, corresponding to its melting. A broad endotherm in the range of 100-120°C in case of MβCD indicated release of water molecule from its cavity. PM and CG systems showed retention endothermic peaks corresponding to drug whereas it was shifted to lower temperature in the case of CE system. KN

system showed a significant shift in location of endothermic peak of IRB to 158.9ºC with substantial broadening which can be attributed to complex formation [5].

Thus kneading method can be utilized for complexation of IRB using MβCD.

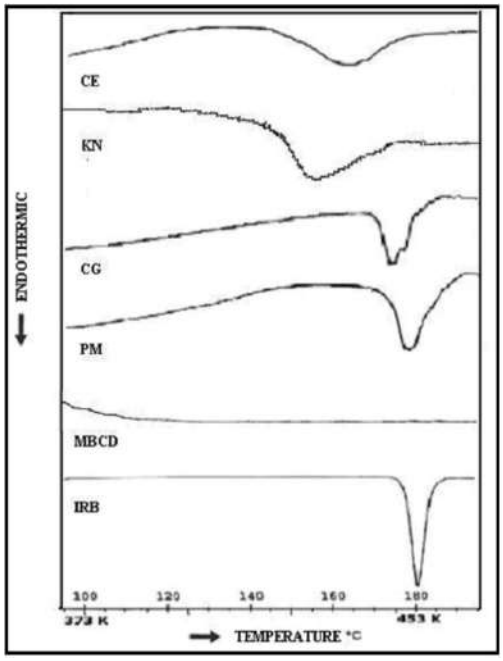


Figure 2: DSC thermograms of IRB-MβCD systems: Irbesartan (IRB), Methyl-β-cyclodextrin (MβCD), physical mixture (PM), co-ground (CG), co-evaporated (CE) and kneaded (KN) systems

XRD patterns of IRB, PM and KN complex are illustrated in Fig. 3. Two broad diffused peak of MβCD indicated its amorphous nature. PM and CG systems showed peaks attributable to IRB. The KN and CE systems exhibited more diffused diffraction patterns, indicating loss of crystallinity. Similar disappearance of crystalline drug peak was observed by Bandi et al in case of HPβCD complexation of some drugs [5].

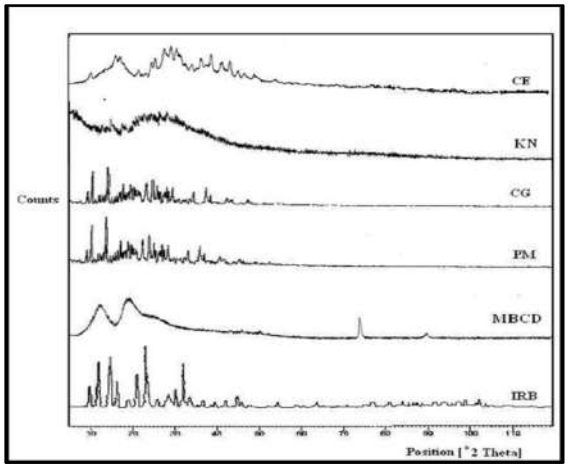


Figure 3: X-Ray Diffractograms of IRB-MβCD systems: Irbesartan (IRB), Methyl-β-cyclodextrin (MβCD), physical mixture (PM), Co-ground (CG), co-evaporated (CE) and kneaded (KN) systems

Dissolution Studies

Amongst the IRB-MβCD systems, KN system showed the highest improvement in the dissolution rate. Thus it can be concluded that KN method was more efficient in improving the solubility when using MβCD. The KN method is of the particular interest for industrial scale preparations because of the low cost and the simple process, which involves less energy, time and equipment.

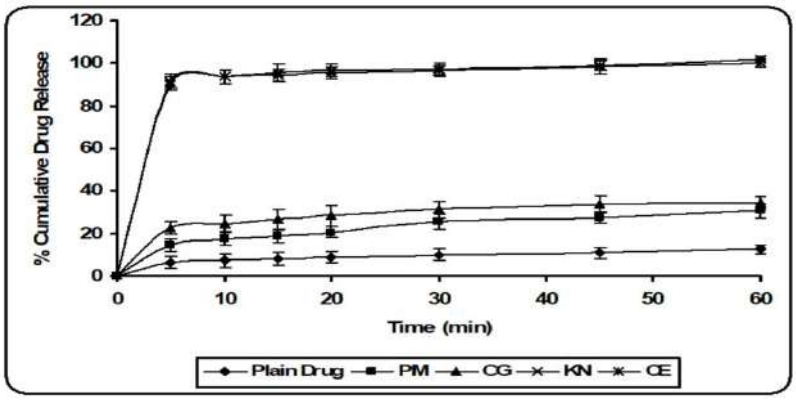


Figure 4: The dissolution diagram of IRB-MβCD systems

Irbesartan (IRB), physical mixture (PM), co-ground (CG), co-evaporated (CE) and kneaded (KN) systems

Conclusion:

From instrumental methods of characterization, it can be concluded that IRB forms inclusion complexes with MβCD. The complexes exhibited improvement in solubility and dissolution rate of drug.

Acknowledgement:

Authors are thankful to Aarati Drugs Ltd. and Wacker fine chemicalsfor providing the gift samples.

References:

[1] Irbesartan In: (2004). (Ed.), Martindale's: The complete drug reference, 34rded, Pharmaceutical Press, London, pp. 940.

[2] Pouleur HG (1997) Am J Hyperten 10:318-324.

[3] Beker O, Uijtendaal EV, Beijnen JH, Butt A & Underberg WJM (1991) Drug Dev Ind Pharm 17: 1503-1549.

[4] Higuchi T & Connors KA (1965) In: C. N. Reilly (Ed.), Advances in Analytical Chemistry and Instrumentation, Interscience, New York, pp117-212.

[5] Bandi N, Wei W, Roberts CB, Kotra LP & Kompella UB (2004) Eur J Pharm Sci 23:159-168.

PA-35

An Approach for Designing of Transdermal Patches for Prophylaxis and Treatment of HIV/AIDS

Ravikant Gupta[1], Alok Pal Jain[1], Akash Yadav[2], Awdhesh Maheshwari[3]
[1]Institute of Science & Technology, SRK University, NH-12, Hoshangabad Road, Misrod, Bhopal, M.P., India
[2]IPS Academy College of Pharmacy, A.B. Road, Rajendra Nagar, Indore, Madhya Pradesh, India
[3]Mylan Laboratories Limited, Pithampur Industrial Area, Kalibillod, Pithampur, Madhya Pradesh, India
E-mail address: ravikant491990@gmail.com

Abstract: Transdermal drug delivery is an alternative route for systemic drug delivery system, which minimizes the absorption of drugs and increases the bioavailability through systemic circulation. Currently available anti-HIV drugs bear some significant drawbacks, such as relatively short half life, low bioavailability, poor permeability and undesirable side effects. The purpose of this work is to formulate and evaluate transdermal drug delivery system of anti-HIV drug using various polymers such as HPMC, PVP and ethyl cellulose by solvent evaporation technique for improvement of bioavailability of drug and reducing toxic effects. By this mean we can achieve dosage form with reduce dosing frequency, increase the bioavailability, decrease the degradation and metabolism in the gastrointestinal tract, improve the CNS penetration and inhibit the CNS efflux and deliver them to the target cells effectively with minimal side effects. This paper provides details of preformulation study, drug selection criteria, formulation strategies, evaluation perspective, Stability approaches for sustain drug release kinetics.

Introduction:

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV) is an immunosuppressive disease that results in life-threatening opportunistic infections and malignancies. HIV infection is one of the major threats to human health's due to lack of relevant vaccine and drugs to cure AIDS. There are lots of drugs and dosage forms in combination & sustain delivery forms are available in the market but failed to achieve targeting. In this era many scientists working on target drug delivery for ARV. Even novel formulations are also in developmental stages. In this work we will discuss about novel formulations in the form of Transdermal patches for ARV drugs.

The numerous administrations of several drugs in moderately high doses are a main cause of patient incompliance and hurdle towards the fulfillment of the pharmacotherapy. Intracellular and anatomical viral reservoirs are accountable for the perpetuation of the infection. Active transport mechanisms involving proteins of the ATP binding cassette super family prevent the penetration of ARV drugs into the brain and may account for the inadequate bioavailability after oral administration. The proposed work is aimed to formulate and characterize the transdermal patches of antiretroviral drugs for efficient transdermal delivery of drug in pharmaceutical system.

Materials and Methods:

Materials for the study are: Drug, Polymers (Poly vinyl pyrolidine, Hydroxyl Propel methyl cellulose and ethyl cellulose) other chemicals are Analytical grade reagents. Method for preparation of the Transdermal patches will be solvent evaporation/Solvent casting method. Below mentioned steps will follow for experimentation:

- **Selection of Drug and Exicipients**
- **Preformulation Study**

  A concise stepwise account of the various tasks that need to be executed for successful accomplishment of the research envisaged.

  Analytical method selection

    - UV-VIS spectrophotometer
    - FTIR

UV-VIS spectrophotometry is suitable for initial screening studies as the method is convenient and less time consuming. Both qualitative and quantitative estimations can be performed.

FTIR is a good method for estimating drug excipient interactions. Qualitative estimations can be very well carried out

- ✓ Solubility studies
- ✓ Partition coefficient

- For estimation of solubility, partition coefficient etc. solvent selection UV-VIS spectrophotometry method used.

  - Assay method
  - Melting point
  - pH analysis
  - Drug-excipient interaction study

- **Formulation development**
  - ✓ Trial batch formulations for preparation of free films
  - ✓ Preparation of medicated films
- **Evaluation**

  The prepared medicated films will be evaluated for:
  - ✓ Thickness
  - ✓ Weight variation
  - ✓ Percent flatness
  - ✓ Moisture content
  - ✓ Moisture uptake
  - ✓ Tensile strength
  - ✓ Modulus of elasticity

- ✓ Percent elongation
- ✓ Drug content
- ✓ Area variation
- ✓ Folding endurance
- ✓ *In vitro* performance
- ✓ *In vitro* dissolution studies
- ✓ *In vitro* permeation studies
- ✓ *In vitro- In vivo* correlation study
- ✓ Bioavailability Study
- ✓ Analysis of permeation data
- ✓ Data analysis
- ✓ Skin irritation test in animal models.
- ✓ Stability Study

Results and Discussion:

In this work an attempt is to formulate transdermal patches for sustained release of antiretroviral drug by solvent casting method. An ideal dosage regimen in the drug therapy of any disease is one, which immediately attains the desired therapeutic concentration of drug in plasma (or at the site of action) and maintains it constant for prolong period of time. Transdermal drug delivery overcomes the number of drawbacks associated with conventional dosage drug delivery system. The main objective of formulating the transdermal system is to prolong the drug release time, reduce the frequency of administration and to improve patient compliance. Therefore an ideal controlled drug delivery system is the one, which delivers the drug at a predominant rate, locally or systematically, for a specific period of time. In this study, it is intended that the side effects and of the oral administration of elected drug be overcome by delivering drug transdermally and bioavailability will also improve.

Conclusion:

In this study an attempt to make transdermal patches an alternative dosage form against conventional medication for the Anti retroviral drugs. Transdermal drug delivery overcomes the number of drawbacks associated with conventional dosage drug delivery system. The main objective of formulating the transdermal system is to prolong the drug release time, reduce the frequency of administration and to improve patient compliance. Preformulation study reflects the suitability of the drug selection and drug exipient study shows the compatibility of the drug in dosage forms and stability study will reflects the shelf life of the drug. From *In vitro- in vivo* models we can conclude the effective delivery of drug.

Acknowledgement:

It gives me genuine pleasure to express the depth of sincere regards and gratitude to my esteemed guide with vast knowledge Dr. Alok Pal Jain Professor, IST, SRK University Bhopal, for his noble guidance, scholarly supervision, endless motivation and constant support towards my project work. It is a great pleasure in expressing my profound sense of gratitude towards Dr. Alok Pal Jain (Professor and Principal) IST, SRK University Bhopal, for providing all necessary facilities and valuable guidance for this research work in this esteemed institute.

References:

[1] Jain S (2010) RJPBCS, 1:78.

[2] Sosnik A (2010) JCR, 138:2-15.

[3] Frezzini C, Leao JC & Porter S (2005) J Oral Pathol Med (2005) 34:513-531.

PA-37

Arnica Hydrogel: An Oil Replacement for Hair Loss Treatment

Mahima Singh Haihaywanshi[1], Tahera Gouri[2], Apoorva Gupta[1], Sanjay Jain[1]
[1] Indore Institute of Pharmacy, Indore, India
[2] SKRP Gujarati Homeopathic Medical College & Recherch Centre, Indore, India
E-mail address: mahima1810@gmail.com

Abstract: Hair loss problems can affect physical and mental health of human and have particular significance during pregnancy, lactation and aging process. At present a number of oils and shampoos are available in the market to promote hair growth. Applying the medicated hair oils and shampooing the hairs daily reduces the patient compliance. Thus no therapy is giving satisfactory results to the hair loss. Aim of this work is to prepare non sticky, non greasy, quickly absorbable Arnica Hydrogel to stimulate hair. *Arnica montana* is widely used herbal and homeopathic medicine for hair growth stimulation and available as arnica oil and arnica shampoo. It rejuvenate scalp, stimulates the hair follicles by improving the blood flow and prevent premature hair fall. *Arnica montana* also have anti-inflammatory and anti dandruff properties which assist its hair growth property. Hydrogel have been emerged out as one of the most beneficial topical drug delivery system. It is three-dimensional cross-linked polymer network. Hydrophilic structure of which make them capable of holding the drug and water in its three dimentional network. These biomaterials can hold large quantity of water and swell. When swelled, they become soft & spongy and exhibit excellent biocompatibility. Arnica Hydrogel was prepared by incorporating ethanolic extract of *Arnica montana* in hydrogel at a particular step in order to prepare non greasy formulation. Hydrogel was optimized for the concentration of Carbopol 934. Different Hydrogel formulations were evaluated for their physical appearance, viscosity, pH, spreadability and stability.

Introduction:

Hair loss commonly occurs to most of the people of all age groups. Although it is not life frightening disorder but a thought of becoming bald may lead to emotional stress lack of confidence, inferiority and traumatic experience. Hair consists of two parts: (1) Hair follicle, present in the dermis of the skin, it contains stem cells and allows hair regrowth. (2) Hair shaft, hard filamentous part extends above the skin surface. Hairs are derived from ectoderm of the skin and composed of epithelial cells [1, 2]. Growth of hair exhibits a regular cycles of regeneration, known as the hair cycle. Hair cycle consist of three phases. An active growing phase in hair growth cycle in which the previous hair is shed off is known as Anagen phase. This phase is followed by a small transitionary phase known as Catagen phase and this is followed by a dormant resting phase known as Telogen phase in which hair follicle produce different types of hairs. After completing one hair cycle, new cycle restarts and a new hair shaft begins to form [2]. One factor is not sufficient to blame for hair loss. There are many factors responsible for the hair loss are genetic predisposition, pregnancy, lactation, many hormonal factors,

and disease states such as typhoid, malaria, jaundice, and use of chemotherapeutic agents [1, 2]. Some hair disorders are also responsible for hair loss such as dandruff, androgenetic alopecia, and pigmentation. The etiology of hair loss is still not entirely understood and also its absolute medical treatment is not adequately developed [1, 2].

Till now hair growth treatments have covered the multi-billion dollar market worldwidebut still there is no significant improvement in the hair growth. Formulations containing Minoxidil and Finasteride are two approved drugs, usefull in the treatment of Alopecia but their side effects limit their uses. To deal with the side effects of the allopathic drugs pharmacist interest have moved towards the herbal drugs as they are lack of any adverse effects as compare to synthetic formulations [1, 2]. There are no of herbal drugs with proven records to treat Alopecia. These drugs are formulated as hair care product containing hair tonics and hair grooming aids [1]. Hair tonics are herbal hair oil which contains herbal extracts in oil base. Nature of oil is sticky and perfumed. Proper application of these hair oils may use to treat baldness, discoloring of hair, hair falling and dryness of hair. Various patents are existing those claims for the effectiveness of these herbal oils these oils stimulate hair follicles and scalp metabolism by stimulation of dermal papilla, antitestosterone activity and enhanced nutrition to the hair follicle through improved blood circulations to the scalp but their mechanism of action are still not clear [1]. Many herbal oils containing extract of Jatamansi, Sikakai, Ritha, Bhringraj, Shankhapushpi, Amla, Hibiscus, Brahmi and Methi with proven hair stimulating actions, are available in market alone or in combination [1]. Similarly, *Arnica montana* is a widely used herbal preparations for hair growth stimulation It is also used in homeopathy for hair growth stimulation and available as arnica oil and arnica shampoo for years. *Arnica montana* is also used as external application to reduce dandruff. Daily application of Arnica hair oil gives remarkable results but daily application of oil and shampooing the hairs, leads to reduced patient compliance. So the aim of this work is to prepare a non sticky, non greasy, quickly absorbable Arnica Hydrogel to improve patient compliance and thus hair growth.

In recent times Hydrogels have gained significant attention for the delivery of drugs topically. Hydrogels are competent to deliver various active moiety including genetically engineered pharmaceuticals, like protein and peptides and to get better therapeutic efficacy and safety of drugs [4]. Hydrogels are a network of water soluble natural or synthetic polymeric chains that form a colloidal swollen gel in water as dispersion medium. They contain 99% water. Due to this significant quantity of water, they are flexible, similar to natural tissue and biocompatible. Hydrogels are three-dimensional cross-linked polymer network. Hydrophilic structure of Hydrogel make them capable of holding the drug and water in its three dimensional network [5].

Material and Methods:

Materials 10% ethanolic tincture of *Arnica montana* was obtained as gift sample from SKRP Gujarati Homeopathic Medical College & Recherch Centre, Indore. Carbopol 940 and sodium hydroxide were

procured from Loba Chemie, Mumbai, And Isopropyl myristate was procured from Alpha Chemika, Mumbai. Double distilled water was used for all experiments. All chemicals were pharmaceutical grade and used without further modification.

Method of Preparation of Hydrogel

Hydrolgel was prepared by the method reported by Monica AS and Gautami J (2014) with slight variations. Total 4 formulation of Arnica Hydrogel was prepared with varying concentration of carbopol 940 and Arnica Montana. Hydrogel was prepared by dispersing carbopol 940 in distilles water, left it for 24 hrs in dark to allow complete swelling. To this 1% v/v isopropyl myristate and 0.0025% w/v benzalkonium chloride were added under magnetic stirrer, and then drug extract was added to form 2% and 5% strength of the formulation after addition of sodium hydroxide solution. Finally, make up the volume with distilled water under magnetic stirrer to form a homogeneous dispersion of gel.

Characterization of hydrogel [4, 5]

Physical Appearance all the formulations of Hydrogels were inspected visually for their color, homogeneity, consistency.

Measurement of pH pH was measured by digital pH meter in triplicate. 1% aquous solution of prepared hydrogel was prepared and pH was determined.

Spreadability Spreadability is used to explain the scope of area to which topical preparations readily spreads on skin or affected part. Topical formulation should have good spreadability. The parallel-plate method is used for determining and quantifying the spreadability of Hydrogels. It is expressed in terms of time in seconds taken by two slides to slip off. Hydrogel was placed in between the slides and upper slide was tied with certain load. Lesser the time taken for separation of two slides, better spreadability of the Hydrogel. Spreadability was calculated by using the formula.

$$S = \frac{(M \times L)}{T}$$

Where

S= Spreadability,

M= Weight tied to upper slide,

L= Length of glass slides and

T= Time taken to separate the slides completely from each other.

Rheological Study Rheology is the study of flow and deformation of materials under applied forces. The viscosity of different Hydrogel formulations was determined at 37˚C using a brook field viscometer at 100 rpm.

Test for Grease-Test was carried out by placing a drop of formulation on filter paper and compare the test filter paper with standard one. Standard was prepared by placing a drop of Arnica hair oil on filter paper. Observation for translucent or greasy spot was taken.

Accelerated Stability Studies Stability studies were carried out on all Hydrogel formulations according to International Conference on Harmonization (ICH) guidelines. The formulations were packed in different aluminium tubes and were subjected to accelerated stability testing for 3 months as per ICH norms at a temperature (40 ± 2˚C) and relative humidity 75 ± 5%. Samples were taken at regular time intervals of 1month for over a period of 3months and analyzed for the change in pH, spreadability and rheological properties.

Results and Discussion:

Physical Examination The prepared Hydrogel formulations were offwhite to brownish in color and were a smooth, translucent and homogenous appearance.

Table 1: Composition of Hydrogel

Ingredients	Formulations Code			
	F1	F2	F3	F4
Carbopol 940(g)	0.5	1	0.5	1
Arnica Mantana Extract (10%)	20 ml	20 ml	50 ml	50 ml
Isopropyl myristate (mL)	1 ml	1 ml	1 ml	1 ml
Benzalkonium chloride	0.25 mg	0.25 mg	0.25 mg	0.25 mg
Distilled water (q.s.)	100 ml	100 ml	100 ml	100 ml

Spreadability The spreadability of various Hydrogel preparations were measured by parallel plate method and all the values are giving satisfactory results. Formulation F3 showed the Mmaximum spreadibility as it contains optimized polymer concentration and large volume of vehicle.

Test for Grease No formulation was left with a greasy sign on filter paper when compared to standard.

Table 2: Evaluation of Arnica Hydrogel

Formulation code	Visual appearance	Viscosity (cps)	Spreadability	pH	Oil Stain
F1	Offwhite and Translucent	9231±2.0	+++	5.9±0.05	--
F2	Offwhite and Translucent	9715±1.5	++	5.7±0.05	--
F3	Brownish and Translucent	9114±2.0	+++	5.9±0.05	--
F4	Brownish and Translucent	9656±1.7	++	5.9±0.05	--

++ Good Spread ability, +++ Better Spread ability, -- No Oil Stain

Accelerated stability studies All formulations were found to be stable after exposure to accelerated temperature and humidity conditions for a period of 3 months. No significant changes were seen in physical evaluation parameters [Table 2 and 3].

pH The pH value of all prepared formulations was found in the range of 5.7-5.9, which is acceptable for topical praparations.

Table 3: Physical Parameters after Accelerated Stability Study of Formulation F1 and F3

Physical Parameter	Formulation F1				Formulation F3			
	Initial	After 1 month	After 2 months	After 3 months	Initial	After 1 month	After 2 months	After 3 months
pH	5.9±0.05	5.8±0.05	5.8±0.05	5.8±0.05	5.9±0.05	5.8±0.05	5.8±0.05	5.8±0.05
Viscosity (cps)	9231±2	9222±1.2	9915±1.5	9242±1.3	9114±2	9154±2	9212±1.2	9108±1.5

Conclusion:

In present work, attempt was made to formulate a non greasy hair tonic which can be applied on scalp daily without shampooing the hairs, to get satisfactory and distinguished effect. Non sticky and non greasy property of the formulation also enhances its acceptance for daily application. Arnica Montana Hydrogel was prepared by varying the concentration of gelling agent and drug extract. Prepared Hydrogels were evalutes on various parameters and all the data are giving satisfactory results. Optimized formulations F1 and F 3 were obtained on the basis of spreadibility and viscosity although a further study on animal model has to carry out in order to get effective and optimized formulation.

References:

[1] Saraf S, Jharaniya M, Gupta A, Jain V & Saraf S (2014) WJPR 3(2):3279-3294.

[2] Patel S, Sharma V, Chauhan NS, Thakur M & Dixit VK (2015) Curr Drug Dis Tech 12:21-42

[3] Singh M, Hariharan AG Sudhakar CK & Jain S (2016) Int J Pharm Sci Res 7(9):3899-3906.

[4] Das N (2013) Int J Pharm Pharm Sci 5(3):112-117.

[5] Monica AS & Gautami J (2014) Int J Pharm Sci Res 5(5):1973-80.

PA-38

Formulation of Paclitaxel Loaded Nanostructured Lipid Carriers to Study the Effect of Concentration of Liquid Lipids on Drug Release

Arti Majumdar[1], Nidhi Dubey[2]
[1]Smriti College of Pharmaceutical Education, Indore
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore
E-mail address: artijmajumdar10@gmail.com

Abstract: In the present study, stearic acid (SA) nanostuctured lipid carriers (NLC) with different proportions of oleic acid (OA) were successfully prepared by solvent diffusion method in an aqueous system. OA was taken in the concentration of 10%, 20%, 30%, 40% and 50%. Prepared NLCs were evaluated for various parameters like drug entrapment efficiency, drug loading and *in vitro* release. A biphasic drug release pattern was observed; initially a fast release was obtained followed by sustained release at a constant rate. The drug release from nanoparticles was found to be significantly influenced by OA content.

Introduction:

The exponential development in the field of nanotechnology has revolutionized the research in the field of advanced drug delivery. Many times problems such as poor solubility, normal tissue toxicity, poor specificity, high incidence rate of pharmaceutical resistance and the rapid degradation, need of large-scale output procedures, a fast release of the pharmaceutical from its carrier, steadiness issues, the residues of the organic solvents utilized in the formulation method, the toxicity from the polymer and sometimes drug expulsion are encountered in the delivery of anticancer drugs through other colloidal delivery systems. These shortcomings are anticipated to be overcome through use of the nanostructured Lipid Carriers along with the advantage of high drug loading capacity and stability. Many nano delivery systems have been studied for the treatment of skin cancers, including liposomes, dendrimers, polymersomes, carbon-based nanoparticles, inorganic nanoparticles, protein-based nanoparticles and nanostructured lipid carriers [1].

Lipid-based drug delivery systems have been proved as promising carriers for cytotoxic drugs because of their potential to increase the solubility and bioavailability of poorly water-soluble and/or lipophilic drugs [2]. The combination of nanoparticulate delivery system with lipids resulted in the development of a new class of NPs commonly known as solid lipid NPs (SLN). As SLN are composed of solid lipids only. Therefore, during formulation a part of the lipid crystallizes in a higher energy modification (α or β). Further on storage, these modifications can transform to more organised lower energy, β modification which further leads to drug expulsion. Apart from polymorphic transition, SLNs also show some disadvantages as drug carriers including an unpredictable gelation tendency, and low incorporation due to the crystalline structure of solid lipids.

To overcome these limitations of the SLNs, second generation encapsulation systems have been developed by incorporating liquid carrier oil into the solid lipid matrix to form nanostructured lipid carriers (NLCs) thus NLCs were introduced. NLCs have shown to have improved active drug encapsulation and delivery properties compared to SLNs. The major advantage of nanostructured lipid carrier as drug delivery system is its ability to accommodate large quantities of drugs as a result of formation of a less ordered lipid matrix with many imperfections [3, 4].

Materials and Methods:

Materials Stearic acid (Loba Chemie, India) was used as solid lipid material of NLC. Oleic acid (Loba Chemie, India) was used as liquid lipid material for NLC. Paclitaxel was kindly donated by Intas Pharmaceutical Ltd., Ahmedabad. The surfactant, sodium lauryl sulfate, was provided by Merck Specialities Pvt. Ltd., Mumbai. Ethanol, acetone and other chemicals were analytical reagent grade.

Preparation of Paclitaxel loaded NLCs dispersion PTX-loaded NLCs were prepared by solvent diffusion method in an aqueous system as reported earlier with slight modification [5]. Briefly, 290 mg selected lipid (SA and OA) with varing content of OA (0%, 10%, 20%, 30%, 40% and 50%) and 10 mg drug were dissolved completely in a 16 ml mixture of acetone and ethanol (1:1, v/v) in water bath at 70 ◦C. This lipid solution was poured into 100 ml of an aqueous phase containing 10mg of SLS under continuous mechanical agitation (Remi Instruments, Mumbai, India) with 3000 rpm at room temperature (25–28 ◦C) for 5 min. The pH value of the acidic aqueous phase was adjusted to 1.20 by addition of 0.1 M hydrochloric acid to form aggregation of nanoparticles. The aggregate of nanoparticle dispersion was then centrifuged 25,000 rpm for 20 min, to get the precipitate of nanostructuered carriers. The precipitate was collected for drug entrapment efficiency determination.

Particle size measurement The volume average diameter of drug-free or drug-loaded nanoparticles in dispersion was determined with Particle Mastersizer 2000 (Malvern Instruments, UK) after diluted 20 times with distilled water.

Drug entrapment efficiency determination

The precipitate of drug-loaded nanoparticles were dispersed in 100 ml of 1 wt% sodium lauryl sulfate solution and shaken for 3 min to dissolve the free drugs. The resulting dispersions were centrifuged for 20 min at 25,000 rpm. The drug content in the supernatant was analyzed by UV-VIS spectrophotometer (UV-1800 Shimadzu Spectrophotometer) at 228 nm. The entrapment efficacy and drug loading of nanoparticle was calculated using:

$EE = (W\alpha - Ws) / W\alpha \times 100$…….....(i)

$DL = (W\alpha - Ws) / (W\alpha - Ws + Wl) \times 100$…….(ii)

Where, EE is entrapment efficiency, DL is Drug loading, Wα stands for the weight of PTX added to the formulation and Ws is the analyzed weight of drug in supernatant and Wl is weight of lipid.

In Vitro release assay

The drug release profiles from nanoparticles were measured in vitro. 100mg of powdered nano structured lipid carriers were dispersed in 30 ml sodium lauryl sulfate solution (1 wt %) in 50 ml glass test-tube. The resulting samples were shaken for 3 min, and one millilitre of the dispersion was withdrawn from the system at definite time interval and filtrated with 100 nm filter. The filtrate was determined by UV-VIS spectrophotometer (UV-1800 Shimadzu Spectrophotometer) at 228nm as described above.

Results and Discussion:

Preparation of stearic acid SLN (0%OA) and NLC by solvent diffusion method in an aqueous system

The stearic acid SLN (0% OA) and NLC with 10, 20, 30, 40 and 50wt% OA content, respectively, were prepared by solvent diffusion method in an aqueous system.

Drug entrapment efficiency and Drug loading capacity

The effects of OA on drug entrapment efficiency and loading capacity of formulations of all the batches were investigated. The curves of drug entrapment efficiency and loading capacity against OA content are given in Figure.1 and Figure.2. It is clear that the drug entrapment efficiency and drug loading capacity of nanoparticles were increased from 48.92 to 82.86% and from 1.65 to 2.78%, respectively, with increasing the percentage of OA from 0 to 50 wt%.

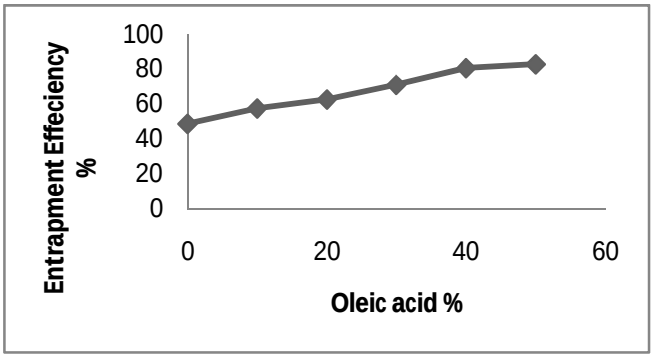


Figure 1: Drug entrapment efficiency (EE) of various batches NLC against oleic acid (OA) content (n = 3).

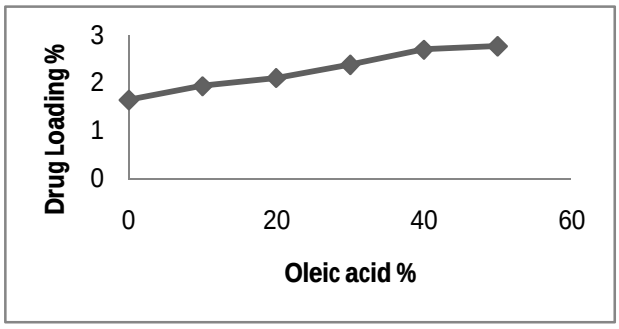


Figure 2: Drug loading (DL) of various batches NLC against oleic acid (OA) content (n = 3)

Drug *In Vitro* release

In Vitro release curves of six type drug-loaded nanoparticles are shown in Fig. 3. A biphasic drug release pattern was observed; initially a fast release was obtained followed by sustained release at a constant rate. The drug released was found to be slowlest from stearic acid SLN ie.0% OA formulation. The release rate became faster when the OA was incorporated to nanoparticles and it increased with increasing the OA content. As shown in Table 1, the nanoparticles with less than 20 wt% OA content had almost similar mean size, but the release rate at the initial stage increased with the increasing OA content in nanoparticles. This means the OA content is a main factor affecting the drug release rate at the initial stage when the OA content was lower than 20 wt%. On the other hand, when the OA content increased up to 50 wt%, the particle size significantly decreased, consequently, the specific surface area was increased. Therefore, the fastest release rate in initial stage, observed in the nanoparticles of 50 wt% OA content, was resulted by both of smaller size and higher OA content. However, later it was noticed that the release profiles of all the batches were almost parallel with each other. This result revealed that the OA almost did not affect the drug release rate of OA incorporated nanoparticles after initial stage. This may be due to non homogenous distribution of OA in nanoparticles. When solvent diffusion method at 70 ◦C was applied to prepare NLC, during cooling down process from the melted lipid droplet in dispersed medium to the formation of a nanostructured lipid carrier at room temperature, because of the different melting point between solid lipid and liquid lipid, the solid lipid (stearic acid) having higher melting point could crystallize first forming a liquid lipid free or little liquid lipid core, finally, most of the liquid lipid located at the outer shell of the nanoparticles which led to drug-enriched shell related with drug burst release at the initial stage observed above. The OA-enriched outer layers possessed a soft and considerable higher solubility for lipophilic drugs character, in which the drug was easily loaded to higher amount and could be easily released as well by the drug diffusion or the matrix erosion manners. Therefore, the OA incorporated nanoparticles showed the burst release at the initial stage and sustained release later.

Table1: Particle size of various batches of Drug free and Drug loaded NLCs

OA Wt%	Particle size (nm)	
	Drug –free nanoparticles	Drug loaded nanoparticles
0	412±2.8	476±6.3
10	409±6.2	471±1.8
20	399±7.9	458±2.6
30	209±1.2	265±3.9
40	189±3.8	228±1.3
50	174±9.1	212±5.2

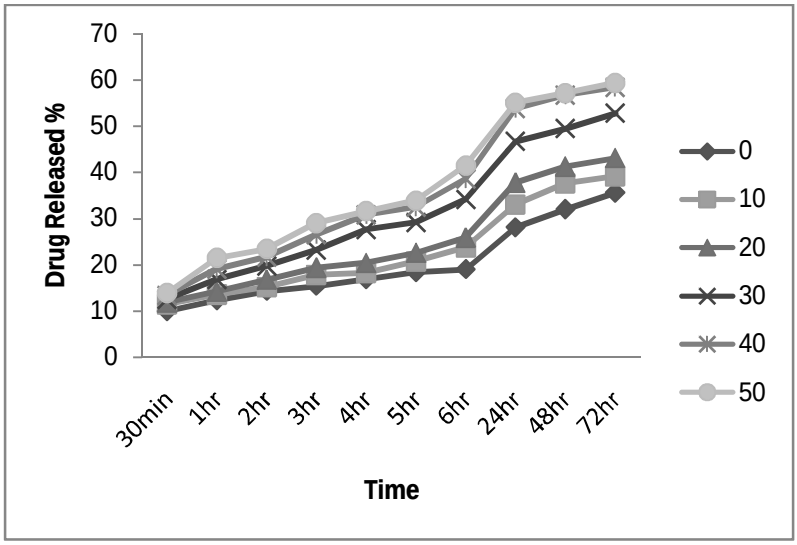


Figure 3: Cumulative *in vitro* drug release from various batches having OA in different proportions

Conclusion:

A solvent diffusion method in aqueous system was employed to prepare the OA–SA NLC with improved drug incorporation and release properties. The drug release characterstics from the NLC exhibited a biphasic pattern with burst release at the initial stage and followed by sustained release at a constant rate. The drug release rate at the initial stage and the drug entrapment efficiency of the NLC were increased with increasing the content of liquid lipid (OA). These results proved that the NLC prepared in the present study can be successfully used as a carrier for therapeutic drug delivery.

References:

[1] Korting HC & Schäfer-Korting M (2010) Carriers in the topical treatment of skin disease. Drug deliv Schäfer-Korting M ed: Handb Exp Pharmacol 435-468.

[2] O'Driscoll CM & Griffin BT (2008) Adv Drug Deliv Rev 60:617–24.

[3] Jenning V, Thünemann AF & Gohla SH (2000) Int J Pharm 199: 167-77.

[4] Radtke M, Souto EB & Muller RH (2005) Pharm Technol Eur 17:45–50.

[5] Hu FQ, Jiang SP & Du YZ (2005) Colloids Surf B Biointerfaces 45:167–73.

PA-40

Development and Evaluation of Hair Gel for the Treatment of Dandruff

Sugan Chouhan, Mahendra Patel, Sudha Vengurlekar
Sri Aurobindo Institute of Pharmacy, Indore
E-mail address: chauhansugan2@gmail.com

Abstract: Dandruff is a very serious problem today; various treatments are available in the market but show temporary effect. An attempt has been made to formulate an antidandruff hair gel of carbopol 940 containing ketoconazole (Antidandruff drug) and *Aloe vera* (Natural antifungal agent), which are effective for number of hours as compared to other marketed hair oils. Hair gel increases the retaining time of drug and improve the effectiveness of antifungal agents. *Aloe vera* keep your hair moisturized, prevent from damage and drying. *Aloe vera* is used in formulation to reduce the dose of ketoconazole and also reduces the adverse and side effect of same.

Introduction:

Fungal infections are common throughout the world. In humans, fungal infections occur when an invading fungus takes over an area of the body and is too much for the immune system to handle. Fungi can live in the air, soil, water, and plants [1]. Ketoconazole is a synthetic imidazole antifungal drug used primarily to treat fungal infections such as in creams (used to treat tinea; coetaneous Candidiasis, including candidal paronychia; and pityriasis versicolor) and in shampoos (used primarily to treat dandruff-seborrhoeic dermatitis of the scalp) [2]. *Aloe vera* has antibacterial as well as antifungal activity against the various types of bacteria and fungus [5]. Hair gel can hold moisture and protect the hair and are perfect for most hair types and can be applied to wet or dry hair [4].

Material and Methods:

Ketoconazole was obtained as gift sample from Alkem pharmaceuticals, Mumbai. Carbopol 940, Triethanolamine, polyethylene glycol, glycerin and ethanol etc. All excipients were of laboratory reagent grade.

Preformulation Studies

The preformulation studies were carried out in term of test for identification (physical appearance, melting point, partion coefficient, solubility profile and qualitative estimation of drug.

Determination of organoleptic properties/description of drug

The organoleptic studies like general appearance like nature, color, odor and state etc. were performed by visual observation [2].

Determination of partition coefficient

Partition coefficient was determined by taking excess amount of ketoconazole in10 ml mixture of n-octanol and water (1:1) in a separating funnel, shaken intermittently for 30 minute and kept

undisturbed for overnight to achieve equilibrium. Then the two phases were separated and centrifuge at 10000 rpm for 15minutes. After centrifugation, the concentration of ketoconazole in both phases was determined by measuring the absorbance at 226 nm on UV-Visible spectrophotometer [2].

Preparation of calibration curve of ketoconazole

Preparation of calibration graph

Varying standard dilutions of 2, 4, 6, 8 and 10 µg/ml of drug in methanol was prepared and absorbance of each solution was measured at 226 nm against methanol blank. A standard graph was prepared by plotting the concentration against the absorbance values [2].

Preparation of hair gel

Measured quantity of methylparabenes, glycerin and polyethylene glycol, were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer. Then carbopol 940 was added slowly to the beaker containing above the liquid while stirring .In another beaker , ketoconazole drug was dissolve in ethanol and added to the above solution by stirring, neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel was formed. Then measured quantity of *Aloe vera* extract was added [3].

Table 1: Formulation of Ketoconazole and *Aloe vera* Hair Gel

Ingredients	HG-1	HG-2	HG-3	HG-4	HG-5
Ketoconazole	1%	1%	1%	1%	%
Alovera	2%	2%	2%	2%	2%
Carbopol 940	1%	2%	3%	4%	5%
Polyethylene glycol 400	5%	5%	5%	5%	5%
Methylparabene	0.01%	0.01%	0.01%	0.01%	0.01%
Triethanolamine	Q.S	Q.S	Q.S	Q.S	Q.S
Glycerin	5%	5%	5%	5%	55
Distilled water (q.s.)	100	100	100	100	100

Evaluation of Anti -dandruff Hair Gels

Psychorheological chracterististic-The psychorheological chracterististic was checked for hair gel formulation (colour, clogging, homogenicity and texture) [2].

Washability-Formulations were applied on the skin and then ease and extent of washing with water checked manually [2].

Extrudability study-The hair gel formulations were filled into collapsible metal tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked [2].

Spreadability-A sample of 0.5 g of each formula was pressed between two slides (divided into squares of mm sides) and left for about 5 minutes where no more spreading was expected. Diameters

of spreaded circles were measured in cm and were taken as comparative values for Spreadability. The results obtained are average of three determinations [2].

Determination of pH-The pH of hair gels was determined by digital pH meter. One gram of gel was dissolved in 25 ML of distilled water and the electrode was then dipped into gel formulation for 30 minute until the constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times [2].

Viscosity-The measurement of viscosity of the prepared gel was done using Brookfield digital viscometer. The viscosity was measured using spindle no.6 at 10 rpm and 25°C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±1°C) before the measurements [3].

Drug content-The drug content was determined by taking 1 g of gel (equivalent to 10 mg of ketoconazole) in 10 ml volumetric flask diluted with methanol. The above solution was suitably diluted and determined using UV-vis spectrophotometer at 226 nm [2].

***In vitro* Drug release study**-*In vitro* drug release of ketoconazole from hair gel was carried out by dialysis test tube method. The donor medium phosphate buffer (pH 7.4) was taken in 250 ml beaker. Then the beaker contain a donor medium placed on magnetic stirrer and stirred at 50 rpm at 37°C ± 0.5°C and hair gel solution was kept in the test tube with egg membrane and sample of 5 ml was withdrawn in each 5 min from donor medium and maintain sink condition .Then these sample were analyzed in UV visible spectroscopy at 226 nm and phosphate buffer ph 7.4 using as blank [3].

Stability Study-The selected formulation were stored at refrigerator (0-8°C), room temperature (25-30°C) and accelerated temperature (45°C) for 4 weeks and observed for any changes in their physical characteristics and drug content [2].

Result and Discussion:

Preformulation Study-ketoconazole is white odourless powder having absorption maximum at 226 nm.

Partition Coefficient-Partition coefficient value of ketoconazole was observed as 0.055 which showed that ketoconazole is lipophilic in nature.

Calibration curve for ketoconazole in Methanol-The absorption of 2 to 10 µg/ml of standard ketaconazole solution in methanol were recorded at absorption maximum (226 nm).

Evaluation of formulation-All the formulation except HG-4 & HG-5 show good psychorheological characteristic. The Carbopol quantity 2% affects the psychorheological characteristic such as presence of clogging and decrease of homogeneity.

Table 2: Psychorheological characteristic

Form	Colour	Clogging	Homogeneity	Texture
HG1	Transparent	Absent	++	Smooth
HG2	Transparent	Absent	+++	Smooth
HG3	Transparent	Absent	++	Smooth
HG4	Transparent	Absent	+	Smooth
HG5	Transparent	Absent	+	Smooth

Excellent (+++) Good: (++) Average: (+) Poor (-)

Table 3: Washability, Extrudability, Spreadabilty, pH and Viscosity of Hair gel

Formulation	Washability	Extrudability	Spreadabilty (gcm/sec)	pH	Viscosity (cps)	% drug content
HG1	+++	++	9.5	6.9	3500	98
HG2	+++	++	8.5	7.0	5300	99
HG3	+++	+++	7.3	7.1	8000	99
HG4	++	+	7	7.1	8500	98
HG5	+	+	6.8	7.4	9000	98

Excellent (+++) Good: (++) Average: (+) Poor (-)

All the formulation except HG-4 &HG-5 showed good wash ability, formulation (HG-1 to HG-5) showed good satisfactory Extrudability. The spreadabilty of formulated gel was decreased as the concentration of gelling agent increased. Formulation HG-1 to HG-3 shows satisfactory spreadibility, pH of all gel formulation was found between 6.9 to 7.4, all formulation showed in increased viscosity as the concentration of the gelling agent was increased. The prepared gel formulation showed uniformity in drug contents.The In-vitro drug release of drug from gel was in the order of decreasing as the concentration of gelling agent was increased. The decrease in vitro –release of drug may be due to the increased viscosity of the gels.

Stability study of optimized formulation The hair gel formulation HG-2 was subjected to stability performance as it was exhibited good drug release and other evaluation parameters.

Table 4: Stability study of optimized formulation HG-2

Temperature	Refrigerator temperature room (0-8^{0}C)	Room temperature (25-30^{0}C)	Accelerated Temperature (45^{0}C)
Period	28 days	28 days	28 days
Viscosity	5300	5280	5130
pH	7.0	7.0	6.9
Spreadability (gcm/sec)	8.5	8.3	8.1
Drug content (%)	99	98	98

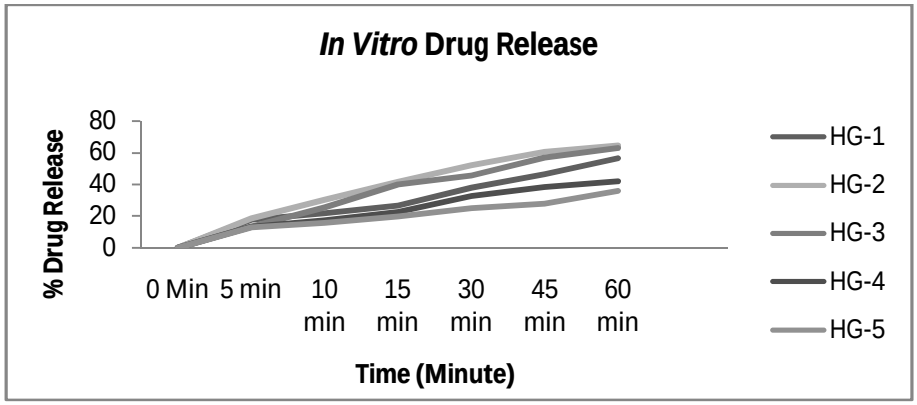


Figure 1: Comparative drug release profile data of all formulation

Conclusion:

Hair gel of ketoconazole and *Aloe vera* were formulated and evaluated for the drug content, viscosity, Spreadability, wash ability, in vitro drug release and stability. Formulation HG-2 was better compare to other. The release rate of drug from HG-2 formulation is best compare to other. The most satisfactory formulation HG-2 did not show any significant change in drug content, viscosity, pH, and spreadability after stability studies at 25-30 °C, 0-8°C and 45^0C for 4 weeks. Thus, the objective of the work of formulation and evaluation of ketoconazole and alovera topical gel has been achieved with success.

References:

[1] Chandel A, Parashar B & Kabra A (2013) IJPRR 2(6):18-28.

[2] Shirsand SB, Para MS, Nagendrakumar D, Kanani KM & Keerthy D (2012) Int J Pharm Investig 2:201-207.

[3] Patil PS, Reddy VM, Biradar KV, Patil CB & Rao KS (2011) IJRPC 1:936-944.

[4] Dhamane SP, Tayade NV & Potnis VV (2016) WJPR 4:1260-1271.

[5] Renisheya JJM, Johnson M, Nancy BS, Laju RS, Anupriya G & Renola JJ (2012) IJBAR 03:184-187.

PA-42

Formulation and Evaluation of Herbal Gel for Wound Healing

Surbhi Jain[1], D. K. Jain[2]
[1]Faculty of Pharmacy, VNS Group of Institutions, Bhopal-462026, Madhya Pradesh India
[2]College of Pharmacy IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, Madhya Pradesh India
E-mail address: surbhijain2425@gmail.com

Abstract: The aim of the present investigation was to formulate and evaluate the herbal gel containing honey and curcumin for wound healing and antimicrobial potential. Curcumin and honey used in the preparation possess an excellent anti-inflammatory, antioxidant and healing potential. Gelling agent used in this study was Carbopol 934P. In the present study five formulations containing different concentration of carbopol and honey were formulated and optimized. The optimized formulations were selected for further studies. Optimized gel was then loaded with active drug. The formulations were characterized for initial physicochemical parameters i.e. surface pH, spreadability, viscosity and antimicrobial susceptibility test to observe toxicity or side effects. The result revealed that the surface pH was within the range of skin pH. The viscosity and spreadability of the gel was appropriate and zone of inhibition was also satisfactory for optimized formulations G4 and G5. The preparations were stable under normal storage conditions and did not produce any skin irritation, i.e., erythema and oedema for about a month, when applied over the skin.

Introduction:

Herbal markets are globally increasing due to safe drug delivery with fewer side effects compared to synthetic drugs. In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. Herbal drugs are used to cure different diseased condition with different dosage form. The treatment of various diseases begins long ago with the use of herbs. Though they have very short half life if not prepared properly with proper precautions then they are not found effective and suitable for use. Topical formulations of gels at pathological sites offer a great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments [1, 2]. A wound has been defined as loss or breaking of cellular and anatomic continuity of living tissue. Wound healing is the process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and extracellular macromolecules that are later remodeled to scar [3].These processes basically divided into three overlapping phases are inflammation, proliferation and remodeling. There are various natural agents, which assist in wound healing process. The herbal moiety selected for the present work is curcumin and honey. Curcumin was used in combination with honey which will enhance the wound healing activity. Both have reported to have anti-inflammatory and anti bacterial activity, which are complementary to wound healing process [4].

Material and Methods:

Materials

Curcumin was received as a gift sample from Ajmera Pharmaceuticals, Indore, M.P., India. Dabur honey was obtained from market. Carbopol 934, triethanolamine, glycerin and all other chemicals were of analytical grade and used without further purification. The bacterial strains used were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 for gram positive and *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 26731 for gram negative were collected from Institute of Microbial Technology (IMTECH), Chandigarh.

Method of preparation of gel

The topical gel was prepared by soaking the Carbopol 934 in water for 24 h. Drug was first dispersed in small quantity of glycerin with gentle heating and then preservatives were dissolved in glycerin and then added to Carbopol solution with stirring the remaining ingredients were added to it and triethanolamine was added to the neutralize the Carbopol gel base [3, 4] The composition of gel is given in Table1.

Results and Discussion:

The present study was aimed to prepare and evaluate the polyherbal gel for topical administration, for the treatment of a severe skin problem known as Wounds and antimicrobial activity. Different parameters studied were carried out for transdermal gel formulations. Carbopol was found to be suitable candidate as it gives better consistency, viscosity, spreadability, pH, homogeneity, and in-vitro drug diffusion. Carbopol concentration was optimized by trial and error method. Triethanolamine was taken as a neutralizer so as to maintain the pH and it also enhance the stability and penetration property of the gel. Honey was added to the formulation to give additive effect along with curcumin.

Table 1: Composition and optimization of gel

S. No.	Ingredients	G1	G2	G3	G4	G5
1.	Carbopol 934P NF (%)	1.5	1.7	2	2.5	3
2.	Honey (%)	50	50	50	50	50
3.	Curcumin (%)	0.5	0.5	0.5	0.5	0.5
4.	Glycerin	5ml	5ml	5ml	5ml	5ml
5.	Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.
6.	Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2
7.	Propyl paraben (g)	0.1	0.1	0.1	0.1	0.1
8.	Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2: Physical properties of formulations

Formulation code	Surface pH	Viscosity cps	Spreadability $Gm.cm^2$	Homogeneity
G1	5.44	4211	11.77	Poor
G2	5.46	4305	11.52	Poor
G3	5.75	4523	10.37	Poor
G4	6.0	4780	9.98	Good
G5	6.68	5370	9.25	Good

Table 3: Physical evaluation of formulations

Formulation code	Colour	Appearance	Feel on application
G1	Faint yellow	Translucent	Less Smooth
G2	Faint yellow	Translucent	Smooth
G3	Faint yellow	Translucent	smooth
G3	Faint yellow	Translucent	Smooth
G4	Yellowish orange	Translucent	Smooth

Table 4: Zone of inhibition diameters of optimized formulation

S.No.	Microbial strains	Zone of inhibition (mm)		
		G4 Formulation	G5 Formulation	Standard
1	*S. aureus*	13± 0.57	13± 0.57	18± 0.74
2	*E. coli*	12±0.45	12±0.45	15±0.58
3	*B. subtilis*	10±0.29	10±0.29	14±0.64
4	*P. aeruginosa*	11±0.75	11±0.75	14±0.81

Various formulations with different concentration of Carbopol and honey were taken and prepared. Formulation G4 and G5 shows significant consistency among all the other formulations. The results of the physical evaluations of the formulated gels showed that all the formulations had a smooth appearance and were uniformly mixed with little or no lumps or gritty texture. They had an agreeable odour (no pungent or irritating smell). Formulations were translucent and faint yellow to yellowish orange in color.

Conclusion:

The study demonstrated the good antimicrobial activities and the desired physical properties of the gel formulations containing the herbal isolates. These could make them potential topical antimicrobial agents effective in the treatment of skin infections with wound healing potential and is safe to apply.

References:

[1] Vure P & Dorl AK (2006) J Ethnopharmacol 107:38–47.

[2] Vyas P, Prajapati PK & Shukla VJ (2013) Phytother Res 3:49-60.

[3] Bhat S, Shankrappa J & Kumar G (2007) J Pharm Sci 2:11-17.

[4] Ramane S, Syed V & Biyani K (2011) Int J Res Pharm Biomed Sci 3(2):39-42.

PA-48

Solubility Enhancement of Clopidogrel Bisulfate by Solid Dispersion Technique Using Carboxymethylcellulose Sodium and Xanthan Gum

Urvashi Sharma, Ankur Joshi, Narendra Vyas, Sapna Malviya, Anil Kharia
Modern Institute of Pharmaceutical Sciences, Indore (M.P.), India - 453111
E-mail address: urvashi.ekta12@gmail.com

Absract: Solid dispersions formulated to improve solubility & dissolution rate of poorly soluble drug clopidogrel bisulfate. Physical mixtures & solid dispersions of clopidogrel bisulfate were prepared with carboxymethylcellulose sodium and xanthan gum in the weight ratios of 1:1, 1:3 and 1:5 using kneading method. The prepared solid dispersions were characterized by solubility determination, drug content, *In Vitro* dissolution and accelerated stability studies. The results revealed that solid dispersions shown improvement in solubility and dissolution characteristics than the physical mixtures and pure drug. The reasons for increase in solubility and dissolution rate is decrease in particle size, increased surface area, amorphous state of the drug in solid dispersions, absence of aggregation and increased wetting of drug molecules. It was also observed that solid dispersions of drug with both carriers showed increased dissolution rate in the ratio of 1:5 (Drug: Carrier) in comparison to pure drug and found to be stable during stability studies.

Introduction:

Solubility behavior of a drug is one of the important determinants of its bioavailability as the rate and extent of dissolution of the active ingredient from any dosage form often determines the rate and extent of absorption of the drugs [1]. About 40% of new chemical entities currently discovered are poorly water soluble. Solubility enhancement is important parameters which should be considered in formulation development of drug with poor aqueous solubility. For improvement of solubility and dissolution, numerous techniques are available. But, solid dispersion is the most promising method to formulators because of its ease of preparation, ease of optimization, and reproducibility [2]. This may be achieved by incorporating the drug in a hydrophilic carrier material obtaining products called solid dispersions [3].Clopidogrel bisulfate is practically insoluble in water, results in poor dissolution and poor bioavailability (50%), used as potent anti-platelet drug, indicated for the prevention of atherothrombotic events in patients with acute coronary syndromes. Also used for the prevention of vascular thrombotic events in patients at risk. Thus increasing the aqueous solubility and dissolution is of therapeutic importance [4]. Literature survey revealed that certain hydrophilic swellable polymers such as carboxymethylcellulose sodium (NaCMC) and xanthan gum had been used for their potential to form solid dispersions [3]. For this reason, the rational of the present study was the preparation of solid dispersion of clopidogrel bisulfate using carboxymethylcellulose sodium and xanthan gum to overcome limited dissolution rate and formulation difficulties.

Materials and Methods:

Materials

Clopidogrel bisulfate was received as gift sample from Ipca Laboratories, Indore. Polymers (Carboxymethylcellulose sodium and Xanthan gum) and all other ingredients were of analytical grade.

Methods

Preparation of physical mixture

Physical mixtures of clopidogrel bisulfate with carriers (carboxymethylcellulose sodium and xanthan gum) in were prepared by taking drug and carriers in ratio of 1:1, 1:3, 1:5 and pulverized by light triturating for 5 m in mortar till a homogenous mixture was obtained. This mixture was passed through sieve no.80 for uniform size and stored in desiccators for further use [1].

Preparation of solid dispersion

Solid dispersions of clopidogrel bisulfate with NaCMC and xanthan gum were prepared by kneading method in ratio of 1:1, 1:3 and 1:5. In this method, accurately weighed quantity of clopidogrel bisulfate and selected carriers were transferred into a mortar and methanol was added to the mixture and triturated to form paste for 30 m. Then the mixture was dried at 50°C in the hot air oven until to get constant weight. The dried mass was pulverized to fine powder, sieved through sieve no.80. The prepared solid dispersion formulations were stored in desiccator for further studies [5].

Characterization of solid dispersions

Determination of equillibrium solubility

Solubility studies were performed according to the method reported by Higuchi and Connors. In solubility study, excess of formulations (drug, PMs and SDs) were added to 10 ml of distilled water in a stoppered conical flasks and subjected to shaking for 8 h at 37±1^{o}C and kept for 24 h after shaking to achieve equilibrium. 2 ml aliquots were withdrawn, filtered and after appropriate dilution with distilled water, filtrate was analysed at 220nm against blank. Readings were taken in triplicate and observations were recorded in table 1.

Drug content

Solid dispersions equivalent to 10mg of clopidogrel bisulfate were weighed accurately and dissolved in the 10ml of methanol. The solution was filtered, diluted suitably and drug content was analyzed at 220nm using UV spectrophotometer and observations were recorded (table 1).

Dissolution rate studies

The dissolution studies were carried out using dissolution apparatus (Rotating Paddle type) at a speed of 50 rpm. Accurately weighed amount of drug, PMs and SDs immersed in a pH 2.0 HCl buffer as dissolution medium at 37±0.5^{o}C. The dissolution was carried out for 1 h and aliquot of 5ml was withdrawn at adequate intervals. The filtered samples were suitably diluted, assayed at 220nm and cumulative percentage of the drug dissolved from the formulations was calculated (Table 2, 3).

Stability studies

The selected solid dispersions of clopidogrel bisulfate with NaCMC (FS3) and xanthan gum (FS6) were subjected to accelerated stability study as per ICH guidelines. The formulations were filled in 10ml glass vials. The vials were plugged, sealed and kept at different temperature conditions such as room temperature (25^{o}C) and 40±2^{o}C/75±5% RH using dessicator containing calcium chloride, for a period of 1 month. At definite time intervals, the samples were visually examined for any physical change. The drug content and dissolution rate was estimated after one month [1].

Results and Discussion:

Determination of Equilibrium Solubility

Solubility of clopidogrel bisulfate was found to enhance in solid dispersion and physical mixture. The formulation prepared with carboxymethylcellulose sodium as carrier showed greater solubility as compared to xanthan gum and result was best in case of formulation FS3 and FS6 which is in the ratio of 1:5 (Table 1).

Table 1: Saturation Solubility Study and Drug Content of Various Formulations

S.No.	Formulation Code	Saturation Solubility at 37±1^{o}C in water (µg/ml)	Percentage Solubility Enhancement (%)	% Drug Content
1	Pure Drug	9.94±0.01	-	
2	FS1	15.49±0.17	155.84	88.58±0.12
3	FS2	17.15±0.09	172.53	96.34±0.25
4	FS3	19.67±0.24	197.89	99.55±0.19
5	FS4	14.72±0.40	148.09	86.52±0.35
6	FS5	16.21±0.14	163.07	94.73±0.19
7	FS6	18.99±0.32	191.05	98.97±0.41
8	FP1	11.63±0.36	117.00	82.54±0.24
9	FP2	13.81±0.07	138.93	92.43±0.15
10	FP3	14.19±0.22	142.75	97.62±0.08
11	FP4	11.03±0.18	110.96	81.69±0.16
12	FP5	12.74±0.41	128.17	91.50±0.26
13	FP6	13.95±0.36	140.34	96.38±0.23

Drug Content

The drug content of prepared formulations of clopidogrel bisulfate with carboxymethylcellulose sodium (NaCMC) was observed to be varying from 88.58±0.12 to 99.55±0.19% and for solid dispersions with xanthan gum, it was found to be in range of 86.52±0.35 to 98.97±0.41% (Table 1).

Dissolution Rate Studies

Solid dispersion of clopidogrel bisulfate showed a significant increase in the dissolution rate than the corresponding physical mixtures and pure drug. The dissolution rate increased on increasing the amount of polymers and maximum drug release was shown by FS3 (SDs with NaCMC 93.37±0.11%) and FS6 (SDs with Xanthan Gum 92.25±0.15%) as shown in Table 2, 3.

Table 2: Cumulative % Drug Release of Solid Dispersion and Physical Mixture with Carboxymethylcellulose Sodium

Time (m)	Drug	FS1	FS2	FS3	FP1	FP2	FP3
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	2.67±0.11	6.98±0.29	8.65±0.21	10.35±0.12	4.67±0.18	5.19±0.27	5.95±0.21
20	5.89±0.25	17.83±0.69	22.50±0.15	25.87±0.54	10.85±0.23	12.36±0.29	15.58±0.34
30	12.46±0.10	29.56±0.21	38.07±0.82	43.20±0.09	19.15±0.93	24.22±0.16	27.94±0.26
40	20.08±0.17	41.83±0.92	50.19±0.27	57.15±0.36	31.09±0.24	36.80±0.41	40.98±0.17
50	27.92±0.47	65.95±0.17	74.42±0.12	77.82±0.22	48.81±0.35	51.69±0.23	62.82±0.43
60	38.17±0.23	81.35±0.10	88.25±0.32	93.37±0.11	60.67±0.08	69.34±0.75	78.59±0.18

Table 3: Cumulative % Drug Release of Solid Dispersion and Physical Mixture with Xanthan Gum

Time	Drug	FS4	FS5	FS6	FP4	FP5	FP6
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	2.67±0.11	6.05±0.12	8.12±0.25	9.87±0.18	4.15±0.11	4.85±0.34	5.36±0.22
20	5.89±0.25	15.21±0.35	20.75±0.11	24.72±0.57	9.52±0.23	11.77±0.42	13.84±0.83
30	12.46±0.10	28.89±0.41	37.71±0.91	42.96±0.23	18.65±0.16	21.46±0.13	23.02±0.19
40	20.08±0.17	41.54±0.18	51.74±0.09	56.89±0.42	30.81±0.10	34.75±0.75	38.90±0.15
50	27.92±0.47	60.33±0.16	72.00±0.21	76.14±0.29	46.73±0.08	50.39±0.17	59.14±0.93
60	38.17±0.23	79.99±0.23	87.05±0.17	92.25±0.15	59.32±0.35	67.50±0.21	76.59±0.09

Stability Studies

The selected formulations FS3 and FS6 showed physical stability at room temperature (25^{o}C) and at 40^{o}C for a period of 1 month i.e no change in colour, no sign of caking. The results of chemical stability studies are showed in the table. This indicated that formulations were physically and chemically stable.

Table 4: Drug Content and Cumulative % Drug Release of Solid Dispersion for Stability Study

Formulation Code	Stability Parameters	Initial	15 Days		30 Days	
			25°C	40 °C	25°C	40 °C
FS3	% Drug Content	99.55±0.19	98.82±0.32	98.27±0.47	97.51±0.12	97.18±0.23
	Cumulative % Drug Release	93.37±0.11	93.30±0.27	93.35±0.36	93.19±0.13	93.24±0.09
FS6	% Drug Content	98.97±0.41	97.65±0.11	97.17±0.25	96.49±0.68	96.07±0.36
	Cumulative % Drug Release	92.25±0.15	92.18±0.31	92.20±0.24	92.09±0.17	91.97±0.44

Conclusion:

The present study has shown that solid dispersions of clopidogrel bisulfate prepared by kneading method enhanced solubility as compared to pure and physical mixture with water soluble carriers i.e. carboxymethylcellulose sodium and xanthan gum. This might be due to amorphous state of the drug in solid dispersions, wettability improvement, reduction in particle size and increase in the effective surface area over which the drug distribution increases. The results of study demonstrated the suitability of selected polymer xanthan gum and NaCMC in the preparation of solid dispersions as effective carrier for increasing solubility. As these swellable polymers are regularly used in conventional solid dose preparations and herbal polymer is readily available at low cost, this ensures about the availability, feasibility in use and cost effectiveness of the formulations. Overall, this research work presents a very simple but effective technique for dissolution enhancement using very common polymers. Further in vivo studies are required to confirm the applicability of these polymers in formulation technology.

References:

[1] Satle A & Agrawal S (2012) Int J Curr Pharm Res 4:67-73.

[2] Sarkar R, Hossain ASM, Islam S & Faroque ABM (2012) Indian J of Novel Drug delivery 4:130-138.

[3] Kaur J, Aggarwal G, Singh G & Rana AC (2012) Int J Pharm Pharm Sci 4:47-53.

[4] Jassim ZE & Hussein AA (2014) Int J Pharm Pharm Sci 6:838-851.

[5] Kaza R, Raju YP & Nagaraju R (2013) Der Pharmacia Lettre 5:126-134.

PA-53

Development and Evaluation of Proniosomes as Drug Carriers for Transdermal Delivery of Ketorolac Tromethamine

Nadeem Farooqui[1], Mousumi Kar[2], Sanjay Jain[3]
[1]Suresh Gyan Vihar University, Mahal Jagatpura, Jaipur (RJ) – India
[2]IPS Academy College of Pharmacy, Indore (MP) – India
[3]Indore Institute of Pharmacy (IIP), Indore (MP) – India
E-mail address: nadeem1712@rediffmail.com

Abstract: Ketorolac tromethamine is a drug with narrow therapeutic index and short biological half-life. This study was aimed at developing and optimizing proniosomal formulation of ketorolac tromethamine in order to improve its bioavailability. The prepared proniosomal gel formulations were evaluated and the effect of the varying composition of non ionic surfactant and cholesterol in various formulations were studied, such as vesicle shape, zeta potential, entrapment efficiency, and *in- vitro* drug release study. The presence of cholesterol made the proniosomes more stable with high drug entrapment efficiency and retention properties. The highest entrapment efficiency was observed with sodium cholate 88.17 ± 0.95 as compared to those formulation prepared with span60 and with sodium deoxycholate. Formulation F1 (LCI-I), zeta potential value was observed -20.0 mV, which is a measure of net charge of proniosomes which made them stable, by preventing aggregation. Formulation F1 which prepared by sodium cholate, showed highest drug release of 94.048 % after 24 hrs as compared to formulation F6 (LDCI-3) and F9 (LSI-3) which were prepared by sodium deoxycholate and sapn60 showed lowest drug release of 76.35% and 69.12%.

Introduction:

Development of a new drug molecule is expensive and time consuming. Improving safety efficacy ratio of "old" drugs has been attempted using therapeutic drug monitoring (TDM) of formulation based on novel drug delivery systems. Proniosomes are based on dry formulation of water soluble carriers that are coated with surfactant. It forms niosomal dispersion immediately during the rehydration to before use on agitation in hot aqueous media within minutes [1]. Proniosomes are physically stable during the storage and transport. Drug encapsulated in the vesicular structure of proniosomes prolong the existence of drug in the systematic circulation and enhances the penetration into target tissue and reduce toxicity. Due to the limited amount of water present, these systems behave as viscous phases. When compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first-pass metabolism and a higher compliance [2, 3]. These 'proniosomes' minimize problems associated with niosome base formulation such as physical stability like aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. The focus of this research work is to

bring out different aspects related to proniosomes preparation, characterization, entrapment efficiency, *in vitro* drug release and *in vitro* permeation studies.

Method of Preparation:

The proniosomes were prepared by rotatory flask method by dissolving cholesterol and various types of surfactants (span60, sodium deoxycholate and sodium cholate) in different concentration in alcohol and thin film was formed along the sides of the flask by continuous vortexing. Drug was dissolved in 10ml of phosphate buffer saline (PBS) pH.7.4 and added to the thin film and then sonicated for 5 min. The proniosomal suspensions were formed, and then these suspensions kept at 4^{o}C.

Table 1: Optimization of Proniosomal Formulation

Batch No.	Formulations	Surfactant: Lipid: Alcohol Ratio (w/w)	PBS pH 7.4 (ml)
F1	LCI-1	2.1:0.3:9	10
F2	LCI-2	1.71:0.3:7	10
F3	LCI-3	0.9:0.3:4	10
F4	LDCI-1	2.1:0.3:9	10
F5	LDCI-2	1.71:0.3:7	10
F6	LDCI-3	0.9:0.3:4	10
F7	LSI-1	2.1:0.3:9	10
F8	LSI-2	1.71:0.3:7	10
F9	LSI-3	0.9:0.3:4	10

C: Sodium Cholate, **D:** Sodium Deoxycholate, **I**: Isopropanol **L:** Lipid**, S**: Span60

Characterization

Determination of Zeta Potential: The zeta potential of the selected batch of proniosomal formulation was determined at 25ºC using Zetasizer (Malvern Instruments). [4] Proniosomal suspension was diluted 100 times with double-distilled water and voltage was set at 50 or 100 V and electrodes were placed in dispersion for the measurement of zeta potential.

Scanning Electron Microscope: The sizes of the vesicles were measured by scanning electron microscopy [5]. Small amount of proniosomal suspension was placed on the specimen stub, coated with carbon and then with gold vapor using Hitachi vacuum evaporator. The samples were examined under scanning electron microscope, and then photographed.

Entrapment Efficiency: The entrapment efficiency was determined after separating the unentrapped drug. Proniosomes (100mg) was hydrated with 10 ml of phosphate buffer saline (pH 7.4) manual shaking for 5 minutes, to form PN dispersion. For the separation of unentrapped drug the PN

dispersion was centrifuged at 15000 rpm for 30 minutes at 20^0C and analyzed by UV spectroscopy at 322 nm. The entrapment efficiency was calculated using the formula:

$$\% \textbf{ Entrapment efficiency} = \frac{\textbf{Total Drug} - \textbf{drug in supernatant liquid}}{\textbf{Total drug}} \textbf{ X 100}$$

***In vitro* Drug Release**: - The release of Ketorolac Tromethamine from proniosomal gel was determined using membrane diffusion technique. The proniosomal gel equivalent to 1mg of Ketorolac Tromethamine was placed in a dialysis bag tied to glass tube acting as a donor compartment. The glass tube was placed in a beaker containing 50ml of phosphate buffer (pH7.4), acting as a receptor compartment. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touching the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 05°C and was agitated at the speed of 100 rpm using magnetic stirrer. Aliquots of 3ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected sample was analyzed by UV spectrophotometer using phosphate buffer (pH 7.4) as blank.

Results and Discussion:

Determination of Zeta potential: - The formulation F1, which was subjected to Zeta potential analysis, had a zeta value of -20.0 mV, which is a measure of net charge of proniosomes. High surface charge provides sufficient electrostatic repulsion between the vesicles, which made them stable, by preventing aggregation. Negative charge leads to rapid blood clearance.

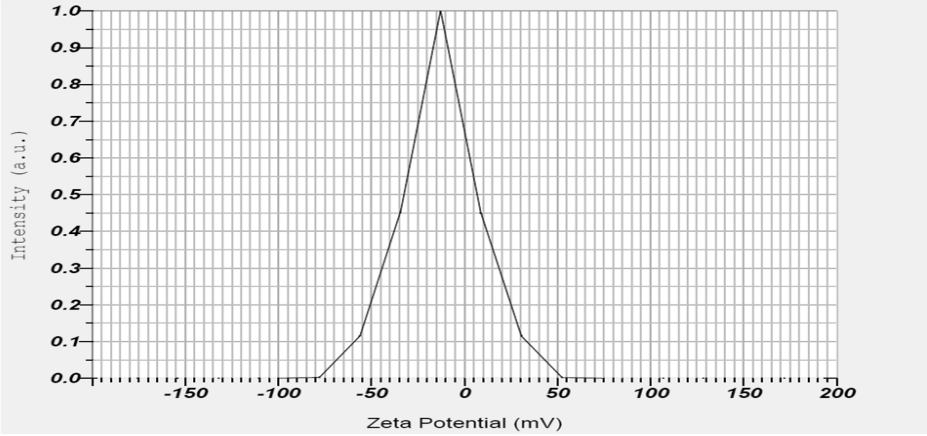


Figure 1: Zeta Potential Analysis

By Scanning Electron Microscope (SEM): - Scanning electron microscopy for the selected formulation F1was carried out. The results are shown in the following SEM photograph.

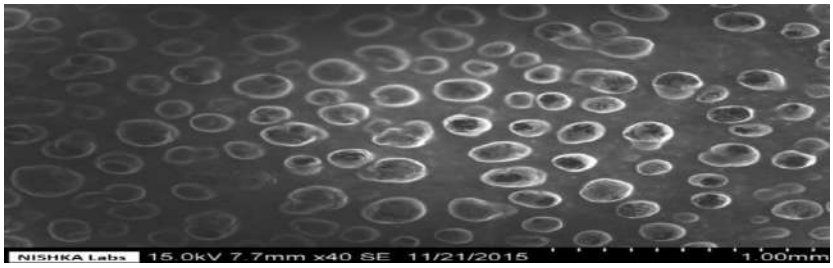


Figure 2: Proniosomes observed under SEM

Entrapment Efficiency: Entrapment efficiency is the percentage fraction of the total drug incorporated into the Proniosomes. As shown in figure no.03. Formulation LCI-1 (88.17 ± 0.95) exhibited very high entrapment efficiency. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the proniosomes.

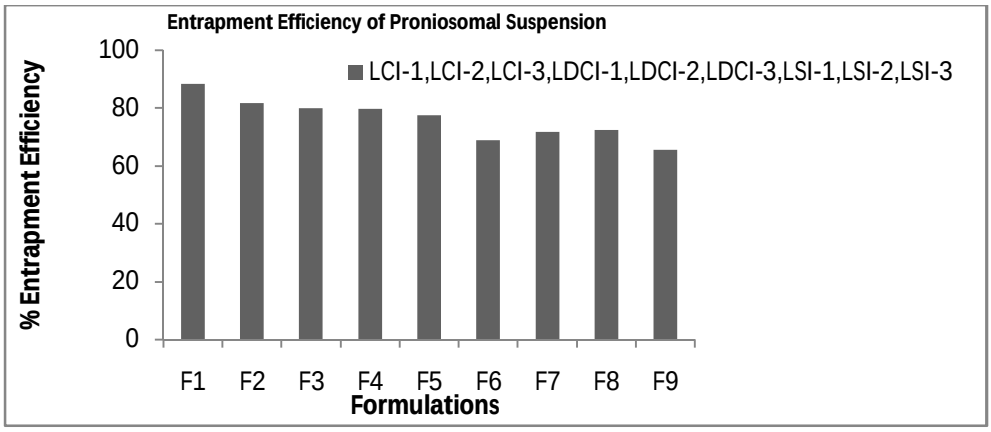


Figure 3: Entrapment Efficiency of Proniosomal Suspension

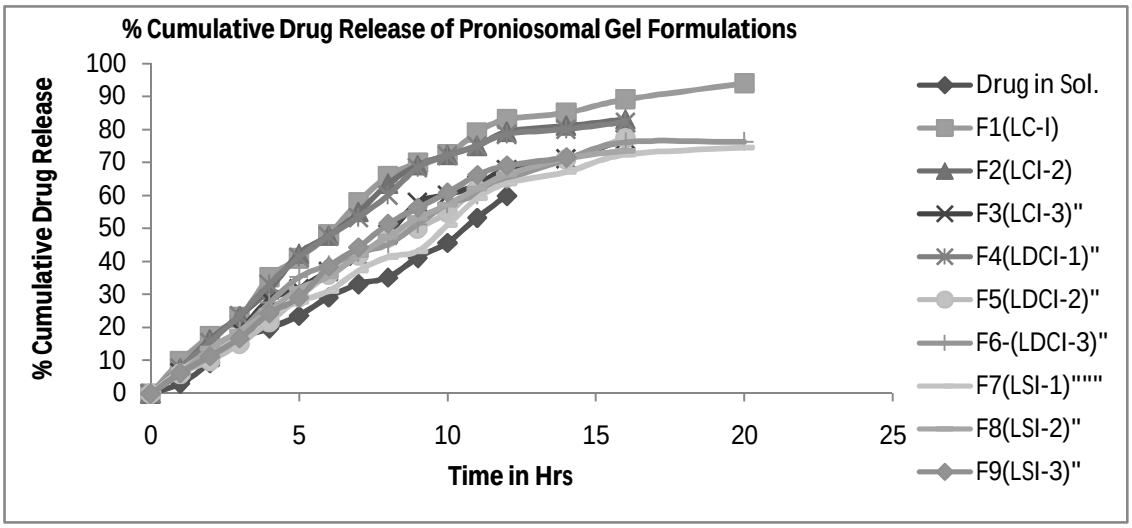


Figure 4: % Cumulative Drug Release of Gel Formulations

***In Vitro* Drug Release of Proniosomal Gel:** Formulation F1 showed highest drug release of 94.011 % in 24 hrs and formulation F9 showed lowest drug release of 71.789 % in 16 hrs (Figure 4). After incorporation of proniosomal vesicles into gel base they show significant delayed in *in-vitro* drug release in 24 hours as compared to proniosomal suspension

Conclusion:

The results of the present investigation showed that the problems associated with the transdermal delivery of KT could be overcome by incorporating it into the new PN drug carrier, proniosomes. Among the nine PN formulations developed for transdermal delivery of KT, LCI-1 (F1) showed promising higher entrapment efficiency, showed highest drug release of 94.048 % after 24 hrs as compared to formulation F6 (LDCI-3) and F9 (LSI-3) which were prepared by sodium deoxycholate and sapn60 showed lowest drug release of 76.35% and 69.12%.

Acknowledgement:

The authors are thankful to Piramal Health Care Pvt. Ltd. (Pithampur, India) for providing gift sample of KT.

References:

[1] Alsarra IA, Bosela AA, Ahmed SM & Mahrous GM (2005) Eur J Pharm Biopharm 59:485-490.

[2] Chen XH, Bai JY, Shen F, Bai AP, Guo ZR & Cheng GF (2004) Acta Pharmacol Sin 25:927-931.

[3] Fry DW, White JC & Goldman ID (1978) Anal Biochem 90:809-815.

[4] El Maghraby GM, Williams AC & Barry BW (2000) Int J Pharm 196:63-74.

[5] Ozgüney IS, Karasulu HY, Kantarc G, Sözer S, Güneri T & Ertan G (2006) AAPS Pharm Sci Tech 7:E1-E7.

PA-55

Solubility Enhancement of Efavirenz (BCS Class II Drug) By Cyclodextrin Inclusion Complex Technique

Mrittunjay Vyas, Harsha Jain, Ravi Sharma, G. N. Darwhekar
Acropolis Institute of Pharmaceutical Education and Research, Indore
E-mail address: mrittunjayvyasbph14@acropolis.edu.in

Abstract: The solubility of BCS class II drugs can be enhanced using inclusion complex techniques. Cyclodextrin (CD) and its derivatives are promising carrier for the enhancement of aqueous solubility of drugs. The use of the Kneading technique to obtain solid inclusion system permitted the formation of a uniform, substantially non-crystalline particle, which increased the solubility and stability of Efavirenz (EFV). The present work shows the enhancement of aqueous solubility of BCS class II drug i.e. EFV by making inclusion complex with β-CD. The aqueous solubility of EFV is calculated to be 5± 0.003 µg/ml which was increased up to 288.9±0.005 µg/ml when complexed with β-CD in ratio of 1:1 and 318.5± 0.03 µg/ml in ratio of 1:2. This shows that, the inclusion complex technique is a promising way to enhance aqueous solubility.

Introduction:

The process of drug absorption, bioavailability and pharmacokinetic profile of orally administered drug substance is highly dependent on factor solubility and permeability of that compound in the aqueous medium.The study of drug synthesis in pharmaceutical researches involves the maximum number of lipophilic drugs which have very low solubility rate [1]. Efavirenz (EFV) [(S)-6-chloro-4-(cyclopropyl ethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one] is a non-nucleoside reverse transcriptase inhibitor approved for the treatment of human immunodeficiency virus type 1 infection. Efavirenz is a drug with crystalline lypophillic solid whose molecular mass is 315.68 and aqueous solubility of 9.0µg/ml. This is a class II drug (low solubility, high permeability) that is highly permeable, poorly soluble drugs often demonstrate poor gastrointestinal (GI) absorption due to inadequate drug solubility in GI fluids [2].

The solubility of these low soluble drugs is being enhanced by various modifications. Cyclodextrins (CD's) are the cyclic oligosaccharides containing 6(α-CD), 7(β-CD), 8 (γ-CD) α- 1, 4-linked glucopyanose units. Each of these CD molecules has the hydrophilic property which is helpful in form of hydrophobic guest molecule [3].

Materials and Methods:

Materials

EFV drug provided by Shagun Pharma, Indore. Cyclodextrin is used of Loba chemie.

Methods

Preparation of Calibration Curve of Drug Efavirenz in distilled water:

The calibration curve was made by finding the absorbance of drug dilutions at different concentration in the distilled water (solvent).Initially 10 mg of drug was dissolved in 10 ml ethanol as a stock solution. Further dilutions were prepared having strength of 5, 10, 20, 25, 30, 40 µg/ml and diluted with distilled water.

Saturated solubility:

The saturated solubility of EFV alone in distilled water was determined. Sufficient excess amount of EFV was added to 10 ml glass vials containing distilled water. The vials were shaken mechanically for 12 h on mechanical shaker (Lab Hosp, Mumbai) at 37 ± 2°C. The solutions were allowed to equilibrate for next 24 h. The solution was transferred into eppendorf tubes and centrifuged for 5 min at 2000 rpm. The supernatants of each vial were filtered through 0.45µ membrane filter and analyzed for drug content by UV visible spectrophotometer (UV-1800, Shimadzu, Japan) at 248 nm after appropriate dilutions. The study was performed in triplicate.

Phase Solubility Studies of Efavirenz with Cyclodextrin:

For the determination of Phase Solubility, different concentration of cyclodextrins in Mili Molar (1,2,4,6,8,10,12 mM) solution form were prepared in 10ml distilled water each containing approximate 30mg of Efavirenz in each solution.

Preparations of Inclusion Complex with help of Cyclodextrin:

Basically inclusion complex is the combination of drug and the cyclodextrins was prepared by kneading method.

Kneading Method:

Initially Cyclodextrin was taken in a mortar with 1 ml of 50% ethanol to prepare a slurry, then Efavirenz in the same equimolar quantity of cyclodextrin was taken and triturated well for mixing. Small amount of water is then added to make slurry having proper consistency and kneaded for 15 min, and dried at 50°C for 24 h. The resultant dry solid mass was powdered well, passed through 60# sieve and stored in a sealed glass vials.Inclusion complexes were prepares in the ration of 1:1 and 1:2 of drug to cyclodextrin ratio [4].

Results and Discussions:

Calibration curve of drug Efavirenz

The λ_{max} of EFV in distilled water was calculated as 248 nm.

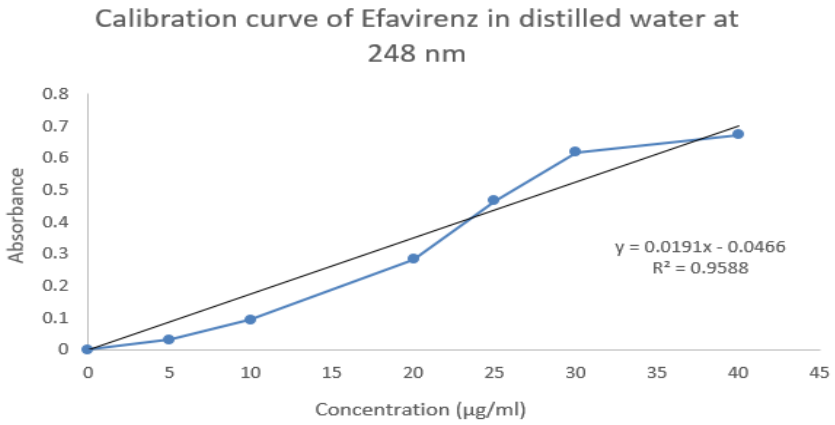


Figure 1: Calibration curve of Efavirenz in distilled water at 248 nm

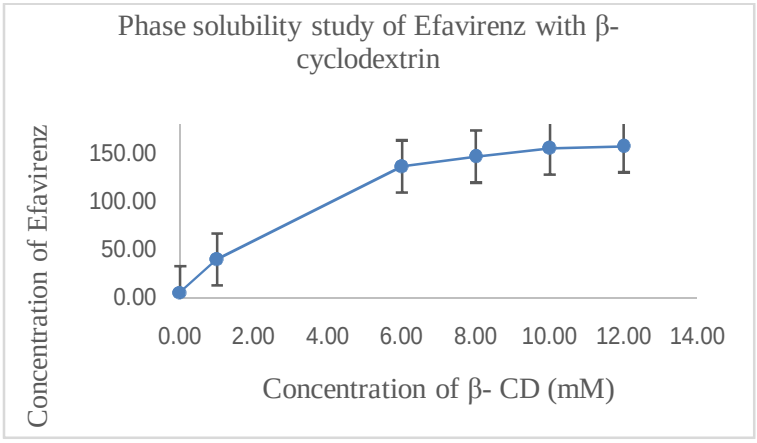


Figure 2: Phase solubility study of Efavirenz with β-CD (K_{st} = 190.05 M^{-1})

Phase Solubility Studies

Table 1: Phase Solubility Studies of Efavirenz with Cyclodextrin

Concentration of CD (mM)	F1	F2	F3	Average	Concentration (in µg/ml)	Concentration (Dilution factor)	SD
0.00	0.066	0.06	0.059	0.062	5.698	5.70	0.0030
1.00	0.708	0.721	0.71	0.713	39.979	39.98	0.0057
6.00	0.203	0.224	0.211	0.213	13.646	136.46	0.0086
8.00	0.222	0.236	0.239	0.232	14.681	146.81	0.0074
10.00	0.249	0.251	0.246	0.249	15.540	155.40	0.0020
12.00	0.258	0.252	0.248	0.253	15.751	157.51	0.0041

Solubility of drug efavirenz inclusion complex (1:1 and 1:2)

The solubility of prepared inclusion complexes in ratio 1:1 and 1:2 were determined by dissolving

complexed overnight into the distilled water. It was observed that more amount of drug solubilized with the increase in amount of cyclodextrin.Following table shows the enhancement in the aqueous solubility of efavirenz with cyclodextrin complex.

Table 2: The Solubility of Drug Inclusion Complexes (1:1 and 1:2)

Complex Solubility	F 1	F 2	F 3	Average	Concentration	Conc* DF	Amount (µg/ml)
Without CD	0.066	0.06	0.059	0.061	5.668	5.668	5.668412
1:1	1.064	1.057	1.051	1.057	57.797	288.987	288.9878
1:2	1.129	1.209	1.173	1.170	63.713	318.568	318.5689

The results show that the initial solubility of efavirenz in water is equal to 5.66 µg/ml whereas, with cyclodextrin in the ratio of 1:1 it was observed as 288.9 µg/ml and 318.56 µg/ml with 1:2 drug: cyclodextrin complex.

Conclusion:

The solubility of BCS class II drugs can be enhanced using inclusion complex techniques. Cyclodextrin and its derivatives due to their high aqueous solubility are promising carrier for the enhancement of aqueous solubility of poorly soluble drugs. The use of the Kneading technique to obtain solid inclusion system permitted the formation of a uniform, non-crystalline particle, which increased the solubility of EFV, and provided an increase in the stability of the drug also.

Reference:

[1] Maurin MB, Rowe SM, Blom K & Pierce ME (2002) Pharm Res 19:517-521.

[2] Srivalli KM & Mishra B (2016) AAPS Pharm Sci Tech 17:272-283.

PA-56

Novel Dry Injection For Reconstitution of Aspirin Using Solid Solubilisers

Anirudha Padiyar, R.K Maheshwari
Department of Pharmacy, Shri G.S Institute of Technology and Science, Indore, India- 452003
E-mail address: anirudhpadiyar23june@gmail.com

Abstract: The concept of mixed solvency is an emerging field which can serve as a milestone for solubility enhancement and therefore deserves an urgent attention of the scientific community to assess its efficiency and applicability. Mixed solvency concept suggested that each substance present on the earth whether solid, liquid or gas has solubilizing power. In mixed solvency concept each substance (gas, liquid or solid) is termed as solubilizer. Solubility enhancement by a single solubilizer in high concentration may raise the toxicity concern. Mixed solvency concept gives solution to this problem. Several solubilizers in small concentration in a blend may give desired solubility for a given drug and hence may be safe (non-toxic). In future, the industries shall use the solubilising properties of different additives for such purpose. The described research is one typical example where safe concentrations of additives have been used to prepare a dry injection for reconstitution of aspirin (a poorly water soluble drug)

Introduction:

As we know that all materials which exist in liquid state are known as solvents like water, chloroform, methanol, propylene glycol, dimethyl formamide, dimethyl sulfoxide, ethanol, benzene etc. No solvent is universal solvent. In other words, we can say that although these all liquids are known as solvent but they are not good solvents for all solutes. For example we know that water is good solvent for about one third drugs and bad solvent for about two third drugs. Thus we can say that all liquids are good solvent for some solutes and bad solvents for other solutes. Similarly, in mixed solvency concept [1-6] each and every substance (gas, liquid or solid) is good solubilizer for some solutes and bad solubilizer for other solutes.

Materials and Methods:

Aspirin was procured as a gift sample from Shree Pharmaceuticals Indore. Lignocaine hydrochloride and niacinamide were procured from Modern Laboratories, Indore.

Experimental

Solubility studies

Approximate solubility studies were carried out to determine the solubilities of seven drugs in an aqueous blend containing safe solid additives of injection in safe concentration (reported in literature). The solubilising efficiencies of these additives were evaluated. The aqueous blend (B) contained 5% w/v sodium benzoate (a safe buffering agent), 5% w/v PVP K30 (a plasma expander), 2.5% w/v

niacinamide (a safe stabilizer), 7.5% w/v PEG 4000 (a safe solubilizer) and 5% w/v lignocaine hydrochloride (a safe local anesthetic). Approximate solubilities of drug were determined by shaking the excess of drug with 10 ml of the blend for about 20 minutes in a bottle and then filteration was done. Approximately saturated solution of aspirin was analysed by titration with 0.1N NaoH using phenolphthalein indicator. For remaining drugs, spectrophotometric estimation were done. Approximate solubilities of all seven drugs are reported in Table 1.

Table 1 also gives solubilities of drug in distilled water at room temperature. Approximate solubility of aspirin in distilled water at room temperature was obtained by shaking excess of aspirin with about 60 ml of distilled water in a bottle for about 20 minutes. It was then filtered and filtrate was analysed titrimetrically. Result is presented in Table 1. The solubilities of remaining six drugs were taken from research papers.

Table 1: Solubilities and solubility enhancement studies

S.No	Name of drug	Solubility in distilled water at room temperature (% w/v)	Solubility in blend (B) at room temperature (%w/v)	Solubility enhancement ratio
1	Aspirin	0.331	8.811	26.6 fold
2	Norfloxacin	0.088	0.652	7.4 fold
3	Naproxen	0.009	5.745	638.3 fold
4	Tinidazole	0.538	1.206	2.2 fold
5	Piroxicam	0.040	0.994	24.8 fold
6	Frusemide	0.064	2.013	31.4 fold
7	Indomethacin	0.036	3.009	83.6 fold

Out of seven drugs studies, aspirin was selected for futhur study. A model dry injection of aspirin was developed. Although aspirin has about 8.811% w/v solubility in the blend (B), an injection was developed having 5% w/v strength of aspirin. Thus 2ml of such solution contains 100 mg of aspirin.

Formulation development of dry injection

Approximate solubility of aspirin in distilled water is 0.33 % w/v at room temperature. Approximate solubility of aspirin in an aqueous solution (a mixed solvency blend) containing 5% w/v sodium benzoate, 5% w/v lignocaine hydrochloride, 5% w/v PVP K30, 7.5% w/v PEG 4000 and 2.5% w/v niacinamide is 8% w/v. Thus a 5% w/v solution of aspirin can be made easily in the above mentioned mixed solvency blend. Hence, 2ml of such solution shall contain 100 mg of aspirin. It is evident from the literature that 5% w/v sodium benzoate is safely employed buffering agent in injections. PVP K30 is a plasma expander, therefore, 5% w/v PVP K30 is safe in injections. In this case lignocaine hydrochloride is an additive (local anesthetic to reduce the pain of injection). PEG 4000, an additive (solubilizer) is safe in 7.5% w/v concentration in injections. Niacinamide (2.5% w/v) is a safely used

stabiliser in injections. Thus, all five solid additives are present in safe concentrations in mixed solvency blend. Because of the solubilising effect of all five solids, the solubility of aspirin is enhanced tremendously and an aqueous injection can be developed to contain 100 mg aspirin in 2 ml of the blend. A dry injection of aspirin can nicely be developed to have very good chemical stability in the form of dry injection for reconstitution.

Table 2 give a formula for model dry injection of aspirin. Aspirin, 100 mg (sieved through fine sieve), lignocaine hydrochloride, 100 mg (sieved through fine sieve), niacinamide, 75 mg (sieved through fine sieve), PEG4000, 150 mg (fine powder), sodium benzoate, 100 mg (sieved through fine sieve) and PVP K30, 100 mg (sieved through fine seive) are kept in a 5ml vial. When 2 ml distilled water is added in the vial and vial is shaken vigourously, a clear solution is obtained. This experiment explains that a dry injection of poorly water soluble drug, aspirin, can be developed using solubilising power of all five solid solubilizers. Chemical stability studies and toxicological studies shall have to be performed to develop a drug injection for reconstitution of aspirin. All materials used in this formula should be free from pyrogens and microbes. Containers should be sterile and aseptic room shall be employed during its manufacture.

Table 2: Composition of model formulation

S.No.	Ingredients	Quantity
1	Aspirin (sieved through fine sieve)	100 mg
2	Lignocaine hydrochloride (sieved through fine sieve)	100 mg
3	PVP K30 (sieved through fine sieve)	100 mg
4	Niacinamide (sieved through fine sieve)	75 mg
5	PEG4000 (powder)	150 mg
6	Sodium benzoate (sieved through fine sieve)	100 mg

Conclusion:

Mixed solvency concept can further be utilized for development of dry injections as well as dry syrups of various poorly water soluble drugs. In above research work it is important to note that drug selected is a model drug and solublizers are model solubilizers. Formulations of various insoluble drugs can be developed using mixed solvency technique. Similarly, several combinations of safely used additives may be used to make innumerable safe blends giving enhanced solubilities of poorly soluble drugs.

References:

[1] Maheshwari RK (2009) Delving J. Tech Eng Sci 1:39-43.

[2] Maheshwari RK (2010) J Pharm Res 3: 411-413.

[3] Maheshwari RK (2010) Asian J Pharm 4:60-63

[4] Maheshwari RK, Padiyar A & Putliwala M (2015) Int J Pharm Res Ana 5:01-03.

[5] Maheshwari RK & Fouzdar A (2014) European J Biom Pharm Sci 1: 424-430.

PA-58

Liquisolid Technique as a Promising Tool to Enhance Solubility and Dissolution of Poorly Water Soluble Drug Valsartan

Madhavi Kasturi, Neelesh Malviya
Research Scholar, Department of Pharmaceutics, Smriti Collge of Pharmaceutical Education, Indore– 452010
E-mail address: madhavi2386@gmail.com

Abstract: "Liquisolid Technique" considered as new technique to enhance solubility and dissolution rate of poorly water soluble drugs. These formulations are prepared by mixing drug in liquid state (solution, suspension or emulsion using non-volatile solvent) with carrier and coating material to form dry, free-flowing, readily compressible powder. In the current research work liquisolid technique is employed to enhance solubility and dissolution of antihypertensive drug Valsartan, which is poorly water soluble (0.021mg/ml) possessing very low bioavailability of 23%. Liquisolid formulation VLS9, containing Tween 80 (non-volatile solvent), Avicel PH102 (carrier) and Aerosil 200 (coating material) showed better flow properties and high *in-vitro* dissolution profile.

Introduction:

Solubility is one of the key parameters to achieve desired concentration of drug in systemic circulation for showing affective pharmacological response. Low aqueous solubility is the major problem with formulation development of new chemical entities. Solubility enhances dissolution which in turn may increase bioavailability of poorly water soluble drugs. "Liquisolid Technique" also known as "Powder Solution Technology" is considered new, safe and economic technique to enhance solubility and dissolution profile of poorly water soluble drugs. Liquisolid formulations are prepared by converting liquid drug or drug in liquid state (solution, suspension or emulsion using non-volatile solvent) into dry, non-adherent, free-flowing, readily compressible powder by blending liquid medication with carrier and coating materials. Due to their significantly improved wetting properties a greater drug surface area is exposed to the dissolution media, resulting in increased dissolution rate and bio availability [1]. Valsartan is an angiotensin II receptor antagonist used in the management of hypertension [2]. Valsartan is an antihypertensive drug having low aqueous solubility of 0.021mg/ml and low bioavailability of 23%. The aim of current research work was to enhance solubility and dissolution of poorly water soluble drug.

Materials and Methods:

Valsartan was gift sample received from Hetero Drugs, Hyderabad. Other excipients include Avicel PH 102, Aerosil 200, Propylene glycol (PG), polyethylene glycol 600 (PEG600), Tween 80. All reagents used were of analytical grade.

Methods

Saturation solubility studies Saturation solubility studies of Valsartan were carried out in distilled

water, propylene glycol, PEG 600 and Tween 80. Saturated solutions prepared in above vehicles were kept in an orbital shaker (Remi motors Pvt. Ltd Mumbai, India.) for 48 h at 25 °C. The solutions were then filtered and drug content was determined using UV- VIS spectrophotometry (Shimadzu 1800, Japan) at 250 nm. . From these results, the solubility of valsartan in the respective liquid vehicle was calculated. Each experiment was carried out in triplicate [3].

Preparation of liquisolid formulations

This drug solution or suspension is incorporated into specific quantity of carrier material which should possess sufficient absorption properties. The resulting wet mixture is then converted into a dry–looking, non adherent, free-flowing and readily compressible powder by the simple addition and mixing of a calculated amount of coating material having high adsorptive properties.

Evaluation of liquisolid formulations

Characterization of flow properties of liquisolid formulations

Rania *et al*. (2008) suggested the pre compression evaluation parameters of Liquisolid powder systems. Flow properties of liquisolid systems were determined by estimating angle of repose, Carr's index and Hausner's ratio. Angle of repose was measured by fixed funnel method. Bulk density and taped density were also determined for the calculation of Hausner's ratio and Carr's index.

Calculation of liquid load factor (L_f): Load factor is calculated by dividing weight of liquid medication (W) to weight of carrier material (Q) and is given by: $Lf=W/Q$

Percentage yield of liquisolid formulation:

The percentage yield of the liquisolid system was determined using the following equation:

$$\% \text{ Yield} = L / Lo \times 100$$

Where, L = weight of prepared liquisolid formulation and Lo = total expected weight of formulation.

Drug Content:

Calculated amount of liquisolid powder formulation equivalent to single dose is dissolved in methanol and analyzed for drug content using UV-visible spectrophotometer at 257nm.

***In vitro* Dissolution Study**:

In-vitro drug release from liquisolid formulations is determined using USP Type II Dissolution Apparatus (paddle type). The dissolution study was carried out in 900ml phosphate buffer pH 7.4 at 37°C ± 2 °C and 50 rpm. At regular time intervals, aliquots of 5 ml samples were withdrawn up to 60min and the dissolution medium was replaced with 5ml fresh dissolution medium to maintain sink conditions. The samples were filtered through Whatman filterpaper no. 1 and analysed for drug content after suitable dilution using UV-Visible spectroscopy. Finally, cumulative percentage drug release is calculated for all formulations.

Optimization of prepared liquisolid formulations

The liquisolid formulation with better dissolution profile and good flow properties of powder was optimized and selected for further solid state characterization.

Solid state characterization of optimized liquisolid formulation

Scanning Electron Microscopy

SEM studies are performed to study surface morphology of drug as well excipients also confirm whether drug is present in crystal form or molecularly solubilized form.

Fourier Transform Infrared spectroscopy (FTIR)

FTIR studies are performed to study the compatability of drug and other excipients in formulation by comparing drug and formulation spectra.

Results and Discussion:

Saturated solubility studies for drug

Saturated solubility studies were performed to select suitable non-volatile solvent in which drug dissolves to prepare liquid medication.

Preparation of liquisolid formulations of Valsartan

After screening several non-volatile solvents propylene glycol, polyethylene glycol (PEG) 600 and Tween 80 were selected as liquid vehicles. Total 9 liquisolid formulations were prepared using Avicel as carrier material and Aerosil as coating material and shown in table below.

Table 1: Saturation solubility studies of Valsartan in various non-volatile solvents

Non-volatile solvent	Solubility of valsartan (mg/ml)
Distilled water	0.021
Phosphate buffer pH7.4	0.955
Polyethylene glycol 200	60.87
Polyethylene glycol 400	65.22
Polyethylene glycol 600	72.68
Propylene glycol	83.71
Tween 80	94.26

Table 2: Various Liquisolid formulations of Valsartan

Formulation Code	Valsartan (mg)	PEG 600 (mg)	Propylene Glycol (mg)	Tween 80(mg)	Avicel (MCC) (mg)	Aerosil 200(mg)	Formulation weight(mg)
VLS 1	40	100	-	-	100	60	300
VLS 2	40	110	-	-	120	60	330
VLS 3	40	120	-	-	150	60	370
VLS 4	40	-	100	-	100	60	300
VLS 5	40	-	110	-	120	60	330
VLS 6	40	-	120	-	150	60	370
VLS 7	40	-	-	100	100	60	300
VLS 8	40	-	-	110	120	60	330
VLS 9	40	-	-	120	150	60	370

Evaluation of liquisolid formulations of Valsartan

Table 3: Flow properties characterization of liquisolid formulations of Valsartan

Formulation Code	Bulk density (mg/ml)	Tapped density (mg/ml)	Carr's Index	Haussner's ratio	Angle of repose(θ)	Load factor	Percentage yield(%)
VLS 1	0.331	0.431	23.21	1.302	35.5	1.4	98.2
VLS 2	0.343	0.448	23.41	1.306	31.6	1.25	98.6
VLS 3	0.355	0.451	21.28	1.346	27.8	1.06	99.1
VLS 4	0.324	0.433	25.17	1.336	35.8	1.4	98.1
VLS 5	0.353	0.448	21.20	1.269	32.5	1.25	98.7
VLS 6	0.361	0.459	21.35	1.271	27.2	1.06	99.4
VLS 7	0.336	0.432	22.22	1.285	35.7	1.4	98.6
VLS 8	0.363	0.447	18.79	1.231	31.8	1.25	98.9
VLS 9	0.382	0.458	16.59	1.198	26.8	1.06	99.5

Drug content

Drug content of Valsartan liquisolid formulations were determined and given in below table.

Table 4: Drug content of Valsartan liquisolid formulations

Formulation Code	Drug Content (%)
VLS 1	90.05
VLS 2	91.43
VLS 3	95.33
VLS 4	91.45
VLS 5	93.89
VLS 6	94.19
VLS 7	92.88
VLS 8	94.66
VLS 9	96.78

***In Vitro* Dissolution Study**:

In vitro drug release i.e cumulative percent drug release was calculated for all valsartan capsules prepared by liquisolid technique and was compared with pure drug formulations.

Table 5: *In vitro* drug release of Valsartan liquisolid formulations

Time (min)	Pure drug	VLS 1	VLS 2	VLS 3	VLS 4	VLS 5	VLS 6	VLS 7	VLS 8	VLS 9
5	5.77	28.43	29.65	30.78	31.66	32.55	33.67	34.88	34.98	35.11
10	7.98	30.76	32.66	34.66	35.88	37.23	38.54	39.55	40.66	42.44
15	10.65	45.66	46.32	48.54	49.55	50.43	51.87	53.66	54.75	55.32
30	15.22	67.44	69.65	70.22	71.64	72.15	73.88	76.89	79.54	80.34
45	21.45	71.56	74.71	75.54	76.34	77.97	78.43	79.55	80.43	82.51
60	27.82	83.74	85.52	86.31	87.48	88.76	89.22	93.64	94.43	98.77

From the results of in vitro drug release it was observed that liquisolid formulation VLS9 showed better release profiles compared to that of pure drug. The mechanism for enhanced drug release postulated in liquisolid systems include increased surface area, improved wettability of drug particles due to reduction in interfacial tension which lead to increased aqueous solubility of the drug in

microenvironment surrounding system. The reason may be due to greater wettability property of hydrophilic solvent Tween 80 having surfactant property of reducing interfacial tension. Valsartan within the liquisolid system is completely dissolved in Tween 80 and is located in the powder substrate still as solubilized, molecularly dispersed state. Thus the surface area of drug available for release is much greater than that of drug particles available in directly compressed tablets.

Solid state characterization of optimized liquisolid formulation

Scanning Electron Microscopy

SEM studies were conducted for drug, excipient as well as for optimized liquisolid formulation. The results of SEM studies revealed that crystalline form of drug has been converted to amorphous state.

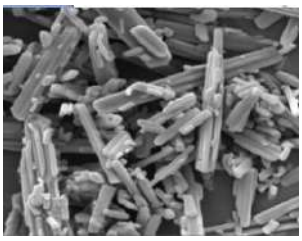


Figure1: SEM of Valsartan

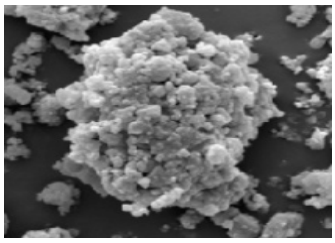


Figure 2: SEM of VLS9 Formulation

Fourier Transform Infrared spectroscopy (FTIR)

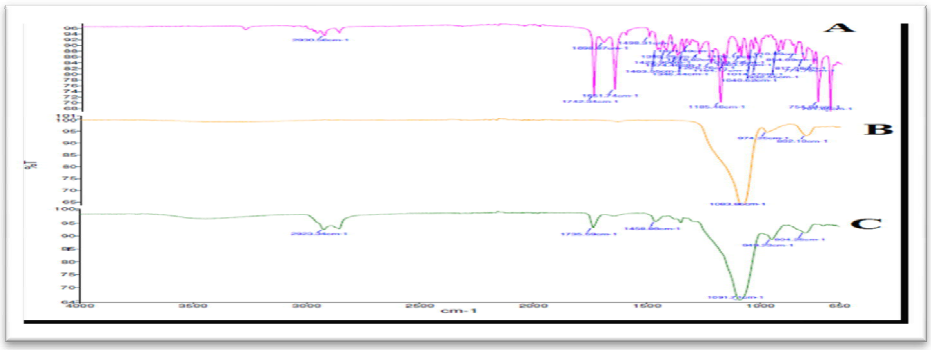


Figure 3: a. Valsartan b. Aerosil 200 c. VLS9 formulation

Results of FTIR showed that characteristic peaks in VLS 9 liquisolid formulation contained both peaks that of pure drug and Aerosil indicating no interaction between drug and excipients occurred.

Conclusion:

Successfully liquisolid formulations of valsartan was prepared using with remarkable improvement in dissolution profile and thus proved the potential of liquisolid technique as safer, efficacious method in enhancing solubility as well dissolution profile of poorly water soluble drugs.

References:

[1] Shashidher B, Madhusudhanrao Y &Venkateswarlu V (2011) Braz J Pharm Sci 47: 475-482.

[2] Leidig M, Bambauer R, Kirchertz EJ, Szabã T, Handrock R, Leinung D, et al. (2008) Clin Nephrol 3:425-432.

[3] Gubbi S and Ravindra J (2010) Asian J Pharm 5:50-60.

PA-59

Diallyldisulfide Containing Polymeric Nanoparticles for Site-Specific Delivery in Colon Cancer

Apeksha Saraf, Nidhi Dubey
School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P.)
E-mail address: saraf.apeksha@gmail.com

Abstracts: Effective delivery of drugs to colon is a challenge, as the drug needs to be protected from gastrointestinal environment and should be released intact in colon. Present study is aimed to develop a site-specific nanoparticulate formulation containing herbal anti-cancer agent in treatment of colon cancer. The Diallyl disulfide nanoparticles (DADS-NPs) were prepared by nanoprecipetation method and optimized formulation was selected using Box-Behnken design (BBD). The particle size of NPs was found to be 108 nm. The encapsulation efficiency was found to be 77.24%. and *in-vitro* release data has revealed controlled and prolonged drug release upto 72h.

Introduction:

Targeted drug delivery systems provides medication intact to targeted site by improving its safety, efficacy and lowering the frequency of dose, due to which there has been increased interest in formulation of site-specific drug delivery system. Colon-specific drug delivery systems can be advantageous to target the drugs directly to the colon in malignancies like cancer of colon and rectum. This site-specific drug delivery system can achieve high concentration of drugs in colon to improve its bioavailability and to lower its systemic toxicity. The potential of DADS as an anticancer agent in colon cancer has extensively been investigated. The present study is aimed to develop a nano-particulate drug delivery system containing a herbal anti-cancer agent, Diallyldisulfide, for colorectal cancer cells as they show enhanced permeation, accumulation and retention in the tumor tissue[1-3].

Material and Methods:

Material

PLGA (75:25) was obtained as gift sample from Corbion Purac (Netherlands). DADS were procured from Sigma Aldrich. All other chemicals and reagents were of analytical grade.

Method

The DADS loaded nanoparticles were prepared by nanoprecipitation method using acetone as a solvent. Briefly, PLGA (75:25) and DADS were dissolved in 5 ml of acetone. The prepared solution was added drop-wise with syringe (G¼22) into 20 ml of distillated water under magnetic stirrer at room temperature. The resulting solution was stirred for complete evaporation of the organic solvent. The nanoparticles were collected by centrifugation and further freeze dried. The effect of independent variables viz. change in stirring speed, polymer to drug ratio, ratio of volume of outer water phase to the organic phase and concentration of surfactant was studied on particle size and entrapment

efficiency(dependent variables). The optimized formulation was selected by application of Box Behnken design using Design expert software.

Results and Discussion:

A Box-Behnken design was applied for optimization of NPs formulation. The optimized formulation of NPs were selected on the basis of criteria of attaining the maximum value of encapsulation efficiency while minimizing the particle size with numerical point prediction optimization method using Design Expert software®. Scanning electron microscopic images have showed that the nanoparticles have regular and uniform shape (Figure 1). The average particle size of nanoparticles was found to be 108nm. The zeta potential of NPs were found to be + 31.5mV ± 2mV, which indicated the physical stability of the formulation. The value of polydispersity index was found to be 0.206. The optimized formulation has shown entrapment efficiency of 77.24%. The in-vitro drug release have shown initial burst release and then prolonged drug release with cumulative drug release of 74.54%. The release kinetics was studied by applying zero order kinetic model, first order kinetic model, Higuchi model and Korsmeyer-Peppas. According to the highest correlation (R^2) value it is evident that the optimized formulation of DADS nanoparticles follows the Higuchi model.

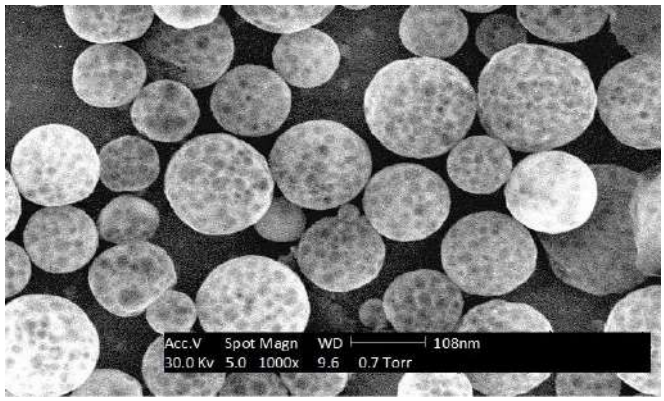


Figure 1: Scanning electron microscopic image of nanoparticles

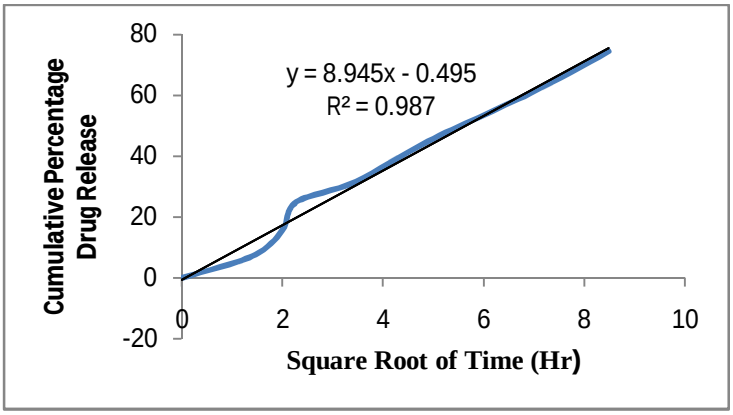


Figure 2: Drug release kinetics of nanoparticles

Conclusion:

The result of the study has shown physical stability, good encapsulation efficiency and prolonged rate of drug release from nanoparticles. Thus, it can be concluded that the PLGA nanoparticles containing DADS could provide a promising strategy for treatment of colon cancer with enhanced bioavailability with further cytotoxicity and *in-vivo* studies.

References:

[1] Lu L, Chen G, Qiu Y, Li M (2016) Sci Bull 61:670–681.

[2] Udompornmongkol P & Chiang B (2015) J Biomater Appl 30: 537-546.

[3] Talluri SV, Kuppusamy G, Karri VVSR, Yamjala K (2017) Artif Cells Nanomed Biotechnol 45:474-488.

PA-69

Formulation and Evaluation of Mouth Dissolving Film of Tadalafil

Pravin Kumar Sharma[1], G. N. Darwhekar[1], Birendra Shrivastava[2], Pankaj Sharma[2]
[1]Acropolis Institute of Pharmaceutical Education and Research, Indore
[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur
E-mail address: praveensharma@acropolis.edu.in

Abstract: This research work was aimed to enhance the oral bioavailability and provide faster onset of action Tadalafil (used for the treatment of the erectile dysfunction (ED) and pulmonary arterial hypertension) by formulating mouth dissolving film (MDF). Tadalafil belongs to BCS class II and the oral bioavailability of it's about 28%. The MDF of Tadalafil was prepared by solvent casting method using HPMC-E5 (film forming agent), Methyl cellulose (thickening agent), Propylene glycol (plasticizer), Tween-80 (solubilizing agent), Microcrystalline cellulose (disintegrating agent), Citric acid (saliva stimulating agent), Sucrose (sweetening agent), Vanillin (flavoring agent), EDTA disodium (preservative). The formulation was optimized by two factors, three level (2^3) full factorial design using concentration of Plasticizer (X1) and concentration of film forming agent (X2) as independent variables. and formulation was evaluated for thickness, uniformity of mass, disintegration, drug content, determination of moisture uptake, in-vitro drug release studies and stability study. Based on results it was concluded that MDF (F5) showed enhanced bioavailability and faster onset of action as compared to available tablet dosage form.

Introduction:

Currently, 90% of the new chemical entities (NCEs) filling belongs to the poorly soluble BCS Class II and IV compounds. Poor aqueous solubility and/or permeability of drug candidates often leads to poor absorption and bioavailability from the gastrointestinal (GI) tract, which presents the formulation scientists with considerable challenges when trying to deliver these drug molecules via oral route. Fast-dissolving drug-delivery systems were first developed in the late 1970s as an alternative to tablets, capsules, and syrups for pediatric and geriatric patients who experience difficulties swallowing traditional oral solid-dosage forms. However, the function and concept of all these dosage forms are similar. There is no need for the administration of water if a solid dosage form that dissolves or disintegrates rapidly in the mouth, and such form is known as an mouth dissolving dosage form [1].

Mouth dissolving film (MDF) is new drug delivery system for the oral delivery of the drugs. MDFs are the most advanced forms of oral solid dosage forms due to more flexibility and comfort. MDFs are strip type preparations with active molecules dissolved or dispersed in film forming materials [2]. It gives quick dissolution, absorption and instant bioavailability of drugs due to high blood flow and permeability of buccal mucosa is 4-1000 times greater than that of skin [3]. Tadalafil is a potent and selective phosphodiesterase-5 inhibitor used for the treatment of erectile dysfunction which was

approved by the FDA. Tadalafil achieves it maximum concentration in plasma after 2-3 hours of administration [4].

Material and Methods:

Materials

Tadalafil was obtained as a gift sample from Shagun Pharma, Indore (M.P.). HPMC-E5, Methyl cellulose and microcrystalline cellulose was obtained from Signet chemicals Pvt Ltd., Mumbai (Maharastra). All other chemicals were purchased from Loba chemicals Pvt. Ltd., Mumbai (MH).

Methods

UV Analysis

Absorption maxima of Tadalafil were determined by scanning its solution on spectrum mode of Solution of UV visible spectrophotometer. Calibration curve of Tadalafil was prepared in methanolic distilled water (2:8) and phosphate-buffered pH 6.8 separately at 281.5 nm by double beam UV visible spectrophotometer (Shimadzu, 1800, Japan). Linearity was observed over a concentration range of 10–50 µg/ml, with an R^2= 0.998.

Solubility determination: (n=3)

Sufficient excess amount of Tadalafil was added to 5 ml glass vials containing distilled water and phosphate buffer (pH 6.8). The content of vials was stirred for 12 h on magnetic stirrer (Remi labs, Mumbai) at 37 ± 2°C. The solution was transferred into eppendorf tubes and centrifuged for 5 min at 2000 rpm. The content of vial were filtered through 0.45µ membrane filter and analyzed for drug content by UV visible spectrophotometer (Shimadzu, 1800, Japan) at 281.5nm after appropriate dilutions.

Formulation of OFDFs of Tadalafil

MDF of Tadalafil was prepared using solvent casting method. Required amount Tadalafil, film forming agent (HPMC-E5), Propylene glycol, Methyl cellulose, Microcrystalline cellulose, Tween-80, Sucrose, disodium EDTA, Citric acid were dissolved in 10ml distilled water under constant stirring at 1100 rpm on magnetic stirrer (Remi labs, Mumbai) on temperature until a clear solution had been obtained. Subsequently, mixture was stirred at room temperature overnight at 100 rpm to allow entrapped air bubbles to disappear. The solution was then casted onto a fabricated glass mould previously lubricated with glycerin. The film layer was dried room temperature and removed from the mould and cut in squares of 2×2 cm yielding four stamp shaped MDFs [5]. Formulation of MDFs was optimized by two factors, three level (2^3) full factorial design as shown in table-1 using concentration of Plasticizer (X1) and concentration of film forming agent (X2) as independent variables.

Evaluation of MDFs

Uniformity of mass Uniformity of mass was determined according to the European Pharmacopoeia. Twenty randomly chosen MDF were weighted individually on an analytical balance. Subsequently the average mass was calculated.

Table 1: Formulation optimization of MDFs of Tadalafil (n=3)

Ingredients	Batch Number								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Tadalafil (mg)	20	20	20	20	20	20	20	20	20
HPMC-E5 (mg) X1	700	700	700	500	500	500	300	300	300
Propylene glycol (ml) X2	0.025	0.05	0.075	0.025	0.05	0.075	0.025	0.05	0.075
Methyl cellulose (mg)	10	10	10	10	10	10	10	10	10
Microcrystalline cellulose (mg)	100	100	100	100	100	100	100	100	100
Tween-80 (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sucrose (mg)	5	5	5	5	5	5	5	5	5
EDTA disodium (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Citric acid (mg)	20	20	20	20	20	20	20	20	20
Vanillin (mg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Thickness

The thickness of the film was measured by micrometer screw gauge (Acculab) at three different places; averages of three values were calculated. The uniformity in the thickness of the film is directly related to the accuracy of dose in the film.

Folding endurance

This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. Folding endurance of the MDF was determined by folding repetitively one film at the same place till it breaks.

Drug Content uniformity

The drug content uniformity of each film was determined by dissolving the film in 10 ml of phosphate buffer pH 6.8, followed by filtering through 0.45 µm membrane filter. The filtrate was appropriately diluted, and the content of Tadalafil was determined at 281.5 nm using UV spectroscopy.

***In Vitro* disintegration test:**

Disintegration time of MDFs was determined by drop method. Five randomly chosen MDFs from each run were tested. The MDF was placed onto a small glass petridish and subsequently a volume of 0.2 ml distilled water was placed repetitively onto the film until the film disintegrates and time was noted.

***In Vitro* dissolution study**

In Vitro dissolution test was carried out for Tadalafil MDF and marketed Tadalafil tablet for comparative drug dissolution study in a paddle type dissolution apparatus (Electrolab). Each film was

fixed to a piece of metal wire slab and placed at the bottom of the dissolution vessel. The dissolution medium was 250 ml of phosphate buffer pH 6.8, maintained at 37±0.5°C and stirred at 50 rpm. Samples of 5 ml were withdrawn at 30, 60, 90, 120,150 and 180 seconds and were filtered through a 0.45-µm membrane filter and analyzed by UV–spectroscopy at 281.5 nm.

Stability Studies

Stability studies was conducted for optimized F5 formulation prepared films to assess their stability with respect to their physical appearance, drug content and drug release characteristics after storing them at 40° C/75 % RH for 3 months. Samples were withdrawn at 0, 30, 60 and 90 days.

Results and Discussion:

Solubility determination

The saturated solubility of Tadalafil was found to be 0.243±0.042 mg/ml in distilled water and 0.312±0.025 mg/ml in phosphate buffer pH 6.8 and it confirms the low aqueous solubility of Tadalafil.

Evaluations of MDF

The results of various evaluation parameters are shown in Table -2. Thickness and Uniformity of mass for all films were noted to increase by increasing the fraction of polymer as expected. The maximum force and the tensile strength were higher for the HPMC-E5 films in general which clearly justify in results of folding endurance and was found to be more than 195 times for all the formulations, which shows that the prepared films are robust in nature. In general all formulations were found to be fast disintegrating and showed different disintegration time because of different polymer concentration. The result of *in vitro* dissolution study showed almost complete (upto 96%) drug release within 3 minutes time. This indicates fast drug release from the thin MDF delivery system. Furthermore, in comparison with Tadalafil tablet, MDF showed 2 fold fast dissolution rate upto 3 minutes as shown in figure1.

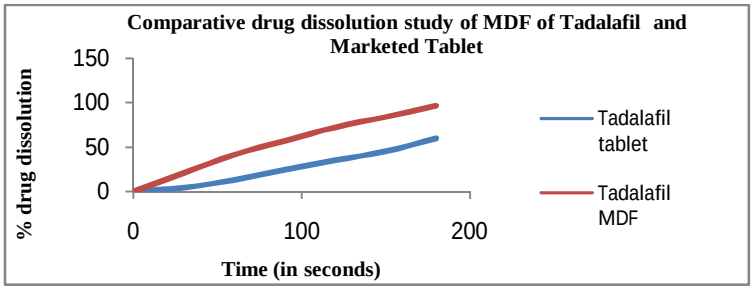


Figure 1: Comparative drug dissolution study of MDF of Tadalafil and Marketed Tablet

Almost all batches met the criteria regarding the content uniformity and the observed results of drug content in the films were found to be in the range 89-97% which indicates that the drug was uniformly dispersed in the film. Formulation F5 was rated as the best formulation with respect to the drug release and content uniformity.

Stability Studies

The result of stability study indicates that the drug product falls well within the proposed stability specification. The data indicates that there is no significant physical or chemical change indicating that the formulation would maintain its efficacy and quality throughout its proposed shelf life.

Table 2: Evaluation data of mouth dissolving film of Tadalafil (n=3)

Batch No.	Uniformity of mass (mg)±SD	Thickness (mm)±SD	Folding endurance (times)	*In vitro* disintegration time (Seconds)	% *In vitro* dissolution	% Drug Content
F1	213.9±0.02	0.15± 0.04	236 ±1	62±2	85.57±0.78	93.5±0.04
F2	214.5 ±0.05	0.12±0.02	230 ±2	71±2	80.82 ±0.07	89.7±0.08
F3	212.4±0.04	0.14±0.01	227±1	65±1	91.42±0.06	93.4±0.07
F4	160.2±0.06	0.10±0.06	228 ±2	32±2	94.87±0.05	95.0±0.05
F5	162.3±0.04	0.11±0.78	230±2	29 ±1	96.30± 0.04	96.8 ±0.02
F6	162.4±0.09	0.10±0.05	225 ±1	27 ±2	92.23± 0.04	94.4±0.04
F7	113.2±0.08	0.09±0.05	198 ±3	25 ±3	89.56 ±0.02	94.5±0.06
F8	113.8±0.05	0.09±0.07	197 ±2	29 ±2	82.56 0.71	89.8±0.09
F9	112.6±0.07	0.10±0.04	195±2	27 ±3	88.50±0.08	92.5 ±0.07

Conclusion:

Formulation of MDF of Tadalafil with desirable properties was achieved by optimization approach through factorial design. The results showed that formulation F5 showed desired properties as compared to other batches. Results of comparative dissolution study of MDF with Tadalafil tablet showed 2 fold fast dissolution rate upto 3 minutes. Stability study also suggests the good shelf life of the MDF. On the basis of above presented experimental work we can conclude that MDF of Tadalafil improve its oral bioavailability and may provide faster onset of action.

Acknowledgements:

The author is especially thankful to Shagun Pharma, Indore for providing Tadalafil as a gift sample.

References:

[1] Naziya K, Raghavendra R and Mahipal R (2013) Int J Chem Pharm Sci 1:63-75.

[2] Hema C, Samita G, Permender R & Kumar V (2013) Bull of Fac of Pharm, 51:193–201.

[3] Hiroyoshi S, Kazumi T, Misao N, Katsuhiko M, Tadao T, Hirotaka Y (2009) Eur J of Pharm and Biopharm 73:361–365.

[4] Hitendra SM & Vinayak BK (2015) Indian J Novel Drug Delivery 7:100-107.

PA-70

Formulation and Evaluation of Bilayer Tablet of Metronidazole and Dicyclomine Hydrochloride for Treatment of Irritable Bowel Disease

Avinash Sharma, Pravin Kumar Sharma, Gajanan Darwhekar
Acropolis Institute of Pharmaceutical Education and Research, Indore (M.P.)
E-mail address: avinashsharmamph15@acropolis.edu.in

Abstract: The aim of the present study was to prepare bi-layer tablet of Metronidazole and Dicyclomine Hydrochloride for the effective treatment of Irritable Bowel Disease. Metronidazole and Dicyclomine Hydrochloride were formulated as immediate and sustained release layer respectively. The rational for formulation of bi-layer tablet of these two combinations was Metronidazole (immediate release) is strong antibiotic usually applied for antidiarrheal to treat inflammation of the large intestine and Dicyclomine Hydrochloride (Sustained release) is anticholinergic drug primarily used for irritable bowel disease. Bilayer tablet was suitable for maximize the efficacy of drugs in irritable bowel disease.

Introduction:

In the last two decades, Irritable Bowel Syndrome (IBS) has gained considerable attention in the health-care field due to its increasingly high prevalence, sometimes debilitating effects and diverse symptom representation [1]. Bi-layer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances, and also for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose [2]. Metronidazole 2-(2-methyl-5-nitroimidazol-1-yl)ethanol is a potent antibiotic usually applied for treatment of anaerobic infections and mixed infections, surgical prophylaxis requiring anaerobic coverage, Clostridium difficile-associated diarrhea and colitis diarrhea. The drug has potent activity and Well absorbed (at least 80%) with peak plasma concentrations achieved in 1-3 hours following oral administration of therapeutic doses of immediate release formulation.Dicyclomine Hydrochloride 2-(diethylamino)ethyl 1-cyclohexylcyclohexane-1-carboxylate;hydrochlorideis a muscarinic antagonist used as an antispasmodic and in urinary incontinence. It antagonizes muscarinic receptors on smooth muscle in the gastrointestinal (GI) tract, thereby preventing the actions of acetylcholine and reducing GI smooth muscle spasms.

Material and Methods:

Materials

Dicyclomine Hydrochloride was a gift sample obtained from Shreepati Pharma, Indore, Metronidazole was a gift sample obtained from Modern Laboratories, Indore, Sodium Starch

Glycolate, Poly vinyl Pyrollidone (PVP K-30), Magnesium stearate, Talc, Isopropyl alcohol were procured from Kasliwal Brothers Indore. All chemicals were used of analytical grade.

Methods

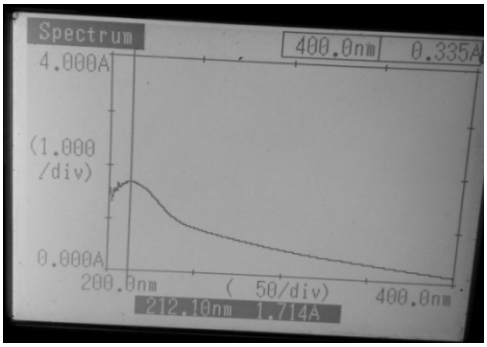
UV Analysis

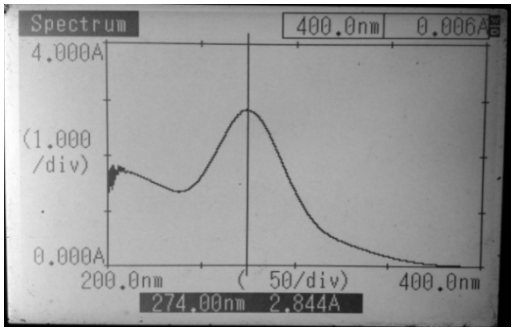
Calibration curve of Metronidazole

The calibration curve of Metronidazole was prepared in distilled water and 1.8 pH phosphate buffer by using Shimadzu 1800 UV visible spectrophotometer. Accurately weighed 50mg of Metronidazole was transferred into a 50ml volumetric flask and the volume was made up with methanol to obtain a 1000µg/ml stock solution of Metronidazole. From the stock solution 1ml was taken and transferred into a 10ml volumetric flask and rest of the volume was made up with methanol to obtain a 100µg/ml of solution from which 1 to 10µg/ml dilutions were prepared [3].

Calibration curve of Dicyclomine Hydrochloride

The calibration curve of Dicyclomine Hydrochloride were prepared in distilled water and 7.2 pH phosphate buffer by using Shimadzu 1800 UV visible spectrophotometer. Same procedure as mentioned above is utilized in phospate buffer 7.2 respectively.





Solubility studies

The solubility of both drugs in various medium was determined by shake flask method. In this method 2ml of each solvent was taken into a vial and an excess amount of drug was added. The vials were sealed properly and stirred for 10min. They were then kept on orbital flask shaker at 37°C for 24h. After solubilization of drug, an extra amount of drug was added to the vials containing drug-solvent mixture. The process was repeated until saturation solubility of drug, indicated by presence of undissolved drug. The mixtures were then kept at room temperature for 24 h. and centrifuged using Remi12C micro-centrifuge at 3000RPM for 15min. The supernatant were separated and diluted with respective solvents. The drug concentration was analyzed spectrophotometrically at 212 and 274 nm using UV-visible spectrophotometer (Shimadzu-1800) [3].

Preparation of Metronidazole Immediate release layer by Direct Compression

Weigh all Ingredients as per the quantities shown in table no. 1. Pass all the ingredients through sieve #80 and collect individuals in polybags. Mix measured quantity of Metronidazole, SSG and PVP-K30 for 15 minutes in a polybag. Magnesium stearate and talc was added to it and blend for 5 min in pastle

mortar. Compress final blend using B-Tooling, multiple rotatory compression machine using 8 mm round shaped punches and corresponding dies.

Table 1: Formula for immediate release layer

S. No.	Ingredients	Quantity
		Per 10 Tablets (in mg)
1.	Metronidazole	2500
2.	Sodium starch glycolate	300
3.	PVP K-30	150
4.	Magnesium Stearate	50
5.	Talc	50

Preparation of Dicyclomine Hydrochloride Sustained release layer by Wet Granulation

Weigh all Ingredients as per the quantities defined in tablet no. 2. Pass all the ingredients through sieve no #80 and collect in separate polybags. Prepare binder solution by dissolving PVP-K30 in Isopropyl alcohol. Mix all material expect lubricant for 15 min. Add binder solution to the above step and mix until uniform dough mass granules are formed. Pass all granules through #12 no. sieve. Dry all the granules at 50-55^{O}C temperature. Add Magnesium stearate and Talc and blend it for 5 Minute. Compress final blend using B-tooling, multiple rotatory compression machine using 8 mm round shape punches and corresponding dies.

Selection of polymers and suitable experimental design

A central composite design for two factor three level was selected to optimize the variable response. The two factors, viz. Polymer X_1Eudragit RSPO and Polymer X_2 PVP-K30 of each polymer blend, were required by the experimental design and the factor level were suitably coded. The amount of Magnesium stearate was kept constant, while Isopropyl alcohol was taken in a sufficient quantity to maintain a constant tablet mass of 200mg. time taken to release 50% of drug were taken as the variable response [4].

Table 2: Formulation optimization data

S.No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Dicyclomine Hydrochloride	200	200	200	200	200	200	200	200	200
2.	Eudragit RSPO	400	400	400	500	500	500	600	600	600
3.	Microcrystalline cellulose	800	800	800	800	800	800	800	800	800
4.	PVP-K30	80	100	120	80	100	120	80	100	120
5.	Magnesium Stearate	50	50	50	50	50	50	50	50	50
6.	Talc	50	50	50	50	50	50	50	50	50
7.	Isopropyl alcohol	QS	QS	QS	QS	QS	QS	QS	QS	QS

Evaluation of Granules

To find out physiochemical properties and release characteristics of the granular blend, all formulations are subjected to pre-formulation studies like bulk density, tapped density, Angle of repose, compressibility index and Hausner's ratio.

Preparation of Bilayer tablets of Metronidazole and Dicyclomine Hydrochloride

Optimized batch of Metronidazole and Dicyclomine Hydrochloride were selected for formulation of bilayer tablet. As previously reported procedure for granules of immediate and sustained release layer were blended separately. One by one both layer was filled in rotatory compression machine and compressed.

Evaluation of compressed tablets

The tablets prepared were evaluated for weight variation, disintegration test, dissolution test, thickness, hardness of individual dose and friability.

Weight variation

The weight variation was performed by weighing 20 tablets individually, then individual weight of tablet is compared with average weight of 20 tablets.

Hardness

The hardness of each batch of tablet was checked by using Monsanto hardness tester. The hardness was measured in terms of kg/cm2.

Friability

The friability was determined by first weighing 10 tablets before placing in friability tester (Roche friabilator), which was rotated for 4 min at 25 rpm. After dusting, the remaining weight of tablet was determined.

Thickness

The thickness of tablet was determined by vernier caliper.

Disintegration

The test was performed by introducing one tablet in each tube and adds a disc to each tube. Suspend the assembly in the beaker containing purified water and operate the apparatus until the tablet completely disintegrates.

***In Vitro* Dissolution test**

The in-vitro dissolution studies were carried out using USP apparatus type II at 50 rpm. The dissolution medium (900 ml) consisted of simulated gastric fluid (pH 1.2 HCL buffer) was used for the first 2 hour and then replaced with phosphate buffer (pH 7.2) for 3 to 8 hours (900 ml), maintained at 37±0.5ºC. The drug release at different time interval was measured by UV-visible spectrophotometer at 212 nm and 274 nm. The release studies were conducted on six tablet in each batch and the mean values were plotted versus time [4].

Result and Discussion:

Metronidazole and Dicyclomine Hydrochloride Bilayer tablet was formulated. Total nine batches were prepared for sustained release layer. All the formulations were subjected to evaluation, Tablet weight of sustained release layer varied from 155 to 189 mg, and thickness 3 to 4.1 mm. All the tablets exhibited friability values between 0.22 to 0.3, all immediate release layer disintegrated in less than 1 minute.

Conclusion:

The present study was carried out to prove that bi-layer tablet of Metronidazole and Dicyclomine Hydrochloride as Immediate and Sustained release layer can be formulated. The concept explains the application of IR/SR from single dosage form which results in cost effectiveness and reduces the problems of irritable bowel disease.

Acknowledgment:

The author is especially thanks to Acropolis institute of pharmaceutical education and research, Indore for supporting to this project also thankful to Shreepatipharma and Mordern laboratories for providing gift samples of drug for this project work.

References:

[1] Spiller R, Aziz Q, Creed F, Emmanuel A, Houghton L, Hungin P, Jones R, Kumar D, Rubin G, Trudgill N, Whorwell P, (2008) Gut 56:1770-1798.

[2] Admassu A, Akseli I, Sprockel O, Kottala N, Cuitino AM (2013) Int J Pharm 461:549-558.

[3] Kumar ES, Ishaq BM, Muneer S, Shreedhar V, Ahed HA, Prakash KV (2014) Indo Am J Pharm Sci 2014, 1-10.

[4] Shiyani B, Gattani S, Surana S (2008) AAPS Pharma Sci Tech 9:818-827.

PA-72

Non-Ionic Surfactant Vesicles (Niosomes) Based Novel Ophthalmic Formulation of Timolol Maleate

Prakash Kumar Soni, T.R. Saini
Nanotechnology Research Lab., Department of Pharmacy, Shri G.S. Institute of Technology and Science, 23-Park Road, Indore-452003, (MP) India
E-mail address: soniprakashpharma@gmail.com

Abstract: The objective of the present work was to develop drug loaded niosomal ophthalmic formulation of timolol maleate (an antiglaucomal drug) for enhanced trans-corneal drug permeation and better ocular bioavailability. Timolol loaded niosomes were prepared by thin film hydration method using rotary evapoarator and ultra-sonicated for size reducation using probe sonicator. The nano-vesicle (niosomal) formulation was optimized by selecting surfactant content, cholesterol content and sonication time as independent variables and particle (vesicle) size, drug entrapment efficiency and % drug release as response variables for optimization studies. The timolol maleate niosomal (TMN) formulation was evaluated for particle size, pH and osmolality and was found to possess the desired properties. The developed TMN formulation was studied for % cumulative in-vitro drug release using bottle rotating apparatus (electrolab) and was found to be 79.98% over 8 hr period exhibiting sustained drug release profile. The ex-vivo trans-corneal drug permeation profile of developed TMN was studied using modified franz-diffusion cell apparatus (Permegear) and the % cumulative drug permeation across freshly excised goat cornea was found to be 65.90 % in 8 hrs duration, which was approximately 1.5 times higher than the conventional eye drop formulation. The developed TMN was also proved to be isotonic and non-irritant in HET-CAM ocular irritancy test. It was therefore, concluded from above studies, that the developed timolol maleate niosomal (TMN) formulation is better than conventional eye drops due to longer corneal retention, sustained drug release and better trans-corneal drug permeation and thereby higher ocular bioavailability, hence, would need less frequent administration.

Introduction:

Timolol maleate, a non-selective beta blocker, is widely used for treatment of glaucoma in the form of eye drops, however, the conventional eye drop formulations suffer from inadequate ocular bioavailability and need to be frequently administered, due to their short pre-corneal residence, naso-lacrymal drainage and poor trans-corneal permeation. It was, therefore, aimed to develop nano-vesicular (niosomal) ophthalmic formulation of timolol maleate for enhanced trans-corneal permeation and ocular bioavailability. Niosome based novel formulations are being extensively explored due to their inherent advantages in drug delivery system [1].

Material and methods:

Material

Timolol maleate was received as gift sample from M/s. Piramal Healthcare Ltd. Pihampur (MP). Span-60 (Loba Chemie) and Cholesterol (Merck) was purchased from local market. All other chemicals, reagents and solvents used were of analytical grade.

Formulation of Timolol maleate niosome (TMN)

Timolol maleate loaded niosomes were prepared by thin-film hydration method [2] using rotary evaporator (Buchi®).

Determination of particle size and entrapment efficiency

The niosomal dispersion obtained as above was sonicated for 2-3 minutes using ultra-probe sonicator (sonics) for converting the niosomes to nanometric size range and the particle (vesicle) size distribution of TMN formulation was determined by Nanotrac nano-particle size analyzer (Microtrac Inc.). The timolol maleate loaded niosomes were separated from free drug (unentrapped drug) by centrifugation at 13,000 rpm and 4°C using refrigerated centrifuge (Eppendorf). The clear supernatant so obtained was analyzed for estimation of drug content and the amount of unentrapped drug was calculated. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug amount taken [3].

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug taken}} \text{ X } 100$$

In vitro drug release study

In vitro drug release study of the developed TMN formulation was performed in Bottle rotating apparatus. In this method, an accurately weighed quantity of timolol maleate niosomes (equivalent to 2 mg drug), was suspended in 10 ml phosphate buffer (pH 7.4) filled in the capped glass bottle (tube) and rotated at 25 rpm and maintained at 37^0C. One test sample bottle (tube) was taken out every one hour and was analyzed for estimation of drug release till 8 hour duration and thereby, the % cumulative drug release was calculated for a period of 8 hr.

Ex vivo Trans-corneal Drug Permeation Study

Trans-corneal drug permeation study of developed TMN formulation was carried out under ex-vivo condition across freshly excised goat cornea using modified franz-diffusion cell apparatus and was compared to that of conventional eye drop product. The exposed area of goat's cornea was 1.130 cm^2 [4].

Isotonicity evaluation

Red blood cells were separated from freshly collected blood from slaughter by centrifugation applying centrifugal force of 14000G for 10 min using cooling centrifuge (Eppendorf). The recovered RBCs were suspended in appropriate amount of developed TMN formulation, 0.9% saline (isotonic)

solution (negative control), and hypotonic & hypertonic saline solution (positive control) was vortexed for 5 min and was kept aside for 30 min and subsequently observed under the polarizing microscope (Leica) at 40X magnification.

HET-CAM Ocular irritancy test

The purpose of this test to evaluate the potential ocular irritancy of a test substance as measured by its ability to induce toxicity in the chorio-allantoic membrane (CAM) of a chicken embryo in fertilized hens egg. In this test, irritancy is assessed by the onset of hemorrhage, coagulation and vessel lysis. These assessments are considered individually and then combined to derive a score; irritation score (IS) which is used to classify the irritancy level of the test substance.

Results and Discussion:

The various physico-chemical properties i.e., particle size, polydispersity index, pH, osmolality and % entrapment efficiency was studies and the results observed are presented in Table 1

Table 1: Physico-chemical properties of developed TMN formulation

S. No.	Properties	Result
1	Particle size	226.29 nm
2	Osmolality	298 mOsm/kg
3	% Entrapment efficiency	42.88%

The developed TMN formulation was studied for % cumulative in-vitro drug release using bottle rotating apparatus (electrolab) and was found to be 79.98% over 8 hr period exhibiting sustained drug release profile. The ex-vivo trans-corneal drug permeation profile of developed TMN was studied using modified franz-diffusion cell apparatus (Permegear) and the % cumulative drug permeation across freshly excised goat cornea was found to be 65.90 % in 8 hrs duration, which was approximately 1.5 times higher than the conventional eye drop formulation, as shown in figure 2.

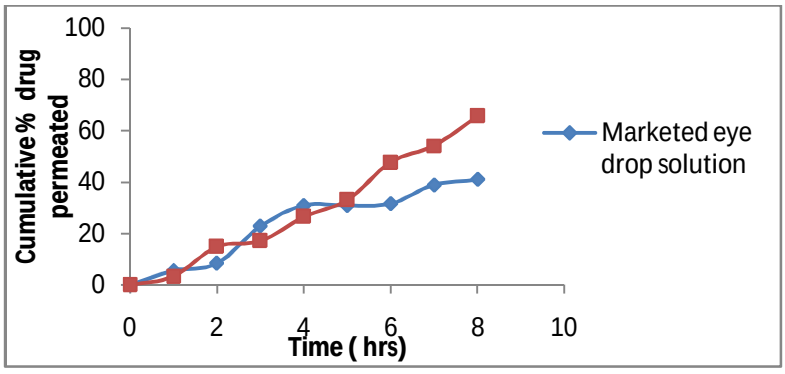
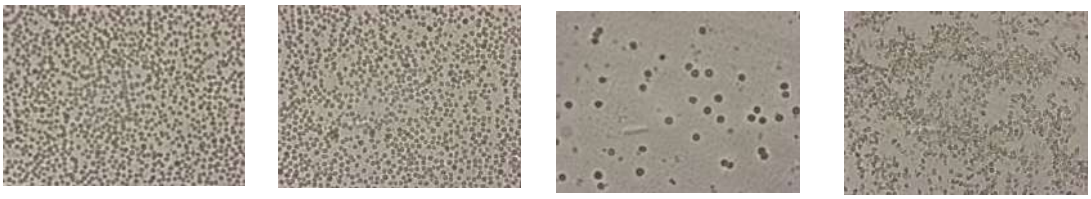


Figure 2: Trans-corneal Drug Permeation Profile of Timolol Maleate Niosomal Formulation

The developed TMN was also proved to be isotonic in isotonicity studies, as the isolated RBCs were normal in size and shape on exposure with developed TMN and isotonic saline solution, while the RBCs were shrinked in hypotonic and swollen in hypotonic solution and are shown in figure 3.



(a) **(b)** **(c)** **(d)**

Figure 3: (a) RBCs with Developed Timolol Maleate Niosomal Eye Drop, (b) RBCs with Isotonic Solution (c) RBCs with Hypotonic Solution (d) RBCs with Hypertonic Solution

The developed timolol maleate niosomal formulation was also proven to be non-irritant in HET-CAM ocular irritancy test securing 0 score, as there were no signs of hemorrahage, lysis and coagulation in CAM of fertilized hens egg with developed TMN and isotonic saline solution (negative control), while hemorrahage was observed with 1% NaOH solution (positive control), as shown in fig.4.

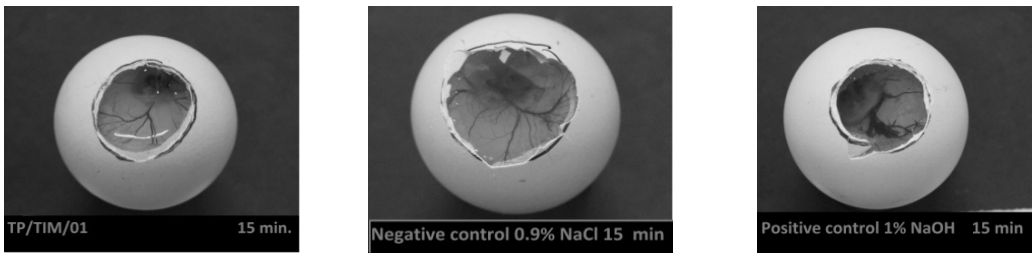


Figure 4: HET-CAM response to exposure with TMN formulation, negative control and positive control

Conclusion:

It can be concluded from above studies, that the developed timolol maleate niosomal (TMN) formulation is better than conventional eye drops due to longer corneal retention, sustained drug release and better trans-corneal drug permeation and thereby higher ocular bioavailability, hence, would need less frequent administration.

References:

[1] Uchegbu IF & Vyas SP (1998) Int J Pharm 172:33-70.

[2] Mancon M, Sinico C, Valenti D, Loy G & Fadda AM (2002) Int J Pharm 234:237-248.

[3] Aggarwal D & Kaur I (2005) Int J Pharm 290: 155-159.

[4] Zhanrong Q, Zeng W (2014) Eur J of Pharm Sci 62:115-123.

PA-89

Application of Taguchi Orthogonal Array Design for Optimization of Chitosan Nanoparticles of Hydrophobic Cardiovascular Drugs

Mayank Sharma[1], Rajesh Sharma[1], Dinesh Kumar Jain[2]
[1]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore, India
[2]IPS Academy College of Pharmacy, Indore, India
E-mail address: mayank2306@gmail.com

Abstract: Nanotechnology based drug delivery system have shown to improve solubility, rate of dissolution and oral bioavailability of poorly water soluble drugs. Present study is aimed to develop Nebivolol loaded chitosan nanoparticles (NB-CS-NPS) for enhancing its oral bioavailability. The NPs optimized by implementation of Taguchi orthogonal array design. The optimized batch (NB-CS-NPS-1) of NPs exhibited average particle size of 91 ± 45 nm , entrapment efficiency of 70.98% and with zeta potential of + 36.8mV ± 2mV and stability at 25 ± 2◦C/ 60 ± 5% RH. The *in vitro* release data studies have shown improved solubility and oral bioavailability of Nebivolol.

Introduction:

Poor aqueous solubility of the pharmaceutical entity is one of the major limitations in successful oral drug delivery. Among various approaches available to improve problems related to poor solubility of drugs, nanotechnology based drug delivery system offers advantages to overcome the problems associated with the oral delivery of these drugs. Nanoparticles exhibit increased surface area which results in increased dissolution rate, enable to control pharmacokinetic properties of a dosage form, ease of administration and readily penetrate through capillary and epithelial membrane, which permits an effective oral delivery of poorly soluble drugs. The present study is aimed to develop Chitosan nanoparticles containing poorly soluble antihypertensive drug, Nebivolol, in order to improve its solubility and oral bioavailability.

Material and Methods:

Material

Chitosan (CS) was obtained as gift sample from Central Institute of Fisheries Technology (Cochin, India). Sodium tripolyphosphate (TPP) was procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Nebivolol (NEB) was obtained as a gift sample from Lupin Ltd. (Pitahmpur, Indore, India). All other chemicals and reagents were of analytical grade.

Methods

The NB-loaded NPs were fabricated according to the procedure reported. Chitosan solutions of different concentrations were prepared by dissolving chitosan in 1% aqueous acetic acid solution. Tween 80 (2% v/v) was added as a surfactant to it under constant stirring at room temperature. Subsequently, drug (2.5%) was dissolved in dichloromethane (2.5 mL), and then this oil phase was

added dropwise to the aqueous phase. This addition was accompanied by continuous stirring for 5 minutes at different speeds using high speed homogenizer. Finally, 10ml TPP solution of different concentration was added drop wise into o/w emulsion to induce cross-linking of the particles under magnetic stirring at 500 rpm. The stirring was continued to ensure complete evaporation of dichloromethane, it was kept overnight at 40°C. Nanoparticles were collected by centrifugation at 15,000 rpm for 25 minutes at 20°C using cooling centrifuge. The supernatant was subjected for the determination of presence of free Nebivolol by UV spectrophotometer (UV 1700, Shimadzu, Japan).

Optimization of nanoparticles By Taguchi orthogonal array design

The optimization of the NPs formulation was carried out by using Taguchi orthogonal array design. Based on the number of factors and their levels, L_9 (3^4) orthogonal array was employed. Four factors i.e. polymer concentration (%w/v), TPP concentration (%w/v), CS: TPP ratio (%v/v) and stirring speed (rpm) were selected and assigned three levels i.e. low, medium and high. The optimum conditions with optimal desirability were determined with the minimum possible effect of the noise factor.

Characterization of nanoparticles

Transmission electron microscopy (TEM)

The morphology of nanoparticles was observed under transmission electron microscopy (Morgagni 268D TEM instrument, AIIMS, New Delhi). The diluted and filtered sample was plunged on the 200 mesh carbon coated copper grids and was allowed to dry completely in the air. After drying, sample grid was loaded onto a specimen holder and viewed under a transmission electron microscope.

Drug-excipient compatibility studies by differential scanning calorimeter (DSC)

The nanoparticles and drug powder were subjected to previously calibrated differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan). The sample was sealed hermetically in an aluminum pan and subjected to nitrogen gas at a flow rate of 50 ml/min. The thermograms were obtained at scanning temperature range of 50-250°C at a heating rate of 10°C/min. DSC thermograms were recorded for CS, NEB and NEB-CS NPs.

Measurement of particle size, polydispersity index (PDI) and zeta potential (ZP) of nanoparticles

Particle size, PDI and ZP of nanoparticles were determined through Dynamic light scattering (DLS) analysis with Malvern Zetasizer Nano S (Malvern, UK). About 100 μL of the prepared nanoparticles dispersion was diluted to 5ml with double distilled water and analyzed with zetasizer. The analysis was performed in triplicate at a temperature of 25 $^{\circ}$C.

Determination of entrapment efficiency

The entrapment efficiency of the nanoparticulate formulation was determined in triplicate using *ultra-violet spectrophotometer*. The nanoparticles were separated from the aqueous medium (containing unentrapped NEB) by centrifugation at 25000 rpm for 30 min (REMI CPR-24 Plus, Remi Elektrotechnick, India). The supernatant was diluted with an appropriate amount of 0.1 N HCl and

analyzed for the amount of unentrapped drug by UV-Visible spectrophotometer (*Shimadzu 1700, Japan*) at 285 nm.

The percentage drug encapsulated was determined by following the formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total drug (mg)} - \text{free drug (mg)}}{\text{Total drug (mg)}} \times 100$$

In vitro drug release studies

The *in-vitro* drug release of nanoparticles was studied by using dialysis membrane (Himedia, India) with a pore size of 2.4nm and molecular weight cut-off between 12,000–14,000 in phosphate buffer saline (PBS) pH 7.4 at 37 ± 2˚C. Dialysis membrane was soaked overnight in double distilled water prior to the release studies. The drug-loaded nanoparticles were placed into a dialysis bag and were suspended in a beaker containing PBS under magnetic stirring while maintaining perfect sink condition. Aliquot samples were withdrawn periodically and replaced with fresh dissolution medium in the same volume. The amount of drug released was analyzed spectrophotometrically at 285 nm for NEB. For comparative purpose, the *In vitro* drug release study was also performed for the marketed formulation using USP paddle type dissolution apparatus.

Accelerated stability studies

Nebivolol loaded nanoparticles were subjected to a stability testing for three months as per International Conference on Harmonisation (ICH) Q1A (*R* 2) guidelines. Freshly prepared nanoparticles were transferred to 5 ml glass vials sealed with plastic caps and were kept in stability chamber (Remi SC-12 Plus, Remi Instruments. Ltd. Mumbai, India) maintained at 25 ± 2◦C/60 ± 5%RH for a period of total 3 months. The formulations were monitored for changes in particle size, zeta potential and entrapment efficiency.

Result and Discussion:

Particle size analysis by transmission electron microscopy (TEM)

The structural morphology of nanoparticles was examined by TEM. TEM image showed that the optimized formulation is nearly spherical in shape and a smooth surface distributed throughout the sample (Figure 1).

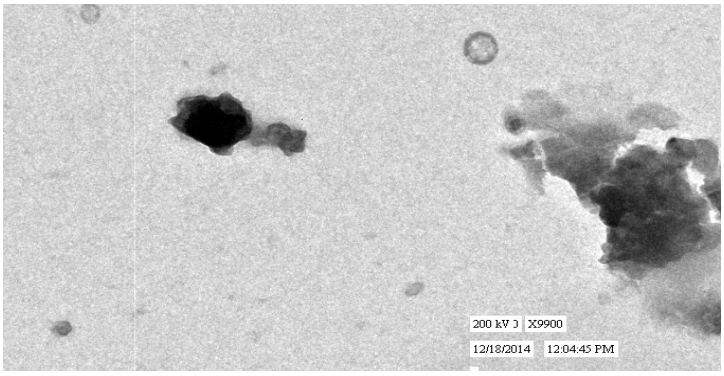


Figure 1: *Drug-excipient compatibility studies*

The pure drug, Nebivolol, showed a sharp endothermic peak at 221°C corresponding to its melting temperature. Chitosan showed broad endothermic peaks at 102°C corresponding to its glass transition temperature. NEB-CS NPs showed both broad and sharp endothermic peaks at 102°C and 221°C which corresponds to chitosan and Nebivolol respectively which predicts that the drug is homogenously dispersed in polymer matrix.

Particle size, poly dispersity index (PDI) and zeta potential of nanoparticles

The average particle size of the optimized batch (NB-CS-NPS-1) of nanoparticles was found to be 91 ± 45 nm. Particle size along with zeta potential (ζ) is the critical factor that affects the biological performance of chitosan nanoparticles. The zeta potential of NB-CS-NPs were found to be + 36.8mV ± 2mV, which indicate the physical stability of the formulation. The zeta potential also tends to affect particle stability and mucoadhesivity.

Entrapment efficiency

The entrapment efficiency acts as an important factor influencing the drug release, as well as the overall efficacy of the formulation. All the formulations were analyzed for entrapment efficiency by using UV-Visible spectrophotometer (*Shimadzu 1700, Japan*) at 285 nm and. The entrapment efficiency of the optimized batch (NB-CS-NPS-1) of nanoparticles was found to be 70.98.

In vitro drug release

The *In vitro* drug release studies were carried out for NEB -CS NPs and marketed formulation in PBS 7.4 at 37 ˚C± 2˚C . The drug release profile of NB-CS-NPs showed biphasic release pattern with an initial burst release in the first 2 h followed by a controlled release over a period of 72 hours and cumulative percentage of drug released was obtained to be 71.24 %.

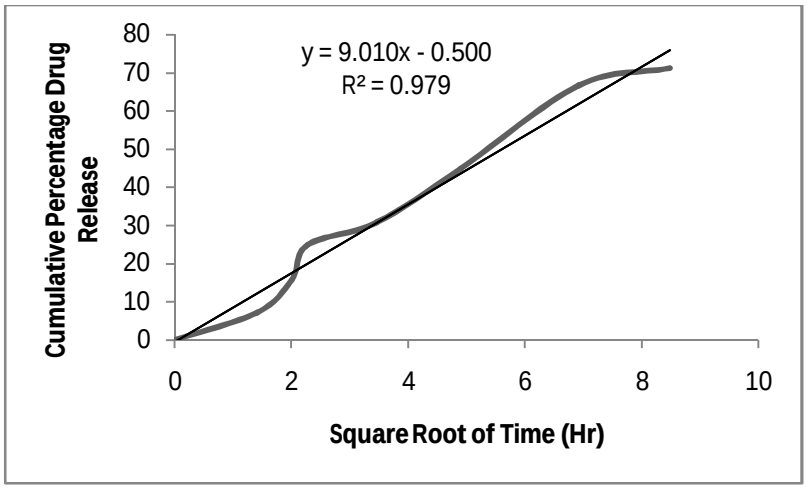


Figure 2: *In vitro* drug release

Accelerated stability studies

Stability studies were conducted in triplicate for optimized formulation which showed slight variations in particle size, zeta potential, and drug entrapment during 3 months of storage. The

obtained results indicated no significant change in the particle size, zeta potential, and drug entrapment during 3 months of storage that ensured the stability of nanoparticles.

Conclusion:

The major challenge in the formulation development is the poor aqueous solubility of the new chemical entity or existing drug molecules. The formulation of these molecules by the application of conventional approaches is difficult and associated with several pharmacological or therapeutical performance issues. The nanoparticles provide a promising approach for enhancing solubility and oral bioavailability of water insoluble drugs. In conclusion, formulation of chitosan nanoparticles could be an effective strategy for enhancing oral bioavailability of nebivolol and other lipophilic drugs upon further *in vivo* pharmacokinetics and pharmacodynamics studies.

References:

[1] Bhatt AD & Pethe AM (2010) J Pharm Res 3:1748-1751.

[2] Nekkanti V & Rueda J (2016) J Drug 1**:**18-27.

[3] Kalepu S & Nekkanti V (2015) Acta Pharm Sin B 5:442-453.

[4] Gulsan T, Gursoy RN & Oner L (2009) FABAD J Pharm Sci 34:55-65.

[5] Soumya M, Gupta S, Jain R & Mazumder R (2013) Int J Res Dev Pharm Life Sci 2:642-649.

PA-90

In Vitro and *In Vivo* Evaluation of Mucoadhesive Microspheres for Treatment of *Helicobacter pylori* Using Factorial Design

Anu Hardenia[1], Arun Kumar Gupta[2], R. P. Singh[3]
[1]Research Scholar, Department of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India.
[2]RKDF Institute of Pharmaceutical Sciences, Dr. A. P. J. Abdul Kalam University, Indore, Madhya Pradesh,
[3]Suresh Gyan Vihar University, Jaipur, Rajasthan, India.
E-mail address: anu.hardenia7@gmail.com

Abstract: The research involves characterization of *in vitro* and *in vivo* activity of mucoadhesive microspheres for *Helicobacter pylori* eradication. Amoxicillin and Famotidine were used as model drugs so that the dual therapy gives better *H. pylori* eradication. Preparation was carried by an emulsion-solvent evaporation method and 27 batches were prepared individually using 3^3 factorial designs to study the effect of independent variables on dependent variables. The *in vitro* mucoadhesion test and *in vivo* studies (Bacterial clearance study, *in vivo* mucoadhesion and *in vivo* ulcer index studies) were performed. A27 batch showed 66% mucoadhesion after 10 h and F24 showed 74% of mucoadhesion after 10 h. In the bacterial clearance studies, the mean bacterial count (log colony forming units) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58. The drug-loaded microspheres formulation exhibited better clearance from infection than plain drugs solution at the same doses. Drug microspheres formulation was found to be effective in the treatment of *H. pylori* infections effectively, and in *in vivo* mucoadhesion studies, the developed system was well taken up and processed by the cells of gastric mucosa of the stomach.

Introduction:

More than 50% of drug delivery systems available in the market are oral drug delivery systems. These systems have the obvious advantages of ease of administration and patient acceptance. Attempts to develop a single-dose therapy for the whole duration of treatment have focused attention on controlled or sustained release drug delivery systems. Gastro retention helps provide a better availability of new products with new therapeutic possibilities and substantial benefits for patients. GRDFs greatly improve the pharmacotherapy of the stomach through local drug release drug concentrations at the gastric mucosa (eradicating *H. pylori* from the sub mucosal tissue of the stomach), making it possible to treat stomach and duodenal ulcers, gastritis, and esophagitis. This can be achieved by coupling bioadhesion characteristics to microspheres and developing characteristics to microspheres and developing mucoadhesive microspheres [1]. *H. pylori* is a major gastric pathogen in the worldwide distribution; these are spiral-shaped bacteria found in the stomach, which destroy stomach along with the duodenal tissue, Several treatment regimens are emerging for *H. pylori* infection from the early 1990s, when monotherapy was first recommended. Even though *H. pylori* is highly sensitive to most of the antibiotics, its elimination is not easy even with the best currently available therapy[2].Therapeutic regimens directed against *H. pylori* infection will continue to evolve.

Amoxicillin (-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers [3]. Famotidine is a histamine H2-receptor antagonist. It is mostly prescribed for gastric ulcers, duodenal ulcers, also for gastro esophageal reflux diseases. With low bioavailability (40-45%) and short biological half-life (2.5-4.0 h), it favors the development of a sustained release formulation [4]. Thus, an attempt was made in the present investigation to prepare mucoadhesive microspheres. The microspheres were characterized by *in vitro* and *in vivo* tests and factorial design was used to optimize the variables.

Methods:

The mucoadhesive microspheres were prepared by emulsion solvent evaporation method using Carbopol 934 and ethyl cellulose as polymers. In the first step, ethyl cellulose was dissolved in 200 ml of ethanol and then drug and polymer were dispersed in the solution of ethyl cellulose under stirring. The preliminary trial batches were prepared and optimized using 3^3 factorial design earlier by varying the drug-to-polymer-to-polymer (amoxicillin/famotidine-ethyl cellulose-carbopol-934P) ratio in the range of 1:3:1% to 1:3:3%. The final mixture was extruded through a syringe (gauge No. 20) in 500 ml of liquid paraffin (mixture of heavy and light, 1:1 ratio) containing Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. The stirring was done for 3 h. In preliminary trial batches, the amount of emulsifying agent (1-3%), the drug:polymer concentration 1:3:1% to 1:3:3%, and stirring speed (500-1000 rpm) were varied.

Results and Discussion:

Optimization of amoxicillin-loaded microspheres

For preparation, a full factorial design was employed. A design model with 3 factors, 3 levels, and 27 runs was selected for the optimization study. The dependent variables obtained at various levels of the 3 independent variables (X1, X2, and X3) were subjected to multiple regression to yield a second-order polynomial equation obtained coefficient. The polynomial equation generated by this experimental design (using Design Expert 7.1.6) was as follows:

$$Y= b0 +b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X1X1 + b22X2X2 + b33X3$$

Where, Y is the dependent variable; b0 is the intercept; b1 to b33 are the regression coefficients; and X1, X2, and X3 are the independent variables. Response surface graphs for amoxicillin are shown in Figures 1-4 and for famotidine are shown in Figures 5-8.

***In vitro* Mucoadhesion**

The *in vitro* mucoadhesiveness test was carried out among the factorial design batches based on good entrapment efficiency and the batch A27 and F24 showed that even after 10 h, 66% and 74% microspheres were adhered to the gastric mucous layer. The mucoadhesive microspheres were

spherical and free flowing.

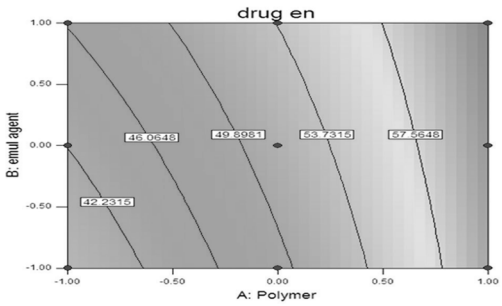


Figure 1: Contour plot for drug entrapment

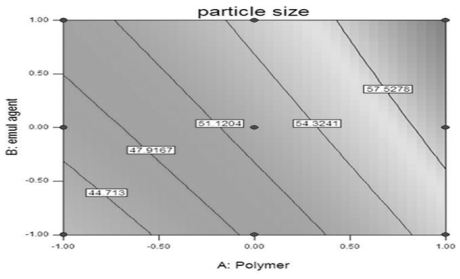


Figure 2: Contour plot for particle size

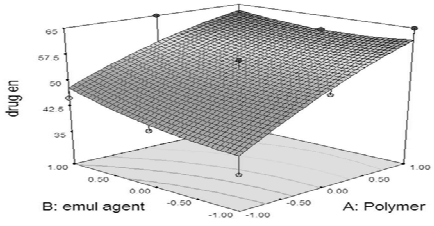


Figure 3: Response surface plot for drug entrapment

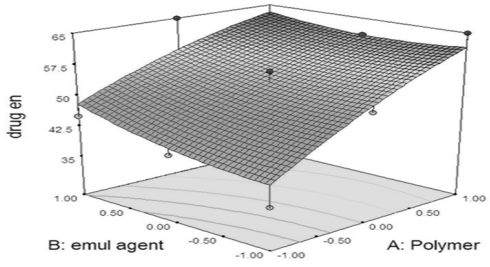


Figure 4: Response surface plot for particle size

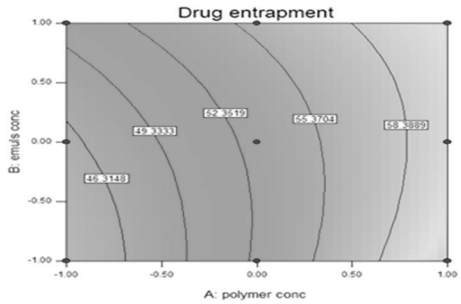


Figure 5: Contour plot for drug entrapment

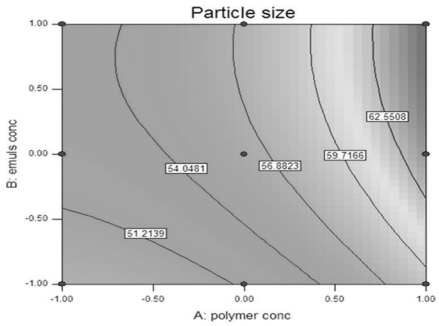


Figure 6: contour plot for particle size

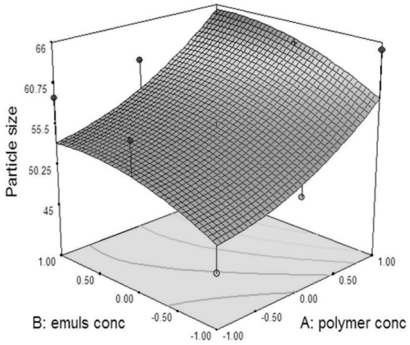


Figure7: Response surface plot for drug entrapment

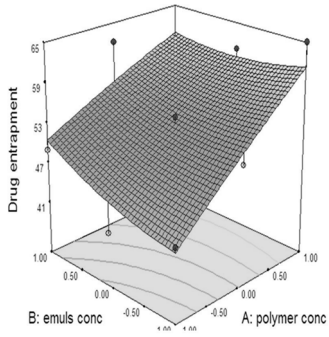


Figure 8: Response surface plot for particle size

***In vitro* drug release studies**

A sustained drug release was obtained for more than 10 h.

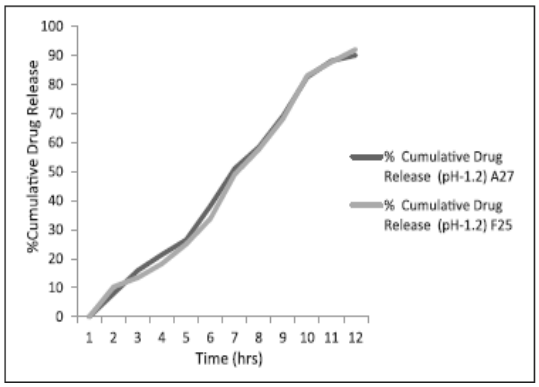


Figure 9: Dissolution profile

***In vivo* studies**

Bacterial clearance count

Infected animal model, i.e, male albino rats with *H. pylori* was used for *in vivo* study. The control group of rat received only physiological saline. The mean bacterial count (log CFU) was found to be 9.64 ± 0.35. The mean bacterial count (log CFU) after oral administration of plain drugs solution (amoxicillin) was found to be 5.83 ± 0.23, which is due to unavailability of 100% drugs and short residence. The mean bacterial count (log CFU) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58.

***In vivo* mucoadhesion study**

The mucoadhesive behavior of the microspheres was determined after 2 h of incubation of films pieces in the stomach and intestine. This muco-adhesivity was also confirmed *in vivo* using FITC dye with the formulation. This shows that the system could attach the mucosal gel layer where *H. pylori* resides [Figures 10 and 11].

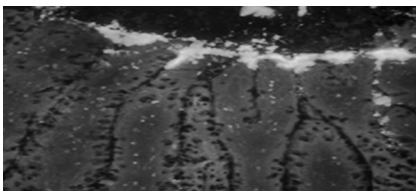


Figure 10: Plain fluorescence isothiocyanate solution

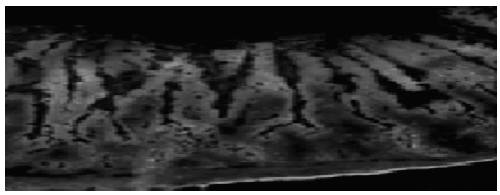


Figure11: Fluorescence isothiocyanate-labeled microspheres in gastric tissue (after 2 h)

***In vivo* ulcer index studies**

Mucoadhesive microsphere dispersion showed a significant decrease in ulcer index (0.46 ± 0.011) when compared with the control group (3.61 ± 0.14) and famotidine suspension-treated animal (0.66 ± 0.035) (Figure 12).

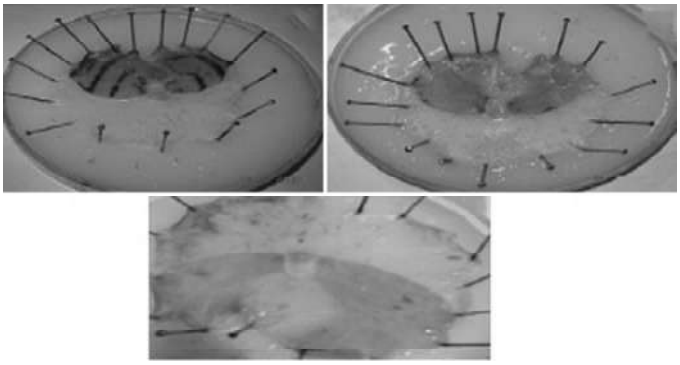


Figure 12: (a) Ulcer-induced rat stomach. (b) Famotidine suspension-treated. (c) Mucoadhesive microsphere-treated

Conclusion:

The mucoadhesive microspheres were developed using a 3^3 factorial design and showed a high percentage of mucoadhesion, drug entrapment efficiency, and exhibited a sustained release property. The formulation showed more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres compared to amoxicillin and famotidine mucoadhesive microsphere dispersion showed a significant decrease in ulcer index when compared with the control group and famotidine suspension-treated animal, which might indicate a potential use of mucoadhesive microspheres in treating *H. pylori* infection.

References:

[1] Brunton LL, Lazo JS & Parker KL (2006) Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw Hill Medical Publishing Division 1119-1122.

[2] Jain SK, Gupta M, Sahoo AK, Pandey AN & Jain AK (2014) Curr Sci 106:267-276.

[3] Brown LM (2000) Epidemiol Rev 22:283-297.

[4] Akiyama Y, Nagahara N, Nara E, Kitano M, Iwasa S & Yamamoto I (1998) J Pharm Pharmacol 50:159-166.

PA-95

Formulation and Characterization of Allicin-Amphotericin-B Liposomal Gel for the Treatment of Fungal Infections

Darshan Jamindar, Nidhi Patidar, Sanjay Jain
Indore Institute of Pharmacy, Indore, (M. P.) India
E-mail address: darshan.jamindar@indoreinstitute.com

Abstract: Spherical multilammelar vesicles of liposome consisting of lipid egg phosphatidylcholine, soya lecithin and cholesterol were prepared by thin film hydration technique. Further liposomal gel was prepared by egg phosphatidylcholine and cholesterol of molar ratio 7:3 and attained slow drug release with Allicin- Amphotericin B liposomal based gels as compared to the plane gel. Liposomal gel was characterized for drug release kinetics, antifungal activity (disc diffusion assay). Formulation F8 A shows highest drug release. Allicin (*Allium sativum* Linn.) showed a minimum inhibitory concentration (MIC) at the dose range from 0.195 µg/ml to 10 µg/ml of broth against all fungal strains (F2B Formulation). These lower concentrations were achievable for fungistatic effect and the reduced adverse effects. The results suggest that liposomal gel of Amphotericin B may be useful in the treatment of Topical Fungal Infections.

Introduction:

Topical liposome formulations are more effective and less toxic than conventional formulations.The type and concentration of the polymer, which forms the gel matrix, could influence the stability as well as the release rate of the incorporated drug [1]. Fungal infections are usually more difficult to treat than bacterial infections, because fungal organisms grow slowly and because fungal infections often occur in tissues that are poorly penetrated by antimicrobial agents. Allicin as active constituent of Garlic (*Allium sativum* Linn.), is a bioenhancers capable for improving the bioavailability of drug, reducing the dose of Amphotericin B, adverse reaction and shorten period of treatment [2]. Combination of Allicin and Amphotericin B show synergistic effect at low concentration. Incorporation of Amphotericin B into Liposome offers a potential means to reduce the toxicity of this antifungal drug [3].

Methods:

Preparation of Liposomal gel

Carbopol (934) 2% w/v was allowed to hydrate for 3-4 hr. 5 ml of liposomal suspension was added in gel base and mix by stirrer. The mixture was stirred until thickening and neutralized by the drop wise addition of triethanolamine to achieve the homogeneous gel. The gel was sonicated for 15 minute and then, kept overnight to remove the air bubbles.

Characterization of Liposomal gel

Drug content in liposomal gel

Specific quantity of the prepared gel was taken and dissolved in 100 ml of phosphate buffer of pH 5.5. Volumetric flask containing gel solution was shaken for 2 hr. on mechanical stirrer, filtered and drug absorbance was recorded by UV-visible spectrophotometer at OD 270 nm using phosphate buffer as blank.

***In vitro* Drug Release**

Dialysis membrane was employed in two sides open ended cylinder. One g of liposomal gel containing known amount of drug was placed in a dialysis membrane 2 ml of acetonitrile was added to each aliquot to precipitate the lipids and dissolve the entrapped Amphotericin-B and then the samples were analyzed by UV spectrophotometers at a λ max of 270 nm.

Antifungal Activity

Sabouraud Dextrose Broth (SDB Liquid Medium) Sabouraud Dextrose Agar (SDA Solid Medium) Sabouraud Dextrose Broth (Sabouraud Liquid Medium) was used for cultivation of yeasts, moulds and fungi microorganisms..The sample of strain of *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* were cultured and colony forming units were calculated by following formula [4].

$$\text{CFU/ML} = N \times \frac{1}{\text{SAMPLE VOLUME}} \times \frac{1}{\text{D. F.}}$$

Two fold serial dilution and cup fold method were implied to test for zone of inhibition.

Result and Discussion:

Surface morphology by Scanning Electron microscopy (SEM)

The samples are examined under scanning electron microscope.

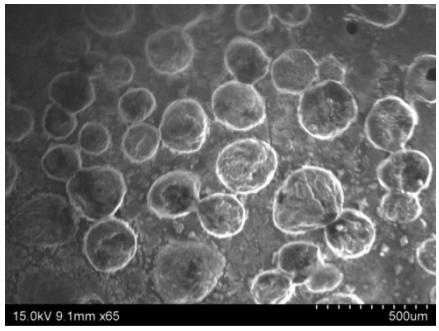


Figure 1: Large unilamellar vesicles

Drug content in liposomal gel

The formulated liposomal gel was analyzed for percentage of drug contents Compared to plane gel. The formulation code, F 2 A and F 2 B has showed the highest drug content (84.06 ± 0.33 and 87.05 ± 0.0.33).

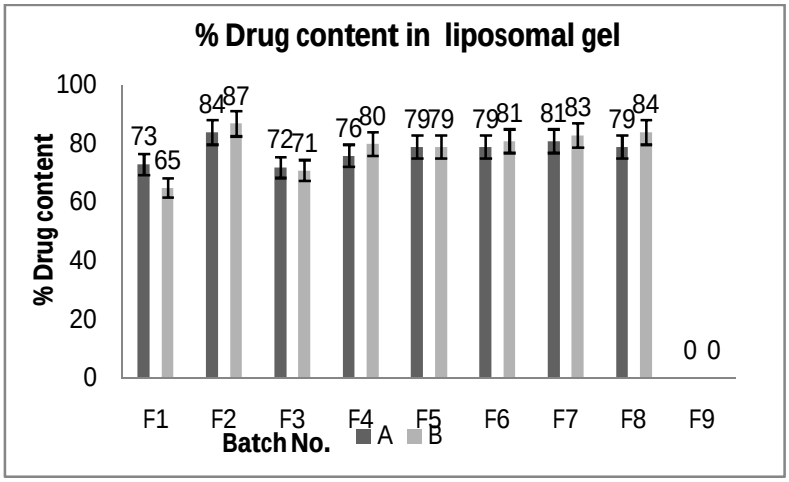


Figure 2: Drug content of liposomal gel ± S.D. mean (n=3)

***In vitro* drug release-** Formulation F8 shows high drug release after plane gel.

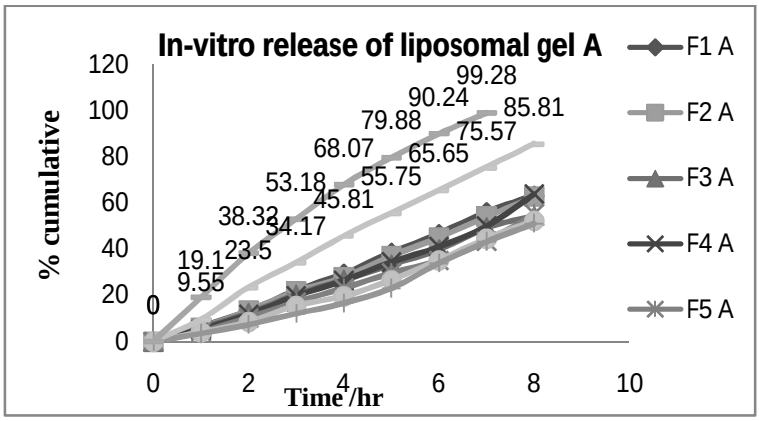


Figure 3: Drug release of liposomal gel (A) ± S.D. mean (n=3).

Antifungal Activity

The result revealed that the Allicin (*Allium sativum* Linn.) showed a minimum inhibitory concentration (MIC) at the dose range from 0.195 µg/ml to 10 µg/ml of broth against all fungal strains. The lowest activity was found with F7A and the greatest activity was found with F2B.

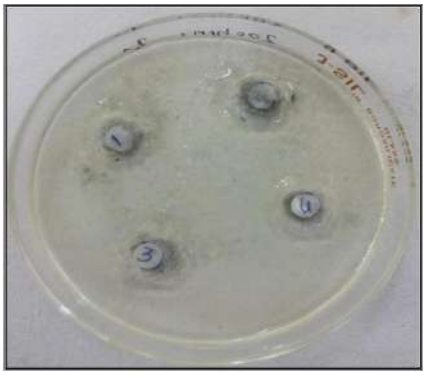


Figure 3: Antifungal activity

Conclusion:

Amphotericin B can be successfully incorporated into the liposomal gel formulations. The use of Allicin increase bioavailability and potential to minimize its dose related Adverse Effect of Amphotericin B. Allicin loaded liposomes provide a control release of Amphotericin B. This is due to the robustness and rigid nature of the egg phosphatidylcholine when compared to the soya-lecithin and cholesterol. The liposomal gel shows promising results for targeting of antifungal drugs.

References

[1] Jithan AV & Swathi M (2010) Int J Pharm Sci and Nanotech 3:1-8.

[2] Borjihan H, Ogita1 A, Fujita KI, Hirasawa K & Tanaka T (2009) J Antibiotics 62:691–97.

[3] Sodhchand Y & Segal E (1999) J Antimicrob Chemother 44:787-90

[4] Cutler RR & Wilson P (2004) Br J Biomed Sci 61(2):1-4.

PA-96

Development and Evaluation of Buccal Dosage Forms of *Garcinia cambogia*

Chetan Ghulaxe[1], Mousumi Kar[2], Sujit Pillai[3], Indrajeet Singhvi[1]
[1]Pacific College of Pharmacy, Pacific University, Udaipur, Rajasthan
[2]IPS Academy College of Pharmacy, Indore, Madhya Pradesh
[3]GRY Institute of Pharmacy, Borawan, Khargone, Madhya Pradesh
E-mail address: chetanghulaxe@gmail.com

Abstract: Medicated jelly formulations are more suitable for pediatric, geriatric and dysphagic patients, which offer rapid dissolution and absorption of drugs thereby early onset of action. The aim of study was to develop and evaluate oral jelly formulation of *Garcinia cambogia* extract using pectin as the natural gelling agent. The primary objective was formulation of unit moulded jelly containing herbal medicaments and also to optimize the dosage form that will have extra beneficiary for hepatoprotective and weight-loss supplement effect without any side effects. The crude extract or constituents from the plant also exerted hypolipidemic, antidiabetic, anti-inflammatory, anticancer, anthelmintic, anticholinesterase and hepatoprotective activities. All the formulations exhibited good physiochemical properties and found to be stable.

Introduction:

Many therapeutic agents are absorbed in the oral cavity. For the drugs having significant buccal absorption, dosage forms such as Medicated Jelly and Chewing Gums permit more rapid therapeutic action as compared to oral dosage forms. Medicated Jelly has been very well received by the parents for their use in children with full dentition. Children in particular may consider chewing gum as a more preferred method of drug administration compared with oral liquids and tablets. These are used for medication, lubrication and some miscellaneous applications [1-3]. Studies on the fruit rind of Garcinia gummi-gutta, commonly known as *Garcinia cambogia* (syn.), have shown that the extracts as well as (−) hydroxycitric acid (HCA), a main organic acid component of fruit rind, exhibited anti-obesity activity including reduced food intake and body fat gain by regulating the serotonin levels related to satiety, increased fat oxidation and decreased de novo lipogenesis [5]. HCA is a potent inhibitor of adenosine triphosphate-citrate lyase, a catalyst for the conversion process of citrate to acetyl-coenzyme A, which plays a key role in fatty acid, cholesterol and triglycerides syntheses.

Materials and methods:

Materials

The fruit rind of *Garcinia cambogia* was obtained from the evergreen forests of Western Ghats, from Konkan southwards to Travancore and authenticated. Pectin was purchased from Loba Chem. Mumbai, India. Citric acid was purchased from Seva Fine Chemicals, Ahmedabad. All the other chemicals used were of analytical grade.

Preparation of *Garcinia cambogia* extract

The extraction was done by Soxhlet extraction method. After 24 hours, the solvents were distilled off, the extract was concentrated on water bath and collected.

Preparation of oral jelly

All the ingredients were weight and added to syrup. Pectin was added with constant stirring followed by propylene glycol and citric acid. Scum was removed and sodium benzoate was added and mixed. Herbal drug extracts were weight accurately, dissolved in water, added, transferred to moulds and allowed to cool and settle. Garcinia cambogia extract with betacyclodextrin was prepared by triturating for 1 hr, and then concentrated & free flowing drug powder was obtained. Then it was subjected to same above procedure. After the jelly was set, it was wrapped in to the butter paper and stored in dry place.

Evaluation parameters for formulation

Appearance

The prepared jelly was inspected visually for clarity, colour and presence of any particle.

pH

The pH of all the jelly was determined using digital pH meter. 0.5 g of the weighed formulation was dispersed in 50 ml of distilled water and the pH noted.

Determination of viscosity

Viscosity of the jelly was carried out by using (LV) Brookfield viscometer (Dial type).

The viscosity was calculated by following relation:

Viscosity in centipoises = Dial reading × Factor

Stability Studies

Stability study was conducted as per ICH (International Conference on Harmonization) guidelines at room temperature (25±2 °C, 60±5% RH) and accelerated temperature condition (40±2°C, 75±5% RH) for one month.

Table 1: Formulation batches of different jelly products

Ingredients	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)	F5 (%w/w)	F6 (%w/w)	F7 (%w/w)	F8 (%w/w)
Pectin	2%	2%	2.5%	3%	3%	3%	3%	3%
Citric acid	1%	1%	1%	1%	1%	1%	1%	1%
Sugar syrup	60	60%	67	67	67%	70	80	90
Propylene glycol	3%	3%	3%	3%	3%	3%	3%	3%
Sodium benzoate	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
G.Cambogia extract	1%	1%	1%	1%	1%	1%	1%	1%

Table 2: Evaluation parameters for formulation

Formulations	Appearance	pH	Viscosity (cps)
F1	Transparent	3.5	640000
F2	Syrupy	3.8	600000
F3	Opaque	3.4	588000
F4	Opaque	3.6	580000
F5	Opaque	3.5	624000
F6	Transparent	3.2	590000
F7	Transparent (Sugar crystallization)	3.4	552000
F8	Transparent (Sugar crystallization)	3.3	604000

All the eight batches of prepared jelly were subjected to the evaluation for the appearance, pH, and viscosity.

Table 3: Stability of the optimized formulation (f6)

Days	Appearance	Viscosity	pH	Stiffness	Sugar crystallization
Room Temperature					
15	Transparent	585000	3.40	Yes	No
30	Transparent	568300	3.95	Yes	No
Accelerated Temperature					
15	Transparent	495000	3.30	Yes	No
30	Transparent	413600	3.20	Yes	No

As per stability data of the jelly, F6 formulation was found to be stable at room temperature. It became very sticky when kept at accelerated temperature, but even at the accelerated temperature if it is kept in covered or enclosed condition; there was no or little change in consistency.

Conclusions:

The present study demonstrates the herbal extracts of *Garcinia cambogia* were successfully formulated in the jelly formulations. In formulation development, taste masking of bitter herbal drugs was a big challenge and that was solved by making complex with betacyclodextrin. All drugs extracts, which are used in the dose range are safe for consumption and can be swallowed without any risk of systemic side effects.

Acknowledgements:

The authors are grateful to GRY Institute of Pharmacy, Borawan and the management to provide such facilities and stage to carry out the research work in a smooth way.

References:

[1] Ninomiya H, Shimizu T, Dairaku M, Komagata T, Misawa M. Unites States Patent, Patent number US 5932235.

[2] Kim MS, Kim JK, Kwon DY & Park R (2004) Biofactors 22:193–196.

3] Kim KY, Lee HN, Kim YJ & Park T (2008) Biosci Biotechnol Biochem 72:1772–1780.

[4] Altiner A, Ates A, Gursel FE & Bilal T (2012) J Anim Plant Sci 22:872–877.

[5] Rawlins EA (1995) Bentley's text book of pharmaceutics, Bailleere Tindall, London, Edition 18:19-24.

PA-97

Incredible Taste Masking of Clarithromycin

Yitesh Jagwani[1], Siddhraj Sisodia[2], Akanksha Jagwani[3]
[1]Pacific Academy of Higher Education & Research, Udaipur, India.
[2]BN College of Pharmacy, Udaipur, India.
[3]Pacific Academy of Higher Education & Research, Udaipur, India
E mail address: jagwaniyitesh@gmail.com

Abstract: Clarithromycin has poor acceptance by pediatric and geriatric patients due to bitter taste. Usually, in dry syrup formulations, high concentration of sugar is used for taste masking. But high consumption of sugar is related to several chronic diseases. Therefore, this study aimed to investigate taste masking capacity of excipients other than sugar. For this purpose, several granulating material were evaluated for masking bitter taste of clarithromycin. The best way to achieve pleasant taste was developed by using combination of aspartame and hydroxyl propyl cellulose which provided high intensity sweetness and miraculously reduced product bitterness.

Introduction:

The macrolide antibiotic, clarithromycin is extremely bitter in taste and effective in treatment of various infections in children and elderly patients, which often experience difficulty in swallowing solid oral dosage forms. For these patients, the drugs are mostly provided in liquid dosage forms, which lead to perceptible exposure of active ingredient to the taste buds. Taste masking is an important factor in these dosage forms for better patient compliance [1].

Traditionally, taste masking in dry syrup formulations is done by using high high concentration of sucrose, however, high sugar consumption has been a matter of great public and scientific interest. These adverse effects have been associated with obesityand risk of cronic disease like type 2 diabetes and cardiovascular diseases. As a result, many alternative taste masking agents have been extensively investigated. Non nutritive sugar can facilitate reduction in added sugar intake and promote beneficial effects on metabolic related parameters [2, 3]. Aspartame has a taste profile very close to that of sucrose, presenting very low level of bitterness and sourness [4]. The study was planned to mask bitter taste of clarithromycin without use of sucrose. Different granulating materials were used to prepare clarithromycin granules by wet granulation technique and were dried, milled to obtain granule of desired particle size. Extent of bitterness was evaluated by taste panel. It was recognized that clarithromycin granules prepared by HPC+ Aspartame mixture not only masked the bitter taste but also created a pleasant taste.

Materials and Methods:

Clarithromycin was received as gift sample from Amneal Laboratories Ltd.

Two different techniques were followed for taste masking:

(1) Solid dispersion technique: Solid dispersions of clarithromycin were prepared using Polyethylene glycol 4000 (PEG 4000) and with Polyvinyl Pyrollidone K 30 (PVP 30).

Using PEG 4000:

Solid dipersion of clarithromycin with PEG 4000 were prepared in 4 different ratios i.e. 1:1, 1:2, 1:3 and 1:4. Clarithromycin was mixed with PEG 4000 in the ratio of 1:1, 1:2, 1:3 and 1:4. Sufficient quantity of acetone was added to dissolve the mixture and mixture was stirred on magnetic stirrer at 60°C-70°C to allow formation of solid dispersion. Solid dispersion obtained were further dried in fluid bed processor to obtain dried material which was further milled and passed through #16 to obtain granules.

Similarly, solid dispersions were also prepared using Polyvinyl pyrollidone K30 in the ratio of 1:1, 1:2, 1:3 and 1:4.

(2) Granulation Technique 5 Different excipients were used separately for preparation of taste masking granules v.i.z. lactose, mannitol, sorbitol, starch, hydroxyl propyl cellulose. Using each excipient, granulation was performed in the ratio of 1:1, 1:2, 1:3 and 1:4.

Granulation with Lactose: Clarithromycin and lactose were sifted through # 30 sieve. Sieved lactose along with clarithromycin granulated using purified water as granulating agent. Wet granules were passed through # 10 sieve and were dried in fluid bed processor to form dried granules. Dried granules were milled and passed through # 16 sieve. Similarly granules were prepared in the ratio of 1:2, 1:3 and 1:4 ratio.

Also granules were also prepared separately using mannitol, sorbitol, starch and hydroxyl propyl cellulose. With each excipient, granules were prepared in the ratio of 1:1, 1:2, 1:3 and 1:4.

Taste Evaluation: The bitterness evaluation was performed on a taste panel of 6 human volunteers with mean age of 30 years. The volunteers rinsed their mouth thoroughly before and after the tasting. Granulated powder equivalent to 250 mg of clarithromycin was held in the mouth for 30 sec and then expectorated. Taste was evaluated and was assigned a numerical value ranging from 1 to 5 as mentioned in table 1, where high score indicated better taste masking. Results observed for taste evaluation score are mentioned in table 2.

Further different combinations of hydroxyl propyl cellulose were used along with intense sweetener aspartame to further conceal the bitter taste. Results of taste evaluation mentioned in table 3.

Results:

Solid dispersion were prepared by solvent evaporation method in which drug and inert excipients were dissolved in common solvent which was evaporated leading to the formation of solid dispersion. However, solid dipersion prepared with PEG 4000 or with PVP K 30 were unable to mask the bitter taste.

Taste masking by granulation technique was found to be comparatively better, inexpensive and quicker technique. Granulation lower the effective surface area of the bitter substance that comes in

contact the tongue. However taste masking effectiveness of different granulating agents was observed in the following order

Clarithromycin + PEG 4000 ≤ Clarithromycin + PVP K 30 < Clarithromycin + Sorbitol ≤ Clarithromycin + Mannitol ≤ Clarithromycin + Lactose Clarithromycin + Starch <Clarithromycin + Hydroxy Propyl Cellulose.

Reason for different taste masking capacity can be attributed to the solubility of granules in the saliva. Granules prepared with sorbitol and mannitol being easily soluble in saliva were not able to effectively mask the bitter taste, while granules prepared with hydroxyl propyl cellulose being less soluble had better ability to prevent the interaction of clarithromycin with taste receptors.

An interesting development in the taste masking was observed by using mixture of hydroxyl propyl cellulose with aspartame. Miraculously, granules were found to have no bitter taste when formulated with mixture of hydroxyl propyl cellulose with aspartame. Instead granules were having sweet and pleasant taste.

Table 1: Taste scale

Value	Parameter
5	Tasteless
4	Very Less Bitter
3	Moderate Bitter
2	Bitter
1	Strongly Bitter

Table 2: Results of taste evaluation score

Combination	Score				Mean Score
	1:1 ratio	1:2 ratio	1:3 ratio	1:4 ratio	
Clarithromycin + PEG 4000	1	1	1	1	1
Clarithromycin + PVP K 30	1	1	1	1	1
Clarithromycin + Lactose	2	2	2	3	2.25
Clarithromycin + Mannitol	2	2	2	2	2
Clarithromycin + Sorbitol	2	2	2	2	2
Clarithromycin + Starch	2	2	3	3	2.5
Clarithromycin + Hydroxy Propyl Cellulose	4	4	4	4	4

Table 3: Results of taste evaluation score

Combination	Score			Mean Score
	1:3+1 ratio	1:2+2 ratio	1:1+3 ratio	
Clarithromycin : Hydroxy Propyl Cellulose + Aspartame	4	5	5	4.66

Conclusion:

From the taste evaluation studies, it was concluded that miraculous taste masking for clarithromycin was achieved by granulation technique using combination of hydroxypropyl cellulose along with aspartame. These granules can be used for further formulation of tablets, dry syrups and can also be taken for scale-up studies.

Acknowledgment:

The authors are humbly thankful to Amneal Pharmaceuticals, Ahmedabad, for providing gift sample of Clarithromycin API.

References:

[1] Ashish K, Narendra S & Deepak K (2014) Int J Pharm Tech Res 6 (1):203-211.

[2] Hu F (2013) Obe Rev 14:606-619.

[3] Gardner C, Wylie-Roset J, Giddin SS, Stefen LM, Johnsn RK & Reader D (2012) Diabet Care 35:1798-1808.

[4] Rodrigues JF, Da Silva Andrade R, Bastos SC, Coelho SB & Pinheiro ACM (2016) Apetite 1:21.

PA-98

Sustained Release Delivery of Repaglinide by Biodegradable Microspheres

Gajanan Engla[1], L.K. Soni [1], V.K.Dixit [2]
[1]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore.
[2]Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar
E-mail address: gajanand54@gmail.com

Abstract: The primary objective of the present study was to prepare repaglinide microspheres for the sustained delivery of the drug for better patient care in the management of diabetics. The biodegradable microspheres of repaglinide is prepare using poly (lactic-co-glycolic acid) (PLGA) by emulsion solvent evaporation technique. The microspheres are prepared with different drug-to-carrier ratios and considering other variables (i.e. solvent, surfactant and stirrer speed) as well. The evaluation of microspheres prepared are perform on the basis of various parameters like particle size, percentage yield, drug entrapment efficiency, surface morphology, drug-polymer interaction (FT-IR study), *in vitro* drug release kinetics and stability studies. SEM reveals that microspheres are spherical and has nearly smooth surface morphology. The percentage yield and drug entrapment efficiency is quite well for all the formulations. FT-IR spectra show that there is no chemical interaction between the drug and the polymer. The *in vitro* release study data shows that the repaglinide release from all the formulations are slow and sustained upto 7days. The various kinetic equations indicate that the *in vitro* drug release is of zero order release with initial burst from repaglinide microspheres. There is no appreciable difference is observed in the stability study observations.

Introduction:

The recent era is of biodegradable polymers era and in the recent time more importance was given to modified release dosage forms to achieve and maintain therapeutic amount of drug in the blood or tissue to improve pharmacokinetics of drug and increase patient compliance as well as reducing side effects for a prolong period of time. Microspheres comprise matrix systems which contain drug throughout their structure and are potential candidates for oral controlled release. Microspheres can be defined as solid spherical particles ranging from 1 to 1000 μm in size [1]. These particles consist of the drug which is the core material and a polymeric coating material. The coating material can be of various types ranging from natural polymers (chitosan, albumin, gelatin,) to synthetic polymers (PVA, PLGA, PEG, poly (ε-caprolactone), blok copolymers etc) [2]. Among the various coating materials used for the development of sustained release formulations, PLGA has been reported to be advantageous as it is biodegradable, biocompatible, and has a very low glass temperature. Apart from single PLGA now-a-days polymeric blend or diblock copolymer with protein repellents (like Poloxamer, Ploxamine and PEG) have been used to impart a stealth characteristic to polymeric micro/nano-particles and ultimately achieved controlled release of the drug. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanoparticles,

and implants. Repaglinide belongs the meglitinide class of drugs is a fast- and short-acting drug with a very short plasma half-life (about 1 hr) and low bioavailability (50%) [3]. Repaglinide was chosen as the model drug in the present study for formulation of microspheres to achieve the controlled drug release profile suitable for peroral administration.

Materials and Methods:

Materials

Repaglinide and polymer were received as a gift sample from M/S Torrent Pharmaceuticals, Ahmedabad, India. Dichloromethane, Methanol, PVP, Polysorbate 80 was purchased from Loba Chem. Pvt. Ltd. And SD fine chemicals, Mumbai, India. All other reagents used were of analytical grade.

Preparation of Microspheres

Solvent evaporation method was used for the preparation of repaglinide microspheres. Total 36 formulations were prepared by using different drug-to-carrier ratios (1:2,1:1& 2:1), different stirrer speed (500rpm, 1000rpm & 1500 rpm), different surfactant (PVP & Polysorbate 80) and different solvent (Methanol & DCM) An accurately weighed quantity (calculated) of the polymer was dissolved in 10 mL of dichloromethane/methanol and weight amount of repaglinide was dissolved in this polymer phase. This solution was emulsified in 100 mL of 0.5% PVP/Polysorbate 80 using continuous stirring for two hours at 500/1000/1500 rpm. The microspheres formed were filtered and washed three times with 50 mL of distilled water to remove surface adhered surfactants and dried at room temperature for 6 h. The dried microspheres were weighed and the % yield of the microspheres prepared was calculated using the formula [4].

Percent Yield = Amount of Microspheres Obtained (g)/ Theoretical Amount (g) × 100

Determination of the mean particle size and surface morphology

Particle size analysis was carried out by using optical microscopy. About 100 microspheres were selected from each formulation randomly and their size was determined using an optical microscope fitted with a standard micrometer scale. Surface morphology and topography of the microspheres were examined by scanning electron microscopy (S-3000N, magnification=x5.0k, WD=33.3mm) and SEM photomicrographs of suitable magnification obtained.

Determination of percentage drug entrapment

For determination of drug content a weighed quantity of the microspheres was crushed and suspended in phosphate buffer, pH 7.4 to extract the drug from the microspheres. After 24 h, the filtrate was assayed by HPLC with mobile phase of methanol: ammonium acetate buffer (pH-4) (80:20*)* at 242 nm for drug content. Corresponding drug concentrations in the sample were calculated from the calibration plot and the drug entrapment efficiency was calculated using the formula:

% Entrapment Efficiency = Quantity of drug in Microspheres/Theoretical drug loading × 100

FT-IR Study

FT-IR spectra of repaglinide and microspheres were recorded in an FT-IR spectrophotometer to check the drug-polymer interaction and chemical integrity of the drug in the microspheres.

Stability studies

For the purpose of stability studies all the formulations were packed in 0.044 mm laminated aluminum foil and subjected to storage at elevated temperature and humidity conditions of 40°±2°C/75±5% RH in an environment chamber. Samples were withdrawn at the end of 1, 3 and 6 months and evaluated for physical properties, encapsulation efficiency, drug content, particle size and *in-vitro* drug release.

***In vitro Drug* release studies**

Drug release studies were carried out using a USP type II dissolution apparatus and the dissolution vessel was filled with 900 mL of 0.1 N HCl and the temperature was kept constant at 37±0.5°C. Samples were withdrawn at predetermined time intervals with the same volume of fresh medium being added after each withdrawal. The sample was suitably diluted and assayed by HPLC method using PDA detector at 242 nm.

Kinetic modeling of drug release

The dissolution profiles of selected formulations were fitted to zero order, first order, Higuchi's and Kresmeyar-Peppas model to ascertain the kinetics of drug release. The regression coefficient (r^2) was calculated for the curves obtained by regression analysis of the above plots.

Results and Discussion:

Repaglinide microspheres with varying proportions of drug & polymer were prepared by the solvent evaporation method. The particle size was determined by optical microscopy and was found to increase with increasing polymer proportions. The % entrapment efficiency, % Drug content and mean particle size of the microspheres is shown in Table 1. Electron microscopy revealed that the microspheres were spherical with a nearly smooth surface [Figure 1]. The yield obtained for all batches was good and in the range of 83.54 ± 2.42 to 88.28 ± 2.54%. The microspheres exhibited an increase in drug entrapment with an increase in the polymer ratio. As the stirrer speed goes high the particle size was lower and the PVP showed the better result as compare to polysorbate 80 in terms of release profile also.

The FT-IR spectra of repaglinide-loaded microspheres showed characteristic absorption peaks that were identical with the drug's reference spectrum. This clearly indicated the stability of the drug during the microencapsulation process and revealed the absence of any drug-polymer interaction [Figures 2].The stability studies did not reveal any remarkable change in the drug content. This indicated that the formulation was stable in medium storage conditions.

The release of repaglinide mainly depended upon the polymer concentration. The release rate of the drug from the microspheres was found to decrease drastically on increasing the polymer

concentration. Repaglinide release from all the formulations was found to be slow and sustained over 7 days. By the end of 7th day, the formulations RMS6 and RMS24 were found to release 49.6±1.06 and 43.7±1.31 of the loaded drug respectively.

Release pattern from all the formulations showed an initial burst release followed a sustained drug release. The cumulative percentage drug release has been observed at the end of day 7. Various kinetic models applied, revealed that drug release was initially zero order followed by first order in both the formulations. The mechanism of drug release from RPG microspheres was studied by using Higuchi and Krsemeyer-peppas models. The r^2 value shows that drug release was initially zero order (diffusion controlled release system) followed by first order (Diffusion and erosion controlled release). The *in vitro* release kinetics profiles of selected formulations have been shown in table 2 and Figure 3, 4, 5 and 6.

Table 1: Characterization of Repaglinide loaded PLGA Microspheres

Fln Code	Formula	EE (%)	DC (%)	PS (□m)
RMSA6	RPG:PLGA[1:2]+Dichloromethane+1500 Rpm + PVA	58.7±1.2	26.84±2.2	12.68±8.26
RMSA24	RPG:PLGA[1:2]+Dichloromethane+1500 Rpm+ Polysorbate 80	47.44±1.22	22.84±1.32	14.24±10.22

Table 2: Release rate kinetics of Repaglinide loaded PLGA Microspheres

Formulation Code	Zero Order Release Rate		First Order Release Rate		Higuchi kinetics		Krsemeyer-Pappas Kinetics	
	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
RMSA6	y = 6.0241x + 2.25	0.9064	y = -0.0377x + 1.95	0.9495	y = 18.505x + 2.08	0.9854	y = 0.3772x + 1.37	0.9983
RMSA24	y = 6.0033x + 5.15	0.9797	y = -0.0347x + 1.98	0.9931	y = 17.802x - 4.08	0.9924	y = 0.668x + 1.09	0.9967

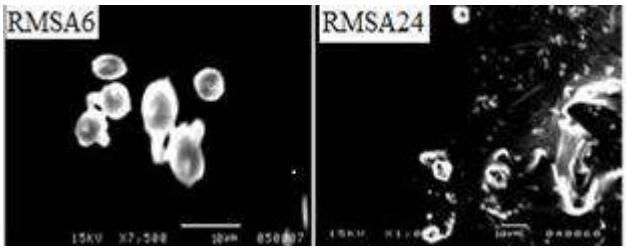


Figure 1: SEM Microphotograph of RMSA6 and RMSA24

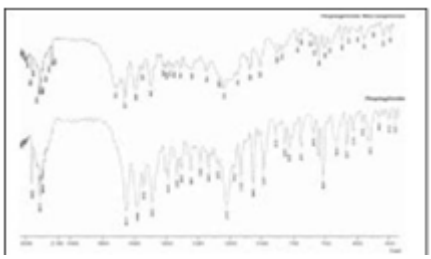


Figure 2: FT-IR spectra obtained for pure Repaglinide and Repaglinide- loaded microspheres

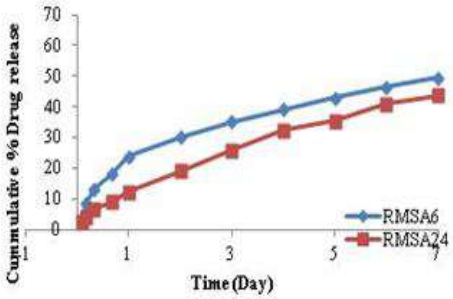


Figure 3: Zero Order drug release kinetics of RPG loaded Microsphere with PLGA

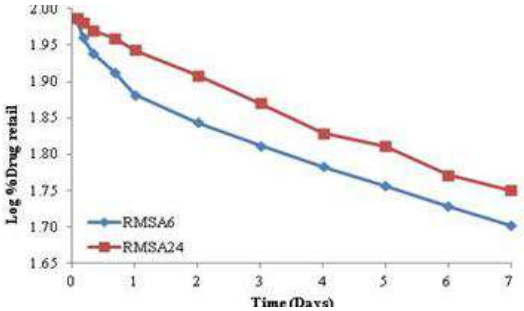


Figure 4 : First order drug release kinetics of RPG loaded Microspheres with PLGA

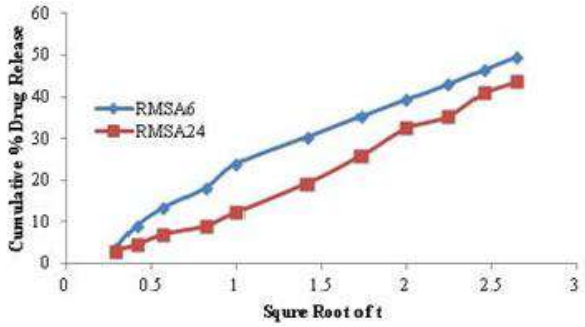


Figure 5 : Higuichi Model drug release kinetics of RPG loaded Micrspheres with PLGA

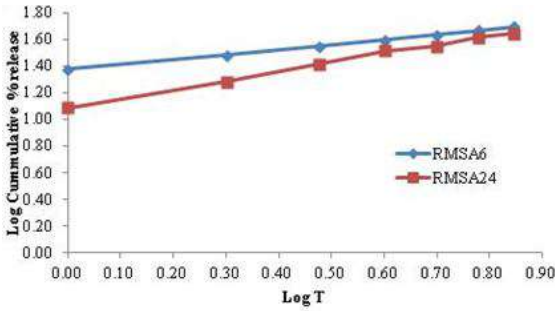


Figure 6: Krsemeyer –Pappas Model drug release kinetics of RPG loaded Microsphees with PLGA

Conclusion:

Present results suggest that biodegradable microspheres of Repaglinide can be rationally employed as long acting formulation. The prepared microparticulate system ensures the sustained delivery of the drug for extended period of time. From the above data, it may be concluded that drug-loaded microspheres appear to be a suitable delivery system for repaglinide and may help in reducing the frequency of medication, improving patient compliance, cost of therapy and reducing side-effects.

Acknowledgments:

The author would like to thank M/S Torrent Pharmaceuticals, Ahmedabad, India for providing gift sample of drug and polymer.

References:

[1] Chowdary KPR, Koteswara N & Malathi K (2004) Indian J Pharm Sci 66:412-6.

[2] Singh D, Saraf S, Dixit VK & Saraf S (2008) Biol Pharm Bull 31:662-7.

[3] Jain SK, Agrawal GP, Jain NK (2006) J Control Rel 113:111-6.

[4] Haznedar S & Dortunc B (2004) Int J Pharm 269:131-40.

PA-99

Development of Clarithromycin Gastroretentive Microspheres

Priya Jain, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Indore (M.P.) 452012
E-mail address: priyajain3878@yahoo.com

Abstract: Gastroretentive microspheres of clarithromycin were prepared on the principle of cation induced gelification using sodium alginate and pectin. These microspheres were investigated for micromeritic properties, drug entrapment, mucoadhesion and drug release properties. Microspheres were found to be discrete, spherical and free flowing. Microspheres were found to adhere to gastric mucosa with high affinity and showed controlled drug release.

Introduction:

Helicobacter pylori (*H. pylori*) infects approximately 50% of the adult formulation; is associated with a wide range of upper gastrointestinal diseases like peptic ulcer and gastric cancer. The widely used drugs for the eradication of *H. pylori* failed to treat the disease fully, might be due to the fact that the drug does not remain in the stomach for long period and do not attain minimum inhibitory concentration in the gastric mucosa. Stomach specific drug delivery systems can prolong the residence time of drug in the stomach, thereby continuously release the drug in the infected area. Mucoadhesive dosage forms have been widely used for site-specific targeting for both local and systemic drug delivery and have also been beneficial for the treatment of *H. pylori* infection. Mucoadhesion involves strong interaction between polymer and mucus lining of the tissue which increases contact time, permits localization, and prolongs drug absorption.

Materials and methods:

Clarithromycin (CLA) was obtained from Alkem labs. (Daman, India) as gift sample. Sodium Alginate and Pectin were procurred of Loba chemie Pvt. Ltd., Mumbai brand (LR grade).

Preparation of Microspheres

Microspheres were prepared employing method of Rajaonarivony *et al.* with some modification on the principle of cation-induced gelification. Aqueous dispersions of sodium alginate and Pectin were prepared in varying ratio of 1:1, 2:1, 3:1, 2:1, 2:2, 2:3, 3:1, 3:2, 3:3 respectively with proper mixing on magnetic stirrer. Clarithromycin 250 mg was added in polymeric dispersion with continuous stirring for 5 minutes. Calcium chloride (1 mL, 18 mmol L^{-1}) was added into 20 mL of the above drug polymer dispersion followed by stirring for 30-45 minutes. The microspheres so prepared were collected by decantation technique, washed repeatedly with deionized water and dried at 45^0C for 12 hr^1.

Characterization of Microspheres

Surface morphology of the microspheres was done using scanning electron microscopy (SEM) (Joel 6100, Japan). All microsphere formulations were sprinkled on a double adhesive tape, which was previously stuck to an aluminum stub. The stubs were coated with gold up to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Prepared samples were randomly scanned and photomicrographs were obtained using electron microscope. Compressibility and flow properties were then determined.

Drug Entrapment Study

Hundred milligrams of the microparticles were placed in 100 mL of phosphate buffer (pH 7.4) and allowed to disintegrate completely for 4 hr. The drug concentration in the buffer was analyzed at 353 nm using UV-visible spectrophotometer (Shimadzu 1800) and percent drug entrapment was calculated [2].

$$\text{Percent Drug entrapment} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100$$

Percent mucoadhesion

The mucoadhesive properties of the mucoadhesive microspheres were evaluated by in vitro wash-off test [3].A 1x1 cm piece of stomach mucosa was mounted on to a glass slide with cyanoacrylate glue and rinsed with 0.1N HCL. Fifty microspheres were spread on to it and kept in USP tablet disintegrating test apparatus containing the 900 ml of 0.1N HCL at 37± 0.5^{o}C. Number of microspheres still adhering to tissue were calculated after 30 min, 1 hr and at the hourly intervals up to 6 hr. The studies were carried out in triplicate.

$$\textbf{Percent Mucoadhesion} = \frac{\textbf{No. of microspheres adhered at the end of 6}^{\textbf{th}}\textbf{ Hour}}{\textbf{No. of microspheres spread}} \textbf{x100}$$

Dissolution Study

The release rate of clarithromycin microspheres was determined in a USP XXIII paddle type 2 dissolution apparatus. Weighed quantity of microspheres equivalent to 100 mg of clarithromycin was filled into a hard gelatin capsule (#2) and placed in the paddle of dissolution apparatus. The dissolution medium (900 mL) of SGF (pH 1.2) was used as dissolution medium. The dissolution fluid was maintained at 37±0.5 ^{0}C and rotation speed of 100 rpm. The sample of 5 mL was withdrawn at the intervals 30 min of and was filtered through 0.25 µm membrane filter. The volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. Samples were analyzed at 353 nm[2].

Statistical analysis

In vitro drug release of CLA from CLAP3b was statistically treated by one-way analysis of variance, ANOVA followed by Dunnet's test where $P<0.05$ was considered statistically significant.

Results and Discussion

Microspheres were prepared by cation-induced controlled gelification of the polymer. The particle size, compressibility index, and angle of repose of the prepared microspheres was found in the range of 106 ±2.13to 180±0.78, 15.26±0.56 to 21.22±0.78, 23.67±0.04 to 27.78±0.03. The particles were found to be discrete, spherical and free flowing. The entrapment effieciency and percent mucoadhesion were found to be in the range of 70.25±0.02 to 62.24±0.02 and 40.1±1.2 to 80.4±3.9 respectively. The formulation CLA3b is opted as best formulation as it has optimum micromeritic properties, maximum entrapment efficiency and percent mucoadhesion.

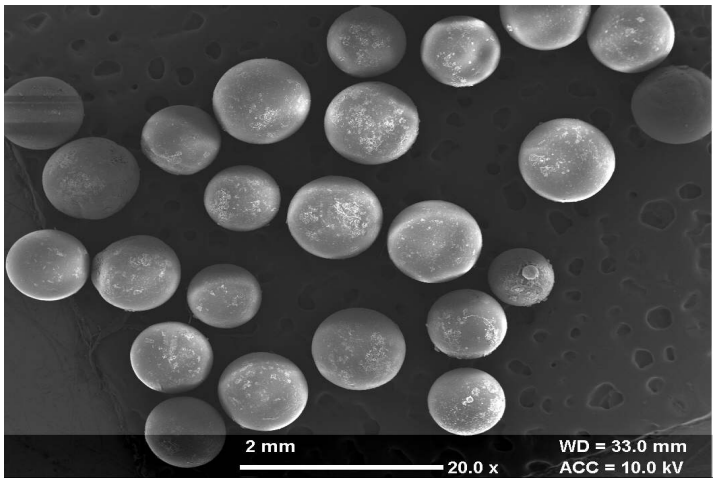


Figure 1: SEM Photograph of formulation CLA3b

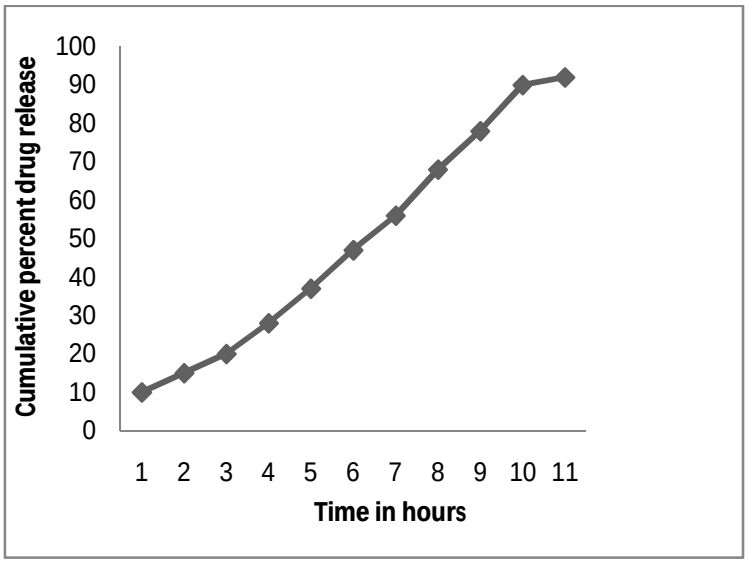


Figure 2: Cumulative % drug release profile of CLA3b in simulated gastric fluid (pH 1.2) at 37°C ± 0.5°C.

Table 1: Micromeritic properties, (%) drug entrapment and (%) mucoadhesion of the microspheres

Formulation code	Sodium alginate : pectin Ratio	Average Particle size (µm)	Compresibi-lity index (%)	Angle of repose in degree (^{o})	Drug Entrapment (%)	Mucoadhesion (%)
CLA 1a	1:1	110±2.13	21.22±0.78	26.27±0.02	62.24±0.02	40.1±1.2
CLA1b	1:2	108±1.43	19.67±0.79	27.78±0.03	65.67±0.04	45.2±0.01
CLA1c	1:3	106±2.13	17.78±0.56	27.98±0.02	62.57±0.02	50.2±0.06
CLA2a	2:1	144±0.34	17.56±0.57	25.98±0.04	67.46±0.01	50.4±0.04
CLA2b	2:2	140±0.27	15.34±0.47	25.45±0.05	69.36±0.03	55.4±1.4
CLA2c	2:3	139±0.45	15.26±0.56	24.67±0.03	66.58±0.02	65.5±0.08
CLA3a	3:1	180±0.78	15.45±0.37	23.67±0.04	65.45±0.04	65.6±0.09
CLA3b	3:2	179±0.68	16.67±0.25	25.01±0.06	70.25±0.02	80.4±3.9
CLA3c	3:3	175±0.86	17.62±0.47	26.24±0.04	64.25±0.02	75.3±0.06

Conclusion:

Gastroretentive Clarithromycin microspheres were successfully prepared with significantly enhanced mucoadhesiveness and controlled release. Such developed formulations could be furthers subjected for *in vivo* studies in future.

References:

[1] Rajaonarivony M, Vauthier C & Couarraze G (1993) J Pharm Sci 82:912–917.

[2] Tripathi GK (2013) Int J PharmTech Res 5:7-16.

[3] Lehr CM, Bouwstra JA, Schacht EH & Junginger HE (1992) Int J Pharm 78:43-48.

PA-108

Enhancement of Transcorneal Permeation and Sustain Release of Timolol Maleate from Developed and Optimized *In Situ* Gel with better Safety Profile

Umesh D. Laddha[1], Archana Nerpagar[2], Savita Mandan[2]
[1]MET's Institute of Pharmacy, Nasik, 422003, (MS)-India
[2]R. C. Patel, Institute of Pharmaceutical Education and Research, Shirpur, 425405, (MS)-India
E-mail address: umeshladdha698@gmail.com

Abstract: Glaucoma is a chronic disease that causes irreversible blindness. Timolol Maleate is used as first line drug in treatment of glaucoma. Poor ocular bioavailability and therapeutic response shown by conventional ophthalmic system can be overcome by use of *in situ* gelling system which undergoes reversible sol to gel transition in cul-de-sac by physical stimulation. Present work describes formulation and evaluation of pH sensitive *in situ* gel system of Timolol Maleate. Carbopol 974P was used as pH sensitive polymer with HPMC K15M as viscosity modifier. 3^2 factorial design was used to study the effect of independent variables viz. concentrations of Carbopol 974P and HPMC K15M on dependent variables like *in vitro* drug diffusion and viscosity. Optimized batch showed 88.48% drug diffusion upto 8h. Optimized formulation was evaluated for various parameters such as drug release study, isotonicity, texture analysis, preservative efficacy studies, sterility testing as per IP 2010, accelerated stability studies. *Ex vivo* transcorneal permeability study was carried out on goat eye cornea which showed that EDTA (0.5%) increases drug penetration by 1.90 fold and showed no corneal damage after histological study. In conclusion, prepared formulation is stable and non-irritant.

Introduction:

Glaucoma is a chronic disease of eye that is characterized by irreversible damage to the ganglionic cells and the optic nerve. Elevated intraocular pressure is most important risk factor for glaucoma. It has been established as the second leading cause of world's blindness, which may affect around 80 million in 2020. The treatment of glaucoma focuses mainly on lowering of IOP. In the last two decades several classes of topical IOP lowering drugs have been made available which includes beta blocker, prostaglandin analogue (PGA), alpha-adrenoceptor agonist (AA) and topical carbonic anhydrase inhibitors (CAI's).

Timolol Maleate (TM) is a non-selective beta-adrenergic receptor blocker used in treatment of open angle glaucoma and occasionally in secondary glaucoma. Conventionally it is available in the form of solution which gets immediately eliminated from the precorneal area. Further, shorter contact time with poor corneal permeability results into poor bioavailability (10%) and decreased patient compliance. Several novel drug delivery systems (NDDS's) have been developed which includes inserts, ointment, nanosuspension etc. However these systems suffer from several drawbacks such as blurred vision associated with ointment, low patient compliance from inserts and high cost of nano suspension. These problems can be overcome by using *in situ* gel forming systems.

In situ drug delivery systems consist of polymers that exhibit sol to gel phase transition in cul-de-sac by several physicochemical parameters. Depending upon method used for sol to gel phase transition three types of *in situ* gels are widely accepted as pH triggered system, ion activated system and temperature dependent system. Pharmaceutically significant gels can be prepared by using various materials. Carbopol 974P is pH sensitive polymer which shows sol to gel transition in aqueous solution when pH is raised above 5.5. It is polyacrylic acid (PAA) which is required in high concentration to form stiff gel. At higher concentration it forms highly acidic solution which is not easily neutralized by buffer action of tear fluid. Reduction in its concentration without affecting the gelling capacity and viscosity was achieved by addition of viscosity increasing polymers such as HPMC.

Material and Methods:

Material

Timolol Maleate and Carbopol 974P were gifted by FDC Limited, Mumbai and Lubrizol advanced material India Pvt. Ltd., Mumbai respectively. HPMC K15M was purchased from S. D. Fine, Mumbai. HPLC grade methanol was purchased from Qualigens Fine chemicals, Mumbai. All other ingredients were of analytical grade. IOTIM (Timolol Maleate eye drops 0.5% by FDC) was brought from local medical shop.

Analytical method development

To quantitate the content of Timolol Maleate in samples reversed phase (RP)-HPLC method was developed and validated as per ICH guidelines Q2 (R1). Shimadzu RP-HPLC instrument (CFR-21) equipped with photodiode array detector (PDA) and C_{18} column of Kromasil (250 mm × 4.6 mm, 5µm particle size) was used. Mobile phase consisted of phosphate buffer: methanol (60:40 v/v) and pH 3.5 was maintained by O-phosphoric acid. Elution was measured at 295 nm with flow rate of 1.0 ml/min.

Full factorial experimental design

For optimization of Timolol Maleate *in situ* gel, 3^2 randomized full factorial design was selected. The design was applied to study the effect of concentration of Carbopol 974P and HPMC K15M on formulation. The amount (%) of pH sensitive polymer, Carbopol 974P (X_1) and the amount (%) of viscosity modifier, HPMC K15M (X_2) were selected as independent variables, in this study. These two factors were evaluated at 3 levels as higher, middle and lower levels. Composition of different batches of Timolol Maleate is shown in table 1.

Evaluation of formulation

Formulation was evaluated for various parameter viz. physicochemical evaluation, viscosity, drug diffusion, *ex-vivo* transcorneal permeation, histological evaluation, *in-vitro* drug release, isotonicity, texture analysis, sterility, preservative efficacy study and accelerated stability study.

Table 1: Composition of different batches of Timolol Maleate *in situ* gel

Name of excipients	Different batches of Timolol Maleate *in situ* gel (%w/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Timolol Maleate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Sodium Chloride	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Carbopol 974P	0.15	0.15	0.15	0.30	0.30	0.30	0.45	0.45	0.45
HPMC K15M	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Water q.s.	100	100	100	100	100	100	100	100	100

Results and Discussion:

The pH triggered Timolol maleate *in situ* gel was successfully formulated by using Carbopol 974P and HPMC K15M. Formulation was optimized by 3^2randomised full factorial design for two responses viz. viscosity at 20 rpm and cumulative percent drug diffused at the end of 8 h. Optimized formulation (Batch f7) was liquid at pH 4 and gel above pH 7 indicating *in situ* transition at physiological pH.Optimized *in situ* gel passes all safety tests used for evaluation of ophthalmic formulation as per regulatory guidelines. *In vitro* drug releasestudy showed sustained release of drug from *in situ* gel over period of 8h as compared to marketed formulation IOTIM (FDC). Prepared formulation was subjected for *ex vivo* transcorneal permeability study and result was compared with IOTIM which showed need of penetration enhancer. EDTA (0.5% w/v) was found to be suitable penetration enhancer which showed significant increase in transcorneal permeability of drug (Fig. 1). Formulation showed less eye irritation as compared to SDS (positive control) during histological study on goat eye cornea (Fig. 2). Stability study performed over a period of 3 month showed that optimized formulation is stable. Hence; prepared formulation is feasible alternative for conventional eye drop for glaucoma treatment but future *in vivo* studies on human is necessary to confirm significant therapeutic effect.

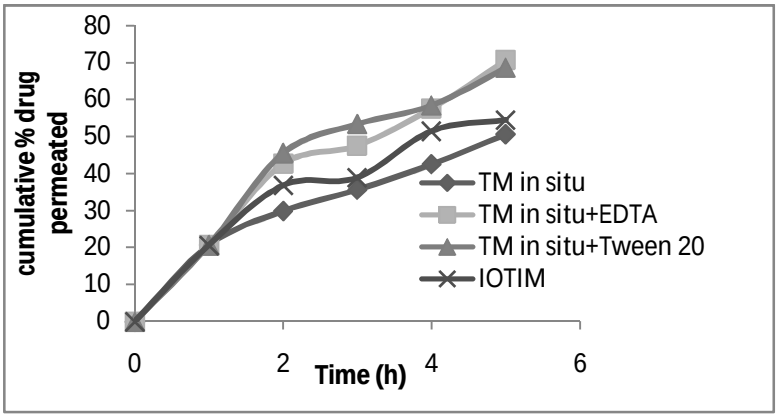


Figure 1: Comparative *ex vivo* transcorneal cumulative % drug release

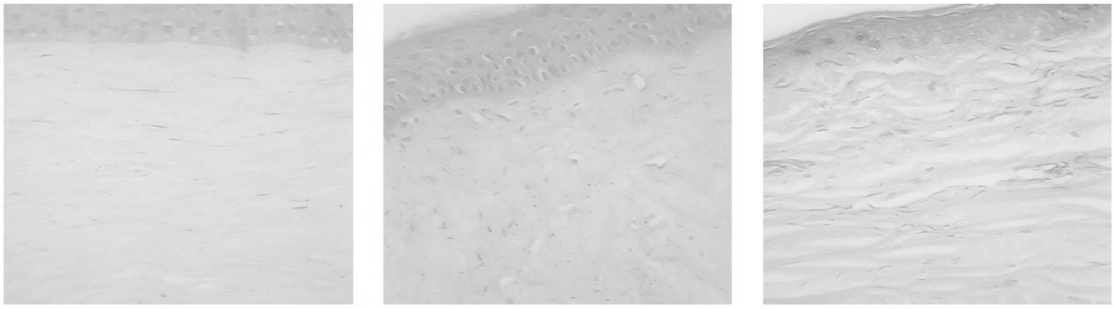


Figure 2: Histological section of goat eye cornea (magnification 40X) a) negative control: untreated cornea, b) test specimen: formulation treated cornea, c) positive control: SDS treated cornea for 5h.

References:

[1] Cheng JW, Cheng SW, Gao LD, Lu GC & Wei RL (2012) Plos ONE 7:1-11.

[2] Bhalerao AV, Singh SS (2011) Int J Pharm Bio Sci 2:7-14.

[3] Gupta S, Samanta MK, Raichur AM (2010) AAPS Pharm Sci Tech 11:322-335.

[4] Nanjawade BK, Manvi FV & Manjappa AS (2007) J Control Rel 122:119–34.

[5] Song J, Bi H, Xie X, Guo J, Wang X & Liu D (2013) Int Immunopharmcol 17:99-107.

PA-109

Formulation and Evaluation of Herbal Lozenges

Kamlendra Kumar Mishra, Kutubuddin Tasneem, Vikas Jain, S.C. Mahajan
Mahakal Instutute of Pharmaceutical Studies, Ujjain
E-mail address: kamleshmishra@gmail.com

Abstract: Throat infections are most common disease in today's world. However, it is not taken too seriously by people. Long term throat infection can lead to severe throat problems like Pharyngitis and also cancer. Lozenges are solid preparations that contain one or more medicaments, usually in a flavored, sweetened base, that are intended to dissolve or disintegrate slowly in the mouth. They are used for medications designed to be released slowly to yield a constant level of drug in the oral cavity or to bathe the throat tissues in a solution of the drug. Since soft lozenges can be made at home using simple ingredients, this formulation is very help in treating throat infection easily using household techniques. Jaggery was melted on water bath and mixed with the other ingredients to form a homogeneous mixture. Subsequently, the mixture was poured into the stainless steel mold. The monograph analysis was performed according to WHO guidelines. The results were found to match with the standards in monograph. From the above investigation it can be concluded that the soft lozenges prepared using pipper longum and glycyrhhiza glabra can be used to treat minor throat infections.

Introduction:

Throat infections are most common disease in today's world. However, it is not taken too seriously by people. Long term throat infection can lead to severe throat problems like pharyngitis and also cancer. Acute sore throat is a symptom often caused by an inflammatory process in the pharynx, tonsils or nasopharynx. Most of these cases are of viral origin and occur as a part of the common cold. A sore throat is pain, scratchiness or irritation of the throat that often worsens when you swallow. The most common cause of a sore throat (pharyngitis) is a viral infection, such as a cold or the flu. A sore throat caused by a virus resolves on its own. Strep throat (streptococcal infection), a less common type of sore throat caused by bacteria, requires treatment with antibiotics to prevent complications. Sore throats may be caused by viral infections, Bacterial infections**,** Irritants and injuries. Signs and symptoms might include pain or a scratchy sensation in the throat, pain that worsens with swallowing or talking, difficulty swallowing, sore, swollen glands in your neck or jaw, swollen, red tonsils, white patches or pus on your tonsils, hoarse or muffled voice.Common infections causing a sore throat might result in other signs and symptoms, including fever, Cough, Runny nose, Sneezing, Body aches, Headache, Nausea or vomiting. Conventional treatment of sore throat Anti-inflammatory drugs, Corticosteroids, Antibiotics, Others.

Lozenges are solid preparations that contain one or more medicaments, usually in a flavored, sweetened base, that are intended to dissolve or disintegrate slowly in the mouth. They can be prepared by molding or by compression of sugar-based tablets. Development of lozenges dates back to 20th century and is still in commercial production. Most of the lozenge preparations are available as over the counter medications. Lozenge provides a palatable means of dosage form administration and enjoys its position in pharmaceutical market owing to its several advantages

Types of Lozenges

Chewable Lozenges, Hard Lozenges, Soft Lozenges, Compressed lozenges.

Methodology:

Formulation Soft lozenges were prepared by melting and mold technique. Jaggery was melted on water bath and mixed with the other ingredients (powder) to form a homogeneous mixture. Subsequently, the mixture was poured into the stainless steel mold.

Table 1: Formula taken to form herbal lozenges

S. No.	Ingredients	Quantity for one lozenge
1	*Piper nigrum*	125 mg
2	*Glycyrhiza glabra*	200 mg
3	Jaggery q. s.	10 gms

Macroscopical evaluation The formulation developed in the laboratory were evaluated for its acceptance based on visual observation for various organoleptice properties.

Table 2: Macroscopic evaluation of formulated lozenges

S. No.	Parameter	Observation
1	Colour	Golden Yellow
2	Odour	Pleasant
3	Taste	Sweet
4	Texture	Smooth
5	Shape	Oval

Determination of ash values The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non-physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Table 3: Determination of total ash value

S.No.	Drugs	Total ash (% w/w)
1	*Pipper longum*	1.5
2	*Glycerhhiza glabra*	3
3	*Lozenges*	6.3

Determination of swelling index The swelling index is the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual herbal material.

Table 4: Determination of swelling index

S.No.	Drugs	Swelling index
1	*Pipper longum*	NIL
2	*Glycerhhiza glabra*	NIL
3	*Lozenges*	NIL

Determination of moisture content

This test is used to determine the water content of a material by drying a sample to constant mass at a specified temperature. To determine the moisture content of the formulation the weighed amount of formulation were processed in hot air oven at 120 degree centigrade till constant weight obtained and the percent moisture content was calculated.

Table 5: Determination of moisture content

S.No.	Dosage form	Moisture content
1	Lozenges	4.35%

Determination of extractable matter

This method determines the amount of active constituents extracted with solvents from a given amount of herbal material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The extractive values were determined as per procedure given in WHO guideline.

Table 6: Determination of extractive values

S.No.	Drugs	Water extractive value	Alcohol extractive value
1	*Pipper longum*	4.458% w/v	0.811% w/v
2	*Glycerhhiza glabra*	4.103% w/v	1.207% w/v
3	*Lozenges*	0.124% w/v	-

Thin-layer chromatography

Thin-layer chromatography is particularly valuable for the qualitative determination of small amounts of impurities. The principles of thin-layer chromatography and application of the technique in pharmaceutical analysis are described in The international pharmacopoeia (5). As it is effective and easy to perform, and the equipment required is inexpensive, the technique is frequently used for evaluating herbal materials and their preparations.

Table 7: Thin Layer Chromatography

S.No.	TLC	Rf value
1	Pure	0.934
2	*Lozenges*	0.958

Figure 1: Thin Layer chromatography

UV Spectrophotometry

UV-Vis spectrophotometric analysis provides both qualitative and quantitative standards. But markers are needed for quantitative analysis. An attempt is made to study UV Vis Spectrometric analysis of some herbal raw materials for understanding qualitative and quantitative parameters without markers.

Preparation of standard solution for calibration curve of piperine

Stock solution of piperine was prepared by dissolving 100 mg of piperine in 100 ml of methanol. Standard solutions of piperine were prepared from stock solution in the concentration range of 1µg/ml to 5µg/ml in 10 ml volumetric flask using methanol as solvent and absorbance were taken at 340 nm.

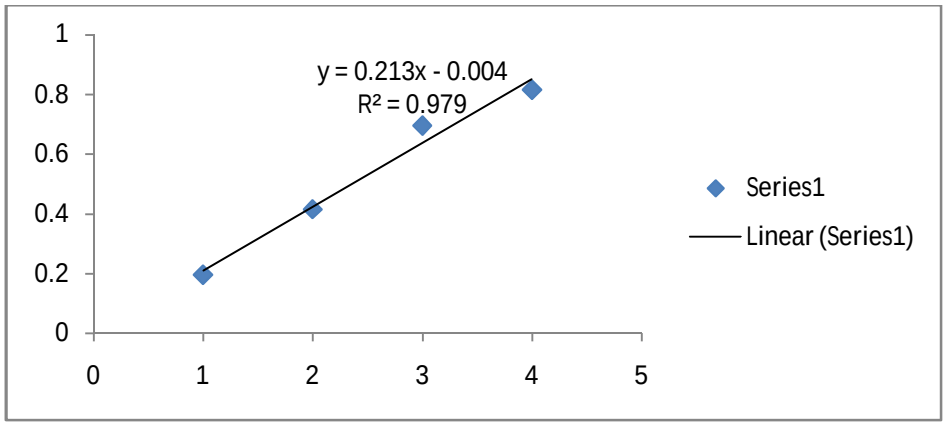


Figure 2: Calibration curve of piperine

Result and Discussion:

The lozenges were prepared with the combination of piper longum and glcyrhhiza glabra using jeggery as the base. The monograph analysis was performed according to WHO guidelines. The

quality control parameter were ash value test, swelling index, moisture content, water and alcohol extractives, thin layer chromatography and UV analysis. On the above investigation it was found that the formulation passage all the parameters of quality control parameters, in uv and chromatographic analysis piperine were found to be present. During the storage it was found that the formulation were gaining the moisture from atmosphere which indicates that proper packing and storage condition is required when the formulation need to keep for future use.

Conclusion:

Lozenges, especially soft lozenges are marketed for throat infection and other buccal infections. From the above investigation it can be concluded that the soft lozenges can be prepared using house hold ingredients using P*ipper longum*, *Glycyrhhiza glabra* and Jaggery as a base very easily and can be used to treat minor throat infections at initial stage of infection.

References:

[1] Paul M (2012) ESCMID Guideline for the Management of Acute Sore Throat, Volume 18, ESCMID: 1-18.

[2] Maheshwari R, Jain V, Ansari R, Mahajan SC & Joshi G (2013) British Biomed Bull 1-9.

[3] Shinde SG, Kadam V, Kapse GR, Jadhav SB, Zameeruddin & Bharkad VB (2014) Indo American J Pharm Res 4:567-570.

[4] Pothu R & Yamsani MR (2014) Int J Adv Pharm Res 1:290-294.

[5] Meghwal M & Goswami TK (2012) Open Acc Sci Rep 1:1-5.

PB-01

CoMFA and CoMSIA Studies on 6, 8-Dibromo–4(3H)-Quinazolinone Derivatives for Anti-Bacterial Activity Against *Salmonella typhimurium*

Rahul Dixit[1], Love Kumar Soni[2], Rajesh Sharma[2]
[1]IPS Academy College of Pharmacy, Rajendra Nagar, AB Road, Indore, M.P. – 452012
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshshila Campus, Ring Road, Indore, M.P. – 452017
E-mail address: rahuldixit1102@gmail.com

Abstract: In order to explore the structure – activity relationship of quinazolinone moiety for anti-bacterial activity against *Salmonella typhimurium*, a series of 4(3H) – quinazolinone derivatives were subjected to Comparative molecular field analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA) methods. The best models for CoMFA and CoMSIA had correlation coefficient of 0.905 & 0.868 and cross-validated correlation coefficient of 0.501 & 0.592 respectively. The information obtained from the above models might be useful in designing of quinazolinone moiety as potent anti-bacterial agents.

Introduction:

Bacterial infections are becoming untreatable due to development of resistance in bacteria against the presently available anti – bacterial agents. This condition presents an immense need to search a new agent which could be effective against the bacteria. Quinazoline and quinazolinone derivatives have been widely studied for its diverse pharmacological activities. Of these, studies reveals 4- (3H)-quinazolinone derivatives as a potential moiety for anti – bacterial activity. There are three main positions (R_1, R_2, R_3) which can be varied in quinazolinone nucleus for variation in biological activity (Fig.1). Literatures also reveal that the variationat R_1position bring changes in anti – bacterial activity gram negative bacteria [1].

Materials and Methods:

The antibacterial activities of 4 (3H) – quinazolinone derivatives (Table 1) against *Salmonella typhimurium* were taken from the work of Mohamed et al. [2]. The activities reported as zone of inhibition (ZOI) were converted to natural logarithm of zone of inhibition (lnZOI) (Table 2). The predictive power of QSAR was analysed by dividing the dataset into training set and test set.

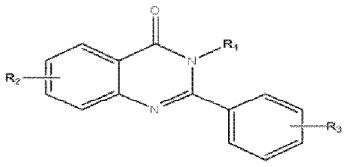


Figure 1: 4- (3H) quinazolinone moiety

Figure 2: Structure alignment of molecules of 6, 8-dibromo-4(3H) quinazolinone derivatives (training)

Structural Alignment: The molecules taken in training set were aligned on the common template of quinazolinone.

CoMFA: Steric (S) and Electrostatic (E) fields were calculated using sp^3 hybridised carbon atom with +1 charge at each lattice of 2 Ansgstrom and the default energy cut off of 30 kCal/mol. Gasteiger, Gasteiger-Huckel, MMFF_94, DelRe and Pullman charges were used to generate the partial charges on the molecules under study and generated models were explored models for the best models.

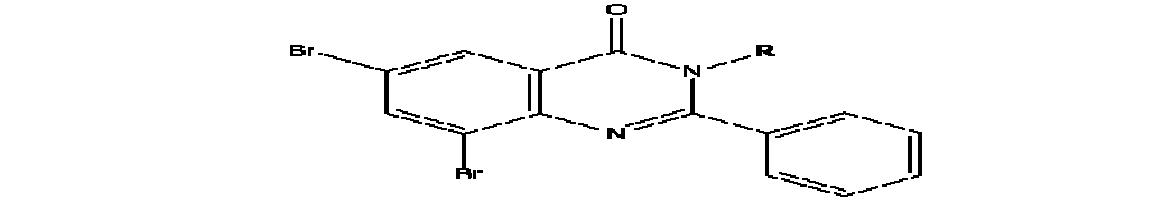
CoMSIA: CoMSIA descriptors i.e. Steric (S), Electrostatic (E), Hydrophobic (H), Hydrogen bond donor (D) and Hydrogen bond acceptor (A), were generated using a sp^3 hybridized carbon atom with +1 charge; the attenuation factor was set to 0.3 and a Vanderwaals radius of 1.4 Angstrom.

Partial Least Square and Predictive r^2 analysis: Leave one out (LOO) validation method was used to calculate optimum number of components while no validation approach was adopted for the predictability of the developed model. R^2_{pred} value is calculated for test set molecules and can be mathematically represented as;

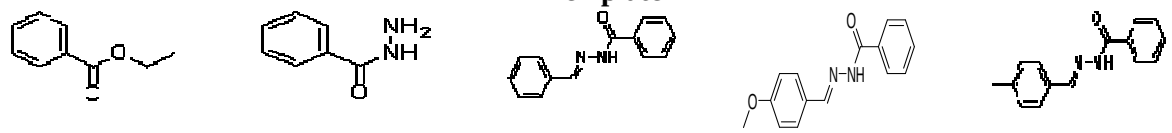
$$\mathbf{R^2_{pred} = 1- (PRESS/SD)}$$

where SD is the sum of squared deviation between the biological activities of the test set molecules to the mean activity of the training set molecules, while PRESS is the sum of squared deviations between the observed the predicted activities of the test molecules.

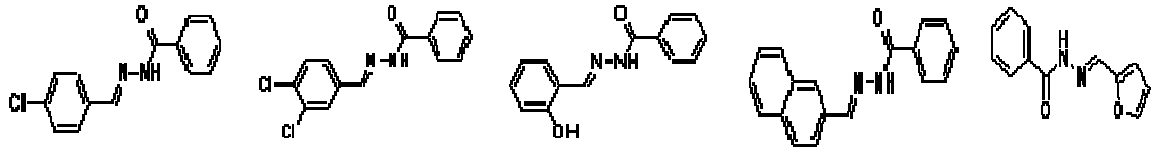
Table 1 : Structures of the molecules considered for QSAR studies



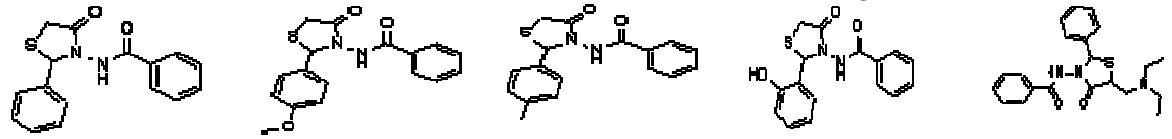
Template



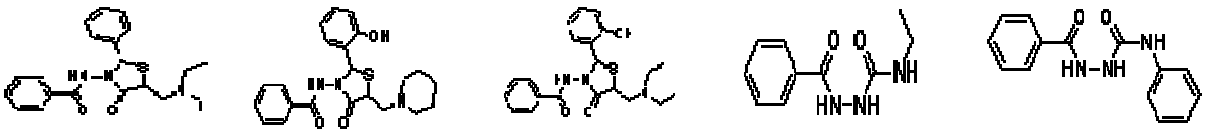
II III Iva IVb IVc



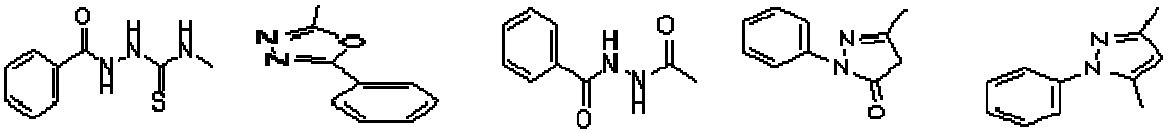
IVd IVe IVf IVg IVh



Va Vb Vc Vd VIa



VIb VIc VId VIIa VIIb



VIIc VIII IX X XI

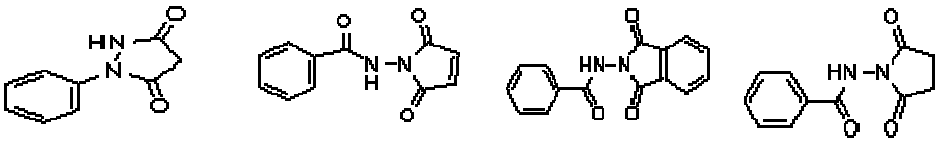


Table 2: Experimental and predicted activities of 6,8-dibromo-4 (3H) quinazolinone derivatives with ZOI and lnZOI values of bacteria *Salmonella typhimurium*

XII			XIII			XIV		
Comp.	**ZOI (mm)**	**lnZOI**	**Comp.**	**ZOI (mm)**	**lnZOI**	**Comp.**	**ZOI (mm)**	**lnZOI**
Training								
II	16	2.7726	**IVg**	12	2.4849	**VIII**	12	2.4849
III	11	2.3979	**IVh**	16	2.7726	**IX**	14	2.6391
IVb	10	2.3026	**Vb**	12	2.4849	**X**	14	2.6391
IVc	15	2.7081	**VIa**	11	2.3979	**XI**	14	2.6391
IVd	10	2.3026	**VIb**	10	2.3026	**XIII**	17	2.8332
IVe	11	2.3979	**VIIb**	16	2.7726	**XIV**	11	2.3979
IVf	13	2.5649	**VIIc**	12	2.4849	**XV**	18	2.8904
Test								
IVa	10	2.3026	**Vd**	12	2.4849	**VIIa**	16	2.7726
Va	16	2.7726	**VIc**	15	2.7081	**XII**	12	2.4849
Vc	15	2.7081	**VId**	18	2.8904			

Results and Discussion:

The best model was screened out on the basis of Q^2 (Crossvalidated R^2) ≥ 0.5 and R^2 > 0.6 (Table 3). The model was validated plotting the graph between observed biological activity versus predicted CoMFA and CoMSIA activity (Table 4). This revealed that the model is validated and contain proper training and test sets for further study.

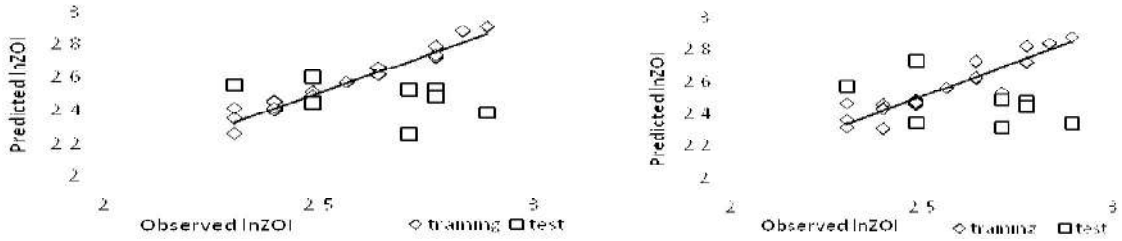
Table 3: CoMFA and CoMSIA statistical results and field contribution of training sets of 6,8-dibromo – 4 (3H) quinazolinone derivatives with for bacteria *Salmonella typhimurium* (Charge: MMFF_94)

Parameters	**CoMFA**	**CoMSIA**	**Parameters**	**CoMFA**	**CoMSIA**
Q^2(Crossvalidated R^2)	0.501	0.592	Steric field	0.407	0.223
R^2	0.905	0.868	Electrostatic field	0.593	0.777
F value	38.218	37.274	(N_1, N_2) for F	4,16	3,17
SEE	0.064	0.073	R^2_{pred}	0.494	0.513
No. of components	4	3			

Table 4: CoMFA and CoMSIA predicted activities of 6,8- dibromo-4(3H)quinazolinone series for bacteria *Salmonella typhimurium*

Comp	CoMFA		CoMSIA		Comp	CoMFA		CoMSIA	
	Predicted	Residual	Predicted	Residual		Predicted	Residual	Predicted	Residual
Training									
II	2.7231	0.0495	2.7224	0.0502	**VIb**	2.2586	0.044	2.314	-0.0114
III	2.4083	-0.0104	2.453	-0.0551	**VIIb**	2.7372	0.0354	2.82	-0.0474
IVb	2.3496	-0.047	2.3632	-0.0606	**VIIc**	2.4493	0.0356	2.4765	0.0084
IVc	2.5231	0.185	2.5345	0.1736	**VIII**	2.4905	-0.0056	2.4654	0.0195
IVd	2.4163	-0.1137	2.4628	-0.1602	**IX**	2.654	-0.0149	2.6223	0.0168
IVe	2.3976	0.0003	2.4301	-0.0322	**X**	2.6626	-0.0235	2.7264	-0.0873
IVf	2.5718	-0.0069	2.5638	0.0011	**XI**	2.6157	0.0234	2.6258	0.0133
IVg	2.5106	-0.0257	2.4552	0.0297	**XIII**	2.8787	-0.0455	2.8399	-0.0067
IVh	2.7853	-0.0127	2.7229	0.0497	**XIV**	2.4157	-0.0178	2.3062	0.0917
Vb	2.4612	0.0237	2.4561	0.0288	**XV**	2.9076	-0.0172	2.8825	0.0079
VIa	2.4541	-0.0562	2.4277	-0.0298					
Test									
IVa	2.5532	-0.2506	2.5722	-0.2696	**VIc**	2.2569	0.4512	2.3145	0.3936
Va	2.5271	0.2455	2.4747	0.2979	**VId**	2.3866	0.5038	2.3424	0.548
Vc	2.5283	0.1798	2.4856	0.2225	**VIIa**	2.4847	0.2879	2.4453	0.3273
Vd	2.4456	0.0393	2.3438	0.1411	**XII**	2.6019	-0.117	2.7322	-0.2473

Graphs:



Contour maps: In steric contour map, yellow colours shows steric group is unfavourable while green colour shows steric group is favourable. In electrostatic contour map, red colour shows electronegative group while blue group favours electropostive group.

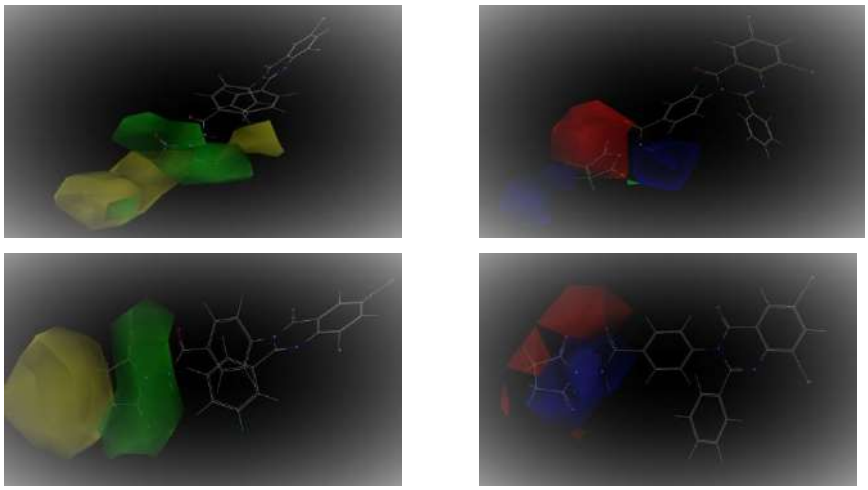
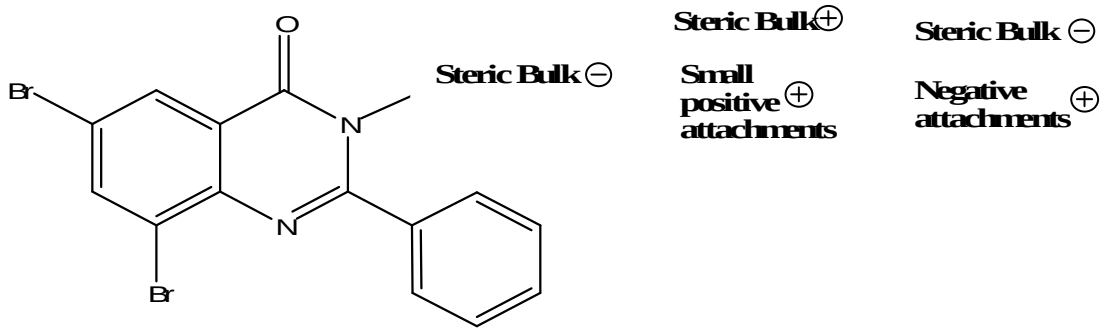


Figure 3: a) CoMFA–Steric b) CoMFA–Electrostatic c) CoMSIA– Steric d) CoMSIA –Electrostatic

Figure 4: Structure activity relationship on the basis of QSAR studies



Region_1: Non steric group with small positive charge attachments on it may be good for activity.

Region_2: Steric group with small hydrophobic and positive attachment may improve the activity.

Region_3: Less bulky group with negative substituents may be beneficial for anti-bacterial activity.

Conclusion:

3D-QSAR models developed in our present study are quite reliable based on appropriate statistical parameters and proper validation of test set. Though CoMFA method afforded slightly better predictability, models from both CoMFA and CoMSIA were utilized in development of SAR. The designed model could serve as a guideline for further study or development of novel anti-bacterial agent against *Salmonella typhimurium.*

Acknowledgement:

The authors would like to acknowledge AICTE and UGC for the work carried out in DAVV.

References:

[1] Srivastava S (2015) Int J of Pharm Sci Res 6:1206-1213.

[2] Mohamed MS, Kamel MK, Kassem EM, Abotaleb N, Abdel-moez S & Ahmeda M (2010) Eur J Med Chem 45:3311–3319.

PB-02

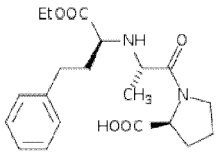
Development and Validation of Stability-Indicating HPTLC Method for Simultaneous Estimation of Enalapril Maleate, Hydrochlorothiazide and Paracetamol in Combined Tablet Dosage Form

Jinendra Sardiya, Ankit Jain, Nitin Dubey, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Indore (M.P.)
E-Mail address: jsindore@gmail.com

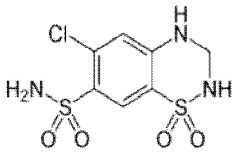
Abstract: A stability-indicating high-performance thin-layer chromatographic (HPTLC) method has been developed for the determination of simultaneous determination of Enalapril maleate (ENAL), Hydrochlorothiazide (HCTZ) and Paracetamol (PARA) in tablet dosage forms, The separation was achieved on TLC aluminum plates precoated with silica gel 60F-254 using chloroform: methanol: toluene : ethyl acetate (20:10:40:30 % v/v/v/v) as the mobile phase. The densitometric analysis was carried out at 230 nm. Compact bands appeared at R_f 0.21 ± 0.01, 0.50 ± 0.02 and 0.73 ± 0.01 respectively, for ENAL, HCTZ and PARA. Linear regression analysis revealed linearity in the range of 1000 – 6550 ng/spot for ENAL, 100-3600 ng/spot for HCTZ and 500 – 3400 ng/spot for PARA. Drugs were subjected to acid and alkali hydrolyses, forced oxidation, thermal and photo degradation treatments. The degraded products were well separated from the pure drugs. Statistical analysis proved that the method is precise, accurate, selective and economical and may be used for routine analysis of ENAL, HCTZ and PARA in tablet dosage forms.

Introduction:

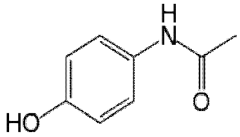
Enalapril Maleate is an angiotensin converting enzyme (ACE) inhibitor used in the treatment of hypertension and some types of chronic heart failure, hydrochlorthiazide, is a first line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water, paracetamol are widely used as an analgesic.[1-5]



Enalapril Maleate



Hydrochlorothiazide



Paracetamol

Experimental:

HPTLC method for simultaneous estimation of ENAL, HCTZ and PARA in combined tablet dosage form was developed using HPTLC (CAMAG (Muttenz, Switzerland). Chloroform: Methanol: Toluene: Ethyl acetate (20: 10: 40: 30 % v/v/v/v) was optimized as mobile phase.

Preparation of standard stock solution: Stock solution of each drug having concentration of 0.1 mg mL^{-1} (100 ng μL^{-1}) was prepared separately in mobile phase.

Analysis of commercial formulation: The method was applied for the quantitative study of drugs in commercially available tablets. For the preparation of the stock solution of tablet dosage form, 20 tablet of INVOZIDE (Ranbaxy Laboratories Ltd, Dewas) were taken and their average weight was determined. 20 tablets were crushed and weighed. Powder equivalent to 5 mg of ENAL in 10 mL volumetric flask and dissolved in 5 mL of mobile phase with vigorous shaking for 5 minutes. The supernatant liquid was transferred to 10 mL of volumetric flask through a whatman no. 41 filter paper. Five replicates of sample solutions were prepared and applied. The concentrations of these drugs were extrapolated from their respective calibration curves by using the peak area of densitogram.

Results and Discussion:

The HPTLC method was developed and validated according to ICH guidelines. [1] ENAL, HCTZ and PARA have shown good linear relationship for ENAL, HCTZ and PARA in the working concentration range (linearity range) of 1000 to 6550 ng per band, 100 to 3600 ng per band and 500 to 3400 ng per band, respectively. Precision of the method was determined in terms of intra-day and inter-day variation (% RSD). The accuracy of the proposed method was evaluated by percentage recovery of all three drugs. (Fig. 1 shows the peaks, overlays and of ENAL, HCTZ and PARA at 215 nm, 270 nm and 245 nm respectively).

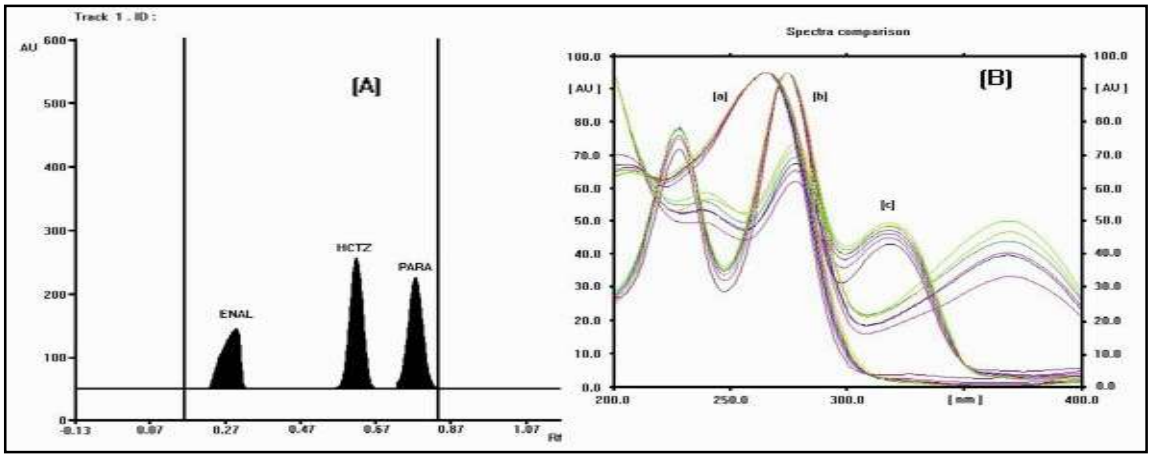


Figure 1: (a) Peaks (b) overlay of ENAL, HCTZ and PARA respectively

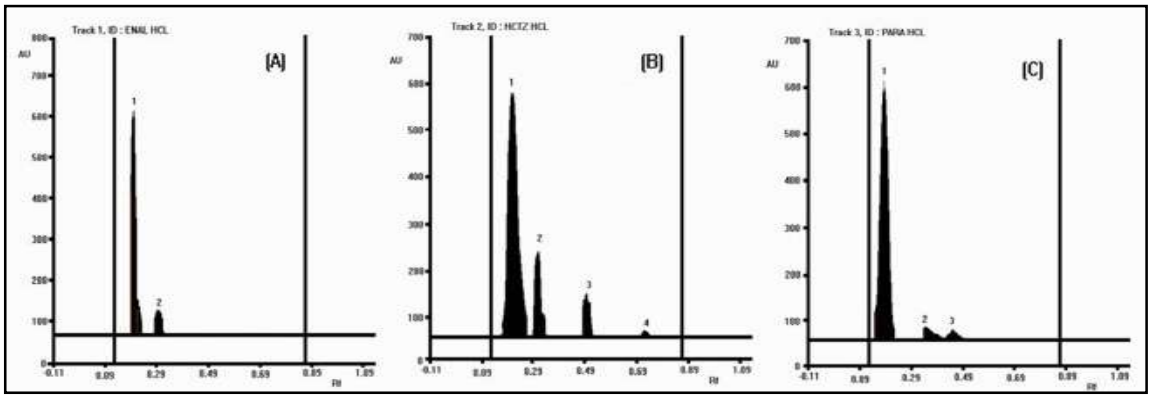


Figure 2: HPTLC chromatogram obtained from acid degradation for (a) ENAL (b) HCTZ (c) PARA

Table 1: Forced degradation study for ENAL, HCTZ and PARA

Parameters	Sample Exposure Condition	No. of Degradation Products (Rf Values)	% Recovery
ENAL	0.1 N HCl, 2 Hours, 60 ºC	1 (0.20)	61.52 ± 1.68
	0.01 N NaoH, 2 Hours, 60 ºC	3 (0.17, 0.33, 0.45)	69.28 ± 3.09
	13 % H_2O_2, 1 Hours, 60 ºC	3 (0.4, 0.78, 0.8)	81.69 ± 3.68
	Photo.chem, 8h	1 (0.32)	71.18 ± 2.70
	Dry Heat, 3h, 55 ºC	2 (0.34, 0.46)	73.35 ± 1.84
HCTZ	0.1 N HCl, 2 Hours, 60 ºC	3 (0.20, 0.42, 0.72)	81.11 ± 5.63
	0.01 N NaoH, 2 Hours, 60 ºC	3 (0.18, 0.45, 0.78)	63.87 ± 2.41
	13 % H_2O_2, 1 Hours, 60 ºC	4 (0.09, 0.15, 0.34, 0.71)	54.10 ± 3.26
	Photo.chem, 8h	2 (0.43, 0.72)	50.79±1.83
	Dry Heat, 3h, 55 ºC	3 (0.16, 0.23, 0.44)	61.42 ± 2.94
PARA	0.1 N HCl, 2 Hours, 60 ºC	2 (0.37, 0.39)	72.80 ± 2.93
	0.01 N NaoH, 2 Hours, 60 ºC	1(0.85)	76.82 ± 2.93
	13 % H_2O_2, 1 Hours, 60 ºC	1(0.89)	62.65 ± 2.14
	Photo.chem, 8h	1 (0.31)	50.76 ± 2.49
	Dry Heat, 3h, 55 ºC	2 (0.33, 0.74)	61.99 ± 2.08

Table 2: Validation parameters of the HPTLC method

Parameters		ENAL	HCTZ	PARA
Linearity range		1000-6550	500-3400	100-3600
Correl. coeff. (r^2)		0.993	0.996	0.995
Slope		2.8398	6.9042	5.079
Intercept		396.89	17.837	971.97
Recovery (%)		99.27 $\pm$ 0.41	99.85 $\pm$ 0.065	99.98 $\pm$ 0.023
Precision	Intraday (n = 3)	3255.1 $\pm$ 5.17	3505.33 $\pm$ 62.51	3274.86 $\pm$ 20.70
	Interday (n = 3)	3237.23 $\pm$ 71.47	3477.533 $\pm$ 21.0003	3284.367 $\pm$ 4.743
Repeatability		3345.65 $\pm$ 12.08	5429.967 $\pm$ 26.01	5077.4 $\pm$ 6.371
LOD		73.24	18.24	82.2
LOQ		221.9	55.3	249.1
Detection Wavelenth		215 nm	270 nm	245 nm
Rf Value		0.20 ± 0.04	0.55 ± 0.03	0.72 ± 0.02

Table 3: Summary of Validation Parameters of HPTLC Method

Particulars	Specifications
Make	CAMAG , Switzerland
Applicator	Camag Linomat 5, Semi automatic application, band application by spray on technique (2-500mL).
Syringe	Hamilton 100 ml HPTLC syringe.
Stationary Phase	Silica Gel 60 G F_{254} coated on aluminium sheet. (Merck, Germany)
Chamber	Camag twin trough glass chamber (10cm´10cm & 20cm´10cm).
Scanner	Camag TLC Scanner 3, scan speed up to 100mm/s, Spectral range 190-800nm.
Software	WinCATS Planner Chromatography Manager, version 1.3.4.
Documentation	Camag Reprostar 3 with digital camera for 254 nm, 366 nm and visible light source.
UV Cabinet	Camag UV cabinet with dual wavelength UV lamp 254 & 366 nm.
Detection Wavelenth	ENAL - 215 nm
	HCTZ - 270 nm
	PARA - 245 nm
Mobile Phase	Chloroform : Methanol : Toluene : Ethyl acetate
	(20 : 10 : 40 : 30, v/v/v/v)
Rf Value	ENAL - 0.20 ± 0.04
	HCTZ - 0.55 ± 0.03
	PARA - 0.72 ± 0.02

The developed HPLC method is simple, precise, accurate and reproducible and can used for simultaneous determination of enalapril maleate, hydrochlorothiazide, paracetamol in tablets. The method was validated as per ICH guidelines.

Conclusion:

As a result, simple, rapid and economical method for the simultaneous analysis of multicomponent formulation, which doesn't require extraction or separation of the analyte from themselves or from the excipients, becomes necessary for the pharmaceutical industry. By keeping all this in mind in the present study a new, simple, rapid, economic, accurate and selective, HPTLC method has been developed for simultaneous quantitative estimation of Enalapril maleate, Hydrochlorothiazide and Paracetamol in bulk and tablet dosage form.

Mobile phase selected for analysis was Chloroform: Methanol: Toluene: Ethyl acetate (20: 10: 40: 30 % v/v/v/v). The retention values for ENAL, HCTZ and PARA were found to 0.2.0.55 and 0.72 respectively.

References:

[1] International Conference on hormonisation (2005) of Technical Requirements for the Registration of Pharmaceutical for Human Use Q 2(R1): 1-13.

[2] Bakshi M and Singh S (2002) J Pharm Biomed Anal 1011-1040.

[3] Sayali SK, Gandhi SV, Ranjane PN and Ranher SS (2009) J Plan Chromatogr: 425-428.

[4] Shethi PD (1996) HPTLC High Performance Thin Layer Chromatography CBS Publishers, New Delhi, 1st edition: 1-68.

[5] Wall PE (2005) Thin-Layer Chromatography A Modern Practical Approach, The Royal Society of Chemistry, Cambridge UK, 1st edition: 6-55.

PB-04

Synthesis and Evaluation of Novel Calcium Channel Blocking Agents

Gaurav Sarsodia[1], Mohammad Laik Mansuri[2], Arvind Sahai[3]
[1]IPS Academy College of Pharmacy, Indore (MP) India
[2]B. R. Nahata College of Pharmacy, Mandsaur (MP) India
[3]Government P. G. College, Mandsaur (MP) India
E-mail address: gauravsarsodia@yahoo.com

Abstract: Benzothiopyran is a heterocyclic compound which contains sulphur as a heteroatom which is responsible for biological and pharmacological activity. Changing heterocyclic ring size will generate derivatives that are not only retained the calcium channel blocking activity but also resulted in several compounds that were more active than diltiazem. A receptor-binding model identifying the benzene ring as a lipophilic group that facilitates transport into the channel and the absolute stereochemistry for the selective binding. Benzothiopyran nucleus is similar to the benzothiazepine nucleuses which are used as calcium channel blockers. In these synthesis series, benzothiazepine nucleus containing nitrogen atom which is replace by bioisosterism of nitrogen.

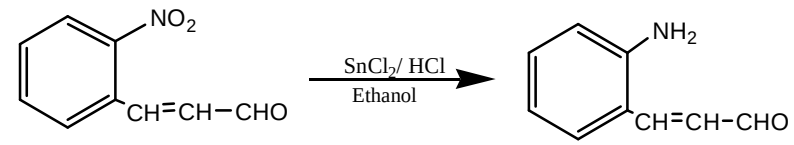
Introduction:

Calcium channel blocking agents (CCBs) inhibit the movement of calcium ions across the cell membrane by blocking the L-type (slow) calcium ion channel. This blockade reduces contraction of both smooth and cardiac muscle, and cells within the sinoatrial (SA) and atrioventricular (AV) nodes [1-3].

Material and Methods:

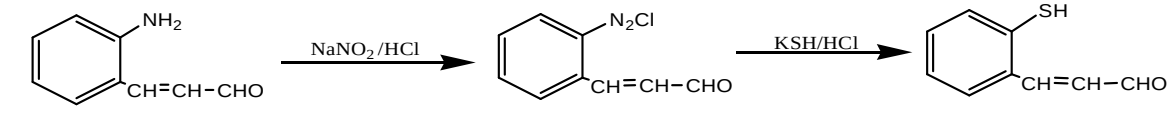
Synthesis of benzothiopyran derivatives The present work comprises synthesis of the benzothiopyran derivatives. The steps involved in the synthesis include the synthesis of.

Step- I



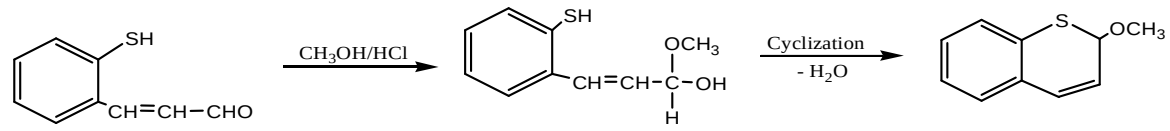
3-(2-nitrophenyl) prop-2-enal 3-(2-aminophenyl) prop-2-enal

Step-II



3-(2-aminophenyl) prop-2-enal Intermediate I 3-(2-sulfanylphenyl) prop-2-enal

Step-III

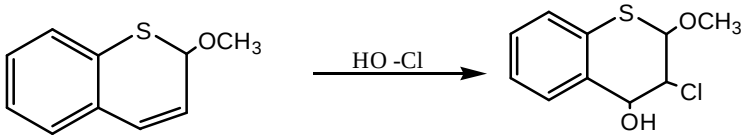


3-(2-sulfanylphenyl) prop-2-enal

Intermediate II

2-methoxy-2H-thiochromene

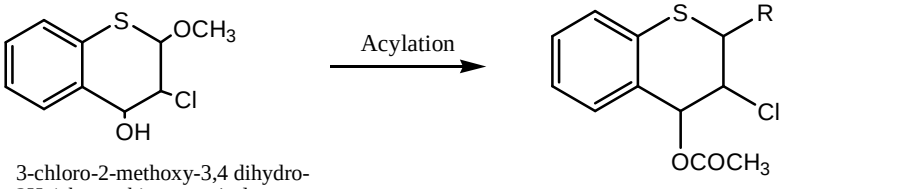
Step-IV



2-methoxy-2H-thiochromene

3-chloro-2-methoxy-3,4 dihydro-2H-1-benzothiopyran-4-ol

Step-V



3-chloro-2-methoxy-3,4 dihydro-2H-1-benzothiopyran-4-ol

3-chloro-2-methoxy-3,4 dihydrothiopyran-4yl acetate

Results and Discussion:

Step	Melting point	TLC	^{1}HNMR	IR SPECTRA (KBr) cm^{-1}
STEP- I	232^0C	Methanol: Ethyl acetate (9:1) R_f for 3-(2-nitrophenyl) prop-2-enal =0.8 R_f for 3-(2-aminophenyl) prop-2-enal = 0.6	δ 3.03(s, 2H, NH_2), 6.66 (d, 1H, -CH=C-), 6.68 (dd, 1H,aromatic proton, ortho to amino group), 7.74 (td,1H, aromatic proton, para to amino group), 7.66 (dd, 1H, aromatic proton, meta to amino group), 7.80 (td,1H, aromatic proton, meta to amino group), 8.12(d, 1H,-C=CH-), 9.77 (s,1H, -CHO).	3228 (N-H Stretch of amine group), 3049.25 (C-H Stretch of aromatic ring),2975.96 (C-H Stretch of alkene)2736.80 (C-H Stretch of -CHO) 1680.56 (C=O Stretch of -CHO) 1625.81 (C=C Stretch of alkene), 1610.43 and1442.66(C=C Stretch of aromatic ring), 1569.95 cm^{-1} (N-H bending of amine group), 1172.64 (C-N Stretch of amine and aromatic carbon), 1037.63-977.84 (C=C bending (oop) of alkene), 806.19 (N-H bending (oop) of amine group),738.69 (aromatic =C-H bending (oop) for ortho-substitution.
STEP-2	248^0C	Methanol: Ethyl acetate (9:1) R_f for 3-(2-aminophenyl) prop-2-enal (1) = 0.5 R_f for 3-(2-sulfanylphenyl) prop-2-enal (2) = 0.29	δ 3.47 (s, 1H, SH), 6.73 (td, 1H, J_o = 7.36 Hz,aromatic proton, meta to SH group), 7.65 (d, 1H, -C=CH-), 7.77 (dd, 1H,aromatic proton, meta to SH group), 7.81 (td, 1H,aromatic proton, para to SH group), 7.92 (dd,1H, aromatic proton,	3045.11 (C-H Stretch of aromatic ring) , 2925.81 (C-H Stretch of alkene), 2735.13 (C-H Stretch of -CHO), 1681.81 (C=O Stretch of -CHO), 1618.17 (C=C Stretch of alkene), 1519.80 and 1456.16 (C=C Stretch of aromatic ring), 671.18 (C-S

			ortho to SH group), 8.02 (d, 1H, -CH=C-), 9.78 (s,1H, -CHO).	Stretch of mercapto and aromatic carbon), 1035.70-964.34 (C=C bending (oop) of alkene), 746.40 (aromatic =C-H bending (oop) for ortho-substitution).
STEP -3	250-252°C	Solvent System = Ethyl acetate : Methanol (8 : 2) R_f Value for Intermediate 3-(2-sulfanylphenyl)prop-2-enal = 0.43 R_f Value for Intermediate 2-methoxy-2H-thiochromene = 0.54	δ 3.54 (s,3H, O-CH_3), 5.71 (d, 1H, -S-CH-),6.72(dd, 1H, -S-CH-CH-), 7.65(d,1H,-S-CH-CH=CH-), 7.79(td, 1H, aromatic proton), 7.81(dd,1H,aromatic proton), 8.06 (td, 1H, aromatic proton), 8.10(dd,1H, J_o = 7.54,aromatic proton).	3047.48 (C-H Stretch of aromatic ring) , 2862.96 (C-H Stretch of alkane), 1595.46 and 1416.10 (C=C Stretch of aromatic ring), 1440.51 and 1375.08 (C-H bending of alkane), 1224.71 and 1043.70(C-O-C Stretch of methoxy),1124.12 (C-O Stretch of methoxy),740.61 (aromatic =C-H bending (oop) for ortho-substitution),785.25 (C-S Stretch of mercapto and aromatic carbon)
STEP- 4	180-185 °C	Solvent System = Methanol : Chloroform (4 : 6) R_f Value for 2-methoxy-2H-thiochromene = 0.56 R_f Value for 3-chloro-2-methoxy-3,4 dihydro-2H-1-benzothiopyran-4-ol= 0.32	δ 2.19 (s,1H, -OH), 3.29(s, 3H, -O-CH_3), 4.49(d,1H, -S-CH-CH-CH-), 4.56 (d, -S-CH-),6.88 (td, 1H, aromatic proton), 7.01 (dd, 1H,aromatic proton), 7.11 (td, 1H, aromatic proton), 7.48 (dd, 1H, J_o = 6.76 Hz, aromatic proton).	3343.34(O-H Stretch of hydroxyl group), 2994.28 (C-H Stretch of aromatic ring) , 2856.38 (C-H Stretch of alkane), 1591.16 and 1512.08 (C=C Stretch of aromatic ring), 1452.30 and 1392.51 (C-H bending of alkane), 1150.24 (C-O Stretch of alcohol),1260.29 and 1047.31(C-O-C Stretch of methoxy),1101.28 (C-O Stretch of methoxy), 1O47.81 (C-Cl Stretch of Ar-chloride), 757.83 (aromatic =C-H bending (oop) for ortho-substitution),669.61 (C-S Stretch of mercapto and aromatic carbon).
STEP- 5	180-185 °C	Solvent System = Methanol : Chloroform (4 : 6) R_f Value for 3-chloro-2-methoxy-3,4 dihydro-2H-1-benzothiopyran-4-ol = 0.62 R_f Value for 3-chloro-2-methoxy-3,4 dihydrothiopyran-4yl acetate = 0.46	δ 2.12(s,3H, -C-(=O) CH_3), 3.63(s, 3H, -O-CH_3), 5.51(d,1H, -S-CH-CH-CH-), 5.98(d ,-S-CH-), s6.97(dd, 1H, aromatic proton), 7.36(dd,1H,aromatic proton), 7.56 (dd, 1H, aromatic proton), 7.59 (d,1H, aromatic proton).	1762.82 (C=O Stretch of ester group), 3082.28 (C-H Stretch of aromatic ring) , 2864.09 (C-H Stretch of alkane), 1627.81 and 1535.23 (C=C Stretch of aromatic ring), 1448.44 and 1388.65 (C-H bending of alkane), 1147.07 (C-O Stretch of alcohol),1147.07 and 1022.31(C-O-C Stretch of methoxy),1147.81 (C-O Stretch of methoxy), 1O74.81 (C-Cl Stretch of Ar-chloride), 761.83 (aromatic =C-H bending (oop) for ortho-substitution),667.32 (C-S Stretch of mercapto and aromatic carbon).

Pharmacological Evaluation

The pharmacological approach for evaluation of calcium channel blocking activity of compounds 5A, 5B derivation guinea-pig ileum by concentration response curve.

Table 2: Effect of Compounds (5A-2) in the Concentration Response Curves

Dose	Concentration (Mm)	Mean± S.E.M. (Compound 5a-2)
KCl	80	1.53
I	60	1.93
II	80	1.43
III	100	2.08
amlodipine	10	2.15

Conclusion:

The main objective of present study was synthesis of novel benzothiopyran derivatives for treatment of cardiovascular disease. In the current research work, substituted 3-chloro-3,4-dihydrothiochromen-4-one and 3-chloro-3,4-dihydro-2H-thiochromen-4yl acetate derivatives (5A-5B) were synthesized, with elaborate characterization by spectral data. Synthesize compounds were obtained in satisfactory yield and were characterized by TLC, FT-IR, ^{1}HNMR. In pharmacological evaluation, synthesize compounds 5A-1, 5A-2, 5B-1, 5B-2 gave *in-vitro* Calcium channel antagonist activity. On the basis of above study it is suggest that substituted 3-chloro-3,4-dihydrothiochromen-4-one and 3-chloro-3,4-dihydro-2H-thiochromen-4yl acetate derivatives having significant calcium channel antagonistic action agents, KCl induce contraction.

References:

[1] Gennaro AR (2000) Remington: The Science and Practice of Pharmacy, 20th ed.; Lippincott Williams & Wilkins, New York, 2: 1290.

[2] Gamble AB, Garner J, Gordon CP, O'Conner SM, & Keller PA (2007) Faculty Science-Papers, 2:11

[3] Furniss BS, Hannford AJ, Smith PWG, Tetchell (1989) 5th ed., 938.

PB-10

Virtual Screening of Derivatives Containing 2-Aminobenzothiazole as Anticonvulsant Agents

Bhagat Singh Chouhan, Love Kumar Soni
School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshshila Parisar, Khandwa Road, Indore 452001, India
E-mail address: chouhan.bhagatsingh7687@gmail.com

Abstract: A new series of 6-subtituted 2-aminobenzothiazole derivatives were designed for their anticonvulsant activity. In order to predict their anticonvulsant activities the virtual screening was performed for all the designed compounds using binding affinities to beta-3 subunit of $GABA_A$ receptor. The data obtained from the virtual screening was analyzed by comparing the scores of designed compound with the score of the reference molecule. In the present study molegro virtual docker (MVD) version 6.0 were used as designing software while the riluzole (2-amino-6-trifluoromethoxybenzothiazole) were taken as reference molecule for the structural similarity with designed compound. Compound 14 showed the highest rerank score (-98.98), mol dock score (-118.98) and h-bond (-3.28) when compared to reference ligand rerank score (-57.74), mol dock score (-76.16) and h-bond (-2.08) for anticonvulsant activities of this series. The results obtained provide information about the most active compounds i.e. compound 14 which could be a useful information for future design and investigation to construct more active analogs.

Introduction:

Epilepsy is a most common hyper synchronous brain disorders which is characterized by recurrent spontaneous seizures of cerebral origin [1]. It is a common neurological condition, affecting 0.5-1% or 45-100 million people of the population worldwide. Some of the conventional antiepileptic drugs like benzodiazepine, phenytoin, primidone, and phenobarbital, are widely used but exhibit unfavorable side effects like headache, nausea, vomiting, blurred vision, insomnia, restlessness, etc. and thus fails to adequate control of seizures. Benzothiazoles and its derivative such as 2-aminobenzothiazole represent a class of heterocyclic scaffold that consists of a five-membered 1,3-thiazole ring fused to a benzene ring. Benzothiazoles contain an extended π-delocalized system that binds to DNA molecules via π–π interactions. Because of this interaction, it demonstrates complex biological properties such as antimicrobial, anticancer, anti-inflammatory, antidiabetic, anticonvulsant activities. $GABA_AR$'s belong to a superfamily of pentameric ligand-gated ion channels (pLGICs) which is also known as the Cys-loop receptors, that include the cation-selective nicotinic acetylcholine receptors (nAChRs) and serotonin type-3 receptors (5HT3Rs), as well as anion-selective glycine receptors (GlyRs). Mol dock score gave the idea of predicting the

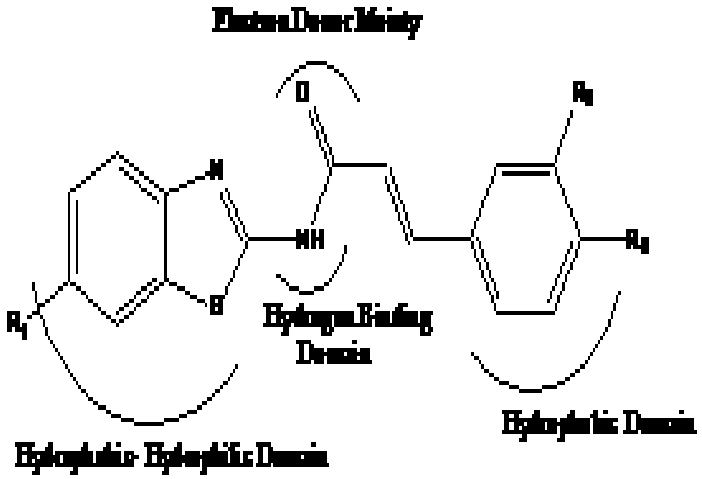


Figure 1: Pharmacophoric features of the titled compounds

Materials and Methods:

Molecular docking Study

The amino acid primary sequence of beta-3 subunit of gamma aminobutyric acid (PDB ID:4COF) was retrieved from the Protein Data Bank. The docking studies were carried out using the Molegro Virtual Docker (MVD), a program for predicting the most likely conformation of how a ligand will bind to a macromolecule. In docking studies the calculated cavity was used as active site.

Structure drawing and energy minimization:

CS Chem Office 8.0 was used for the sketching of molecules. The sketched 2D structures were transformed into 3D structures using module of the program (Chem3D Ultra 8.0). The 3D structures were then subjected to energy minimization using molecular mechanics (MM2) and re optimized via MOPAC until the RMS gradient attained a value smaller than 0.0001 kcal/mol Å.

Docking Procedure:

Protein structure of beta-3 subunit of gamma aminobutyric acid (PDB ID:4COF) [2] was downloaded from the Protein Data Bank. Protein model of $GABA_A$. Protein ligand docking studies were carried out based on the basis of crystal structure of protein Pdb 4COF and ligand binding.

Docking studies shows that Gln224, Gln185, Glu52, Tyr143, Val53, Ser51, Leu268, Thr271, Thr225, Arg216, Ile264 present in the protein structure of A-chain of beta-3 subunit of GABAA are highly conserved and might play a major role in substrate binding. Standard drug riluzole is also found to be bind to these amino acids.

Table 1: Substitution on title compound**,** Mol dock score, rerank score, hydrogen bond energies, of designed compounds and reference ligand

Ligand	R1	R2	R3	Mol Dock Score	Re rank Score	H-Bond
Comp.1	H	H	H	-107.14	-89.04	-1.09
Comp.2	H	H	Cl	-106.10	-84.25	-1.11
Comp.3	H	H	OH	-106.27	-90.71	-0.85
Comp.4	H	OH	OCH_3	-111.80	-93.71	-085
Comp.5	H	H	OCH_3	-107.75	-90.11	-2.54
Comp.6	Cl	H	H	-110.61	-95.64	-4.55
Comp.7	Cl	H	Cl	-106.61	-88.97	-1.04
Comp.8	Cl	H	OH	-107.21	-87.13	-0.99
Comp.9	Cl	OH	OCH_3	-112.40	-92.57	-1.29
Comp.10	Cl	H	OCH_3	-112.65	-92.07	0
Comp.11	NO_2	H	H	-114.28	-96.33	-1.22
Comp.12	NO_2	H	Cl	-116.32	-96.01	-4.95
Comp.13	NO_2	H	OH	-111.80	-98.98	-3.28
Comp.14	NO_2	OH	OCH_3	-118.98	-98.98	-3.28
Comp.15	NO_2	H	OCH_3	-114.70	-84.46	-6.90
Comp.16	CH_3	H	H	-105.42	-87.45	-0.99
Comp.17	CH_3	H	Cl	-104.30	-80.76	-2.41
Comp.18	CH_3	H	OH	-106.08	-85.79	-3.71
Comp.19	CH_3	OH	OCH_3	-111.12	-89.80	-3.33
Comp.20	CH_3	H	OCH_3	-111.99	-89.52	-4.82
Reference				-76.16	-57.74	-2.08

Table 2 Common types of interactions between protein structures of GABAA with most active comp (comp.14) and reference comp (riluzole).

S. No.	Interaction	Residue
1.	Hydrogen Bond Interaction	Tyr143
2.	Steric Interaction	Gln224, Thr271, Tyr143

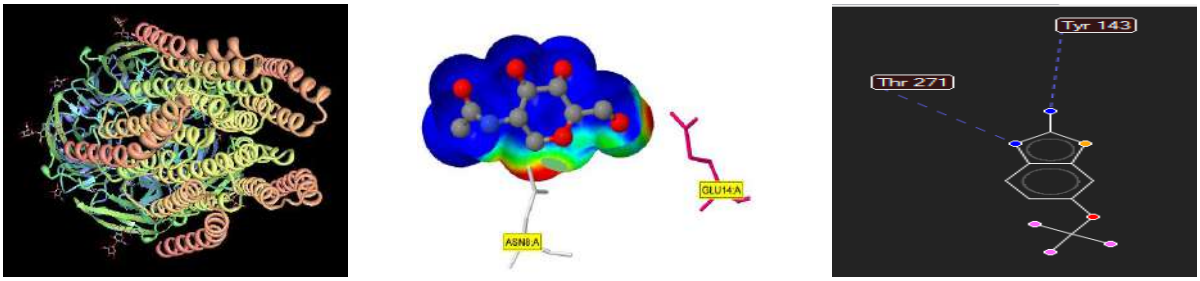
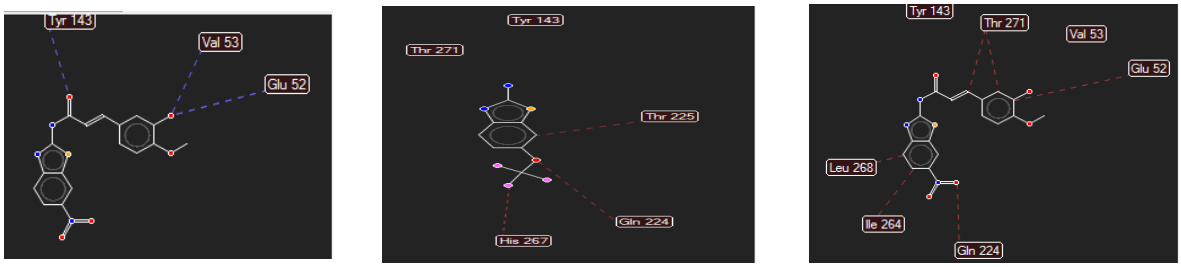


Figure 2: 3D-Structure of $GABA_A$ Enzyme Binding Pocket of $GABA_A$ Enzyme H-Bond Interaction of Reference Ligand



a. b. c.

Figure 3: a. H-Bond Interaction of Most Active Ligand, b. Steric Interaction of Reference Ligand c. Steric Interaction of Most Active Ligand

Results and Discussion:

Evaluation of the docking results was based on protein-ligand complementarity considering steric and electrostatic properties. By analyzing the hydrogen bond formed between the fifteen compounds and the active site of A- chain of protein we observed almost the compounds and riluzole exhibited hydrogen bonds with Tyr143. By analyzing the steric interaction formed between all the compounds and the active site of A-chain of protein we observed:(i) compound 1, 2 and 3 exhibited electrostatic bonds with Gln224, Leu268, Thr271, Glu52, Thr225, Gln185, Gln185 (ii) compounds 4,5 and 6 exhibited electrostatic bonds with Gln224, Leu268, Tyr143, Gln185, Arg216, Glu52, Ile264, Arg216 (iii) compounds 7,8 and 9 exhibited electrostatic bonds with Gln224, Leu268, Gln185, Thr271, Glu52, Ile264 (iv) compounds 10,11 and 12 exhibited electrostatic bonds with Gln224, Ile264, Glu52, Thr271, Gln185, Arg216, Tyr143. Compound 14 experiences a lower intermolecular energy in terms of Mol docks score and rerank score or more stable complex because the distance between the two aromatic rings is larger. The neat results of the above interactions are given in terms of mol dock score, rerank score and hydrogen binding energies toward the active site of A-chain of beta-3 subunit of $GABA_A$ as depicted in Table 1. According to these values, compound 14 presented an estimated affinity to the 4COF active site higher than the standard compound riluzole the most promising reference compound.

Conclusions:

The scoring results reveal the higher negative mol dock score and rerank score of the test compounds (especially compound 14) in comparison to riluzole. It was also observed that the commercial drug riluzole and all the 20 designed compounds binds to the specific binding sites and shows only one hydrogen bond interaction i.e. Tyr143. Here docking study provides an important insight in designing the structures of the most potent compound and subsequent construction of library of such derivatives.

Acknowledgements:

The authors are thankful to School of Pharmacy, Devi Ahilya Vishwavidyalaya Indore, India for providing facility for research work. Also, we are thankful to Dr. Rajesh Sharma, School of Pharmacy, Devi Ahilya Vishwavidyalaya Indore, India for his help and valuable suggestions in manuscript revision.

References:

[1] Saravanan G, Alagarsamy V & Prakash CR (2012) Bioorg Med Chem Lett 22:3072.

[2] Amnerkar ND & Bhusari KP (2010) Eur J Med Chem 15:149–159.

PB -20

Docking Potential of Some N-Substituted-Diaryl-Pyrazoline Analogues Towards The COX-2 Enzyme

Upendra Bhadoriya, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Indore (M.P.), India.
E-mail address: bhadoriyaupendra@yahoo.co.in

Abstract: Some N-substituted-diaryl-pyrazoline analogues were investigated *insilico* for their docking potential towards the Cyclooxygenase (COX)-2 enzyme. The compounds were designed on the basis of rational selection & the PDB entry 1CX2 from RCSB Protein Data Bank was used for mimicking COX-2 binding sites. The Compound (E)-3-(4-(dimethylamino)phenyl)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5 dihydropyrazol-1-yl)prop-2-en-1-one (E5) was found to possess strong binding potential along with two H-bond. The H-bond with Tyr-355 residue suggests desired COX-2 inhibitory potential of compound E5**.**

Introduction:

Cyclooxygenases enzyme catalyzes formation of inflammatory mediators *via* arachidonic acid path way. COX-1 and COX-2 are two major isoforms of cyclooxygenase [1]. COX-1 considered responsible for platelet aggregation & gastric safety while COX-2 is responsible for inflammatory response [2]. Therefore, various COX-2 inhibitors have been developed recently as anti-inflammatory compounds [3].

Molecular docking is a computational approach used to investigate interactions between the ligand and macromolecule thus helps to predict possible drug like effect of ligand. Various researchers investigated different compounds for their *insilico* binding affinity towards COX- 1 and COX-2. The computational chemistry also confirms selectivity of COX inhibitors since ARG513 residue of COX-2 responsible for drug-receptor binding replaced with HIS513 residue of COX-1; therefore selective inhibitor of COX-2 does not inhibit COX- 1 [4, 5].

Experimental:

Selection of docking parameters:

- *Torsion*: 0.2 A^0
- *Rigid-body orientation*: 5 A^0
- *Dihedral angles* : 5 A^0
- *Root Mean Square Deviation Tolerance*: 2.0 A^0

Preparation of Ligand Structures of diaryl pyrazoline analogues were designed using Chem Draw 3D software they were further energetically minimized by MOPAC, with minimum RMS gradient of

0.01 and saved as MDL Mol File. Other parameters such as; pH, partial charges and polar groups were optimized using molecular mechanics. Rotatable bonds and types of atom were also optimized and assigned.

Preparation of macromolecule (protein)

PDB entry 1CX2 was drawn from Protein Data Bank and after selecting protein chain, macromolecule was further optimized for presence of heteroatoms and water molecule. Protein binding sites were selected through co-crystallized ligand binding interaction.

Docking simulation Docking simulation involves docking of designed diaryl pyrazoline analogues with macromolecule (protein) after addition of hydrogen bonds and charge assignment. Auto-Dock online server was used for the same. The docking algorithm finds minimum energy function including interactions of the ligand with the receptor and optimization of best conformational poses. All analogues were compared according to the minimum free binding energy and formation of H-bond.

Result and Discussion:

Designed compounds (diaryl pyrazoline analogues) were investigated for their binding potential with COX-2 enzyme using docking techniques. The designed compounds (Figure 1) docked on the crystal structures of cyclooxygenase-2 available through RCSB Protein Data Bank (PDB entry 1CX2). The binding score of the compounds were calculated from minimized ligand protein complexes (Table 1). The score values showed good binding affinities and stable conformational complexes of ligand-receptor. The co-crystallized ligand Celecoxib reveals hydrogen bonds with Tyr355, His90 and Arg513 (Figure 2), while Diarylpyrazoline derivative E5 showed similar hydrogen bonds with Tyr355 and high binding affinity (Figure 3). Other compounds also exhibited good binding score without hydrogen bond might be due to the other bonding interactions.

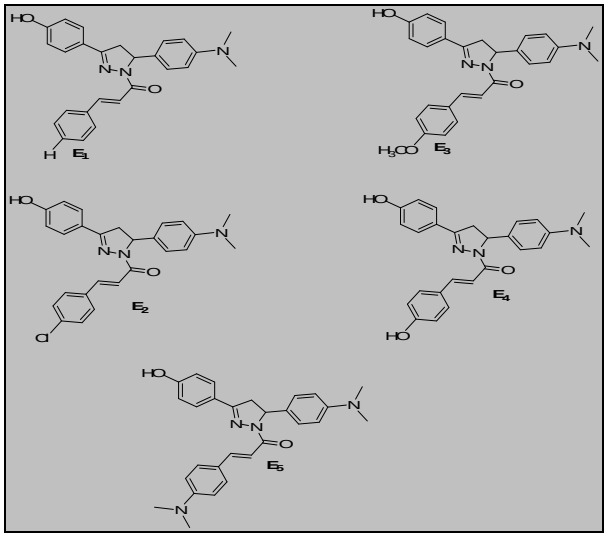


Figure 1: Structures of designed ligands

Table 1: Binding Score of Compounds

S. No.	Designed ligand Code	Designed ligand Name	No. of H-Bond	Binding energy of best pose
1	E1	(*E*)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-phenylprop-2-en-1-one	None	-4.64 kcal/ mol
2	E2	(*E*)-3-(4-chlorophenyl)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)prop-2-en-1-one	None	-4.36 kcal/ mol
3	E3	(*E*)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-(4-methoxyphenyl)prop-2-en-1-one	None	-4.49 kcal/ mol
4	E4	(*E*)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one	None	-4.19 kcal/ mol
5	E5	(*E*)-3-(4-(dimethylamino)phenyl)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)prop-2-en-1-one	02	-4.19 kcal/ mol

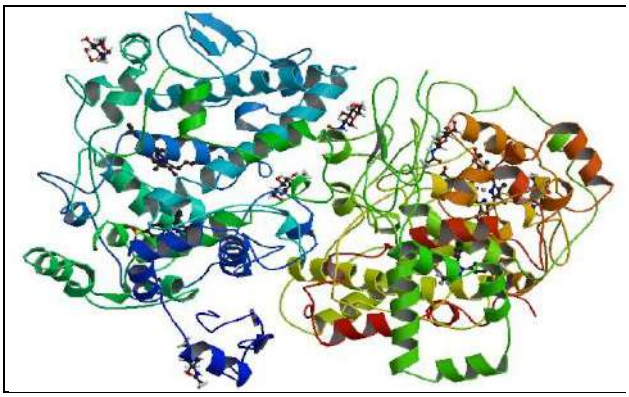


Figure 2: Binding pose of co-crystallized ligand Celecoxib

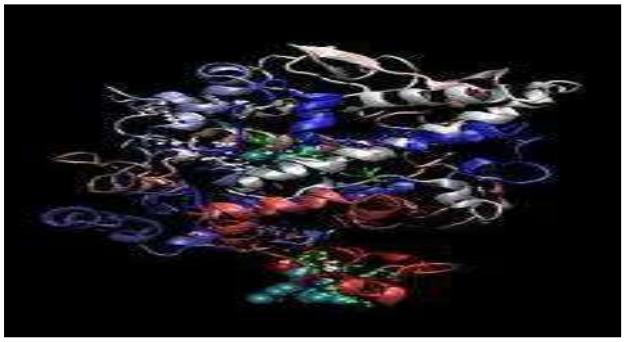


Figure 3: Binding pose of designed compound E5.

Conclusion:

N-substituted-diaryl-pyrazoline analogues were designed and investigated for their *insilico* docking potential towards the COX-2 enzyme. The literature based rational approach was utilized for designing ligand molecule. The designed compounds were docked on the crystal structures of COX-2 (PDB entry 1CX2) obtained from RCSB Protein Data Bank. The Compound (E)-3-(4-(dimethylamino) phenyl)-1-(5-(4-(dimethylamino)phenyl)-3-(4-hydroxyphenyl)-4,5 dihydropyrazol-1-yl)prop-2-en-1-one (E5) was found to form hydrogen bond with Tyr-355 residue. This interaction suggested that designed analogues may be used as potent COX-2 inhibitor after further investigations.

References:

[1] Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van DP & Lipsky PE (1998) FASEBJ, 12:1063-1073.

[2] Kaufmann WE, Worley PF, Pegg J, Bremer M & Isakson P (1996) Proc Natl Acad Sci 93: 2317-2321.

[3] Kawada N, Moriyama T, Kitamura H, Yamamoto R, Furumatsu Y, Matsui I & Takabatake Y (2012) Clin Exp Nephrol 16:25-29.

[4] Patrono C, Patrignani P & Garcia LA (2001) J Clin Invest. 108:7-13.

[5] Gehan HH, Ghaneya SH, Nahla AF & Amal Y (2011) Life Sci 8:387.

PB-23

Validated RP HPLC Method Development for Exemestane in Tablet Dosage Form

Deepak Kumar Gupta, Prakash Panday, Revati Gupta
Dr. APJ Abdul Kalam University, Indore (M.P.) India
E-mail address: Deepak_gupta20072008@yahoo.com

Abstract: The aim of this present work was to develop stability indicating LC method, which is selective, accurate, simple, precise, reliable, cost effective and rapid for the quantification of all possible degradants and determination of exemestane. In addition, to develop and validate Stability Indicating Method for the determination of impurities (degradation products) in exemestane API by RP-HPLC. Finally, validate the developed method as per ICH guidelines.

Introduction: The principle of chromatographic methods consists in the unequal distribution of components of a mixture between the stationary and the mobile phase. The prerequisite for an unequal distribution is the different possibility of diffusion into them.

- **Classification of Chromatography:**

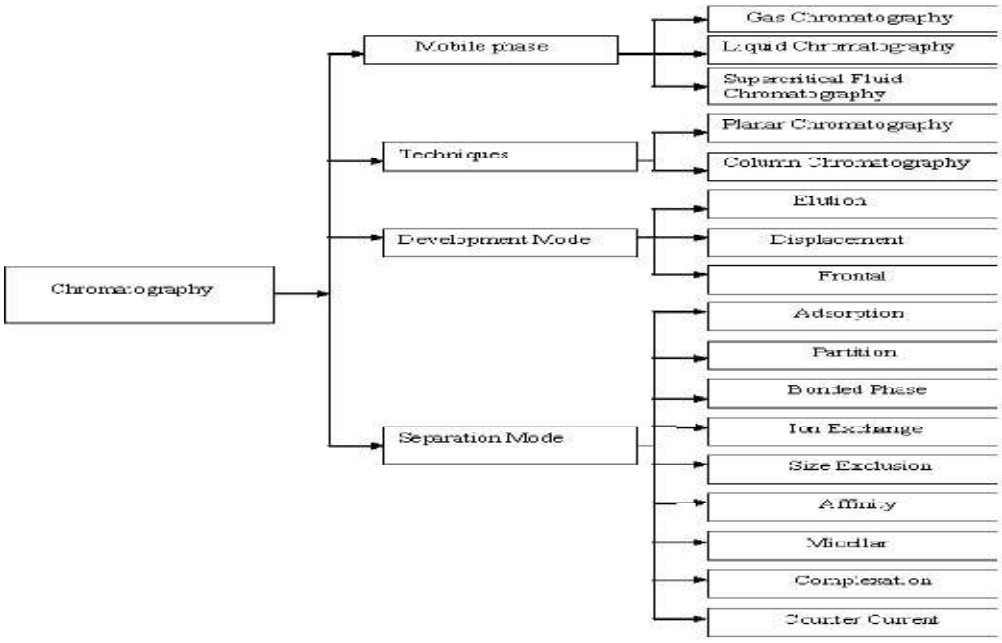


Figure 1: Types of chromatography

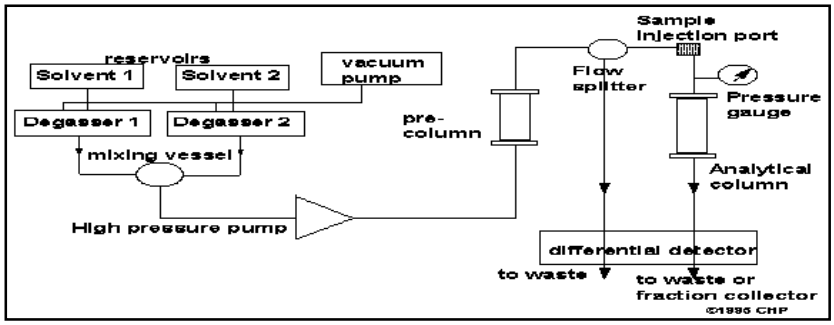


Figure 2: Various components of HPLC are as follows

Method Development in HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. "Optimized column, mobile phase, best detection wavelength, efforts in separation can make a world of difference, while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results and a validated method of separation.

Materials and Method:

Exemestane was received from Cadila Healthcare, Ahmedabad, HPLC grade acetonitrile was purchased from Merck Specialities Private Limited, Mumbai, India. Double distilled water along with acetonitrile was used as diluent throughout the study.

-Selection of Analytical Technique

- HPLC method

- Selection of method for evaluation (either Reverse Phase Chromatography or Normal Phase Chromatography)

-Method development and validate for determination of impurities (degradation products) and of exemestane by using RP-HPLC.

-Selection of different stress studies:

As per ICH guidelines Q1A (R2), different stress conditions were selected and applied to drug for forced degradation studies.

-Validation of method which involves. Specificity

Accuracy. Linearity. Precision.Robustness. Limit of Detection (LOD) ,Limit of Quantitation (LOQ), Compilation of data.

Result and Discussion:

Selection of Mobile phase

Different mobile phase systems like methanol: water, acetonitrile: water were tried in order to determine the best composition for separation of exemestane. It was found that acetonitrile: water (60:40 % v/v) by isocratic elution gives good resolution and satisfactory peak symmetry as compared to others. Finally this mobile phase was found to be most suitable. The chromatogram obtained by using acetonitrile: water (60:40 %v/v) as mobile phase is shown below in Fig. 3.

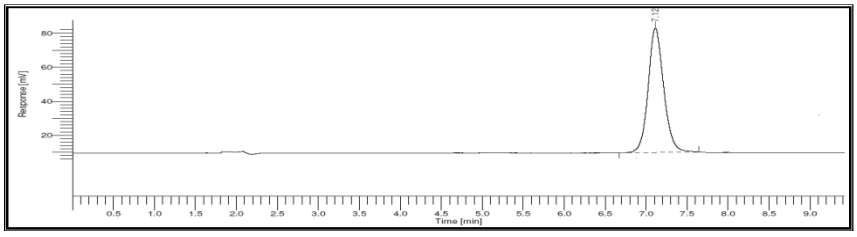


Figure 3: Chromatogram of a 10 µgmL^{-1} solution of exemestane

Calibration Curve

Table shows the peak area observed for various concentrations of standard exemestane solutions. The graph of Peak Area (µV*sec) vs. Concentration (ppm) was plotted in figure.

Table 1: Standard calibration curve for exemestane

Sr. No.	Concentration (ppm)	Peak Area (µV*sec)
1.	4	584173.86
2.	6	792628.08
3.	8	966578.04
4.	10	1171116.28
5.	12	1353103.47
6.	14	584173.86

Method development

Detection wavelength for the HPLC study was selected as 242 nm. The chromatographic conditions were optimized for resolution of the peak of the drug and degradation products under each forced degradation condition by varying the proportion of acetonitrile/water in the mobile phase. Subsequently, a mixture of samples of different stress conditions was used to optimize the chromatographic conditions for resolving exemestane and all the degradation products in a single run. An appropriate blank was injected before the analysis of all forced degradation samples. Such an optimized method was then used to study the forced degradation behaviour of exemestane and was also applied in the determination of exemestane.

Method Validation

Linearity: Peak areas obtained with respective concentrations were subjected to the least square linear regression analysis to calculate the calibration equations and correlation coefficients. The calibration plot for exemestane assay was linear over the calibration range 6–14 µgmL^{-1} and the regression coefficient, slope and intercept were found to be 0.9997,96854 and 4147.6 respectively (In Table). The calibration curve of exemestane is shown below in Fig.

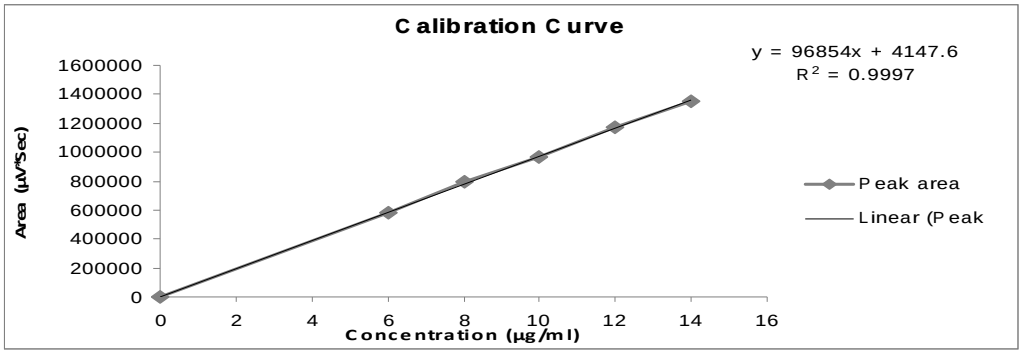


Figure 4: Calibration curve of exemestane

Table 2: Linearity range, slope, intercept and correlation coefficient for Calibration curve

Drug	Linearity range (μgmL^{-1})	Slope	Intercept	Correlation coefficient
Exemestane	4-14	96854	4147.6	0.9997

Conclusion:

The analytical method described in this paper is suitable for determination of exemestane and this method has been demonstrated to be accurate, linear, precise, repeatable, specific, and robust, and therefore suitable for routine analysis of exemestane.

This method is a stability indicating method because it can separate all known degradation products from exemestane (API).

The stability indicating method that has been reported in the literature uses 100% organic modifier as the mobile phase and by this method drug has an retention time of greater than 20 min which is costly and time consuming. So, this present method was found to be cost effective and rapid.

From the results of the forced degradation studies it can be concluded that drug was found to degrade rapidly by oxidation, followed by base hydrolysis, acid hydrolysis, dry heat and neutral catalyzed degradation.

As the method is successfully validated using ICH guidelines, it can be readily implemented in quality control laboratories for the purpose of lot release and stability testing of Exemestane API.

References:

[1] Heftman E (2004) ERDAM, 53: 291.

[2] Conners KA (1999) A Textbook of Pharmaceutical Analysis, 3rd ed., A Wiley- Interscience Publication, 373-374.

[3] Chatwal GR & Anand SK (2004) Instrumental Methods of Chemical Analysis, Himalaya Publishing House, 2:110.

[4] Sethi PD (2001) In; HPLC 'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulations, 3rd ed., CBS Publishers and Distributors, New Delhi, 72: 116-120.

[5] Snyder LR, Kirkland JJ & Glajch LJ (1997) Practical HPLC Method Development, 2nd ed., A Wiley-Interscience publication, New York, 33:35.

PB-28

Synthesis and Antibacterial Activity of Some New Substituted Azetidine Derivatives

Rajni Shah, Deepesh Rathore, Farzana Khan, Nitin Deshmukh, Sujit Pillai
G.R.Y. Institute Pharmacy, Borawan, Khargone MP.
E-mail address: rajni.shah210@gmail.com

Abstract: Four-membered nitrogen heterocycles such as β-lactams and azetidines are useful substrates in organic chemistry for the design and preparation of biologically active compounds. New series of 4(3-Chloro-(Substituted-Phenyl)-4-oxoazetidine1-yl)1-phenylthiosemicaebazide derivative were synthesized by the reaction of Schiff base with 2-Chloro acetyl chloride. Synthesized compounds were evaluated for their Anti- bacterial activity against Gram positive bacteria (Bacillus Subtilis) and gram negative bacteria (Klebsiella pneumonia). In this synthesis 6 derivatives are used named as NM_1, NM_2, NM_3, NM_4, NM_5 and NM_6.Synthesized compounds show significant activity against bacteria strains on agar plate. Their structures were established on the basis of elemental analysis, IR and NMR spectral data. The different substituted azetidine derivatives were synthesized followed by cyclization reaction. The newly synthesized azetidine derivatives were evaluated for their antimicrobial activity. The synthesized compounds NM_1 showed effective antimicrobial activity. This clearly indicates that new azetidine derivatives can be effectively synthesized by method mentioned in this study.

Introduction:

Azetidine is the four membered heterocyclic compound which contain one Nitrogen atom in their ring. β- lactams and azetidines have caught the attention of organic chemists and medical researchers. The azetidines are four-membered nitrogen heterocycles of great interest for fundamental research and useful for practical applications [1].

Scheme: Synthesis of proposed derivatives.

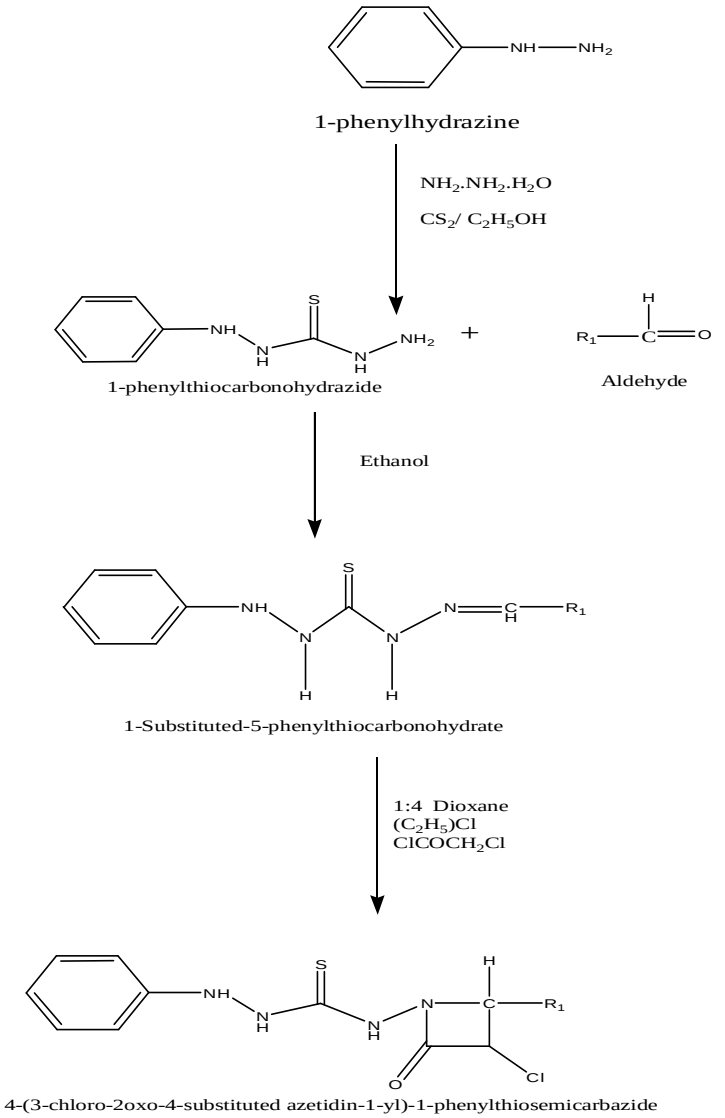
Substituted aldehyde: NM_1= Benzaldehyde, NM2= P-Chloro benzaldehyde, NM_3= 3-Nitrobenzaldehyde, NM_4= Diamino benzaldehyde, NM_5= 4-Bromo benzaldehyde and NM_6= 2-Chlorobenzaldehyde.

Materials and Method:

Synthesis of 1-Phenylthiocarbonohydrazide

Phenyl hydrazine (0.1mole) was dissolved in ethanol (95% 50ml) and ammonia solution 20ml. The CS_2 20ml was added slowly within 15 minute with shaking and solution is allowed to stand for 1hr.

Experimental:



Synthesis of 1-Substituted-5-phenylthiocarbohydrate

Aldehyde (0.01Mole) was added in the solid which obtained by compound A. Ethanol (30-35ml) was added into it. The solution mixture was refluxed for 3hr. The mixture was cooled at the room temperature and allowed to stand it for the 5hrs. The solid product was thus obtained and washed with ice cold water. Then recrystalized by ethanol [2-4].

Synthesis of 4-(3-chloro-2oxo-4-Substitutedazetidine-1-yl)-1-phenylthiosemicarbazide

A solid which is obtained by compound B (0.1mole) is added in the chloroacetyl chloride (0.1mole) in the presence of Et_2N was dissolved in acetone at room temperature and allow to reaction. The reaction mixture was first continuously stirred on a magnetic stirrer for about 2-3hr in the ice bath. Then kept it over night and make it in a powder form in the presence of Ethanol and filter it.

Analytical data of synthesized compound:

NM_1: IR(cm^{-1}): 771(C─Cl), 3034,2936(CH stretching aromatic), 1552(C═C aromatic), 1056(C─N stretching), 1645(C═O ketone), 3420(N─H bending), 3089(CH aromatic ring)

NMR: H^1NMR ($CDCl_3$), δ 6.91-8.21(m,6H, Ar-H), 4.27(s;1H;CH), 7.88(s; NH amide)

NM_2: IR(cm^{-1}): 1554(C═C aromatic), 774(C─Cl), 1059(C─N stretching), 3028, (CH stretching aromatic), 1647(C═O ketone), 3424(N─H bending), 3085(CH aromatic ring)

NMR: H^1NMR ($CDCl_3$), δ 4.23(s;1H;CH), 7.85(s; NH amide) 6.94-8.20(m,6H, Ar-H),

NM_3: IR(cm^{-1}): 3030,2932(CH stretching aromatic), 1550(C═C aromatic), 775(C─Cl), 1058(C─N stretching), 1640(C═O ketone), 3425(N─H bending), 3084(CH aromatic ring)

NMR: H^1NMR ($CDCl_3$), δ 6.90(m,6H, Ar-H), 4.24(s;1H;CH), 7.84(s; NH amide), 1410(NO_2)

NM_4: IR(cm^{-1}): 3431(N─H bending), 3038, (CH stretching aromatic), 1556(C═C aromatic), 773(C─Cl), 1060(C─N stretching), 1652(C═O ketone), 3090(CH aromatic ring)

NMR: H^1NMR ($CDCl_3$), δ 4.26 (s;1H;CH) 8.26(m,6H, Ar-H), 7.83(s; NH amide)

NM_5: IR(cm^{-1}): 1046(C─N stretching), 3041,2942(CH stretching aromatic), 1558(C═C aromatic), 772(C─Cl), 1649(C═O ketone), 3426(N─H bending), 3094(CH aromatic ring)

NMR: H^1NMR ($CDCl_3$), δ 6.97-7.27(m,6H, Ar-H), 4.22(s;1H;CH), 7.82(s; NH amide)

NM_6: IR(cm^{-1}): 1560(C═C aromatic), 779(C─Cl), 1072(C─N stretching), 1654(C═O ketone), 3416(N─H bending), 3096(CH aromatic ring), 3042 (CH stretching aromatic)

NMR: H^1NMR ($CDCl_3$), δ 7.78(s; NH amide) ,4.37(s;1H;CH), 6.82-8.31(m,6H, Ar-H),

Antibacterial evaluation:

Cup- Plate Method: It is one of the official method in IP where the test sample diffuse from the cup through an agar layer in Petri dish or plate to such an extent that growth of added microorganism is restricted entirely to circular area.

Result and Discussion:

Table 1: Zone of Inhibition

S. No.	Solutions	(+)ve bacteria (*Bacillus Subtilis*)	(-)ve Bacteria (*Klebsiella pneumonia*)
1	Test Solution (100µg/ml) NM_1 NM_2 NM_3 NM_4 NM_5 NM_6	 18mm 16mm 15mm 14mm 16mm 12mm	 26mm 24mm 19mm 18mm 11mm 14mm
2	Standard (AmoxicillinTrihydrate) (50µg/ml)	16mm	24mm

Conclusion:

This research work was oriented towards the finding of newer Azetidine derivative with antimicrobial activity. The different substituted azetidine derivatives were synthesized followed by cyclization reaction. The newly synthesized azetidine derivatives were evaluated for their antimicrobial activity. The synthesized compounds NM_1 showed effective antimicrobial activity. This clearly indicates that new azetidine derivatives can be effectively synthesized by method mentioned in this study. These synthesized compounds exhhibit significant antimicrobial activity.

Acknowledgement:

The authors are thankful to GRY Institute of Pharmacy, Borawan, Khargone (M.P.) for providing laboratory facilities.

References:

[1] Rao BG & Prasad AVGS (2013) Int J Inno Res Dev 2: 72-75.

[2] Murendra M & Hussain A (2013) J Applied Pharm Sci 3:135-138.

[3] Deshmukh N (2014) J Chem 3:51-53.

[4] Patel R & Bhandari A (2014) Am J Adv Drug Del 1:104-109.

PB-31

Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for Simultaneous Determination of Atorvastatin and Pioglitazone in Combined Tablet Dosage Form

Tahir Nizami[1], Shrivastava Birendra[2], Pankaj Sharma[2], G.N. Darwhekar[3], Praveen Sharma[3]
[1]Smriti College of Pharmaceutical Education, Indore
[2]School of Pharmaceutical Sciences, Jaipur National University, Jagadapura, Jaipur
[3]Acropolis Institute of Pharmaceutical Education and Research, Indore (Madhya Pradesh), India.
E-Mail address: tahir.nizami@scopeindore.info

Abstract: Simple, accurate, precise, and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of Atorvastatin (ATOR) and Pioglitazone (PIO) in combined tablet dosage form have been developed and validated. The spectroscopic methods employs formation and solving of simultaneous equation at 247 nm and 267 nm as 2 wavelengths for estimation of ATOR and PIO respectively (method 1) While method 2 involves formation of Q- absorbance equation at 233 nm (isoabsorptive point) and 267 nm (λ max of Pioglitazone) with methanol as solvent. Beer's law is obeyed in the concentration range of 5.0–50.0 mcg/mL for ATOR and PIO, respectively. The RP-HPLC method uses HPLC system with a Phenomenex Luna C18 (5 µm x 25cm x 4.6mm i.d) using Methanol, acetonitrile and potassium dihydrogen phosphate buffer, pH 2.5 adjusted with orthophosphoric acid (60:20:20 v/v) at a flow rate of 1.0 mL/min at ambient temperature as the mobile phase. The detection was carried out using an ultraviolet detector set at 233 nm. For the HPLC method, Beer's law is obeyed in the concentration range of 5.0-50.0 µg/ml for ATOR and PIO, respectively. LOD values for ATOR and PIO were found to be 57.12µg/ml and 12.01 µg /ml respectively. All the methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Introduction:

Atorvastatin [R-(R,R)] -2- (4-fluorophenyl) -ß,δ-dihydroxy-5-(1-methylethyl) -3-phenyl-4 [(phenylamino) carbonyl] -1H-pyrrole 1-heptanoic acid is a hypolipidemic agent which is used for treatment of hyperlipidemia and Pioglitazone (±)-5-[[4-[2- (5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-thiazolidinedione monohydrochloride is a hypoglycemic agent which is used in treatment of hyperglecemia.

A literature survey revealed that different analytical methods involving Specrtrophotometric and column high-performance liquid chromatography (HPLC) for determination of ATOR and PIO in biological fluids and in pharmaceutical preparations have been developed. Because of absence of an official pharmacopoeial mehod for the simultaneus determination of ATOR and PIO in

pharmaceutical formulations, efforts were made to develop an analytical method for estimation of ATOR and PIO in their combined dosage form using HPLC method and UV Spectroscopy.

Materials and Methods:

Instruments

A UV-visible (UV-Vis) double beam spectrophotometer (Model 1601; shimadzu) with 1 cm matched quartz cells was used for the spectrophotometric method. For the HPLC method, an HPLC system consisting of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µL. Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Phenomenex Luna C18 (5 µm x 25cm x 4.6mm i.d).

Method I: Simultaneous equation method.

This spectrophotometric technique is employed when the two absorbing drugs in the sample X and Y absorbs at the λ max of each other. The standard solution of strength 100 µg/ml of ATOR and PIO were prepared in methanol separately. From overlain spectra of ATOR (10 µg/ml) and PIO (10 µg/ml) two wavelengths 247nm (λ max of ATOR) and 267 nm (λ max of PIO) were selected for the formation of simultaneous equation. Different aliquots were taken from stock solutions and diluted with the same solvent to prepare a series of concentrations. The calibration curves were found to be linear in concentration range of 5-50 µg/ml for ATOR and PIO respectively. The absorbance of ATOR and PIO were measured at 247nm and 267nm which are respective Wavelength of both the drugs respectively. The concentration of two drugs in mixture can be calculated by using simultaneous equation

Method II: Q-absorbance method

Q-absorbance method uses the ratio of Absorbances at two selected wavelength,one at isoabsorptive point and other being the λmax of one of the two components. The standard solution of strength 100mcg/mL of ATOR and PIO were prepared in methanol separately. From overlain spectra of ATOR (10 µg/ml) and PIO (10 µg/ml) two wavelengths 233nm (Isoabsorbtive point) and 267 nm (λ max of PIO) were selected for the formation of Q-absorbance equation. Different aliquots were taken from stock solutions and diluted with the same solvent to prepare a series of concentrations. The calibration curves were found to be linear in concentration range of 5-50 µg/ml for ATOR and PIO respectively. The absorbance of ATOR and PIO were measured at 233nm and 267nm.

Method III: HPLC

Method Validation

(a) **Linearity.-**Calibration graph was constructed by plotting peak area vs. Concentration of ATOR and PIO and the regression equations were calculated. The calibration graph were plotted over 5 different concentrations (10, 20,30,40,50 µg/ml) using methanol. Aliquots (20µL) of each solution were injected under the chromatographic conditions.

(b) **Accuracy**- The accuracy of the methods was determined by calculating recoveries of ATOR and PIO by standard addition method. Known amounts of mixed standard solution of ATOR and PIO (80,100 and 120%) were added to prequantitated sample solutions of tablet dosage form. The amount of ATOR and PIO were estimated by applying values of peak area to the regression equation of the calibration graph.

(c) **Method precision (repeatability)** - The precision of the instrument was checked by repeatedly injecting (n=6) mixed standard of ATOR and PIO (20µg/ml).

(d) **Intermediate precision (reproducibility)** - The intraday and interday precision of proposed methods were determined by analyzing mixed standard solution of ATOR and PIO at 3 different concentrations, and three times on the same day and on three different days. The results are reported in the terms of Relative standard deviation.(RSD)

(e) **Limit of detection (LOD) and limit of quantitation LOQ**: The LOD with signal-to-noise ratio of 3:1 and with S/N ratio of 10:1 were calculated for both the drugs using the following equations

LOD=3.3*σ/s

LOQ=10* σ/s

Results and Discussion:

The proposed method was found to be simple, accurate, economical, and rapid for routine analysis of ATOR and PIO in combined dosage form. The accuracy of method was determined by calculating the mean percentage recovery. Precision was determined as inter and intraday variation for both the drugs. Both methods were successfully used to estimate the amount of ATOR and PIO present in marketed formulation. For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for ATOR and PIO. Different mobile phases containing methanol, Acetonitrile and 0.05 M Potassium Di hydrogen phosphate were examined (data not shown), and the mobile phase methanol, acetonitrile–0.05 M Potassium di hydrogen phosphate, pH adjusted to 2.5 ±0.1 (60 :20:20, v/v/v) was selected as optimal for obtaining Well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 233 nm, at which the best detector response for both the substances was obtained Straight line calibration curves were obtained for ATOR and PIO in the spectrophotometric and RP-HPLC methods. The proposed methods were also evaluated in the assay of commercially available tablets containing ATOR and PIO. Six replicate determinations were performed on the accurately weighed amounts of tablets. For ATOR, recovery (mean, %, ± SD, n = 6) was found to be 99.48 ± 1.10, 100.05±0.613 and 100.16 ± 0.616 for Methods I,II and III, respectively. For PIO, recovery was found to be 99.89 ± 0.15, 100.10±0.56 and 99.30 ± 0.35 for Methods I, II and III, respectively. For ATOR, the recovery study results ranged from 99.34 to 100.1%, 99.98 %to 100.02% and 100.1 %to 100.94 %for Methods I, II

and III, respectively. For PIO, the recovery results ranged from 99.92 %to 99.75%, 99.27% to 99.23% and 100.30% to 100.33% for Methods I, II and III,

Conclusion:

The results confirmed that the method is simple, precise and accurate. The method can be used for the routine simultaneous analysis of ATOR and PIO in pharmaceutical preparations.

References:

[1] Munson JW (2001) Pharmaceutical Analysis- Modern Methods, Part-3: 16-17

[2] Sharma BK (2005) Instrumental methods of chemical analysis 24th Edition, Goel Publishing house Meerut, 311: 266-291.

[3] Snyder LR, Kirkland JJ & Glajch JL (1997) Practical HPLC method development 2nd Edition, Wiley Interscience Publication, 966-986.

[4] Indian Pharmacopoeia (1996) 1: 347-348.

PB-34

Designing of 2, 5-Disubstituted-1,3,4-Thiadiazole Derivatives for Their Anticonvulsant Potential

Mahavir Chhajed[1], A. K. Shrivastava[2], Atika Chhajed[3], Vijay Taile[4]
[1]Indore Institute of Pharmacy, Pithampur Road, Opp. IIM, Rau, Indore-453331 (MP) India
[2]Nandini Nagar Mahavidyalaya College of Pharmacy, Gonda (UP)India;
[3]Institute of Pharmacy, APJ Abdul Kalam University, Arandiya, Indore (MP) India;
[4]Gangwal Chemicals Pvt. Ltd.,706-707 Quantum Tower, Malad (West), Mumbai, (MS) India
E-mail address: mahavirchhajed@rediffmail.com

Abstract: QSAR is the study of quantitative relationships between biological activity and the physicochemical properties of a common parent structure molecule. CS chemoffice software utilized for QSAR of series. The reported IC_{50} values were converted to negative log IC_{50} values, which were correlated with various descriptors. Upon stepwise, multiple, and sequential regression analysis of descriptor, the statistically significant QSAR equations were obtained. The correlation between the physicochemical parameters and the biological activity were found using the least squares method. The equations having good correlation coefficient (r^2), F-test value, SD values and minimum variance were validated by the cross validation method and IC_{50} and pIC_{50} values were calculated using Valstat. 5-Benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide, was designed as parent structure.

Introduction:

With the evolution of mankind, its confrontation with newer and complex diseases has also been increased. Humanity has always been searching for new ways to overcome the disease, and one of such way is the discovery of the newer drugs for the prophylaxis and/or treating the diseases. Today medicinal chemistry has been trying to reduce the toxicity and increases the therapeutic efficacy of the existing compound by structural modification using computer aided drug design and QSAR [1]. In the early 1960s, one could expect to discover a marketable compound out of 2000-3000 tested molecules, where-as this ratio in now close to 1 in 10000 and biological testing expenses have increased dramatically. Good drug must be replaced by better one, which often seemed to result from a small change in structure of the original or "lead" compound [2]. Drug design is inherently multi-disciplinary and involves the integration of vast amount of complex information, so computational methods can aid in this process. The strategy use in the design of drugs, involved a change in shape such that the new drug had a better 'fit' for receptor. Other strategies involved the change in the physical properties of the drug such that its distribution, metabolism, or receptor binding interactions were affected [3].

The primry objective of the research is to increase the efficiency by designing new drug by making structural modification with the help of QSAR & CADD where the chemical feature of molecules or series of molecules have been correlate to biological activities [4].

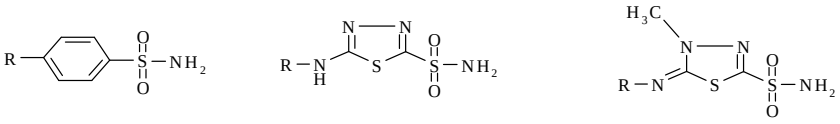
Materials and Methods:

The workstation

All the computational studies were performed using software CS Chem. Office (Version 6.0).

QSAR analysis of 2,5-disubstituted 1,3,4-thiadiazole

QSAR analysis was carried out on all thirty compounds which are reported previously [5].



(I) (II) (III)

Figure 1: Structure of Sulphonamide and 1,3,4-Thiadiazole Derivatives which are reported as Carbonic Anhydrase Inhibitors are as follows

Upon stepwise, multiple, and sequential regression analysis of descriptor, the following statistically significant QSAR equations were obtained as follows. The correlation between the physicochemical parameters and the biological activity were found using the least squares method. The cross correlation matrix for –log IC_{50} and the various physicochemical descriptors were calculated for equation (1, 2, and 3).

- BA = [5.31643(± 0.468307)] + HLC [0.300503(± 0.103401)] + LUMO [-0.701809(± 0.384675)] + VDWE [0.083507(± 0.0521942)] **…..Equation (1)**

  N = 30, r = 0.918647, r^2 = 0.843912, variance = 0.103995, std = 0.322482, F = 46.8575
- BA = [5.31042(± 0.418884)] + HLC [0.287537(± 0.0929893)] + LUMO [-0.703293(± 0.34406)] + VDWE [0.086354(± 0.0467316) **…..Equation (2)**

  N = 29, r = 0.931009, r^2 = 0.866778, variance =0.0828709, std = 0.287873, F = 54.219
- BA = [5.43547(± 0.477882)] +DM [0.0686359(± 0.0675143)] +PMIY [7.44461e-005(± 5.29802e-005)] +NVDWE [-0.144571(± 0.0782467)] +LUMO [-1.06437(± 0.376148)] **…..Equation (3)**

N = 30, r = 0.904767, r^2 = 0.818604, variance = 0.125691, std = 0.354529, F = 28.2049 Out of the several models, equation (2) was selecting as the best equation on considering the criteria for QSAR equations. The equation had better statistical significance viz., r > 0.8, std. deviation (std) should be minimum, statistically significant F value and the correlation matrix was found to be in the limit.

Although the above equations are having good correlation coefficient (r^2), sequential fisher test value (F-test) and standard deviation (Std) values and minimum variance. But in equation (3) shows less correlation than in equation (2). Equations (1, 2, and 3) were validated by the cross validation method and IC_{50} and pIC_{50} values were calculated for these equations using software Valstat. The pIC_{50} calculated and predicted for the equations (1, 2, and 3) by Valstat are covered in the Table 1. Equations (1, 2, and 3) are validated by using different parameters such as cross-validated correlation coefficient, predicted sum of squares, and standard error of prediction and cross-validated equations are reported in the Table 2.

Table 1: Predicted activity data of earlier synthesized compounds

Compd. No.	IC_{50}*	Equation (1)		Equation (2)		Equation (3)	
		pIC_{50}** Cal.	pIC_{50}** Pred.	pIC_{50}** Calc.	pIC_{50}** Pred.	pIC_{50}** Cal.	pIC_{50}** Pred.
(Ia)	3.228892	7.65354	7.67835	7.61418	7.63532	7.51513	7.53411
(Ib)	2.656621	6.62968	6.64967	6.60267	6.61773	6.41428	6.39318
(Ic)	2.734382	6.83843	6.84394	6.80039	6.80293	6.82511	6.83221
(Id)	2.756201	6.83114	6.83419	6.79324	6.79301	6.82664	6.82997
(Ie)	1.930475	6.95766	6.98075	6.92539	6.94687	6.62471	6.62666
(If)	1.737577	6.9574	6.96279	6.92513	6.92846	6.62468	6.59319
(Ig)	1.95221	6.93711	6.95869	6.90509	6.92483	6.61923	6.61737
(Ih)	1.953405	6.90713	6.92897	6.87545	6.89504	6.61699	6.61371
(Ii)	1.951018	6.97728	6.99903	6.94487	6.9654	6.69369	6.70419
(Ij)	1.588046	6.69372	6.69668	6.66516	6.66461	6.73768	6.74897
(Ik)	1.610167	6.71378	6.72227	6.68507	6.6904	6.7148	6.72738
(Il)	1.440459	6.981	6.96894	6.94855	6.93426	6.79528	6.76165
(Im)	1.271854	6.85372	6.53771	6.82119	6.47087	7.33379	7.60966
(In)	1.652787	7.12452	7.10433	7.09076	7.06773	7.07325	7.0487
(Io)	1.762105	8.035	8.13625	7.98791	8.0725	8.44837	8.57969
(Ip)	0.889326	7.30917	7.35963	7.27296	7.32215	7.40561	7.46131
(Iq)	1.25642	7.34023	7.4013	7.29577	7.35054	7.3543	7.45399
(IIa)	1.278754	7.01245	7.00672	6.97175	6.96356	7.22887	7.24159
(IIb)	2.440922	7.06207	7.02058	7.06207	7.02058	7.55027	7.39195
(IIc)	1.66227	7.31066	7.31070	7.2739	7.27228	7.16904	7.15532
(IId)	1.771029	7.26035	7.24107	7.21692	7.19093	7.46718	7.47505
(IIe)	1.963489	7.23979	7.17812	7.19688	7.12543	7.53781	7.53395
(IIf)	2.344829	7.52942	7.48820	7.48955	7.44369	7.74769	7.7162
(IIg)	2.25329	8.1305	8.20568	8.08186	8.1297	8.387	8.54984
(IIh)	2.134695	6.95684	6.94726	6.91702	6.90466	7.73489	8.00052
(IIi)	2.476107	8.5579	8.56907	8.57619	8.59324	8.10879	8.07261
(IIIa)	2.698101	8.86932	8.83303	8.88264	8.85001	8.52344	8.45435
(IIIb)	2.698101	8.90283	8.87316	8.91858	8.89367	8.56855	8.49718
(IIIc)	2.298853	9.03929	9.16298	9.0572	9.1877	8.75267	8.76483
(IIId)	2.476107	8.10072	8.04244	8.09029	8.03088	8.31289	8.08426

IC_{50} =Biological activity, pIC_{50} = Negative log of IC_{50} value, * Determined from systat, ** Determined from validated model in valstat, Cal. = Calculated, Pred. = Predicted

Results and Discussion:

The study of Equation (2) revealed that thermodynamic (HLC, VDWE) and electronic (LUMO) parameters are associates with anticonvulsant activity. The HLC and VDWE contributed positively where as LUMO contributed negatively to biological activity. The equation suggested that HLC of molecule and VDWE are of significance, they are having better correlation with biological activity and have minimum standard deviation. The results shows overall significance level better than 99.9% as it exceeded the tabulated F value (54.219,$F_{3,15}$). The Equation (3) revealed that all three parameters viz., thermodynamic (NVDWE), spatial (PMIY) and electronic (DM, LUMO) are associated with

anticonvulsant activity. The PMIY and DM have better correlation with biological activity and have low value of standard deviation. The data showed overall significant level greater than a 99.9% as it exceeded the tabulated F value (28.2049F3.15). Both of above equation was validated by leave one out cross validation method and bootstrapping method which give statistically significant values of internal equation where as Q^2 was found to be greater than 0.5 also statistically significant.

Table 2: Cross Validation with Different Parameters

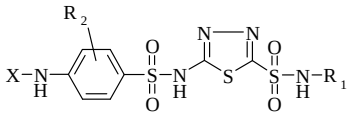
Sr. No.	Parameters	Equations		
		20	**21**	**22**
1	Q^2	0.766858	0.806632	0.719494
2	S_{PRESS}	0.394122	0.363346	0.440867
3	S_{DEP}	0.366908	0.337359	0.402455

Q^2 : Cross validated correlation coefficient[21], S_{PRESS} : Predicted sum of squares, S_{DEP} : standard error of prediction

This statistical parameter represents a high level of significance having high Q^2 values (0.719494). PMIY is depends on the total mass of molecule, mass distribution within molecule and position of the axis of rotation of the molecule equation shows direct relationship between PMIY of molecule and biological activity, whereas DM is an indication of strength and orientation behavior in electrostatic field and shows direct correlation. NVDWE and LUMO energy is directly correlated to stability of molecule.

Conclusion:

On the basis of QSAR study and nucleus present in the reported series, the parent structure was selected. In the series; compounds (Ia-q, IIa-i, IIIa-d), 1,3,4-thiadiazole, benzene and sulphonamide moieties are present and thus, 5-benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide (IV), was selected as parent structure which contains all three aforesaid moieties.



(IV)

On the basis of results and discussion theiron, the parent structure (IV) 1,3,4-thiadiazole will substituted at different position as represented R_1, R_2 and X .The bulkier substitution at R_1 and electronegative group at R_2 will play a significant role. However, X can be either hydrogen or acetyl group.

References:

[1] Ariens EJ (1972) In; Drug Design, A Series of Monograph Vol. I, New York: Academic Press, 2.

[2] Smith HJ (1988) In; Smith and Williams Introduction to Principle of Drug Design, 2nd Edn., Great Britain: Anchor Brendon Ltd 241.

[3] Patrick GL (1989) In; An Introduction to Medicinal Chemistry, 2nd Edn, Oxford: Oxford University Publications, 259.

[4] Kubinyi H (1993) Methods and Principles in Medicinal chemistry. In; QSAR: Hanch Analysis and Related Approaches, Weinheim: VCH Publishers, 19-93.

[5] Supran CT, Scozzafava A, Antel J, Gallori E, Franchi M & Vullo D (2004) J Med Chem 47:1272-1279.

PB-47

Development and Validation of RP-HPLC Method for the Estimation of Olanzapine in Marketed Formulation

Nidhi Jain, Ankur Joshi, Narendra Vyas, Sapna Malviya, Anil Kharia
Modern Institute of Pharmaceutical Sciences, Indore (M.P.), India, 453111
E-Mail address: nidhisinghai232@gmail.com

Abstract: A simple, accurate, precise and rapid stability indicating RP-HPLC method was developed and validated for the analysis of Olanzapine in marketed formulation. Analysis was performed on a C-18 (250mm x 4.60mm, 5 µm) column as stationary phase and using mobile phase which was Potassium di-hydrogen phosphate Buffer (pH 6): Acetonitrile (60:40) (v/v) at a flow rate of 1ml/min with UV detection at 258 nm at constant room temperature. The injection volume was 20 µl and the chromatographic runtime of 5 min was used. Proposed method was found to be linear in the range of 5-25 µg/ml with the correlation coefficient 0.998. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 100% 120%) was ranging from 99.58% - 100.50%. The robustness of developed method was checked by changing temperature, flow rate and mobile phase ratio.

Introduction:

The quantitative estimation of the active constituents is an integral part of developing and manufacturing process of pharmaceutical dosage forms. There is a need for development of better and reliable methods for the estimation of pharmaceutical dosage form. The official methods for the analysis of active ingredients of formulations are few and the most of the methods available for the analysis of active ingredients are applicable only after prior separation that involves tedious and time-consuming procedures.

Literature survey reveals that lesser method development of RP-HPLC method for the determination of Olanzapine in bulk forms or other pharmaceutical dosage form has been reported. Literature survey also indicates that LCID/ MS, [1] TLC-spectrodensitometric method [2], Ultra Performance Liquid Chromatography (RP-UPLC) technique [3] were also been developed for the determination of Olanzapine.

Therefore in this study an attempt is made to develop RP-HPLC methods for the method development of the drug in pharmaceutical formulations. Hence, the proposed method is simple, fast, accurate, precise and reproducible and can be applied for routine quality control analysis of drugs in bulk or in pharmaceutical formulations.

Materials and Methods:

Olanzapine was made available from Bioplus, Bangalore (Purity 99.8%). Methanol (AR Grade) was purchased from Merck Ltd., India were used for sample preparation. Acetonitrile (HPLC), Methanol

(HPLC) and Water (HPLC) were purchased from Merck Ltd., India used for the preparation of mobile phase. Potassium di-hydrogen phosphate, Ortho Phosphoric acid and Triethyl amine used for the preparation of buffer. Olanzapine marketed formulation (Oleanz-10 containing 10mg of Olanzapine which was manufactured by Sun Pharma, Mumbai, India) was procured from a local pharmacy. Chromatographic separation was performed on Waters HPLC system. The output signal was monitored and processed using empowers software. The chromatographic column used was C-18 (250mm x 4.60mm, 5µm).

Analytical Method Development by RP-HPLC

Mobile Phase selection was done by selecting numbers of mobile phase in different ratio. Taking into consideration of the system suitability parameter, the mobile phase found to be most suitable for analysis was Buffer (1.75 gm KH_2PO_4 in 1000 ml water add 1 ml of TEA adjust the pH 6 with OPA): Acetonitrile (60:40). Solubility and FTIR of standard Olanzapine drug was performed.

Selection of wavelength was done by making a solution of 10µg/ml of standard Olanzapine drug and scanned over UV range (200-400nm).

Selection of separation variable was done by preparing Olanzapine solution in different mobile phase and chromatograph was recorded by using different column at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

Separation variables were set and mobile phase was allowed to saturate the column at 1.0 ml/min. After complete saturation of column, three replicates of working standard of Olanzapine 10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram checking the system suitability parameters.

Different working standard solutions were made of concentrations 5, 10, 15, 20, 25 µg/ ml using the diluents Water: ACN (50:50 v/v)**.** Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this calibration curve. A solution was made containing 10 µg/ml of Olanzapine from marketed formulation and the amounts of Olanzapine in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated three times with tablet formulation.

Validation

Linearity was observed by plotting the calibration curve after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve were noted and the response ratio (response factor) was found.

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision was observed by repeatability and intermediate precision (Intra-day Precision, Inter-day Precision Analyst to Analyst). Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Robustness was performed by altering the pH, temperature and concentration of the mobile phase.

Results and Discussion:

Olanzapine was yellow powder with melting point 195 ^{o}C. Solubility study shows that Olanzapine was freely soluble in acetonitrile, 0.1 N HCl, benzene, methanol, slightly soluble in ethyl alcohol and insoluble in water, 0.1 N NaOH, phosphate buffer pH 7.4. The interpretation by FTIR shows that the drug was Olanzapine; the peaks were at 3565.69 cm^{-1}, 2171.25 cm^{-1}, 1964.27 cm^{-1}, 1889.97 cm^{-1}, 1771.24 cm^{-1}, 1646.19 cm^{-1}, 1515.62 cm^{-1}, 1540.45 cm^{-1} and 1139.06 cm^{-1}. Maximum absorbance was found at λmax 258.00 nm. Taking into consideration the system suitability parameter the mobile phase found to be most suitable for analysis was Buffer (1.75 gm KH_2PO_4 in 1000 ml water add 1 ml of TEA adjust the pH 6 with OPA): Acetonitrile (60:40). The separation variables were set at flow rate 1ml/min, wavelength at 258 nm, the injection volume was 20 µl and the chromatographic runtime of 5 min was used at room temperature. The system suitability parameters study was done in three replicates and the mean was calculated; retention time 3.476 min, theoretical plate 3077.333 and tailing factor 1.176667. An assay of marketed formulation was performed and mean reading of three batch of formulation was 10.076 mg. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards 80%, 100% 120% was ranging from 99.56%, 100.10% and 90.75% respectively. By the Repeatability study the amount of Olanzapine in marketed formulation was found 9.95mg i.e. 99.50%. By Intra-day Precision after 6 hr the amount of Olanzapine in marketed formulation was found to be 98.97%, by Inter-day Precision the amount of Olanzapine in marketed formulation was found to be 97.10% and by Analyst to Analyst precision the amount of Olanzapine in marketed formulation was found to be 99.84%. The robustness of developed method by altering the pH, temperature and concentration of the mobile phase were made and the method capacity remains unaffected.

References:

[1] Zhang M, Jia J & Chuan LU (2010) Acta Pharm Sin, 45 (6): 767-771.

[2] Mahmoud AT, Nagiba YH, Nariman AE & Mohamed A (2013) JAR (4): 173–180.

[3] Raja A & Venkateshwar R J (2013) Asian J Pharm Clin Res 6 (3): 178- 181.

PB -54

Development and Validation of Bioanalytical Method for Estimation of Rivaroxaban Using HPLC-PDA in Human Blood Plasma

Satyendra Yadav, Nidhi Dubey
School of Pharmacy, Devi Ahilya Vishwa Vidyalaya, Indore (MP) India.
E-mail address: syadav8584@gmail.com

Abstract: Rivaroxaban is oral anti-coagulant drug, used in the treatment of various blood disorders. A sensitive and accurate liquid chromatographic methodfor the quantification of rivaroxaban in human plasma was successfully developed. Protein precipitation was used for sample preparation. Quantification of analyte was achieved on phenomenexluna C_8 (5µm x 25cm x 4.6mm i.d.) column using methanol:water:dimethyl sulfoxide (50:45:5, v/v/v) as mobile phase at a flow rate of 1ml/min. Detection was achieved at 252nm over the linearity concentration range of 5-40µg/ml. Retention time was found 6.2 min. The LOD and LOQ were found to be 1.5 µg/ml and 4.5 µg/ml, respectively.The developed method was validate as per US FDA guidelines.

Introduction:

Anticoagulants are given to prevent the blood clotting or to prevent existing clots from getting larger. Clots can block the blood flow to the brain or to the heart muscle.These blockages may cause a heart attack or a stroke.Rivaroxaban (RIV)is potent and effective oral anti-coagulant drug, which is widely used in the treatment of thromboembolic ailments, myocardial infarction, stroke, angina pectoris and various blood clotting related disorders. IUPAC name of RIV is (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5-yl]methyl}thiophene-2-carboxamide (Fig.1).RIV is a small molecule(molecular weight 436 $gmol^{-1}$) and almost insoluble in water. It is rapidly absorbed through gastro intestinal tract and attains maximum plasma concentrations at 2–4 h after oral administration. Oral bioavailability of RIV is high (92–98 %) with the dose of 10 mg, irrespective of food intake. RIV acts as anticoagulant by inhibiting the factor Xa. Inhibiting factor Xa,it activates the generation of thrombin molecules. The factor Xa inhibition occurs mainly as a result of RIV binding with S1 and S4 pockets of the serine endopeptidaseenzyme with high selectivity.

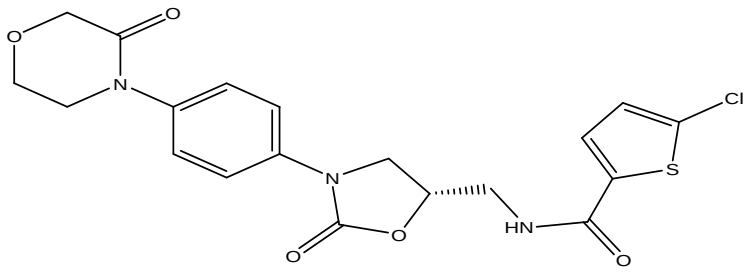


Figure 1: Chemical structure of Rivaroxaban

The literature review revealed that there are few bioanalytical methods reported for the determination of rivaroxaban in human blood plasma [1-5]. There is only one HPLC-UV method till date for

RIVquantification in human plasma [3]. The sample preparation in above reported method is very tedious, further above method utilized acetonitrile and water as mobile phase in the ratio 55:45 % v/v. Method requires quite high concentration of acetonitrile. However, present studies attempts to develop simplified and ecofriendly sample preparation procedure and mobile phase to implement greener approach for the quantification of RIV in human plasma.

Material and Methods:

Materials Rivaroxaban was procured as a gratis sample from Mehta API Pvt. Ltd., Mumbai, India. Chloramphenicol was procured from Panchsheel organics, Indore, India. HPLC grade water was procured from Medicaps Pvt. Ltd. Dhar, India. Methanol, acetonitrile and dimethyl sulfoxide (DMSO) were purchased from spectrochem Pvt. Ltd, Mumbai, India. Human plasma wascollected from Greater Kailash Hospital, Indore, India. The required chromatographic solvents and solutions were first filtered through 0.45µmmembrane filters (Pall life sciences, India) and sonicated prior to use.

Method *Preparation of standard solutions:* Stock solutionsof RIV (1000µg/ml) and Chloramphenicol, internal standard (IS) (1000µg/ml) were prepared inDMSO.Serial dilutions of stock solutions were prepared and used as working standard for analysis.

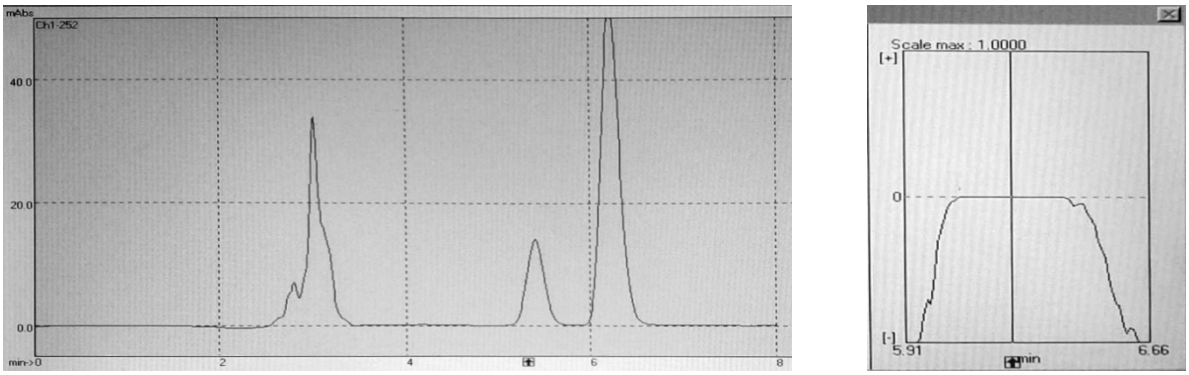
Sample preparation: Human plasma (0.1ml) was taken in a 2ml poly propylene tube, 20µl of standard drug and IS were spiked into it, then 0.5 ml of acetonitrile was added. These solutions were vortexed for 1 min and then centrifuged for 10 min at 10000 rpm, supernatant was injected to chromatographic system.Sample preparation was done in triplicate.

Chromatographic conditions: The mobile phase consisted methanol: water: DMSO (50:45:5, % v/v/v) at a flow rate of 1.0 ml/min. The required chromatographic solvents and solutions were first filtered through 0.45 µm membrane filters (Pall life sciences, India) and sonicated prior to use. Chromatographicseparation was achieved at ambient temperature on HPLC system having a pump (Shimadzu LC $10AT_{VP}$)with 20 µL Rheodyne injector, Phenomenex Luna C_8 (5 µm x 25cm x 4.6mm i.d) column and SPD-10 A_{VP} photodiode array (PDA) UV-Visible detector set at 252nm and equipped with *CLASS-VP* software (Shimadzu, Japan). The run time was kept 8 min. Calibration curve is in the range of 5 µg/ml to 40 µg/ml. Peak purity is checked by obtaining ratio chromatogram.

Sample analysis: The responses of sample solutions were measured at 252 nm for quantitation of RIV by using the HPLC method as described above. The amounts of RIV present in sample solution were determined by applying value of peak area to the regression equation of the calibration graph.Validation was done as per US FDA guidelines. The specificity of the developed method was determined by comparing test results obtained from RIV solution containing plasma with that of results obtained from standard RIV solution. The linearity equation was obtained by linear regression analysis method by plotting a graph between mean peak response area and concentration. LOD and LOD were determined using dilution method. The recovery experiments were performed by the standard addition method.

Results and Discussion:

The literature revealed that RIV is insoluble in water and less soluble in various solvents. DMSO was used as solvent for RIV. Several trials were performed to remove the interferences of biological matter in the sample. Finalsample preparation involves precipitation of undesirable matrix component by protein precipitation using acetonitrile.



A B

Figure 2: RP-HPLC Chromatogram of Rivaroxaban with IS in human plasma

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of RIV with good peak symmetry and steady baseline was obtained with the mobile phase methanol: water: DMSO (50:45:5, %v/v/v) at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 252nm based on peak area. Complete resolution of the peak with clear baseline separation was obtained (Fig. 2). The system suitability test parameters are shown in Table 1.

Table 1: System suitability test for the proposed HPLC method

Parameters	**Rivaroxaban ± % RSD[a]**
Retention time (R_t), min	6.2 ± 0.03
Tailing factor	1.16 ± 0.04
Resolution (Rs)	2.19 ± 0.04
Theoretical plates (N)	4249 ± 0.8

[a]% Relative standard deviation, (n=6)

Method validation parameters:

System suitability: System suitability parameters were found to be satisfactory. All the parameters, theoretical plate count (N) >4100, resolution (Rs) >2 and tailing factor >1.2 were within acceptable value.The relative standard deviation (RSD) of peak area was found to be <2percentage.

Specificity: There is no chemical interaction between the RIV and IS, and both the RIV and IS was resolved well. There were no interfering peak/s found in the chromatogram obtained from the blank plasma at the retention times of RIV and IS.

Linearity: The linear regression correlation coefficient of 0.975 was obtained over the six different concentration levels ranging from5 µg/ml to 40 µg/ml for RIV. The average slope and interceptof

linearity equations were 26709.03 and -21174.56 respectively. Regression analysis data are summarized in Table 2.

Table 2: Regression analysis of calibration graphs for RIV for the proposed HPLC methods

Parameters	**HPLC**
Conc. Range	5-40 µg/ml
Slope	26709.03
SD of slope	110.98
Intercept	-21174.56
SD of intercept	1154.79
correlation	0.9735
Regression equation	y= 26709x-21174

Y = peak area

x = Concentration of analyte in µg/ml

Limit of detection (LOD) and limit of quantitation (LOQ): The concentration of RIV for determination of LOD was 1.5 µg/ml, which indicates the sensitivity of the method. Similarly, LOQ was found 4.5 µg/ml, which proves that RIV can be estimated at low concentration.

Accuracy and Precision: The interday and intraday precision values of RIV for various concentrations ranged from 0.57% to 1.15% RSD and0.46% to 0.88% RSD, respectively. The valuesfor accuracy were also found within acceptable limits at the same concentrations. The data are presented in Table 3.

Table 3. Method validation parameters for estimation of Rivaroxaban

Parameters	**HPLC**
Limit of detection	1.5 µg/ml
Limit of quantification	4.5 µg/ml
Recovery	99.38-100.44%
Repeatability (%RSD, n=6)	0.64
Intermediate Precision	
Interday (%RSD, n=3)	0.57-1.15
Intraday (%RSD, n=3)	0.46-0.88
Robustness (%RSD)	< 2%

[a]%RSD = Relative standard deviation

Robustness: The developed method was found to be robust during robustness studies, the %RSD was found to be <2 in each case. The low values of %RSD showed the robustness of the method (Table 3).

Sample and standard stability: The stability of RIV sample in human plasma and the standard solutions were determined. Peak area and retention time variation were found to be <1%. Also, no significant change in peak area was observed during 24 h.

Conclusions:

In the present study, an attempt was made to develop a simple, precise, selective and sensitive validated bioanalytical method of RIV using RP-HPLC. This method is quite simple, economic, less time-consuming method up to date for the determination of RIV in plasma with RP-HPLC.

Acknowledgement: We are highly grateful to Department of higher education for providing financial support for facilitation research.

References:

[1] Rohde G (2008) J Chromatogr B 872: 43-50.

[2] Kuhn J, Gripp T, Flieder T, Dittrich M, Hendig D & Busse J (2015) PLoS ONE 10(12):78.

[3] Çelebier M, Reçber T, Koçak E, Altınöz S & Kır S (2015) J Chrom Sci 7:1-5.

[4] Zhang W, Lou D, Zhang DT, Zhang Y & Huang HJ (2016) J Thromb Thrombolysis: 1-7.

[5] Reddy GS, Prasad RSLN & Reddy LSK (2016) Orient J Chem 32(2):1145-1154.

PB-63

In Silico Homology Modeling and Validation of α-Glucosidase Enzyme

Navin Sainy, Nidhi Dubey
School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (MP)-452017
E-mail address: nsainy23@gmail.com

Abstract: In this research work we have derived three dimensional (3D) structure of α-glucosidase enzyme through homology modeling and the predicted structure was validated with the help of PROCHECK analysis in the form of Ramachandran plot. According to Ramachandran plot statistics the derived model was found to be of good quality.

Introduction:

Diabetes mellitus type 2, include 90% of all cases of diabetes mellitus is increasing considerably and affecting about 5% of the world population. The burden of diabetes is driven by vascular complications such as cardiovascular disease, stroke, nephropathy, retinopathy and renal failure. Type 2 diabetes mellitus is often associated with abnormalities in plasma lipid and lipoprotein profiles, and postprandial hyperlipidemia has been shown to be an independent risk factor and predictor of atherosclerosis [1,-3].α-glucosidase inhibitors delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. Thus inhibition of enzyme α-glucosidase is a new and promising approach for the treatment of diabetes mellitus.

Material and Methods:

As the crystal structure of α-glucosidase enzyme is not available in the protein data bank (PDB), therefore the 3D structure of α-glucosidase was derived through homology modeling. The protein sequence (FASTA sequence) of enzyme α-glucosidase was retrieved from Uniprot/Swissprot database. The sequence was submitted to modweb [4] web server to find a suitable template with sufficient query sequence coverage and sequence identity. The model derived through modweb web server was selected on the basis of maximum sequence similarity (46.00 %) with the template (crystal structure of Human intestinal maltase-glucoamylase; PDB code: 2qmjA) [5].

Result and Discussion:

Structure of modeled enzyme (Figure 1) was validated through PROCHECK analysis. For this analysis, the PDB format file of modeled α-glucosidase enzyme was submitted to PDB SUM web server of European Bioinformatics Institute. The results of PROCHECK analysis was obtained in the form of Ramachandran plot (Figure 2) which resolve stereo chemical aspects along with main chain

and side chain parameters with widespread analysis.The Ramachandran plot (PROCHECK) statistics are summarized in Table 1.

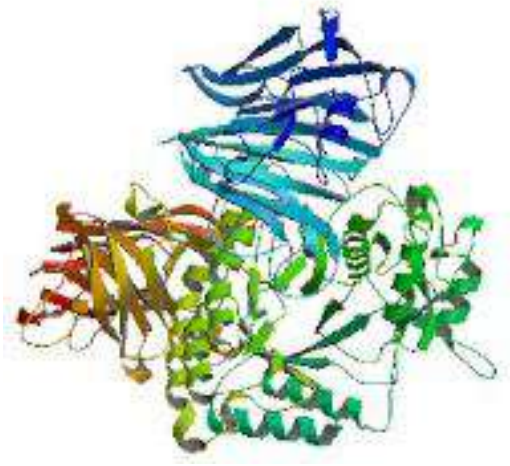


Figure 1: 3D structure of derived model of α-glucosidase enzyme

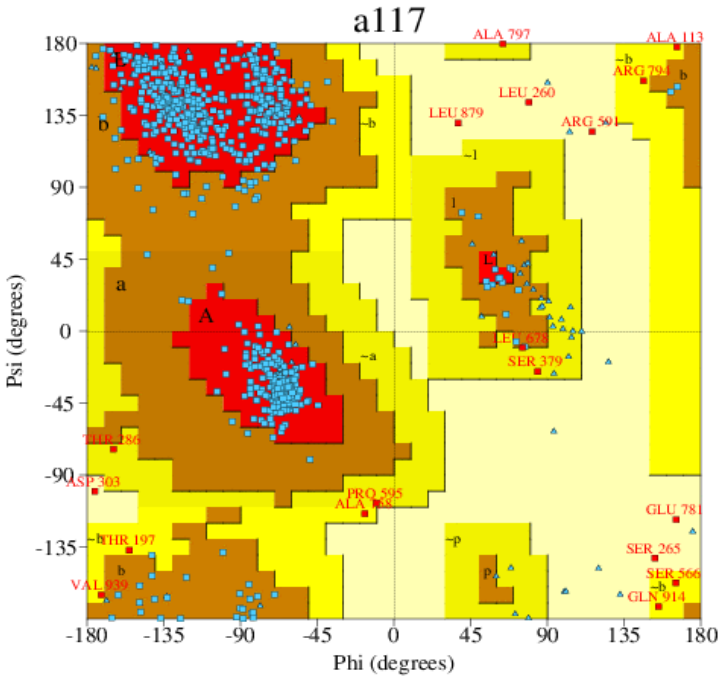


Figure 2: Ramachandran plot of homology modeled α-glucosidase enzyme

Table 1: Ramachandran plot (PROCHECK) Statistics of homology modeled α-glucosidase enzyme

Residue	**No. of residues**	**Percentage**
Most favoured regions [A,B,L]	631	87.0%
Additional allowed regions [a,b,l,p]	77	10.6%
Generously allowed regions [~a,~b,~l,~p]	11	1.5%
Disallowed regions	6	0.8%
Non-glycine and non-proline residues	725	100.0%
End-residues (excl. Gly and Pro)	2	
Glycine residues	67	
Proline residues	69	
Total number of residues	**863**	

Conclusion:

In this research work we have successfully derived the 3D structure of enzyme α-glucosidase. The modeled enzyme was validated through PROCHECK analysis (Ramachandran plot). The Ramachandran plot indicated that phi(φ) and psi(ψ) angles to contribute in conformations of amino acids excluding glycine and proline with 87.0 % residues in most favored region, 10.6 % in additional allowed region, 1.5 % in generously allowed region and 0.8 % residues in disallowed region. The above statistics of Ramachandran plot indicated that derived model was found to be of good quality and can be used for further molecular modeling study.

References:

[1] Ceriello (1998) Diabetic Med 15:188–193.

[2] Temelkova-Kurktschiev T, Koehler C, Henkel E, Leonhardt W, Fuecker K & Hanefeld M (2000) Diabetes Care 23:1830–1834.

[3] Bastyr E J, Stuart, C A & Brodows (2000) RG Diabetes Care 23: 1236–1241

[4] Fiser A & Sali A (2003) Meth Enzym 374: 461–491.

[5] Moche M, Dehesh K, Edwards P & Lindqvist Y (2001) J Mol Biol 305: 491-503.

PB-64

Docking Studies on Imidazolidine Analogues for Management of Diabetes

Sumeet Prachand[1], Ritu Gilhotra[1], Arun Gupta[2], Sanjay Jain[3],
[1] Suresh Gyan Vihar University, Jaipur
[2]School of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[3]Indore Institute of Pharmacy, Indore
E-mail address: sumeet_prachand@gmail.com

Abstract: Glycogen synthase kinase-3β (GSK-3β) has recently emerged, in the field of medicinal chemistry, as one of the most attractive therapeutic targets for type II diabetes. Phenylmethylene hydantoins (PMHs) forms strong interactions with the hinge region of GSK-3β; carbonyl oxygen at position 2 form a H-bonding with backbone nitrogen of Val135 and the NH at position 3 to the carbonyl oxygen of Asp133. The hydantoin ring was sandwiched between Ala83, on top, and Leu188, on the bottom. The aromatic ring is rotated out of plane from the hydantoin plane, allowing extensive interactions with the nucleotide-binding loop. Furthermore, the substituted benzylidene ring system builds an H-bonding interaction with the guanidine moiety of Arg141. Targeting Arg141 is important to improve the activity in the process of designing new derivatives because it is considered the selectivity residue for GSK-3β.

Introduction:

The insulin insensitive form of diabetes, type 2 diabetes mellitus characterized by hyperglycaemia which is also known as elevated blood glucose concentrations, most frequently arises as a consequence of obesity, represents approximately 95% of the overall incidence of diabetes-I. Additionally, diabetes related complications exert a heavy toll on patients with poor metabolic control [1-5]. Most of kinase inhibitors act by competition with either ATP or metal-binding sites that are involved directly in the catalytic process. Over the past 15 years, there have been extensive efforts to understand and reduce the high attrition rates of drug candidates with an increased focus on physicochemical properties. The fruits of this labour have been the generation of numerous efficiency indices, metric-based rules and visualization tools to help guide medicinal chemists in the design of new compounds with more favourable properties. This deluge of information may have had the unintended consequence of further obfuscating molecular optimizations by the inability of these scoring functions, rules and guides to reach a consensus on when a particular transformation is identified as beneficial. In spite of the early discovery of insulin and its subsequent widespread use in the treatment of diabetes mellitus, and later discovery and use of sulfonylureas e.g. chlorpropamide, tolbutamide and biguanides viz. phenformin as oral hypoglycemic agents, the treatment of diabetes mellitus remains less than satisfactory. Insulin can only be administered intravenously due to its chemical nature, and therefore, is troublesome and inconvenient to use. Oral hypoglycemic agents tend to promote side effects such as excessive hypoglycemia or lactic acidosis. Glycogen synthase

kinase-3β (GSK-3β) has recently emerged, in the field of medicinal chemistry, as one of the most attractive therapeutic targets for Type II diabetes. The full potential of GSK-3β inhibitors is yet to be realized and the number of drug candidates being developed by both academic centers and pharmaceutical companies has increased exponentially in the last few years. Glycogen synthase kinase-3β (gsk-3β) is a unique multifunctional serine/threonine kinase that is inactivated by phosphorylation in response to insulin binding; PKB/AKT phosphorylates GSK-3β on serine9, which prevents the enzyme from phosphorylating glycogen synthase. Unphosphorylated glycogen synthase is active & able to synthesize glycogen.

Phenylmethylene hydantoins (PMHs) forms strong interactions with the hinge region of GSK-3β; carbonyl oxygen at position 2 form a H-bonding with backbone nitrogen of Val135 and the NH at position 3 to the carbonyl oxygen of Asp133. The hydantoin ring was sandwiched between Ala83, on top, and Leu188, on the bottom. The aromatic ring is rotated out of plane from the hydantoin plane, allowing extensive interactions with the nucleotide-binding loop. Furthermore, the substituted benzylidene ring system builds an H-bonding interaction with the guanidine moiety of Arg141. Targeting Arg141 is important to improve the activity in the processof designing new derivatives because it is considered the selectivity residue for GSK-3β. Design of potent and selective GSK-3β inhibitors should consider the following important hot spots

- H-bonding interaction with the hinge region of Asp133 and Val135.
- Targeting Arg141 and Gln185 amino acids.
- Filling the Val70, Lys85 and Cys99 hydrophobic pocket. For example, keeping the hydantoin ring and chemical moiety at benzylidene ring system can afford potent and selective GSK-3β inhibitor.

The wide chemical diversity ofpossible inhibitors and the existence of multiple sites forpotential inhibition encourage researcher topursue the development of GSK-3β inhibitorsas potential drugs. Therefore it is worthwhile to develop further substituted GSK-3β inhibitorsas antidiabetic agents.

Material and Methods:

Docking of Designed Compounds The molecular docking was performed using Molegro Virtual Docker (MVD) 2006. 1.5 and CS Chem Office version 11.0. The docking scoring function of MolDock that we use is based on a piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms introduced. Structures of all the compounds were sketched using builder module of the program. The sketched structures were subjected to energy minimization using molecular mechanics (MM2) until the RMS gradient value became smaller than 0.1 kcal/molA°. The energy minimized molecules were subjected to re-optimization via Austin model-1 (AM1) method until the RMS gradient attained a value smaller than 0.01 kcal/mol °A using MOPAC The descriptor values for all the molecules were calculated using “compute properties” module of program. The minimized molecule was saved as MOL file format.

Following Steps were used for docking through MVD:

1) **Importing and Preparing Molecules**

- File Import: - MVD supports PDB, Mol2, SDF, and its own XML-based format,
- MVDML.
- Adding a Molecular Surface
- Predicting the Binding Site

2) Running the Docking Simulation: -

- By selecting **Docking** | **Docking Wizard**
- Choosing Structures
- Defining the Region of Interest

3) Viewing the Results

These designed analogs were docked in the ATP binding site of GSK-3β by Molegro Virtual Docker 2006.1.5.

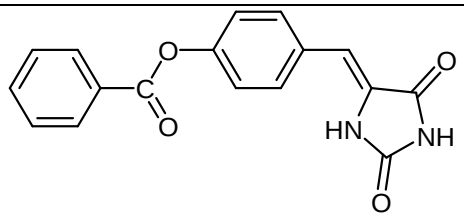
Results and Discussion:

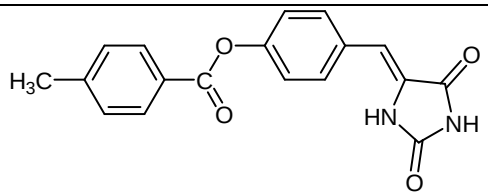
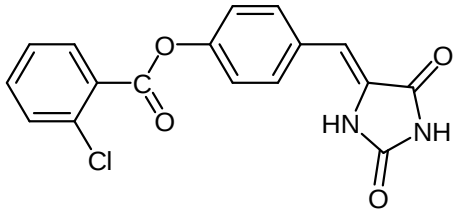
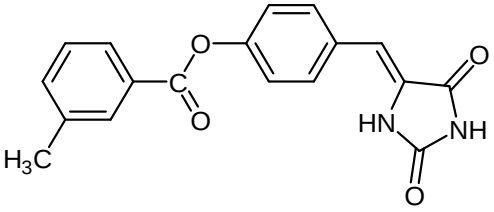
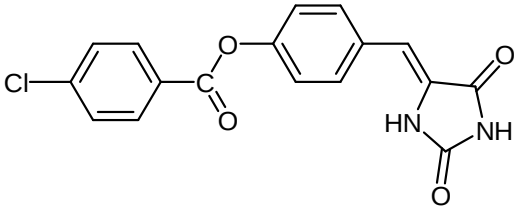
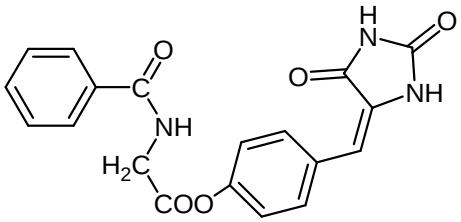
On the basis of literature study hydantoin analogs were designed. These designed analogs were docked in the ATP binding site of GSK-3β by Molegro Virtual Docker 2006.1.5.

Phenylmethylene hydantoins (PMH) forms strong interactions with the hinge region of GSK-3β; carbonyl oxygen at position 2 form a H-bonding with backbone nitrogen of Val135 and the NH at position 3 to the carbonyl oxygen of Asp200.

Furthermore, the substituted benzylidene ring system builds an H-bonding interaction with the guanidine moiety of Arg141. Targeting Arg141 is important to improve the activity in the processof designing new derivatives because it is considered the selectivity residue for GSK-3β. Substitution at benzylidene ring system can afford potent and selective GSK-3β inhibitors as H (a-f).

Table 1: Docking Score of Synthesized compound

Comp. Code	Synthesized Compound	Mol Dock Score (E-Total)	H-Bonds Interactions With Amino Acids
H-a		-113.32	Arg 141 Pro 136 Tyr 134

H-b		-126.06	Asp 200 Lys 183 Asp 200
H-c		-129.62	Tyr 134 Arg 141 Pro 136 Cys 199
H-d		-125.06	Arg 141 Pro 136 Tyr 136 Cys 199
H-e		-127.93	Val 135 Asp 200 Phe 201 Glu 97
H-f		-147.53	Arg 141 Asn 64 Ile 62 Glu 97

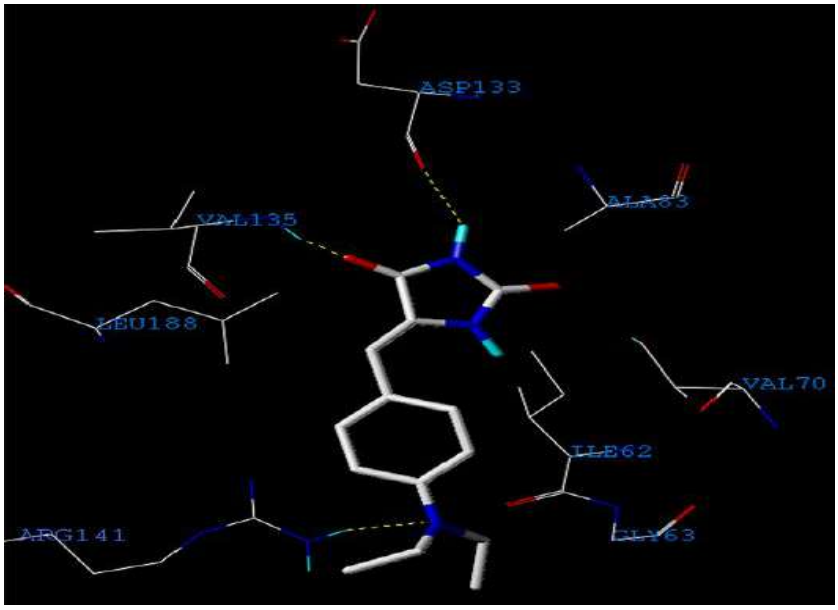


Figure 1: The prototype pose of docked structure of a Hydantoin analogue.

Conclusion:

Glycogen synthase kinase-3β (GSK-3β) is a unique multifunctional serine/threonine kinase that is inactivated by phosphorylation in response to insulin binding; PKB/AKT phosphorylates GSK-3β on serine9, which prevents the enzyme from phosphorylating glycogen synthase. Unphosphorylated glycogen synthase is active & able to synthesize glycogen.

Phenylmethylene hydantoins (PMH) forms strong interactions with the hinge region of GSK-3β; carbonyl oxygen at position 2 form a H-bonding with backbone nitrogen of Val135 and the NH at position 3 to the carbonyl oxygen of Asp133.

The hydantoin ring was sandwiched between Ala83, on top, and Leu188, on the bottom. The aromatic ring is rotated out of plane from the hydantoin plane, allowing extensive interactions with the nucleotide-binding loop. Furthermore, the substituted benzylidene ring system builds an H-bonding interaction with the guanidine moiety of Arg141. Targeting Arg141 is important to improve the activity in the processof designing new derivatives because it is considered the selectivity residue for GSK-3β. Encouraged by the above literature we tried to prepare the analogs in which keeping the hydantoin ring, and substitution at benzylidene ring system, apart from this hydantoin ring is replaced with bioisoesteric substitute ring (like rhodadine, oxindole) can afford potent and selective GSK-3β inhibitor.

References:

[1] Bastaki S (2005) Int J Diabet Metab 13: 111-134.

[2] Khanfar AM, Bilal AA, Mudit M & Kaddoumi A (2009) Bioorg Med Chem 17:6032–6039.

[3] Sivaprakasama P, Aihua X & Robert JD (2006) Bioorg Med Chem 14:8210–8218

[4] Denise MF & David KD (2000) Biol 225:471–479

[5] Vats RK, Kumar V, Kothari A & Mital A (2005) Curr Sci 88: 241-249.

PB-73

Docking Study of Chrysin Derivatives on Different Targets for Anticancer Activity

Suresh Chand Dangi, Sudha Vengurlekar
Sri Aurobindo Institute of Pharmacy, Indore
E-mail address: suresh91dangi@gmail.com

Abstract: Some anticancer molecules using natural flavonoid chrysin were designed and docking studies were performed using Molegro Virtual Docker (MVD) software. Of the available crystal structures of the non- mutated *Homo sapiens*, five were selected for the final docking studies. The docking results with selected crystal structures shown that designed legends forms hydrogen bonds with at least two out of three key active site residues (Asp, Val and Lys). It also form hydrogen bonds to other active site residues, in particular Glu. The average MolDock score and the MolDock Re-rank score were obtained as -156.704 Kcal/mol and-125.649 Kcal/mol respectively. The docking results shown that some molecules fit well in the active site and interact with the residues in the active site which are crucial for their biological activity.

Introduction:

Cancer is a group of diseases characterized by uncontrolled growth and multiplication of abnormal cells that invade and metastases to other parts of the body [1]. Several techniques have been adopted for the treatment and eradication of cancerous cells. These techniques involved surgery, radiation, immunotherapy, chemotherapy and chemoprevention. Ideal anticancer drugs would eradicate cancer cells without harming normal tissues. Chrysin, also known as 5, 7-dihydroxyflavone with an IUPAC name of 5, 7-dihydroxy 2-phenyl-4H-chromen-4-one, belongs to the flavone sub-class of flavonoids. Its chemical structure is essentially based on a three ring nucleus with a phenyl ring attached to position 2 of the fused bicyclic and rings. Flavonoids including flavones, flavonols, and flavones possess various biological activities as antioxidant, anticancer etc. chrysin as a flavonoids shown activity for anticancer activity [2].

Binding of a small molecule (ligand) with a large molecule (protein) is called docking. Docking is the process by which two molecules fit together in 3D space. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [3].

Materials and Methods:

Designing of compounds

New molecules were designed by combining chrysin and cycloalkane derivatives. Four Molecules were designed on the basis of literature of their biological response towards cancer cells (Figure 1)

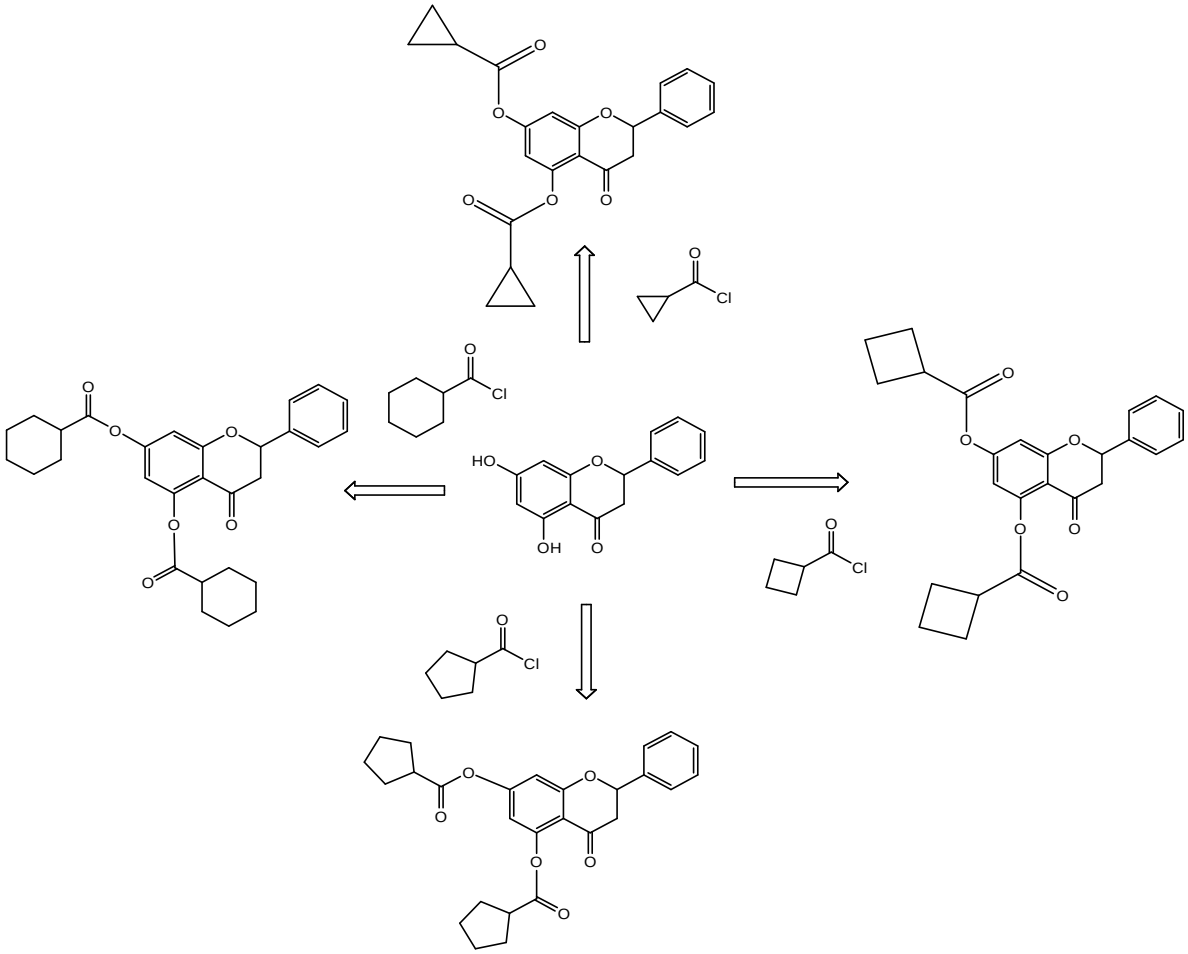


Figure 1: Designed compounds

Target Selection

Three targets (Table 1) were selected for performing docking studies to check the binding affinity of designed molecules with the target and to predict the potential molecules to be synthesized with the aim to have anticancer activity [4].

Software

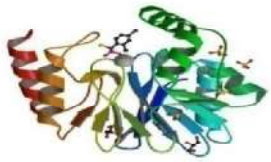
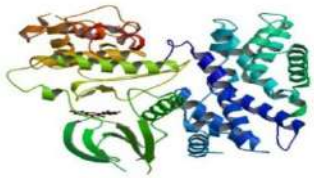
Ligand Preparation

The structures of Chrysin with cyclic compounds were converted into suitable chemical information using Chemdraw ultra v 10.0 (Cambridge software), copied to Chem3D ultra v 10.0 to create a 3D model and, finally subjected to energy minimization using molecular mechanics (MM2). The minimization was executed until the root mean square gradient value reached a value smaller than 0.001kcal/mol [5].

Protein Selection

The selection of protein for docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.0-2.5Ao, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure.

Table 1: Selected anticancer drug targets with PDB ID

S. No.	Name of anticancer target protein	PDB ID	3D structure of target
1	Crystal structure of CphA N220G mutant with inhibitor 18	3IOG	
2	Crystal structure of a human cyclin-dependent kinase 6 complex with a flavonol inhibitor, fisetin	1XO2	
3	Structure determinants of phosphoinositide 3-kinase inhibition	1E8W	

Protein Preparation

All the X-ray crystal structures of the selected target proteins were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). Subsequent to screening for the above specific standards the resultant protein targets.

Software Method Validation

Software method validation was performed in MVD Molegro Virtual Docker 6.0 2013 and Marvin Sketch Product version 6.2.3 2013 were using Protein Data Bank (PDB) protein 3IOG, 1XO2, 1E8W.

Results and Discussion

Dockings results reveals that compound no. 4 has shown best affinity towards all the targets selected for the study.

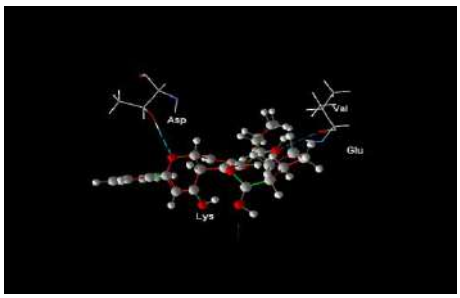


Figure 2: Biding affinity of Compound (a) with target (where carbon is grey, oxygen is red, nitrogen is blue and sulphur is yellow and hydrogen in white). Green lines represent the hydrogen bonds in between the ligand and the active site).

PDB code	Ligand name	MolDock Score	Rerank Score	HBond
3IOG	Compound (a)	-135.548	-106.292	-5.66085

(b)

Figure 3: Biding affinity of Compound (b) with target (where carbon is grey, oxygen is red, nitrogen is blue and sulphur is yellow and hydrogen in white). Green lines represent the hydrogen bonds in between the ligand and the active site).

PDB code	Ligand name	MolDock Score	Rerank Score	HBond
1XO2	Compound (B)	-155.357	-124.456	-3.68857

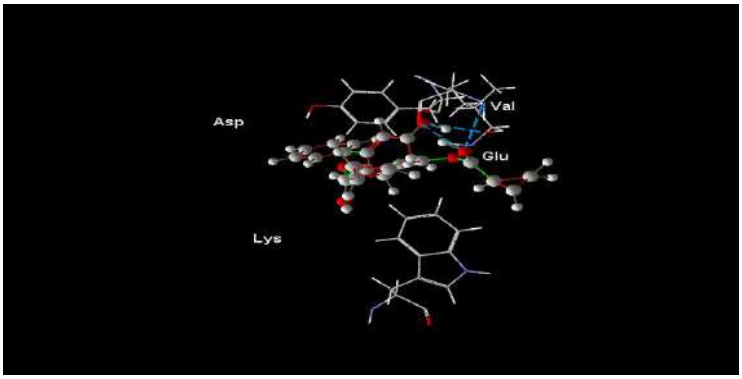


Figure 4: Biding affinity of Compound (b) with target (where carbon is grey, oxygen is red, nitrogen is blue and sulphur is yellow and hydrogen in white). Green lines represent the hydrogen bonds in between the ligand and the active site).

PDB code	Ligand name	MolDock Score	Rerank Score	HBond
1E8W	Compound (b)	-126.682	-105.89	-4.50612

References:

[1] Ahmedin J, Siegel R, Ward E, Hao Y, Xu J, Murray T & Thun MJ (2008) Cancer J Clin 58: 71–96.

[2] Mohammed HA, Ba LA, Bhurkhloz T, Schumann E, Diesel B, Zapp J, Kiemer AK, Riesc, Hartmann RW, Hosny M & Jacob C (2011) Nat Prod Commun 6: 31-34.

[3] Gehlhaar DK, Verkhivker G, Rejto PA, Fogel DB, Fogel LJ & Freer ST (1995) Proceedings of the Fourth International Conference on Evolutionary Programming, 123-124.

[4] Ramachandran GN & Sasisekharan V (1968) Adv Protein Chem 23:243-438.

[5] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN & Bourne PE (2000) Nucleic Acids Res 24: 235-242.

PB-82

A Stability Indicating Micellar Liquid Chromatographic Method for the Determination of Deferasirox in Solubilized System

Hitesh Malvia[1], Ashok Kumar[1], Pratibha Sharma[1], Ritesh Mishra[2]
[1]School of Chemical Sciences, Devi Ahilya University, Takshashila Campus, Khandwa Road, Indore 452 001 (M.P.), India
[2]API Research & Development Department, Medilux Laboratories Pvt. Ltd., Plot No. 98-99 Sector-I, Pithampur, Dist. Dhar, 454775, (M.P.), India
E-mail address: drashoksharma2001@yahoo.com

Abstract: A simple, isocratic and stability indicating micellar liquid chromatographic (MLC) method containing a surfactant solution above its critical micellar concentration (CMC) was developed and validated for determination of Deferasirox (BTBA) content in active pharmaceutical ingredient and its pharmaceutical formulations. The analysis was performed in on isocratic mode at 40^0C temperature through Thermo Hypersil ODS 100mm×4.6mm, 3 µm particle size columns. The UV detection wavelength was set at 250 nm in the mobile phase containing 0.1 M Cetyl trimethyl ammonium bromide (CTAB) buffer (pH – 2.5) and 10% 1-butanol. The method was successfully validated following as per the requirements of ICH guidelines. The proposed method was successfully applied as stability indicating method for determination of BTBA under different stressed conditions. The method allows accurate and reliable determination of BTBA for drug stability assay in pharmaceutical investigations.

Introduction:

Deferasirox [4-[3,5-bis (2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl] benzoic acid (BTBA)] (Figure 1) is an oral iron chelator and well known antidote. BTBA is an orally active chelator that is selective for iron (as Fe3+). It is a tridentate, oral, iron-chelating agent used in the management of chronic iron overload secondary to various RBC transfusions, i.e., transfusional iron overload such as β-thalassemia sickle cell disease, other unusual anemias, and myeloproliferative disorders4. It is an initial oral medication prescribes for such patients in the USA. The affinity and selectivity of the molecule is very high towards iron with high affinity in a 2:1 ratio. BTBA can be efficiently processed as chronic iron toxicity such chelation therapy. BTBA is the first FDA approved oral drug for chronic iron overload [1].

A comprehensive survey of the literature for BTBA analysis reveals that only a few systematic methods are available in favor of BTBA, By Liquid Chromatography Mass Spectrophotometry (LC–MS) [2]. Terbium - sensitized fluorescence method, LC method for pharmaceutical formulation, a stability representative LC method for bulk drug and pharmaceutical dosage forms, comparative bioavailability, dispersion and dispersed in various drinks, Pharmacokinetics, allocation, metabolism, and emission and its iron complex, were reported. In this present study an attempt was made to

develop a rapid and economical micellar liquid chromatographic method for assessment of BTBA in bulk and pharmaceutical formulation with improved sensitivity, precision and accuracy using Cetyl trimethyl ammonium bromide (CTAB) as surfactant above its critical micellar concentration. Micellar liquid chromatography is successfully used in the analysis of variety of compounds in several types of samples [3, 4].

Material and Methods:

Chemicals and reagents

BTBA standard was obtained as a gift samples from Medilux Laboratories Pvt. Ltd and it's formulate table "Desirox-250 mg" (Cipla pharmaceutical Ltd) were procure from a viable source. All Reagents were of analytical grade and solvents were of HPLC grade.

Instrumentation

An analytical balance, pH, vortex shaker were used to prepare the standard and sample solution. Chromatographic separations were performed in an Agilent and Waters Separation HPLC. The photo stability chamber utilized during forced degradation studies was controlled by a temperature controller. All measurements were carried out at 25±20C temperature.

Micellar liquid chromatographic conditions

Mobile phase was prepared by mixing of 0.1 M CTAB buffer solution at pH 2.50 and n-butanol in ratio of 90:10 v/v and filtered through 0.22 mm nylon filter paper. The stationary phase was Thermo Hypersil ODS column 100mm X 4.6mm, 3µm. Mobile phase flow rate was 1.0 mL/minute and column temperature 400C temperature as well as detection wavelength of 250nm. The total run time was set as 15 minute and injection volume was 10 µl. Under these optimized analytical conditions, BTBA was eluted at about 3.6 minute as shown in Figure 2 [5].

Results and Discussion:

Selection of appropriate detection wavelength

Analysis of reference and test solutions were performed and peak purity spectra were obtained over a wavelength range 200-400 nm. It was found that 250 nm is the optimum detection wavelength to maximize the sensitivity of the BTBA. A typical UV-Vis absorption spectrum obtained with the present method is depicted in Figure 3.

Surfactant concentration

Various types of surfactants were used to optimize the surfactant selection. Each individual surfactant exhibited different type of selectivity for BTBA. The increased selectivity is achieved in CTAB surfactant media. The effect of Cetyl trimethyl ammonium bromide (CTAB) buffer concentration on asymmetry, retention time and efficiency of BTBA was studied. After optimization of these variables,

best shape and lowest peak tailing were achieved with well-defined peaks and good sensitivity within a reasonable analytical run time.

Effect of the pH variation

To study the effect of pH on Peak response, Retention time, Peak tailing, Peak area, Peak height and Theoretical number of plate, Injections (each time) were made using 0.1 M CTAB buffered solution between pH 4.8 to pH 2.0. It was observed that 0.1 M CTAB buffer pH 2.50 gave good combination between peak symmetry and analysis time.

Method Validation

The validation of the proposed method was performed according to ICH guidelines17 pertaining to the Specificity (Identification and force degradation), system suitability solution, linearity, range, LOD, LOQ, accuracy (recovery), precision, sample solution stability and robustness18. All the measurements for validation were performed using BTBA standard solution in mobile phase.

Specificity

Specificity of proposed method was determined by injecting sample and standard solutions. It was checked that no interference was created by blank and placebo at the retention time of BTBA peak. Peak purity of stressed samples was checked and confirmed the spectral purity of BTBA. The obtained results indicated that the proposed method is selective and able to determine BTBA and its degradation impurities.

Instrumental precision

The instrumental precision was checked by injecting six replicates of standard solution containing BTBA (0.1 mg mL-1) and % RSD, Peak asymmetry and Number of theoretical plate counts were determined. %RSD was found 0.37%, Peak asymmetry-1.34 and Theoretical plate – 1916, respectively.

Linearity and range

Linearity of assay test method was carried out progressively diluting BTBA standard stock solution. A calibration curve was obtained by plotting area response against concentration in ppm and calculate residual sum of the squares (r2), slope and y-intercept using the plot.

Accuracy

The accuracy of the method was ascertained by calculating recoveries of BTBA by the standard addition method. Known amount of standard of BTBA was spiked in three different levels (80%, 100% and 120%) and prepared three spiked samples at each level (Total 9, determinations are as per ICH guideline). These spiked samples were analyzed against working standard and recovery of BTBA in three different levels was calculated. The obtained recovery found in ranged between 99.28% - 99.63%.

Precision study (repeatability and reproducibility) the method precision of the proposed method was determined by preparing six different sample solutions of same batch and analyzed

against working standard solutions. Assay values of these all six samples were calculated. % RSD of assay values were found 0.21%. The low % RSD values indicate that the proposed method is precise and repeatable. Intermediate precision study was performed by same concentrations as prepared in method precision and analyzed against working standard solutions on different days. % RSD of assay values of 12 samples (Method and Instrument precision sample) were found to be 0.30%. The closeness of assay results and % RSD values indicate that the proposed method is reproducible.

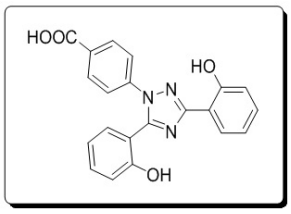


Figure 1: Structure of Deferasirox (BTBA)

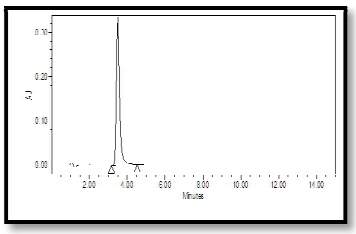


Figure 2 : Typical chromatogram of BTBA after Optimized chromatographic condition

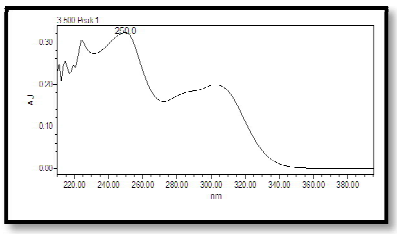


Figure 3 : Absorption spectrum of BTBA

Conclusions:

Micellar liquid chromatography described the optimization strategy used to select the best mobile phase for the resolution and determination of compounds in pharmaceutical preparations, serum, urine, and food samples. The projected method was found accurate, simple, precise, rapid and economical. Method validation parameters meet the specifications laid down in ICH guidelines with satisfactory results in the linearity, selectivity, precision, accuracy (recovery) and robustness. It is worth to mention that proposed methodology meets the fundamentals of the "green chemistry" exploring the use of environment-friendly reagents. Also the strategy is relatively inexpensive compared to other methods, thus making it more attractive and highly useful for many pharmaceutical analysis studies.

Reference:

[1] United States Food and Drug Administration (FDA) approves first oral drug for chronic iron overload, press release (2005) 1-4.

[2] Berthod A & García-Alvarez-Coque MC (2000) Micellar Liquid Chromatography, New York, Marcel Dekker 1-5.

[3] Ruiz-Angel M J, García-Alvarez-Coque M C & Berthod A (2009) Separ Purif Rev 38-45.

[4] Sechaud R, Dutreix C, Balez S, Pommier F, Dumortier T, Morisson S & Brun E (2008) J Clin Pharmacol Ther 46 :102.

[5] Bruin GJM, Faller T, Wiegand H, Schweitzer A, Nick H, Schneider J, Boernsen KO & Waldmeier F (2008) Drug Metab Dispos 36:25-23.

PB-84

QSAR and Docking Studies of Indene N-Oxide Derivatives as PPARγ Agonists

Ganesh Prasad Mishra[1], Rajesh Sharma[2], Hemant M Joshi[1]
[1]Ujjain Institute of Pharmaceutical Sciences, Chandesara, Ujjain, Madhya, Pradesh, India –452 017
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshshila Campus, Indore, M. P., India-452 001
E-mail address: gmrdmishra@rediffmail.com

Abstract: Indene N-oxide derivatives were used for docking and three dimensional quantitative structure activity relationship studies. Molecular docking and validation studies were carried out for all compounds on peroxisome proliferator activated receptor γ active site. The reliability of the docking results was acceptable with good root mean square deviation value (ranging from 0.96 to 2Å). The three dimensional quantitative structure activity relationship studies were also carried out by advanced technique (Stepwise forward-backward variable selection method) using training set of 19 compounds and test set of 7 compounds. A statistically reliable model with good predictive power (q2 = 0.8820, Pred r2= 0.7063) was achieved. Both above approaches illustrated insights into the structure activity relationship of these compounds which may helps in the design and development of potent indene N-oxide derivatives as PPARγ agonists.

Introduction:

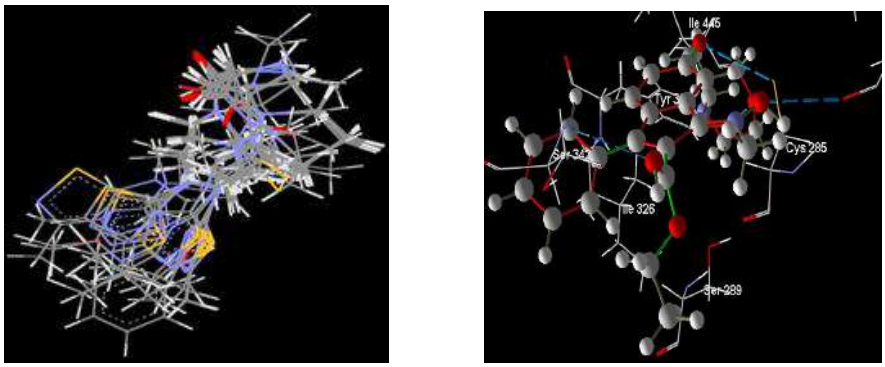
Type-2 diabetes has emerged as one of the biggest problems facing the world today. It is now forecasting that by the year 2025, 300 million people will suffer from diabetes worldwide, with 90% of these cases attributed to non-insulin dependent (type-2) diabetes [1]. Peroxisome proliferators-activated receptor gamma (PPARγ) is a nuclear receptor and transcription element that plays a crucial role in glucose homeostasis, insulin sensitization and lipid storage [2]. Currently, increasing interest on PPARγ research, a number of relevant quantitative structure-activity relationship (QSAR) studies were done. Most of them employed three dimensional QSAR field methodologies, such as Comparative Molecular Field Analysis and Comparative Molecular Similarity Indices Analysis [3]. In this paper, Non-TZDs (indene N-oxide derivatives) have been studied as PPARγ agonistic activity using docking and QSAR approach.

Material and Methods:

Dataset and molecular alignments

26 PPAR γ agonists were taken from the literature [5] and the reported EC_{50} values (μM) have been changed to the pEC_{50} for docking and QSAR study. The dataset was divided into a training set of 19 molecules and test set of 7 molecules as external set for validation of developed model using sphere exclusion methods. Test set is used to challenge the QSAR model developed based on the training set to assess the predictive effectiveness of the model which is not included in model generation. Sphere

exclusion algorithm was used for creation of training and test sets. The alignment of all the indene N-oxide derivatives is shown in Figure 1.



a. b.

Figure 1: Structural alignment of indene N-oxide derivatives used to develop the 3D-QSAR models (a) and docking pose of most active compound 18 with 2xkw (b).

Molecular docking simulations

MOLDOCK program employed to simulate interaction of PPARγ receptor with selected molecules. The most promising poses returned when the docking run was completed and further analyzed in the Pose Organizer. Moreover selected poses were confirmed to be the most stable conformation of each molecule for the binding to the PPAR γ active site. All of the selected poses of the 26 analyzed molecules were visually inspected to demonstrate that they were able to establish the molecular interactions with receptor.

Generation of the three dimensional QSAR model

Here, we performed 3D-QSAR analysis using the Molecular Design Suit Vlife MDS software package, version 4.1; supplied by Vlife Sciences, Pune, India [19]. Structures were sketched using CS Chem Office Version 11.0, Cambridge Soft Corporation, Software Publishers Association USA, the 2D draw application and converted to 3D structures. The sketched structures were subjected to energy minimization using molecular mechanics (MM2) until the root mean square (RMS) gradient value became smaller than 0.1 kcal/mol Å. The geometry optimization of the lowest energy structure was carried out using EF routine. K-Nearest neighbor molecular field analysis (kNN-MFA) is a novel methodology unlike conventional 3D-QSAR regression methods, this methodology can handle non-linear relationships of molecular field descriptors with biological activity by which it leads to improved models resulting in better predictive ability.

Results and Discussion:

Molecular docking studies

26 compounds were docked into the crystal structure of PPARγ and the highest scoring pose was selected for each compound. The best docking poses are predicted to be the most stable conformation

of each compound for binding to the PPAR γ receptor active site. Fig. 1 indicates that amino acid residue Ser289 and Cys285 were formed hydrogen bond with carboxylic part of the compound 18. A compound 18 with highest docking score has shown highest activity among indene N-oxide derivatives probably because of morpholine substituent fully interact with phenyl ring of Tyr 327 amino acid residue.

SW-kNN MFA studies In the development of 3D-QSAR model stepwise (SW) variable selection method was used. The compounds of both, training and test set were aligned using the indene N-oxide template (Figure 1). Descriptor range for the selected model of the Series indicates that; phenyl alkyl substituent is essential for the effective binding with the hydrophobic pocket of the active site of receptor. The presence of electrostatic field with positive coefficient (E_1212) suggests that polar group must be favorable and forming H-bond with the head group of the active site. Moreover, acidic carboxylic moiety of the ligand also plays an important role in the ligand-receptor interactions. The descriptor S_4412 exhibiting range analogously negative suggests that steric descriptors are provided less contribution than the others. The positive hydrophobic potential is favorable for increase in activity and hence more hydrophobic substituent group is preferred in tail region. Among the indene N-oxide derivatives, compound 18 is most potent; reason could be an optimum mopholine alkyl group is substituted 3rd position of the indene N-oxide ring is fully accommodate the hydrophobic pocket of the receptor.

Conclusion:

In the present study, we have successfully established the use of computational approaches to identify indene N-oxide derivatives as PPARγ agonists. It was found that, SW-kNN-MFA 3D-QSAR model and the docking interactions between the agonists and the active site of PPARγ are complementary. Moreover, these models match well with the known features of the different parts of the PPARγ binding site and approved that the binding portion of the PPARγ receptor is essential for agonistic activity. These results provide crucial clues that the positive hydrophobic potential is favorable for increase in activity. Therefore hydrophobic groups might be suitable substituents for designing of PPARγ agonists.

References:

[1] Seidell JC (2000) Br J Nutr 1: S5-S8.

[2] Desvergne B & Wahli W (1999) Endo Rev 20: 649-688.

[3] Rathi L, Kashaw SK, Dixit A, Pandey G & Saxena AK (2004) Bioorg Med Chem 12: 63-69.

[4] Liao C, Xie A, Shi L, Zhou J & Lu X (2004) J Chem Inf Comp Sci 44: 230-238.

[5] Ahn JH, Shin MS, Jung SH, Kim JA, Kim HM, Kim SH, Kang SK & Kim SS (2007) Bioorg Med Chem Lett 17: 5239-5244.

PB-85

Designing of New Benzotriazole Analogs using Molecular Docking Studies Against Receptor 1EA1.Pdb & 1IYL.Pdb for Treatment of Fungal Infection

Mohammad Rizwan Khan[1], Love Kumar Soni[2], R.K. Nema[1], Neelam Balekar[3]
[1]Lakshmi Narain College of Pharamcy (RCP), Indore MP India 453331
[2]School of Pharmacy, DAVV, Indore MP India 452012
[3]IPS Academy College of Pharmacy, Rajendra Nagar, Indore MP India 452012

Abstract: Infectious diseases raise consciousness of our global liability and the aptitude of mankind to prepare medicinally significant molecules during the past century has lead to decrease in the mortality rate from numerous infectious diseases. Benzotriazole derivatives have showed activities like antibacterial, antifungal, antiviral, antiprotozoal and anthelmentic action. These investigations propose the option of emerging a lead compound of benzotriazole having a potential antifungal activity. Molecular docking simulation based virtual screening with ligand library having benzotriazole derivatives have been performed to identify possible lead molecules to inhibit 1EA1.pdb & 1IYL.pdb receptor for treatment of fungal infection. Further the docked conformation of ligand should be perfectly overlayed with the crystal structure of the downloaded protein. This testing is completed successful and the docked confirmation of the fluconazole is perfectly superimposed with reference structure of fluconazole used by the Autodock docking algorithm.

Introduction:

Infectious disease are of major concern now a days as the infectious diseases are responsible for more than 9.5 million deaths people around the world. Infectious diseases are also destructive to the health of adults, causing disability, a diminished quality of life, decreased productivity or death. Co-infection is also a matter of great concern as people infected with one infectious disease become more susceptible to other diseases. Examples include: HIV/AIDS co-infection with tuberculosis or malaria. Treatment of these infections with rapid development of resistance in organisms has added fuel to worsened situation.

Recent study showed that several benzotriazole and 1,2,4-triazole derivatives represented an interesting class of heterocycle and became the most rapidly expanding group of antifungal compounds with the advantage of low toxicity, high oral bioavailability and broad spectrum activity. Moreover, a variety of benzotriazoles have been reported to inhibit the growth of some microorganisms and some benzotriazole derivatives show anti-inflammatory properties. Benzotriazole moiety has distinct property on biological system as antifungal, antibacterial, diuretics etc. Benzotriazole, benzimidazole and Imidazole moiety and to screen their diverse activity like antifungal, anti-inflammatory and antimicrobial activity. Tuberculosis is also the top most cause for the death of HIV-infected individuals, as immune compromised HIV-infected individuals are highly prone to get infected with *Mycobacterium tuberculosis* bacteria [1]. Multi drug-resistant tuberculosis

(MDR-TB) plus resistance to a fluoroquinolone and at least one second-line injectable agent, such as amikacin, kanamycin and/or capreomycin, is called extensively drug-resistant TB (XDR-TB) [2-3].

Materials and Methods:

The compounds were designed on the basis of virtual screening and quantitative structure activity relationship studies [4]. The designed benzotriazole compounds was validated by molecular docking simulations using Molegro as docking tool on various receptor model like 1EA1.pdb and 1IYL.pdb obtained from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb).

Molecular docking simulations of designed compounds

All the calculations were carried out by using *Molegro* as docking tool. The visualization and other programs necessary for docking studies were performed out by means of *Pymol*, *Chimera*, *DS visualizer*, *MMP Plus*.

Docking of MTCYP51 complex

Docking studies was performed with crystal structure (1EA1 & 1IYL) of protein consisting of receptor associated with bound ligand for antifungal activity of the designed compounds.

Crystal structure of 1EA1 & 1IYL.pdb

The crystal structures of the protein consisting of receptor associated with bound ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1EA1.pdb & pdb id: 1IYL) registered in the Protein data bank was used (**Figure 1 & 2**).

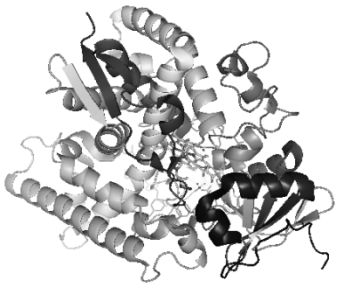


Figure 1: Crystal structure of 1EA1.pdb

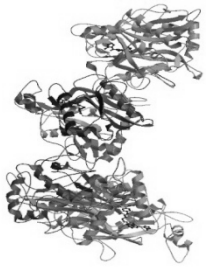


Figure 2: Crystal structure of 1IYL.pdb

Processing of Protein

Receptor Preparation**:** The X-ray crystal structures of (1EA1.pdb & pdb id: 1IYL) was obtained from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Chain A of 1IYL.pdb was selected for docking by parsing the protein structure in *Chimera* (UCSF, CA, USA).

Ligand: The ligand is separated from the receptors1EA1.pdb & 1IYL.pdb by the means of Chimera software than it was saved as pdb file and then this ligand was docked into receptors using the Molegro program.

Grid Box: The regions of interest used by AUTODOCK were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present

in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimension (Figure 5).

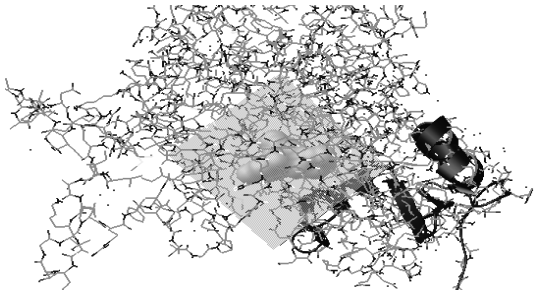
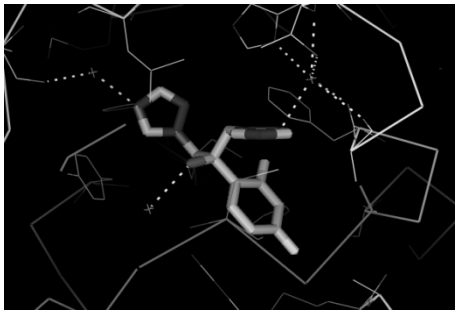


Figure 3: Grid box covering all active sites in receptor

Validation of Docking Process

Internal validation

To ensure that the ligand orientations and positions obtained from the docking studies represent valid and reasonable potential binding modes of the inhibitors, the docking methods and parameters used were validated by redocking the crystallized DCKA and overlaying the docked and crystallized DCKA chemical structures and calculating the rms value.



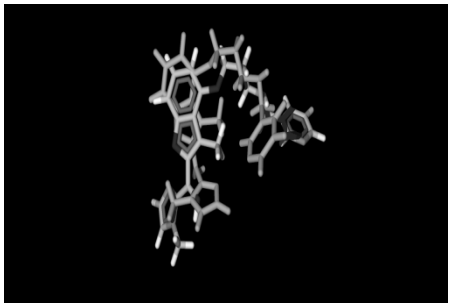


Figure 4: 1EA1- ligand interaction rcsb data base **Figure 5:** 1IYL.pdb- ligand interaction rcsb data base.

The internal method validation is performed to obtain the orientation of the ligand and fluconazole molecule. Further the designed molecules were validated by taking the overlay of the three molecules i.e. the ligand, fluconazole internal reference and the designed molecule to obtain orientation and position of the site of attachment to give maximum effect.

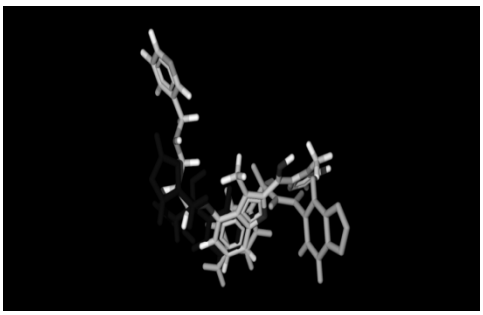


Figure 6: 1IYL.pdb- ligand, fluconazole & designed interaction rcsb data base.

Result & Discussion:

Docking Studies

To strengthen the designing of the compounds further the docking studies were performed of the designed compounds. For the docking studies the protein molecule selected for inhibition was 1EA1.pdb and 1IYL.pdb was into consideration. The docking studies were performed using Molgro Virtual Docker (MVD) software. All the compounds as well as fluconazole were docked into the active site of 14a-demethylase which was obtained from Protein Data Bank using molegro virtual docker. Docking score showed that these compounds docked to the active site of the enzyme comparable to fluconazole. All new azole compounds plus fluconazole were characterized by a docking mode in the active site of the cytochrome P450 14a-sterol demethylase.

Table 1: Docking studies of designed compounds.

Comp. No.	Benztriazole Analogs	1EA1.pdb		1IYL.pdb	
		MolDock Score	Rerank Score	MolDock Score	Rerank Score
MRK2	N-(4-chloro-2-nitrophenyl)-1H-benzo[d][1,2,3]triazole-5-carboxamide	-124.826	-102.933	-127.367	-104.427
MRK3	N-(2,4-dinitrophenyl)-1H-benzo[d][1,2,3]triazole-5-carboxamide	-132.387	-100.089	-130.291	-108.232
MRK7	N-(4-aminophenylsulfonyl)-1H-benzo[d][1,2,3]triazole-5-carboxamide	-115.437	-94.3645	-126.319	-107.959
MRK8	N-(2,6-dimethylphenyl)-1H-benzo[d][1,2,3]triazole-5-carboxamide	-119.713	-100.962	-119.246	-99.8017
MRK9	N-(4-nitrophenyl)-1H-benzo[d][1,2,3]triazole-5-carboxamide	-118.428	-96.2889	-122.037	-102.098
MRK11	N-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-chloro-4-nitrobenzenamine	-119.276	-97.7265	-121.333	-102.304
MRK12	N-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-4-chloro-2-nitrobenzenamine	-123.532	-97.9336	-126.359	-102.057
MRK13	N-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2,4-dinitrobenzenamine	-119.554	-96.4469	-130.782	-106.987
MRK19	N-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-4-nitrobenzenamine	-118.188	-95.8836	-130.981	-103.662

Conclusion:

There is some correlation between antifungal activity and docking energy. Thus for the compounds MRK02, MRK03, MRK07, MRK09, MRK11, MRK12 and MRK19 show potent growth inhibition and have good docking energy. All of these compounds are found to be active but docking studies shows that compound 3 and 19 are the most active compounds with better biological activity.

References:

[1] Zhang Y (2005) Annual Rev Pharmacol Toxicol 537: 38.

[2] Almatarneh MH, Altarawneh M, Poirier RA & Saraireh IA (2014) J Comp Sci 5: 568–575.

[3] Johnson DR & Lovas FJ (1972) Chem Phys Lett 15: 65–68.

[4] Hamada Y, Hashiguchi K, Tsuboi M, Koga Y & Kondo SJ (1984) J Mol Spectr 20:70–80.

PB-86

Synthesis and Antimicrobial Activity of 1,4-Dihydropyridine Derivative

Tamanna Narsinghani, Love Kumar Soni, Shikha Chourey
School of Pharmacy, Devi AhilyaVishwavidyalaya, Takshashila Campus, Khandwa Road, Indore
E-mail address: kashishnarsinghani@rediffmail.com

Abstract: A series of substituted 1,4-dihydopyridine derivatives (SC1-SC10) was synthesized via condensation of acetoacetanilide /4-chloro acetoacetanilide and substituted benzaldehyde in methanol with excess amount of ammonia. The synthesized compounds were characterized using FT-IR, NMR and Mass spectroscopic techniques.The anti-bacterial and anti-fungal activity of title compounds was evaluated utilizing paper disc diffusion method. The anti-bacterial activity was determined by using *S. aureus* and *E.coli as* the gram-positive and gram negative strains, while *Candida albicans* was used to evaluate the anti-fungal activity of synthesized compounds. 1,4-dihydropyridine derivative (SC8) with bromo group at para position of phenyl ring attached to dihydropyridine ring and chloro group linked to para position of carbamoyl phenyl ring was found to be the most active anti-bacterial agent, with its activity observed more on gram negative strain (81.76%) as compared to gram positive strain (75.94%). The most active anti-fungal agent was found to be SC1 (86.85%); 1,4-dihydropyridine derivative with hydroxy group at 2^{nd} position and bromo group at 5^{th} position of phenyl ring attached to dihydropyridine ring while chloro group linked to para position of carbamoyl phenyl ring. This suggests the requirement of electron withdrawing group at 3^{rd} and 5^{th} position of dihydropyridine ring for anti-bacterial and anti-fungal activity.

Introduction:

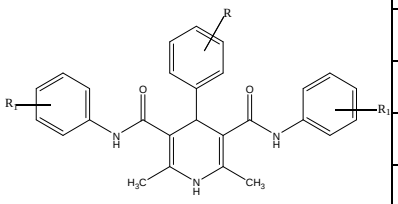
1, 4 Dihydropyridines are nitrogen containing heterocyclic compounds with nitrogen as the hetero-atom. This nucleus is associated with varied pharmacological activities, viz. anti-microbial, anti-tubercular, anti-cancer, antioxidant, vasodilator, calcium channel blocker, analgesic, anticonvulsant, anti-inflammatory, anti-ulcer activities [1]. Extensive literature survey revealed its potential for antimicrobial activity. The present work was thus focused to explore the anti-microbial activity of this nucleus. In this regard, it was planned to synthesize a series of 1,4dihydropyridine derivatives (Table 1) by utilizing different substituted aromatic aldehydes group with acetoacetanilide in presence of ammonia and methanol.

Material and Methods:

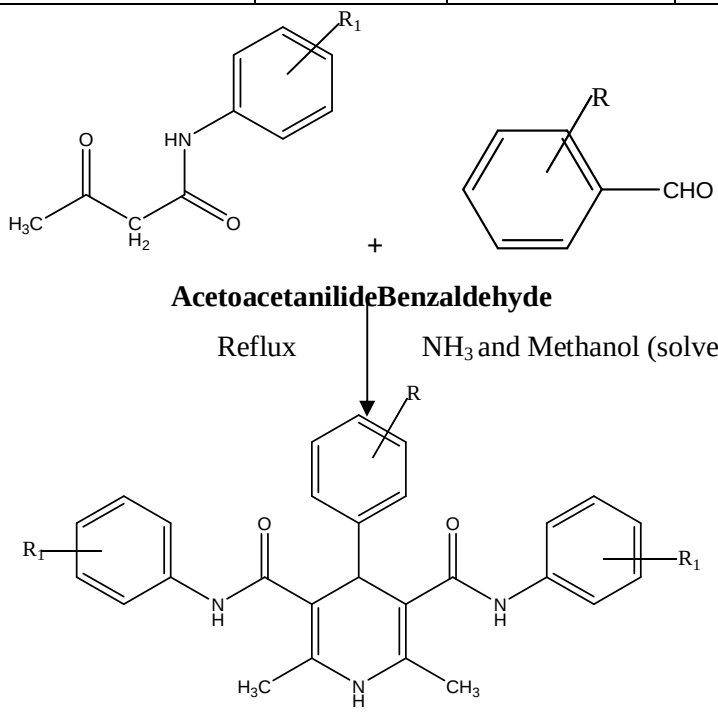
Synthesis A mixture of acetoacetanilide /4-chloro acetoacetanilide (0.02 mol) and substituted benzaldehyde (0.01 mol) was dissolved in methanol. 5-7 ml of ammonia (25%) solution was added, followed by refluxing for 5–6 h (Scheme 1). 2-3 ml ammonia was added at an interval of 3-4 h and the refluxing was continued for 24 h [2-3]. The reaction mixture was kept overnight and

the crystalline product was separated out.It was then filtered, washed 2–3 times with chilled methanol, dried and recrystallized using methanol. The synthesized compounds were characterized using FT-IR, NMR and Mass spectroscopic techniques.

Table 1: Substituted 1, 4 dihydropyridine derivatives



Comp. Code	R	R_1
SC1	2-OH, 5-Br	4-Cl
SC2	4-OH	H
SC3	3,4,5-trimethoxy	H
SC4	2-NO_2	4-Cl
SC5	3,4-dimethoxy	4-Cl
SC6	2-NO_2	H
SC7	3,4-dimethoxy	H
SC8	4-Br	4-Cl
SC9	4-NO_2	4-Cl
SC10	4-NO_2	H



1, 4-dihydropyridine derivatives

Scheme 1: Synthesis of 1, 4-dihydropyridines derivatives

Anti-bacterial activity assay The cultures of gram positive and gram negative strains were obtained in Mueller–Hinton Broth after incubating them at 37 ± 1°C for 18–24 h. The anti-bacterial activity

was performed on Mueller–Hinton Broth at pH 7.4 and twofold dilution technique was applied. The growth of microorganism was recorded to inhibition zone diameter expressed in percent of relative inhibition zone diameter after incubation for 18–24 h at 37 ± 1°C.Ciprofloxacin was used as standard .

Anti-fungal activity assay The yeasts were maintained in Sabouraud Dextrose Broth pH 7.4 after incubation for 48 h at 25 ± 1°C. Controls tubes contained only inoculated broth. The growth of microorganism was recorded to inhibition zone diameter expressed in percent of relative inhibition zone diameter after incubation for 48 h at 37 ± 1°C. Fluconazole was used as standard drug.

Calculation of percent of relative inhibition zone diameter The percent of relative inhibition zone diameter (% RIZD) is the calculation of percentage of relative inhibition zone obtained for control as compared to zone of inhibition obtained from standard drug at same concentration. The antimicrobial activity was calculated by applying the expression:

$$\% \text{ RIZD} = \frac{(IZD\ sample - IZD\ negative\ control)}{(IZD\ standard - IZD\ negative\ control)} \text{ X } 100\%$$

where RIZD is the percent of relative inhibition zone and IZD is the inhibition zone diameter (mm).

Results and Discussion:

The title compounds were synthesized via condensation of acetoacetanilide /4-chloro acetoacetanilide and substituted benzaldehyde in methanol. These newly synthesized compounds were assayed for their antimicrobial activity against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram negative bacteria) and the fungal strain *Candida albicans*.The antibacterial activity of compounds was determined by the paper disc diffusion method using Mueller-Hinton agar. Ciprofloxacin was used as the reference antibacterial agent. The antifungal activity of compounds was determined by the paper disc diffusion method using Sabouraud dextrose agar growth medium. Fluconazole was used as the reference antifungal agent. All synthesized compounds exhibited higher inhibitory activity against gram negative bacteria than gram positive bacteria (Table 2, Fig.1).

Staphylococcus aureus (gram positive) All the compounds were inactive towards *S. aureus* at minimum concentration of 6.25 µg/ml whereas the compounds showed a significant inhibitory activity towards *S. aureus*at concentration ranging from 12.5-50 µg/ml. Compound SC8 exhibited highest inhibitory activity. Following is the antibacterial efficacy of synthesized compounds against *S.aureus* in decreasing order - SC8 > SC1 > SC7 > SC10 > SC6 > SC3 > SC4 > SC9 > SC2 > SC5

E. coli (gram negative) All the compounds were inactive towards *E. coli* at minimum concentration of 6.25 µg/ml whereas the compounds showed a significant inhibitory activity towards *E. coli* at concentration range12.5, 25, 50,100 µg/ml, amongst all these compounds, SC8 (81.76%) showed highest percentage of relative zone of inhibition. Following is the antibacterial efficacy of synthesized compounds against *E. coli* in decreasing order

SC8 > SC3 > SC4 > SC10 > SC9 > SC5 > SC1 > SC2 > SC6 > SC7

Candida albicans (fungi) all the compounds were inactive towards *C. albicans*at minimum concentration of 6.25 µg/ml and 12.5 µg/ml. Whereas the compounds showed a significant inhibitory activity towards *C. albicans*at concentration range 25, 50,100 µg/ml.Amongst all these compounds, SC1 (86.85%) exhibited highest percentage of relative zone of inhibition (Table 3, Figure 1).

Table 2: Anti-bacterial activity of synthesized 1,4-dihydropyridine derivatives in terms of RIZD

Bact.strain	***Escherichia coli***					***Staphylococcus aureus***				
Conc. in (µg/ml)	**6.25**	**12.5**	**25**	**50**	**100**	**6.25**	**12.5**	**25**	**50**	**100**
Comp. no.	**Relative inhibition zone diameter in percentage**									
SC1	-	75.15	79.37	80.05	75.45	-	75.00	71.76	71.85	73.04
SC2	-	74.33	73.76	75.79	71.97	-	71.81	66.64	65.45	64.38
SC3	-	80.49	81.05	80.51	75.05	-	72.44	67.59	65.94	69.15
SC4	-	79.73	74.66	75.09	77.51	-	72.16	70.87	67.79	67.33
SC5	-	77.44	74.94	75.79	74.35	-	71.12	67.36	67.52	68.22
SC6	-	74.20	78.02	74.70	73.69	-	73.83	67.30	67.21	65.46
SC7	-	70.64	80.54	75.34	75.00	-	74.93	66.46	68.79	65.07
SC8	-	81.76	76.34	78.42	71.22	-	75.94	66.34	65.62	67.87
SC9	-	77.70	76.45	76.04	74.57	-	71.88	69.62	67.74	73.33
SC10	-	78.65	80.66	83.58	77.51	-	74.39	68.25	65.83	64.58

Table 3: Anti-fungal activity of synthesized 1,4-dihydropyridine derivatives in terms of RIZD

Fungal strain	***Candida albicans***				
Conc. in (µg/ml)	**6.25**	**12.5**	**25**	**50**	**100**
Comp. No.	**Relative inhibition zone diameter in percentage**				
SC1	-	-	86.85	78.63	79.53
SC2	-	-	74.98	77.27	78.62
SC3	-	-	79.31	78.38	75.85
SC4	-	-	82.82	84.57	82.76
SC5	-	-	81.70	80.15	75.56
SC6	-	-	76.92	79.77	76.30
SC7	-	-	80.58	76.61	77.38
SC8	-	-	75.35	79.73	78.00
SC9	-	-	74.98	77.37	78.62
SC10	-	-	82.00	79.77	76.36

Following is the antimicrobial efficacy of synthesized compounds against *C. albicans* in decreasing order: SC1 > SC4 > SC10 > SC5 > SC7 > SC3 > SC8 > SC2, SC9

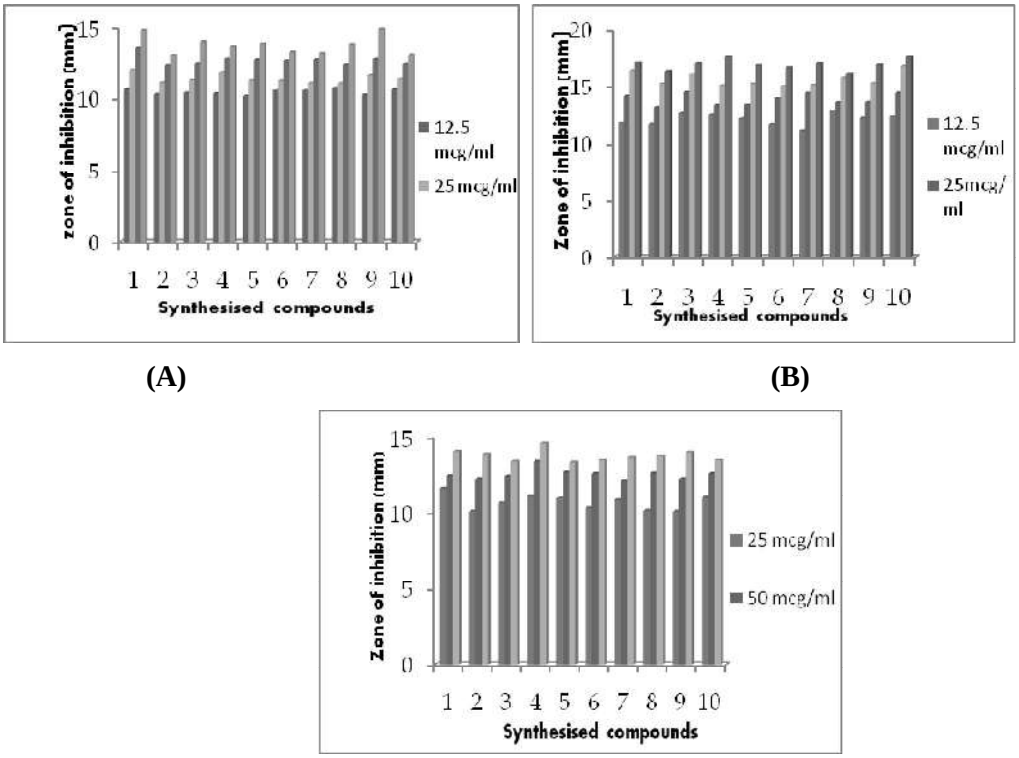


Figure 1: Antimicrobial activity of synthesized compounds against (A) *S. aureus* (B) *E. coli* (C) *C. albicans*

Conclusion:

1,4-dihydropyridine derivative (SC8) with bromo group at para position of phenyl ring attached to dihydropyridine ring and chloro group linked to para position of carbamoyl phenyl ring was found to be the most active anti-bacterial agent, with its activity observed more on gram negative strain (81.76%) as compared to gram positive strain (75.94%). The most active anti-fungal agent was found to be SC1 (86.85%); 1,4-dihydropyridine derivative with hydroxy group at 2nd position and bromo group at 5th position of phenyl ring attached to dihydropyridine ring while chloro group linked to para position of carbamoyl phenyl ring. This suggests the requirement of electron withdrawing group at 3rd and 5th position of dihydropyridine ring for anti-bacterial and anti-fungal activity.

References:

[1] Swarnalatha G, Prasanthi G, Sirisha N & Chetty CM (2011) Int J Chem Tech Res 3: 75-89.

[2] Desai B, Sureja D, Naliapara Y, Shah A & Saxena AK (2001) Bioorg Med Chem 9: 1993-1998**.**

[3] Sirisha K, Achaiah G & Reddy VM (2010) Arch Pharm Chem Life Sci 343: 342-352.

PB-87

Development and Validation of HPLC Method for Estimation of Brimonidine Tartrate as Bulk Drug and in Ophthalmic Formulation

S.S. Mandan, A.V. Nerpagar, U. D. Laddha,
MET's Institute of Nashik Shirpur, India (MH)
E-mail address: ssmandan@yahoo.com

Abstract: The optimized reverse phase high performance liquid chromatographic method was developed for estimation of Brimonidine Tartrate in bulk drug and pharmaceutical dosage form. Chromatography was performed on Kromasil C 18 (250 mm X 4.6 mm i.d. , 5 µm particle size) column with mobile phase citric acid monohydrate buffer:water:methanol (30:50:20 v/v/v) and pH 3 was maintained by using triethylamine. The flow rate was 1.0 ml/min. Elute was detected at 246 nm and it effectively separated at Retention Time of 5.96 min. The LOD and LOQ was 1.47 and 4.47 µg/ml respectively. A linear response was observed over the concentration range 40-80 µg/ml for Brimonidine Tartrate. Thus the proposed HPLC method was found accurate, specific, precise, robust and reproducible.

Introduction:

Brimonidine Tartrate is used in the treatment of open-angle glaucoma or ocular hypertension. It is selective alpha-2 adrenergic receptor agonist. Chemically it is 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate. No significant HPLC method reports were found for estimation of Brimonidine Tartrate in pharmaceutical formulation while few HPTLC, HPLC, LC-MS, HILIC (Hydrophilic interaction liquid chromatography) methods reported for the estimation of Brimonidine Tartrate in blood serum and in ocular fluids (Rahore, 2010, Phogat, 2011, Jiang, 2009, Sethi 2001).

The aim of study was to develope and validate simple, specific, sensitive, accurate and precise HPLC method for determination of Brimonidine Tartrate in ophthalmic formulation as per International Conference on Harmonization (ICH) guidelines (ICH, 2005).

Material and Methods:

Apparatus

A Shimadzu RP-HPLC instrument (LC -20 AD as Per 21 CFR) equipped with an photodiode array detector, manual injector with 20 µl loop, and Kromasil C18 column (250 mm × 4.6 mm id, 5 µm particle size) and LC- solution software was used. Contech CB-50 analytical balance and ultra sonic cleaner (Spetralab, UCB-40) were used during the study.

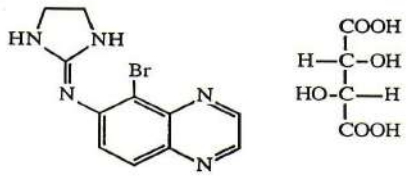


Figure 1: Structure of Brimonidine Tartrate

Reagents and materials

Brimonidine Tartrate was received as gift sample from Cipla Ltd., (Mumbai, Maharashtra). HPLC grade methanol (Qualigens), citric acid monohydrate buffer was of AR grade and water for RP-HPLC was prepared by double glass distillation and filtered through nylon membrane filter 0.45 µm (Pall Lab Sci).

Chromatographic conditions

Kromasil C18 Column (250 mm × 4.6 mm id, 5 µm particle size) was used at ambient temperature. The mobile phase consisted of citric acid monohydrate buffer: water: methanol (30: 50:20 v/v/v) and pH 3 was maintained by triethylamine. Flow rate was 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before used. The elution was monitored at 246 nm and injection volume was 20 µl.

Preparation of solutions

Citric Acid Buffer

Accurately weighed Citric acid monohydrate (1.05 gm) was transferred to a beaker (500 ml) and dissolved in double distilled water (500 ml).

The mobile phase was citric acid monohydrate, pH 3: water: methanol (30: 50:20)

Preparation of Standard Stock Solution

Standard stock solution of Brimonidine Tartrate was prepared by dissolving 10 mg of drug in10 ml of methanol to get the concentration of 1 mg/ml from which 1 ml was further diluted to 10 ml with methanol to obtain a working standard having a concentration of 100 µg/µl.

Determination of wavelength of maximum absorbance

The standard solutions of Brimonidine Tartrate were scanned in the range of 200-400 nm against buffer solution as a blank. Brimonidine Tartrate showed maximum absorbance at 246nm. So the wavelength selected for the determination of Brimonidine Tartrate was 246 nm.

Method Validation (ICH, 2005)

Calibration curve (Linearity)

Calibration curve was plotted over concentration range of 40-80 ug/ml for Brimonidine Tartrate. Accurately measured standard stock solution of Brimonidine Tartrate (4, 5, 6, 7 and 8 ml) was transferred to a series of 10 ml volumetric flask and volume in each flask was adjusted 10 ml with mobile phase. Resulting solution were injected into the column and the peak area obtained at flow rate

of 1.0 ml per minute for Brimonidine Tartrate. Calibration curve was constructed for Brimonidine Tartrate by plotting peak area versus concentration at 246 nm. Each reading was average of three determinations.

Accuracy (% Recovery)

To check the accuracy of the method, recovery studies were carried out by addition of formulation to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Chromatogram was obtained and the peak areas were noted. At each level of the amount, three determinations were carried out.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of Quantification (LOQ) for Brimonidine Tratrate was derived by calculating signal-to-noise ratio (S/N, i.e. 3.3 for LOD and 10 for LOQ) and using following equation as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the responses and

S = Slope of calibration curve.

Specificity and System Suitability

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix etc.

One blank and one standard preparation was injected and chromatograms were recorded which is further calculated for system suitability parameters.

Robustness

Robustness studies were carried out by examining the effect of small, deliberate variation of the analytical conditions on the peak areas of the drug. Factors varied were volume of mobile phase (± 3 ml), wavelength (±2 nm) and flow rate (± 0.2 ml/min). One factor at a time was changed to study the effect.

Intra-day and Inter-day precision

The intra-day precision was determined by analyzing standard solution of Brimonidine Tartrate at 60 ug/ml concentration for six replicates on the same day while inter-day precision was determind by analysing corresponding standard on two different days over a period of one week.

Result and Discussion:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of citric acid monohydrate buffer pH 3, methanol and water (30:20:50) at 1.0 ml/min flow rate. As it was shown in Figure 2, the optimum wavelength for detection was set at 246 nm at which much better detector responses for Brimonidine Tartrate was obtained. The retention time was 5.962 min as reported in Figure 3.

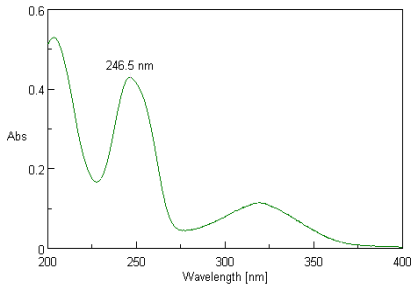


Figure 2: Maximum detection wavelength of Brimonidine Tartrate

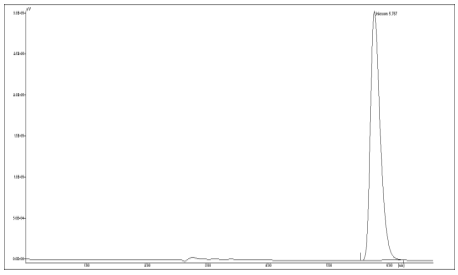


Figure 3: Typical RP- HPLC Chromatogram Brimonidine Tartrate (40 ug /ml) with corresponding retention time.

Chromatographic conditions are outlined in Table 1. The calibration graph for Brimonidine Tartrate was constructed by plotting the peak area versus their corresponding concentrations, good linearity was found over the range 40-80 µg/ml. The calibration graph is shown in Figure 4.

Table 1: Chromatographic conditions

Parameter	Observation
Mobile phase	0.01 Mol/L Citric acid monohydrate: Methanol : Water (30:20:50) pH 3 maintained by using triethylamine
Column	Kromasil – C 18 Column (250 mm × 6.5 mm × 5 um)
Flow rate	1 ml/min
Detection wavelength	246 nm
Injection volume	20 µl
Run time	10 Minutes
Retention time	5.96 mins

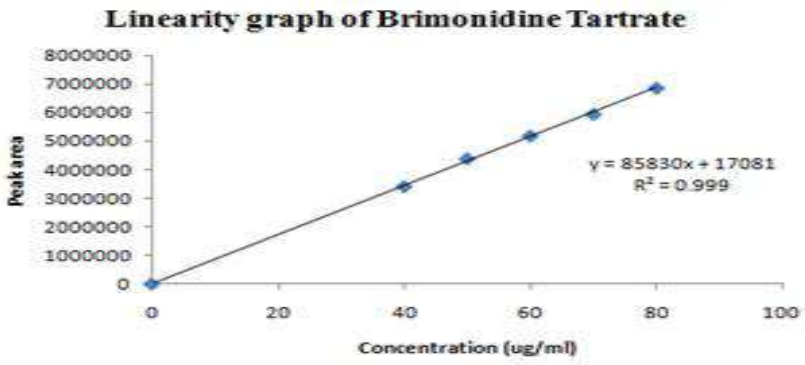


Figure 4: Calibration Curve of Brimonidine Tartrate

The proposed method has been applied to the assay of Brimonidine Tartrate in pharmaceutical dosage form. The results obtained indicate the additives present do not interfere with analysis of the studied formulation. System suitability test parameters for Brimonidine Tartrate for the RP-HPLC method are reported in Table 2. The optical and regression characteristics and validation parameters are reported in Table 3. Data of recovery study is shown in Table 4. The robustness study is reported in Table 5.

On the basis of series of investigation the optimized method can be routinely use for analysis purpose.

Table 2: System suitability testing of the HPLC method.

System suitability Parameters	Observation	Acceptance Criteria
Peak Area	3419885	-
Tailing Factor	1.49	NMT 2
Column Efficiency	5861	NLT 2500
% R.S.D.	0.7425	NMT 2

Table 3: The optical and regression characteristics and validation parameters of HPLC method for analysis of Brimonidine tartrate

Parameter	Observation
Calibration range	40-80 µg/ml
Detection limit	1.477 µg/ml
Quantitation limit	4.476 µg/ml
Slope	85830
Intercept	17081
Correlation coefficient	0.999
Intraday RSD, %	0.7425
Interday RSD, %	Day 1 -0.7425 Day 2- 0.4202

Table 4: Data of recovery study for Brimonidine Tartrate by HPLC method

Amount taken (µg/ml)	Amount added (µg/ml)	Amount found	% Recovery
25	20	45.17	100.39
25	25	49.48	98.96
25	30	54.18	98.52

Table 5: Data of robustness study for Brimonidine Tartrate by HPLC method

Experiment	% RSD	Theoretical plates	Tailing factor
(+) Wavelength	0.244	5713	1.50
(-) Wavelength	0.167	5992	1.49
(+)Flow rate	0.549	5760	1.40
(-) Flow rate	1.300	6091	1.61
(+) Mobile phase volume	0.303	7065	1.46
(-) Mobile phase volume	0.653	5041	1.51

References:

[1] Rathore K, Kumar R & Sisodia S (2010) Int J Pharm Sci Rev Res 3:23-29.

[2] Phogat A, Kumar M & Mahadevan N (2011) Int J Recent Adv Pharm Res 3:31-36.

[3] Jiang S, Chappa A & Proksch J (2009) J Chromatogr B Anal Technol Biomed Life Sci 3:107-114.

[4] Sethi PD (2001) High performance liquid chromatography, Quantitative analysis of pharmaceutical formulation, 1st edition: 38-70.

[5] ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1) (2005).

PB-88

Synthesis and Antimicrobial Screening of Some Imidazolidine derivatives of Isonicotinamide

Rakesh Patel[1], Pritesh Paliwal[1], Anil Bhandari[2]
[1] Indore Institute of Pharmacy, Indore (MP)
[2] Goenka College of Pharmacy, Lachhmangarh, Sikar (Raj) India
E-mail address: patelcip@gmail.com

Abstract: New series of *N* (2-alkyl/aryl-5-oxo-imidazolidine-1-yl) isonicotinamide derivatives were synthesized by the reaction of Schiff base with amino acetic acid in the presence of 1:4 dioxane. Synthesized compounds were evaluated for their Anti-bacterial activity against *Staphylococcus aureus* and *Echerichia coli*, Antifungal activity against *Candida Albicans* and Anti-tubercular activity against *Mycobacterium tuberculosis*. Synthesized compounds show significant activity against bacterial, fungal and mycobacterium strains. Their structures were established on the basis of elemental analysis, IR, ^{1}H NMR and Mass Spectral data.

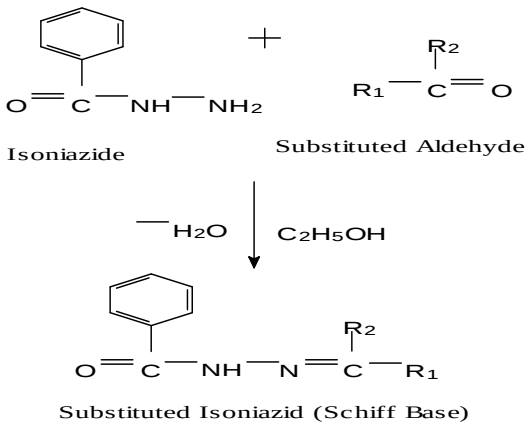
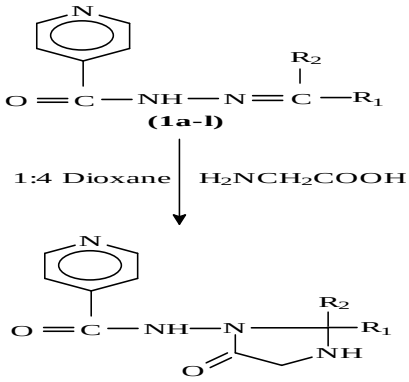
Introduction:

Antimicrobial agents act against microbial organisms and inhibit their growth and reproduction. Antibiotics and antimicrobial term used synonymously but there is a difference between both the terms. Antibiotic refers to substances produced by microorganisms that act against another microorganism where antimicrobial agent may be synthetic. Thus, antibiotics do not include antimicrobial substances that are synthetic (sulfonamides and quinolones), or semisynthetic (methicillin and amoxicillin), or those which come from plants (quercetin and alkaloids) or animals (lysozyme) [1]. Microbial infection is the major cause of death in the world, although deaths from bacterial and fungal infection have dropped currently. Natural, synthetic and semi synthetic antimicrobial agents have been used since a long time against the life threatening infectious diseases [2]. Although deaths from bacterial and fungal infection have dropped currently, still those are the major cause of death in the world. Over the few past decades the bacterial resistance to antibiotics, anti-fungal and anti-tuberculotic drugs has become one of the most challenging problem in the infections treatments. Tuberculosis (TB) is the world's oldest known infectious disease that kills three million deaths each year [3-4].

Material and Method:

All the chemicals used were purchased from E Merck, S D Fine and Loba Chem and were purified by established methods (whenever needed). Various Substituted Isoniazid (Schiff Base) derivatives were prepared according to the procedure outline in scheme-I. imidazolidine derivatives were synthesized by formation of imines (from Schiff base) and ketenes (from amino acetic acid) followed by cycloaddition of ketenes to imines, in the presence of 1:4 Dioxane, in a single step reaction. Melting points were determined by open capillary tube method and are uncorrected. Purity of synthesized

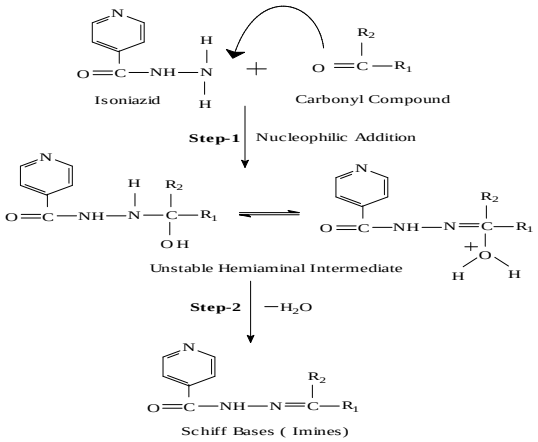
compounds was checked by TLC plates (Silica Gel G) and visualized by iodine vapor. The infra red absorption spectra of the synthesized compounds were recorded using KBr disc on FTIR 8010 Shimadzu model. 1H NMR spectra were recorded on Brucker Spectrospin DPX 300 spectrophotometer. Mass spectra were recorded on Jeol SR-102 FAB Mass spectrometer. CHN analyses of synthesized compounds were done on Perkin-Elmer-240 analyzer.

1(a-f)	N (2-alkyl/aryl-5-oxo-imidazolidine-1-yl) isonicotinamide (2a-f)
	
Part A: Synthesis of various Schiff Bases by the reaction of isoniazid with substituted aldehydes	**Part B:** Synthesis of N-(5-oxo-2-alkyl/aryl imidazolidine-1-yl) isonicotinamide derivatives

Mechanism of reactions involved in synthesis of titled compounds

Part A: It involves synthesis Schiff Bases by the reaction of isoniazid and substituted carbonyl compounds which proceeds in two steps; 1) Nucleophilic addition forming unstable hemiaminal intermediates and 2) Hemiaminal intermediate formation followed by dehydration to form Schiff Bases (imines).

Part B: This part of synthesis of titled compounds is attributed to synthesis of N-(5-oxo-2-alkyl/aryl-imidazolidine-1-yl) isonicotinamide derivatives, 2 (a-f)



The stepwise reaction mechanism is as follows:

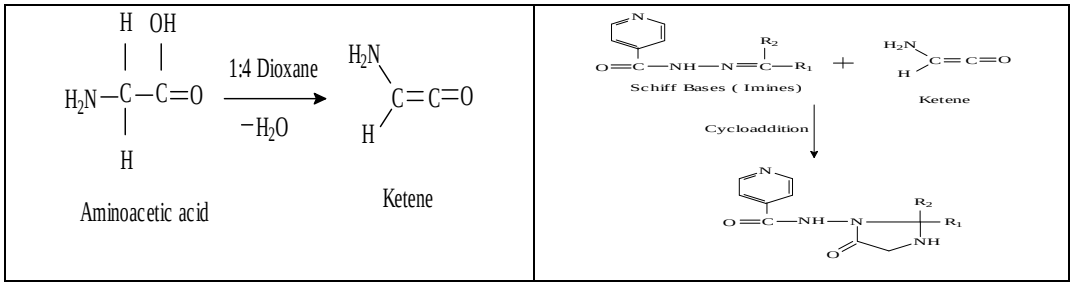
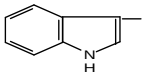


Table 1: Physical Characteristics of synthesized compounds:

Comp.	R_1	R_2	Molecular Weight	Yield (%)	Melting Point (°C)	Rf Value
2a	CH_3	C_6H_5	296.32	1.95gm (65.88%)	215-7	0.7
2b	C_6H_5	C_6H_5	358.39	2.46gm (68.72%)	242-4	0.7
2c		H	272.26	1.56gm (57.35%)	276-8	0.7
2d		H	283.29	1.74gm (61.48%)	275-7	0.5
2e		H	321.33	2.17gm (67.60%)	276-8	0.8
2f		H	288.33	1.83gm (63.54%)	244-6	0.8

General Procedure for Synthesis of substituted isoniazid, (Schiff Base) 1(a-f):

In a round bottomed flask, isoniazid (0.1mol), substituted aldehyde (0.1mol) and ethanol (30-35 ml) was taken and refluxed for three hours. The solution was cooled at room temperature and allowed to stand for 5 hours. Solid product was separated out, filtered, washed with ice cooled distilled water, dried and recrystalised with ethanol.

General procedure for synthesis of N-(2-mehtyl-5-oxo-imidazolidine-1-yl)isonicotinamide, 2(a-f)

0.01 mol of substituted isoniazid 1(a-f) (Schiff Base) and amino acetic acid (0.75gm, 0.01 mol) was dissolved in 1:4 dioxane (25ml) with constant stirring. The content was transferred to round bottom flask and heated under reflux for 5 hours. The mixture was allowed to cool at room temperature. The solid product was filtered, washed with ice cold water, dried and recrystalised from ethanol.

Compound 2a: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3040 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1420 (CH_3), 1340 (C-NH imidazolidine). H^1 NMR (DMSO-d_6 δ ppm): 9.6 (m, 1H NH cyclic), 8.1 (s, 1H, NH amide), 7.6-7.8 (m, 4H, CH pyridine), 7.2 (m, 5H, aromatic H), 3.3 (d, 2H, -CH_2-aromatic), 2.2 (s, 3H, CH_3). Mass Peaks: 296.7 (M^+), 219.5, 204.7, 121.1, 84.7, 78.1. Elemental

analysis% found: C-64.85%, H-5.44 %, N-17.69%

Compound 2b: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3050 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1340 (C-NH imidazolidine). H^1 NMR (DMSO-d_6 δ ppm): 9.6 (m, 1H NH cyclic), 8.1 (s, 1H, NH amide), 7.6-7.8 (m, 4H, CH pyridine), 6.9-7.6 Mass Peaks: 358.7 (M^+), 281.5, 204.7, 121.1, 84.7, 78.1. Elemental analysis% found: C-70.31%, H5.06 %, N-15.62%

Compound 2c: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3050 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1420 (CH_3), 1340 (C-NH imidazolidine), 1310 (cyclic C-O Stre). H^1 NMR (DMSO-d_6 δ ppm):9.6 (1H NH cyclic), 8.1 (1H, NH amide), 7.6-7.8 (4H, CH pyridine), 5.8 (3H furfural), 3.3 (2H, -CH_2 - aromatic), 2.5 (1H, aromatic –CH-). Mass Peaks: 272.3 (M^+), 205.8, 121.1, 84.7, 78.1. Elemental analysis% found: C-57.35%, H 4.44 %, N-20.58%

Compound 2d: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3050 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1420 (CH_3), 1340 (C-NH imidazolidine). H^1 NMR (DMSO-d_6 δ ppm) 9.6 (1H NH cyclic), 8.1 (1H, NH amide), 7.6-7.8 (8H, CH pyridine), 3.3 (2H, -CH_2 - aromatic), 2.5 (1H, aromatic –CH-). Mass Peaks: 283.4 (M^+), 205.8, 121.1, 84.7, 78.1. Elemental analysis% found: C-59.36%, H 4.63 %, N-24.72%

Compound 2e: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3050 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1420 (CH_3), 1340 (C-NH imidazolidine), 790 (N-H wag). H^1 NMR (DMSO-d_6 δ ppm) 9.6 (1H NH cyclic), 8.1 (1H, NH amide), 7.7-7.8 (4H, CH pyridine), 7.6 (4H, CH benzene), 7.2 (1H NH indole), 6.4 (1H, CH pyrole), 3.3 (2H, -CH_2 - aromatic), 2.5 (1H, aromatic –CH-). Mass Peaks: 321.2 (M^+), 205.7, 121.1, 84.7, 78.1. Elemental analysis% found: C-63.54%, H 4.71%, N-21.79%

Compound 2f: IR (KBr, Cm^{-1}) 3390 (N-H Stre. Secondary Amide), 3350 (N-H Stre imidazolidine), 3050 (Aromatic -C-H Stre.), 1660 (C-N Stre pyridine ring), 1610 (acyclic C=O stre.), 1490 (CH_2 bend.), 1420 (CH_3), 1350 (C-NH imidazolidine), 1310 (C-S Stre). H^1 NMR (DMSO-d_6 δ ppm) 9.6 (1H NH cyclic), 8.1 (1H, NH amide), 7.7-7.8 (4H, CH pyridine), 7.2-7.3 (3H, CH thiophene), 3.3 (2H, -CH_2 - aromatic), 2.5 (1H, aromatic –CH-). Mass Peaks: 288.5 (M^+), 205.8, 121.1, 84.7, 78.1. Elemental analysis% found: C-54.15%, H 4.20%, N-19.43%

Antimicrobial Activity All the synthesized compounds were evaluated for their invitro antimicrobial activity against gram positive bacteria *staphylococcus aureus* (ATCC-24392), the gram negative bacteria *Echerichia coli* (ATCC-24391) in nutrient agar media, fungi *C Albicans* (ATCC-436) in sabouraud dextrose medium and *mycobacterium tuberculosis* (ATTC-27286) in tween-albumin medium. The zone of inhibition values were determined and compared with well known (standard) antibacterial (Ofloxacin), antifungal (Ketoconazole) and antituberculotic (Isoniazid) drugs. Table: 2 shows data obtained from the biological screening of synthesized compounds and reference drugs.

Table 2: Antimicrobial screening data of compounds 2a-2f.

Compounds	Zone of Inhibition (in mm) at concentration of 20 µg/mL)			
	S. aureus	***E. Coli***	***C. Albicans***	***M. tuberculosis***
2a	17	19	23	28
2b	18	19	24	31
2c	19	20	32	32
2d	23	22	28	29
2e	25	27	31	34
2f	19	19	27	31
Ofloxacin	17	19	-----	-----
Ketoconazole	-----	-----	25	-----
Isoniazid	-----	-----	-----	28

Results and Discussion:

Yield of synthesized compounds were found to be satisfactory. The purity of synthesized compounds and completion of reactions were checked by TLC on silica Gel G plates in the solvent system methyl chloride: methanol (8:2 v/v) and visualized spots in iodine vapor. Proposed structures were confirmed by Spectral and microanalysis data. IR spectra showed presences of various functional groups that were further supported by the H^1 NMR and Mass spectral data. Furthermore elemental analysis data were also found in agreement with calculated values from proposed structures. Antibacterial, antifungal and anti tuberculotic screening data of synthesized compounds showed good to moderate activity as compared to reference drug. Compound 2c and 2d showed moderate and 2b, and 2e showed good activity against tested strains. Amongst these compounds 2e showed best antibacterial, antifungal and anti tuberculotic activity against all strains. The antimicrobial potency of synthesized compounds is due the presence of pharmacological active isonicotinamide moiety and increased by the addition of imidazolidine moiety.

Conclusion:

On the basis of above research work; the results and discussion showed that the synthesized compounds showed good antimicrobial activity as compared to reference antimicrobial drugs. These results concluded the need of development of such type of compounds in future for the progress of drug synthesis area.

References:

[1] Patel R & Bhandari A (2016) Der Pharmacia Sinica 7: 28-33.

[2] Patel R & Bhandari A (2016) Der Chemica Sinica 7: 8-13.

[3] Patel R & Bhandari A (2014) Asian J Res Chem 7: 950-953.

[4] Patel R & Bhandari A (2014) Am J Adv Drug Delivery 2: 104-109.

[5] Ramuz H (1981) U S Patent No. 4244957.

PB-100

Docking, Synthesis and Evaluation of Novel Derivatives of Substituted Chalcones as Antihyperglycemic Agents

Ankit Jain, D.K. Jain
IPS Academy College of Pharmacy, Indore-452012 (M.P.) India
E-mail address:ankkitjain@hotmail.com

Abstract: Various substituted chalcone derivatives (4A-4E) were synthesized. The structures of these compounds were established by spectral (IR, ^{1}H-NMR, Mass) analysis. The synthesized compounds were screened for their antihyperglycemic activity. Compound 4-C (2-(3-(4-methoxyphenyl)-1H-pyrazol-5-yl) phenol) was found to possess potent antihyperglycemic activity.

Introduction:

Diabetes mellitus (DM) is a heterogeneous metabolic disorder that is characterized by high levels of blood glucose with disturbances of carbohydrate, lipid, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Various heterocyclic derivatives of substituted chalcone compounds possess différent biological activities such as Protein Tyrosine Phosphatases (PTP)-1B inhibitory, antifungal, anti-inflammatory, anticonvulsant, antitubercular and immunomodulatory activity. The deregulation of PTP activity contributes to the pathogenesis of several human diseases, including cancer, diabetes, and immune disorders [1–3]. PTP1B plays an important role in down-regulating insulin signaling cascades via tyrosine dephosphorylation of the insulin receptor, which renders it inactive, or dephosphorylation of insulin receptor substrates 1 and 2, which inhibits their interactions with downstream signaling molecules (Figure 1) [2-5]. The present investigation involves docking, synthesis and evaluation of novel derivatives of substituted chalcone *via* cyclization of substituted chalcone and their screening for antihyperglycemic activity.

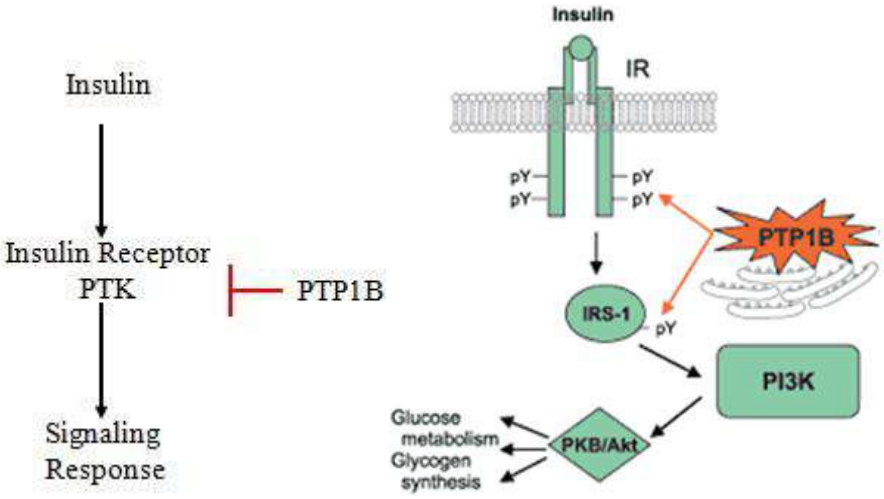


Figure 1: Role of PTP1B in hyperglycemia.

Materials and Methods:

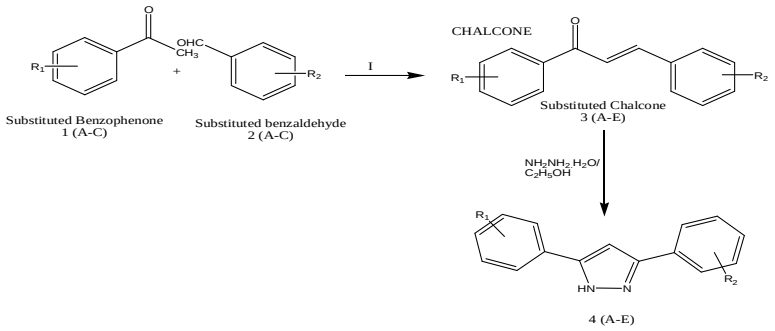
Melting points were determined on a capillary melting point apparatus (Lab Hosp). IR spectra were determined with a Thermo-Electron FT-IR spectrophotometer within range 400-4000 cm^{-1}. ^1H NMR spectra were recorded on a Bruker's AVANCE-III 400MHz FT NMR spectrometers. The mass spectra were measured on a Bruker microTOF QII mass spectrometer coupled to waters acquity LC system.

General Procedure for Synthesis of Substituted Chalcones: 3 (A-E)

A solution of sodium hydroxide (30%) in water and rectified spirit (100 mL) was continuously stirred in mechanical stirrer under ice cooled condition. Substituted acetophenone (0.015 mol) followed by substituted benzaldehydes (0.025 mol) was continuously added. The mixture stirred until it became thick enough (Approx. 6 hr). The reaction mixture was kept at 8^oC overnight. The product was filtered and recrystallized from ethanol.

General Procedure for Synthesis of Heterocyclic Derivatives of Substituted Chalcones: 4 (A-E)

A mixture of substituted Chalcones 4 (A-E) (0.01 mol) and hydroxylamine hydrochloride (0.02 mol) in ethanol (50 mL) was refluxed for 6 hrs on a water bath followed with addition of ice cold water at room temperature. The mixture was kept overnight at 8^oC. The precipitates were filtered, washed with distilled water and dried. The product was recrystallized with ethanol to obtain the final product 4 (A-E).



Scheme 1. Synthesis of proposed compounds.

Table 1. Details of synthesized compounds.

S. No.	Compound code	R1	R2
1.	4A	4-OH	4-OMe
2.	4B	3-OH	4-OMe
3.	4C	2-OH	4-OMe
4.	4D	4-OH	3,4-Dimethoxy
5.	4E	4-OH	3,4-Methylenedioxy

Table 2. Spectral analysis of the synthesized compounds.

Compound code	Mol. formula	Mol. weight	M.P. (ºC)*	IR spectra (cm^{-1})	Mass Spectra (Molecular ion peak)	^{1}H NMR Spectra (δ) in ppm
4 A	$C_{16}H_{14}N_2O_2$	266.29	170-175ºC	3246 (O-H str.), 3236 (aromatic N-H str.), 3229 (C-H str.), 3022 (C-H str. aliphatic), 1522 (C=C str.), 1420 (C=N str. aromatic), 820 (C-H bend, aromatic).	268	7.5 (r, 4H, aromatic ring), 6.7-7.37 (m, 4CH, aromatic ring), 5.0 (d, COH aromatic), 2.87-3.7 (s, OCH3), 13.7 (r, NH).
4 B	$C_{16}H_{14}N_2O_2$	266.29	160-165ºC	3246 (O-H str.), 3236 (aromatic N-H str.), 3229 (C-H str.), 3025 (C-H str. aliphatic), 1690 (C=C str.), 1425 (C=N str. aromatic), 901 (C-H bend).	268	7.6 (r, 4H, aromatic ring), 6.8-7.37 (m, 4CH, aromatic ring), 5.0 (d, COH aromatic), 2.87-3.7 (s, OCH3), 13.7 (r, NH).
4 C	$C_{16}H_{14}N_2O_2$	266.29	161-166ºC	3246 (O-H str.), 3236 (aromatic N-H str.), 3229 (C-H str.), 3025 (C-H str. aromatic), 1416 (C=N str. aromatic), 1352 (C=C str.), 910 (C-H bend).	267.80	7.6 (r, 4H, aromatic ring), 6.79-7.37(m, 4CH, aromatic ring), 5.0(d, COH aromatic), 2.87-3.7(s, OCH3), 13.7 (r, NH).
4 D	$C_{17}H_{16}N_2O_3$	296.32	180-185ºC	3082 (O-H str.), 3232 (aromatic C-H str.), 1609 (N-H bend), 1520 (C=C str.), 1120 (C-O-C str. asymmetric), 910 (C-H bend), 876 (C-H bend).	294.12	7.6 (r, 4H, aromatic ring), 6.79-7.31 (m, 4CH, aromatic ring), 5.0(d, COH aromatic), 2.87-3.7 (s, 2OCH3), 13.7 (r, NH).
4 E	$C_{16}H_{12}N_2O_3$	280.28	166-171ºC	3082 (O-H str.), 3232 (aromatic C-H str.), 1609(N-H bend), 1520 (C=C str.), 1495 (C-O-C str. asymmetric), 947(C-H bend), 875 (C-H bend).	282.11	7.6 (r, 4H, aromatic ring), 6.79-7.31 (m, 4CH, aromatic ring), 5.0 (d, COH aromatic), 5.9 (d, CH2 in ring), 13.7 (r, NH).

*uncorrected

Biological Activity:

All the synthesized compounds 4 (A-E) were screened for their antihyperglycemic activities by Sucrose Loaded Diabetic model using albino mice. Fasting blood glucose level of each animal was checked by glucometer using glucostrips (ACCU-CHEK) after 16 h starvation. Animals showing

blood glucose level between 60 to 80 mg/dl at 0 min were finally selected and divided into groups of five animals in each. Mice of experimental group were administered the suspension of the test sample orally prepared in 0.1 % CMC at desired dose levels i.e. 100 mg/kg body weight of compounds and standard antidiabetic drug i.e. glibenclamide. Animals of control group were given an equal amount of 0.1 % CMC. An oral sucrose load of 10 g/kg body weight was always given to each animal exactly after 30 min post administration of the test sample/ vehicle. Blood glucose profile of each mice was again determined at 30, 60, 90 and 120 min post administration of sucrose.

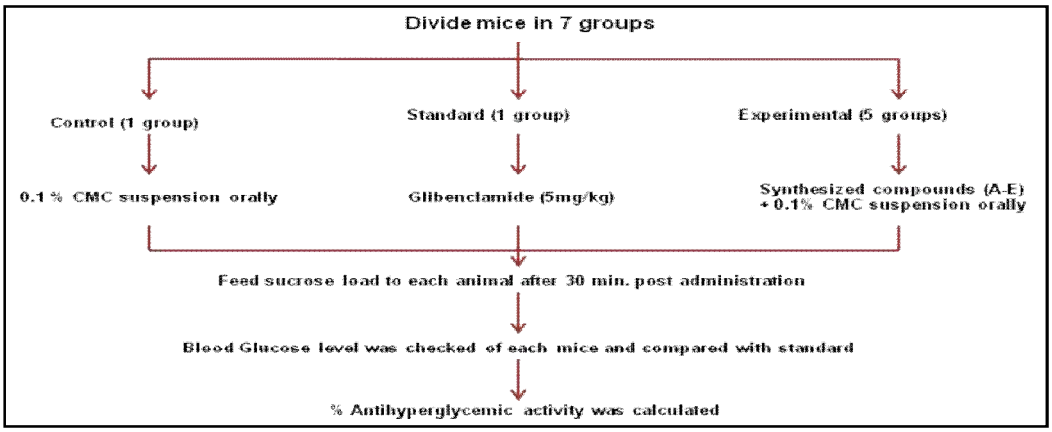


Figure 2. Flow chart for antihyperglycemic activity using "Sucrose Loaded Diabetic Model".

Molecular docking

The protein structure (PDB ID: 2QBP) downloaded from protein data bank was used without any modification. In view of biological activity of pyrrazoles the potential of some substituted Pyrrazoles for PTP-1B inhibition was studied using molecular docking. Some of the designed compounds were docked in the active site of PTP-1B using Python prescription (PyRx). Among these compounds 4-C (2-(3-(4-methoxyphenyl)-1H-pyrazol-5-yl) phenol) was found to have decent binding free energy (-8.4 KJ/Mol), thus this compound can be considered as a hit for development of PTP-1B inhibitors.

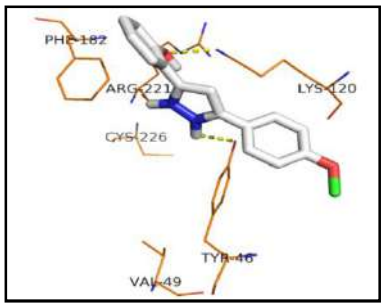


Figure 3. PTB 1B binding sites.

Results and Discussion:

The synthesis of substituted chalcone derivatives 4(A-E) is outlined in Scheme 1. All the synthesized substituted chalcone derivatives 4(A-E) was characterized by 1H NMR, IR and Mass spectral data. In general the IR spectral data of all the substituted chalcone derivatives 4(A-E) indicated the presence

of distinctive functional groups. The mass spectra of compounds showed (M+1) peaks, is in agreement with their molecular formula. The 1H NMR data for the chalcone derivatives 4(A-E) were also is in agreement with the assigned structures. All the compounds were evaluated for antihyperglycemic activity by observing their fall in blood glucose level towards sucrose loaded diabetic model. Pharmacological data of the compounds have been given in table. Docking scores predict that all the synthesized compounds 4(A-E) will show slight difference in activity with 4C having highest activity. These docking results are in accordance with the results of in vivo activity.

Table 3. Result of i*n vivo* Antihyperglycemic activity of synthesized derivatives

S. No.	Compounds	Percentage Antihyperglycemic Activity
1.	4-A	32.5
2.	4-B	34.0
3	4-C*	70.0
4.	4-D	49.2
5.	4-E	34.8
6.	Glibenclamide (Standard)	68.0

Data presented as % anti-hyperglycemic effect on comparison with standard group.

*indicates most potent compound.

Conclusion:

From the results of *in-vivo* antihyperglycemic activity, it is concluded that these molecules can be designed as a potential drugs. Among all the synthesized compound 4-C exhibited remarkable anti hyperglycemic effect which are also, supported by docking study. Therefore results of study suggested that the heterocyclic derivatives of substituted chalcone may be exploited as commercial anti hyperglycemic agents.

References:

[1] Danish A, Mohd IK, Gaurav K, Subhadeep R, Swetlana G, Manjari S, Uma D & Shubhini S (2017) Beni-Seuf Univ J Appl 6: 269–284.

[2] Prasanna AD & Sonali RJ (2015) Int J Med Chem 67: 10.

[3] Sureshbabu D (2013) Eur J Med Chem 63: 347–377.

[4] Cheng AY & Fantus IG (2005) CMAJ 172(2): 213-26.

[5] Satyanarayana M, Priti T, Tripathi K, Srivastava A K & Pratap R (2004) Bioorg Med Chem 12: 883.

PB-101

Molecular Modeling Studies of *N, N* -Disubstituted Derivatives as Potent Urease Inhibitors

Sheetal Gupta, Amritlal Bajaj
School of Chemical Science, Devi Ahilya Vishvavidyalaya, Takshashila Campus, Khandwa Road, Indore.
E-mail address: sheetalgb2b@gmail.com

Abstract: The discovery of *Helicobacter pylori* in the human stomach by Professor W. Jaworski has encouraged many scientists to study these bacteria, their characteristics, and their effect on human health. In 1982, Marshall and Warren showed experimentally that *H. pylori* infection is responsible for human gastritis. The pathogenic urease found in *H. pylori* causes infection not only in the stomach, but also in the duodenum, uterus, and urinary tract, among others. To determine the efficacy and potency of newly synthesized compounds in treating *H. pylori* infection, docking studies were performed with the 1E9y protein. During these studies, the Arg338, Ala365, Asn168, and Hie221 amino acids were observed to actively participate in bond formation with the studied compounds. In this series of compounds, only the R2 –substituted group is responsible for the observed biological activity. In this study, the results have been revaluated with MD simulations, in which the potency of the compounds was assessed by the percent contribution of hydrogen bonds at the end of the simulation. To calculate the free energy, the present study employs MMGBSA and finds the deviation of the RMSD value.

Introduction:

Helicobacter pylori bacteria are gram-negative, spiral/helix-shaped, and [1] micro-aerophilic [2]. *H. pylori*, both genotypically and phenotypically, are highly heterogeneous bacteria that can survive in highly adverse environments [3]. In 1980, Marshall and Warren [4] discovered that *H. pylori* infection is the primary cause of gastric cancer, a discovery for which both scientists were awarded the Nobel Prize in Physiology or Medicine. The author, motivated by this discovery, has become interested in studying the carcinogenic properties of *H. pylori*. In addition to gastric cancer, *H. pylori* have been found to cause gastritis, peptic ulcers, and duodenal ulcers.

N, N'-disubstituted thiourea, a basic and important derivative of thiourea, can be found in either white crystalline leaflets or grey powder. Due to the remarkable number of its potential derivatives, N, N'-disubstituted thiourea has become pharmacologically and industrially significant and continues to be an essential compound for future drug development.

To determine the pharmacological action of these compounds, each must go through the drug design process, of which molecular modelling is an essential step. Though it is the least time-consuming approach to molecular modelling, docking studies, falling within the scope of computational quantum chemistry, provide sufficient explanatory power for study needs and generate results (molecular visualization and graphics) that are easily interpreted. For the newly synthesized series, docking had

to be done initially to determine the protein-ligand binding pose, the orientation of the ligand on the protein binding site, the bonds that form between the protein and the ligand, the contributions to bond formation made by hydrophobic and polar regions of the active site. After for checking the results for accuracy, MD-simulations (advanced docking) were performed to determine the stability and consistency of each bond via its duration as determined from the snapshots. Although MD simulations are time-consuming and costly, their use remains widely accepted and suitable. As long as computer power and advanced quantum algorithms continue to improve, the future of computer-aided drug modelling seems to be very promising, though study should overcome the limitations of the simulations themselves. In particular, simulations are useful for finding the RMSD, RMSF, and Gyration Radii. Following the simulation, MMGBSA, an attractive approach used to revaluate the results obtained by virtual screening or docking, is used to calculate the binding free energy between the protein and the ligand.

Material and Methods:

Structures of compound of Reported series (Table 1) was draw using ChemSketch software, thereafter all structure were optimized. All proteins were downloaded from PDB database and processed with Schrodinger software. Docking was carried by using Extra Precision Glide module and then MMGBSA studies were performed. MD simulation was performed with Desmond software utility.

Results and Discussion:

Active site: Within each protein, there is a specific active space, called the binding site, which is responsible for attaching and fitting incoming ligands. Cytosolic urease, a Ni^{2+}-containing enzyme, is found in *H. pylori*. Structurally, each protein consists of a regular, precise, and systematic pattern of amino acids, forming by two chains, A and B, in which the α and β sub-units are present. In α sub-unit, there exists a catalytic cavity in which the two ligands Ni and HAE are found attached with a coordinated bond. The structural arrangement of ligands, both their geometry and orientation, makes the site suitable to bind incoming ligands.

Docking Studies: To determine the potency of the selected compounds and find the combinations that are most effective as urease inhibitors, three different crystalline proteins, 1E9Y, 4UBP, and 4AC7, were imported from PDB into the Schrödinger glide module. Once the proteins have been imported, the XP glide docking process is applied. Compounds are selected randomly, such that some have an experimental IC50 close to that of standard Thiourea while others have an experimental IC50 that differs significantly from the standard. Compounds 2, 24, 30, and 37 are selected for docking, which was performed with each of the three proteins mentioned above (Table 2). The docking score for each compound-protein pair was recorded and analysed. The first best dock score was determined by docking the compounds with 1E9Y. To ensure the accuracy of the results, the RMSD for each pair of compounds and proteins was calculated by superimposing the initial 3D structure of the ligand and

the docked pose. The average RMSD was then calculated, from which the minimum deviation was found to be when compounds were docked with 1E9Y. Thus, the 1E9Y protein was selected for further detailed study, from which the docking results for the reported series were found.

In Table 3, the XP glide score, docking score, interacting amino acids, and hydrogen bonds are shown. The three compounds with the best dock score (when docked with 1E9Y) and the best experimental IC50 have been selected for detailed analysis. Compound 24 presents the best glide score due to its impressive affinity, perfect bonding, and favourable orientation. The pyridine ring in compound 24 enters the active site cavity, in which ring's nitrogen atom forms a bond with the polar portion of the cavity. Nitrogen, [N···HN^+], makes a side chain H-bond with the Hie221 amino acid. Similarly, the ring makes [Pi- cation ···H^+-N^-] and [Pi-Pi stackingC···N-H] bonds with the Arg338 amino acid. Additionally, the NH group of thiourea forms a backbone hydrogen bond [NH···O=C^-] with Ala338, keeping the compound inside the cavity. The compound's phenyl ring was found to be oriented towards the mouth of the cavity, but no bond was formed.

In the case of compound 2, which has the second best dock score, the phenyl ring, representing the R^2-substituted group, enters the cavity. For this compound, only the phenyl ring participates in the formation of [Pi-cation···H^+-N^-] and [Pi-Pi stacking···C-N-H] bonds with Arg338. The ring could not fit into the cavity due to the inability of its chlorine group to form a bond. The NH group of thiourea also forms a backbone hydrogen bond [NH···O=C-] with Ala365.

Compound 6, which has the next best dock score, enters the cavity primarily vertically, though tilted slightly towards the inner side. The compound's meth-oxy-phenyl ring reaches deep within the cavity, in which the oxy- group makes a side chain hydrogen bond [O---N=C-] with Arg338 amino acids. The phenyl ring forms a [Pi cation···H^+-N^-] bond with Hie322, and the NH group of thiourea forms a backbone hydrogen bond [NH···O=C^-] with Arg168. The compound's second chloro-phenyl ring is oriented away from the cavity mouth, preventing it from participating in bond formation.

For compound 30 (Figure 1), which was found to have the best IC50, the complete pyridine ring reaches deep within the cavity and forms [Pi cation···H^+-N^-] and [Pi-Pi stacking···C-N-H] bonds with Arg338. As before, the NH group of thiourea also forms a backbone hydrogen bond [NH···O=C-] with Ala365. All bonds conveniently keep the compound in the cavity.

Simulation Studies: Over the past decade, computational methods have proven to be instrumental to the field of biochemical and drug modelling, allowing scientists to investigate protein-ligand companionship at the atomic level. In this sense, computational simulations have acted as a bridge between the microscopic world observed in the laboratory and the microscopic length and time scales provided computationally. Fundamentally, MD simulations are merely statistical mechanics, showings all protein-ligand contacts and their stability through an impressive pattern found on each snapshot.

Four compounds, 30, 24, 6, and 2, were selected for detailed study according to factors such as biological activity, docking score, and free energy. Figure 3 shows that the ligand-ligand RMSD for

each compound is 0.96, 1.61, 0.90 and 0.82, respectively, indicating that no more changes in orientation were observed after binding.

For compound 30 (Figure 2), the three bonds were formed primarily through the water bridge, in which only the nitrogen atom of the pyridine ring was seen to participate in bond formation. 80% of the bonds with His138 are preserved, 32% of the bonds with Asn168 are preserved, and 40% of the bonds with Ser363 are preserved. Although Ala365 and Arg338 are also seen to be participating in bond formation at the beginning of the docking simulation, these bonds had disappeared by the last snapshot.

Similarly, only one water bridge formed between compound 24 and Thr251, of which 35% was preserved. The nitrogen atom in the pyridine ring participated in bond formation. For compound 6, one hydrogen bond formed with Asn168 in the starting snapshot but disappeared before the final snapshot. In compound 2, at the beginning of the simulation, compound 2 formed two hydrogen bonds with Arg338 and Ala365. Later in the simulation, approximately half (57%) of the bonds with Ala169 were preserved.

In short, compound 30 was found to be the most biologically active and shows stable bond interactions throughout the simulation. To this end, the analysis presented in this study supports the experimental results. Compound 24 did not show biological activity but displayed the best result during docking. Upon re-examination via simulation, bonds were preserved up to 35%. This result shows that the bioactivity of compound 24 depends only on the behaviour of the hydrogen bond. If the R group is replaced by an activating or electron-donating group, the potency may be improved. Similarly, compound 6, which displays the minimum free binding energy in MMGBSA, did not preserve its potency for the duration of the simulation. This result is comparable to the manner in which compound 2 displayed the second-best dock score, but did not preserve efficacy through the end of the simulation.

Free Energy Studies: MMGBSA was carried out as a post-scoring approach for the reported series. The various energies derived in the course of this study are listed in Table 4 to this end; the present study provides a valuable correlation between the experimentally determined activity of a compound and the activity predicted via docking.

The calculated free binding energies of the N, N'-disubstituted thiourea compounds range from -4.7 to -30.83. According to the energies obtained from this analysis, the primary contributions to bond formation are made by the Van der Waals and non-polar solvation energies (ΔG_{solSA}). Conversely, the polar solvation (ΔG_{solGB}) and Coulomb energies oppose bond formation. The data show that compound 30 presents an MMGBSA term of -30.83k/Cal in addition to its excellent experimentally-obtained activity. Thus, there is a high correlation between the values obtained via different approaches. The polar solvation energy term, which strongly opposes bond formation, should positive

per the table. As such, the value obtained for compound 30 was 65.42 k/Cal; the overall range is from 21.63 to 65.42 k/cal.

As mentioned above, the Coulomb energy term also strongly opposes bond-formation. In this study, however, compound 24 was found to have a coulomb energy of -2.54 k/Cal despite its excellent docking scores. Contrarily, the experimental data show that this compound was not biologically active.

The other contributory energies, Van der Waals and bind lipo or Δ_{solSA}, were found to have energies that range from -27.7 k/Cal to -42.23 k/Cal, and -6.73 k/Cal to-14.90 k/Cal respectively.

Conclusion:

In this manuscript, the ligand-protein docking pose, their geometrical orientation, constituent bonding interactions, and binding free energy were discussed. Each method was performed separately, step by step, using different modules of Schrödinger and subsequently tested. MD simulations were used to determine many of the details that were seen in our initial studies, allowing for the examination of processes that experimental methods have been unable to study. In the field of drug modeling, further research regarding the development of advanced forms of quantum-virtual methods may help in the search for and discovery of novel, potent, and efficacious pharmaceutical agents.

References:

[1] Herrera V & Parsonnet J (2009) Clin Microbiol Infect 11: 971-6.
[2] Bury-Mone S, Kaakoush NO & Asencio C (2006) Helicobacter 4: 296-303.
[3] Marshall BJ & Warren JR (1984) Lancet 1: 1311-5.
[4] Parkin DM, Bray F, Ferlay J & Pisani P (2005) Cancer J Clin 2: 74-108.
[5] Khan KM & Naz F (2014) Euro Med Chem 74: 314-323.

PB-110

Elucidating the Structural Requirements of Novel Pazopanib Derivatives Towards Tyrosine Kinase Inhibitory Activity Through Classical Hansch and *De-Novo* Approach

Nidhi Gupta, Love Soni
School of Pharmacy, Devi Ahilya University, Khandwa Road, Indore, 452001(M.P.)
Email: gnidhi504@gmail.com

Abstract: Quantitative structure–activity relationship (QSAR) and Molecular modeling study have been performed on a series of novel Pazopanib derivatives by using the Mixed Hansch Fujita-Ban approach and employing AM1 calculations in docking study which give insight into the structural requirements of the derivatives towards inhibitory activity against VEGFR-2, PDGFR-α and c-kit tyrosine kinases enzymes. Evaluation of the predictive ability of the resulting models is carried out by using 'Leave-one-out' (LOO) method of cross validation. There is a remarkable agreement in the results of both the approaches. From the QSAR study it can be inferred that methyl substitution at 5^{th} position of the terminal benzene ring and hydrophobicity play a key role in kinase inhibitory activity. Potential binding sites were elucidated by docking study. Docking simulations was performed through Molegro Virtual Docker (MVD) for lead optimization of compound as protein tyrosine kinase inhibitors.

Introduction:

Cancer is the second leading cause of death in the western world. Despite advances in diagnosis and treatment, overall survival of patients still remains poor. This has improved survival in several types of solid tumors, but treatment-related toxicity and emergence of drug resistance have been the major cause of morbidity and mortality. Hence, there is an urgent need to develop newer more effective therapy to improve patient outcomes [1].

Tyrosine kinases (TKs) represent a major subclass of protein kinases, which play a pivotal role for intracellular signal transduction. Crucial cellular processes are regulated by TK signaling, such as adhesion, proliferation, migration, invasion, differentiation, metabolism, angiogenesis, survival and apoptotic cell death [2]. Tyrosine kinase is an enzyme that transfers a phosphate group from ATP to a tyrosine residue on specific cellular proteins. Many growth factors such as insulin, epidermal growth factor, and platelet-derived growth factor mediate their effects via receptor, switches on the kinase activity of catalytic domain. This signaling cascade is altered in cancer cell, which cause overexpression of TK receptors. TKs generally activate downstream target proteins through phosphorylation or provide binding sites for protein–protein interactions [3]. Pazopanib (Votrient, GW786034) is a novel multi-targeted receptor tyrosine kinase inhibitor, having both anti-proliferative and anti-angiogenic properties, targeting the vascular endothelial growth factor receptor (VEGFR-1, -2 and -3), platelet-derived growth factor receptor (PDGFR-α and -β), and c-kit. It was first approved

by Food Drug Administration (FDA) as an agent to treat metastatic renal cell carcinoma in 2009, and again approved by FDA in 2012 to treat soft tissue sarcoma. Clinical experience with Pazopanib demonstrates the advantages of broad-spectrum anticancer potency and less prone to resistance [4].

The aim of this study is to explore the structural requirements of pazopanib derivatives for inhibiting the tyrosine kinase enzyme activity by employing two – dimensional (2D) QSAR approach. Further docking study has been performed on this series by using Molegro Virtual Docker (MVD) 1.2 software. This study gives the idea for rational design of tyrosine kinase inhibitors which will exhibit greater therapeutic efficacy and safety.

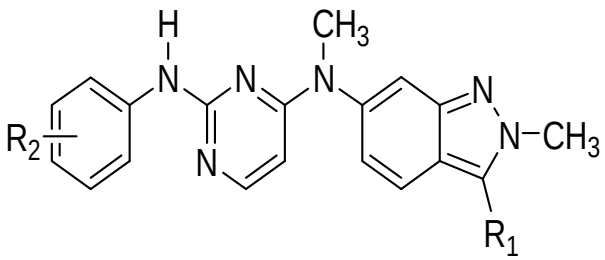
Materials and Methods:

Data Set and Molecular Modeling

Thirteen compounds were taken for the QSAR and docking study as tyrosine kinase inhibitors from the previously reported literature [5]. The biological activity data (IC_{50} in nM) was converted into negative logarithmic dose ($-\log IC_{50}$ or pIC_{50} in mole) to reduce the skewness of data taken as dependent variable, which exhibit the correlation with independent variable or descriptors listed in (table 1).

$$pIC_{50} = -\log_{10} IC_{50} \quad (1)$$

Where, IC_{50} is the nano molar concentration of the kinase inhibitors producing 50% inhibition. Initially series was subjected to QSAR analysis via De Novo contribution of substituent to the activity of molecule by employing Mixed Hansch Fujita-Ban approach. Here physicochemical descriptors and indicator variables which are used in Hansch-FB analysis. Molecular modeling study was carried out via Chem Office Ultra Version 8.0(Cambridge Soft.Corp.), Molegro virtual Docker (MVD) 1.2 and regression analysis study was done by using VALSTAT software. Sequential Multiple Linear regression (SMLR) analysis was used to generate the QSAR model. Validation is a crucial step in any QSAR modeling method. It is needed to establish the predictiveness of a model on unseen data and it helps to determine the complexity of an equation that the amount of data justifies. Model is validated both internally (least square fit R^2, Leave-one out-cross validation or q^2, γ randomization, boots-trapping r^2_{bs}) and externally (predictive ability of correlation coefficient r^2_{pred}).



7a-m

Table 1: Common Structure and Inhibitory activity data (IC_{50}) of pazopanib analogs as anticancer agents

S.No.	Compd.	R1	R2	IC_{50}(nM)			pIC_{50}(M)		
				VEGFR-2	PDGFR-α	C-Kit	VEGFR-2	PDGFR-α	C-Kit
1	7a	CH3	3-F	78	130	102	7.108	6.886	6.991
2	7b	CH3	3-Br	64	97	98	7.194	7.013	7.009
3	7c	CH3	3-Cl	25	85	80	7.602	7.071	7.097
4	7d	CH3	3OCH3	38	96	72	7.420	7.018	7.143
5	7	CH3	3-CH3	42	80	87	7.377	7.097	7.060
6	7f	CH3	3,5-DiMethyl	21	52	40	7.678	7.284	7.398
7	7g	CH3	4-SCH3	51	94	71	7.292	7.027	7.149
8	7h	CH3	4-OCF3	72	104	89	7.143	6.983	7.051
9	7i	H	3-F	93	140	96	7.032	6.854	7.018
10	7j	H	3-Cl	72	95	72	7.143	7.022	7.143
11	7k	H	3-OCH3	108	86	77	6.967	7.066	7.114
12	7l	H	4-OCF3	12	72	83	7.921	7.143	7.081
13	7m	H	3,5-DiMethyl	28	75	61	7.553	7.125	7.215

The docking study was performed to observe the interaction of all compounds with the receptor and to examine the agreement between the docking pattern and predictive activity of the validated pharmacophore. The docking study was carried out on tyrosine kinase enzyme using Molegro virtual docker (MVD) 1.2 software by 64 bit operating system under window 8 with an Intel ®Celeron® Processor N2840. Reported crystal structure of tyrosine kinase enzyme inhibitor was extracted from the Protein Data Bank (PDB Id: 1t46) (http://www.rcsb.org/pdb). Before docking, protein is prepared by using Protein preparation wizard, where water molecule and cofactors are removed from the proteins, optimizing the hydrogen bonds and deleting the ligands present in the crystal structure. For docking, ligand structures were prepared using Cambridge software and were subjected to energy minimization. The active site was generated using grid box. The lowest energy conformation was selected where RMS gradient reaches to 0.01kcal/mol and RMS distance to 0.1A^{o} and were also subjected to energy minimization.

Results and Discussion:

Generation of QSAR Model for Hansch-Fujita ban approach

Model-1 (for VEGFR-2 receptor)

BA= [7.527(± 0.247)] +HA [0.469(± 0.691)] +$\mathcal{R}$ [1.131(± 1.089)]

contribution of parameters to model is HA:$\mathcal{R}$::1:6.168……(1)

n=13, r=0.600, r^2=0.360, r^2adj=0.232, std=0.248, F=2.814

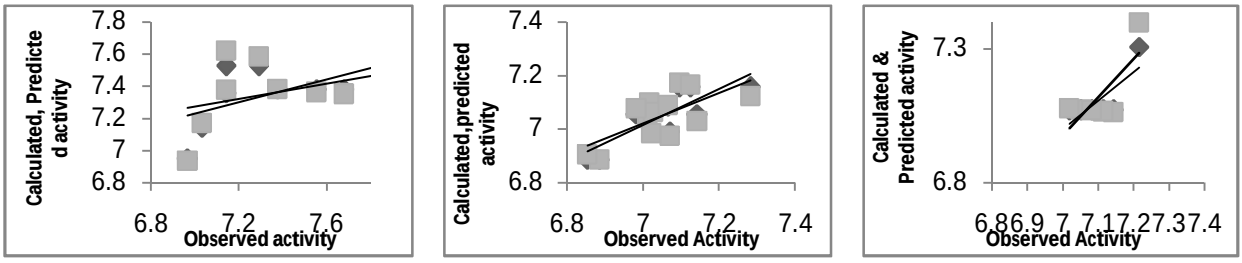
Model-2 (for PDGFR-α receptor)

BA= [7.036(± 0.084)] +MR [0.019(± 0.015)] - $\mathcal{F}$ [0.388(± 0.217)]

contribution of parameters to model is MR:$\mathcal{F}$::1.128:1……(2)

n=13, r=0.818, r^2=0.669, r^2adj=0.603, std=0.069, F=10.117, FIT=1.190,

Bivariant equations were generated for both VEGFR and PDGFR-α receptor, which mediate tyrosine-kinase inhibitiory activity. In model 1, difference between r^2_{adj} and r^2 is less than 0.3, which indicates that model is significant. Internal validity of the model is observed via q^2 or cross validated correlation-coefficient. Model also express S_{PRESS} and S_{DEP} activity. Model 1 suggested that hydrogen acceptor group should be present in the designed molecule, which contribute positively and favored towards the inhibition. Whereas in model 2, MR contribute positively and field effect contribute negatively to the activity, which suggests that less bulky group is optimum for the activity.



Model-1 **Model-2** **Model-3**

Figure 1: Plot between observed vs calculated and predicted activity of model-1,2,3

Model-3 (for c-Kit receptor)

BA= [7.071(± 0.047)] +R_4SMe [0.078(± 0.156)] +R_5C [0.236(± 0.115)]

Contribution of parameters to model is R_4S:R_5C::1:6.022……(3)

n=13, r=0.827, r^2=0.685, r^2adj=0.622, std=0.066, F=10.860

This model gives the idea about the substitution at 4 and 5 position in designing the analogues for c-kit receptor. According to the model, at 4^{th} position thiomethyl substitution and 5^{th} position methyl group should be present there for its inhibitory activity.

Table 2: Observed, calculated and predicted IC_{50} (by the LOO method) via Hansch-Fujita ban approach of pazopanib analogs as anticancer agents

Hansch-Fujita- ban analysis											
VEGFR-2 (Model-1)				PDGFR-α (Model-2)				c-Kit (Model-3)			
Compd.	Obs.	Calc.	Pred.$_{(Loo)}$	Compd.	Obs.	Calc.	Pred.$_{(Loo)}$	Compd.	Obs.	Calc.	Pred.$_{(Loo)}$
1	7.108	7.143	7.151	**1**	6.886	6.886	6.886	**1**	6.991	7.071	7.080
2	7.194	7.335	7.348	**2**	7.013	7.033	7.042	**2**	7.009	7.071	7.078
3	7.602	7.358	7.335	**3**	7.071	6.990	6.974	**3**	7.097	7.071	7.068
4	7.420	7.420	-	**4**	7.018	7.084	7.098	**4**	7.143	7.071	7.063
5	7.377	7.380	7.381	**5**	7.097	7.157	7.172	**5**	7.060	7.071	7.072
6	7.678	7.380	7.351	**6**	7.284	7.157	7.123	**6**	7.398	7.307	7.215
7	7.292	7.527	7.584	**7**	7.027	7.055	7.063	**7**	7.149	7.149	-
8	7.143	7.527	7.619	**8**	6.983	7.055	7.076	**8**	7.051	7.071	7.073
9	7.032	7.143	7.169	**9**	6.854	6.886	6.904	**9**	7.018	7.071	7.077
10	7.143	7.358	7.378	**10**	7.022	6.991	6.984	**10**	7.143	7.071	7.063
11	6.967	6.951	6.933	**11**	7.066	7.084	7.088	**11**	7.114	7.071	7.066
12	7.921	7.527	7.433	**12**	7.143	7.055	7.030	**12**	7.081	7.071	7.070
13	7.553	7.380	7.363	**13**	7.125	7.157	7.165	**13**	7.215	7.307	7.398

Docking Studies

Docking study was performed to gain structural insight into the binding mode of most active compound. This study was carried out via Molegro Virtual Docker program on (Pdb Id:1t46) (fig.2).as tyrosine kinase inhibitors. The best binding model of compound 7f is shown in (fig.3). In the binding model, compound 7f was nicely bound with ATP binding site of kinase inhibitor receptors through hydrophobic , H-bonding and steric interactions. Docking score of compound 7f was found to be -151.594, indicates high binding affinity and better H-Bonding interactions with protein (1t46) residues. From the figure, it was revealed that compound 7f was nicely bound to Asp 810A amino-acid residues by two hydrogen bonds. Among them, One H-bond is formed between amino (-NH-) group of Pyrimdine ring and amino –acid residue of Asp810A. whereas another –NH- group between phenyl and pyrimidine ing also takes part in H-bonding interaction. Data of kinase inhibition study suggested that, H-bonding interaction plays an important role in its inhibitory activity. Rather than H-bonding interaction, compound 7f also exhibited hydrophobic and steric interaction with Glu640, Val668, Val654 and Leu644 amino-acid residues.

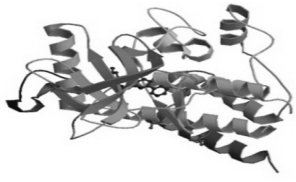


Figure 2 (a) : 3D crystal structure of tyrosine kinase inhibitors pdb code:1t46

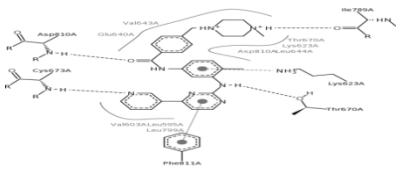


Figure 2(b): 2D view of receptor and their interactions is Shown.

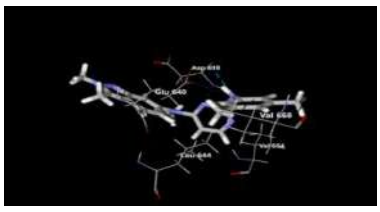


Figure 3: Molegro predicted binding mode of the ligand 7f is shown. Blue colour indicates hydrogen bonding interactions, and red colour shows steric interaction.

Conclusion:

In this paper, QSAR and docking study was performed on a series of pazopanib derivatives as anticancer agents, where inhibitory activity was observed against VEGFR-2, PDGFR-α and c-Kit tyrosine-kinase enzyme. QSAR models were developed by using Sequential multiple linear regression analysis. Mixed Hansch Fujita-Ban approach suggested that R2 substituent in pazopanib ring is more favorable for kinase inhibition and lipophilicity also plays crucial role towards its inhibitory activity. Data also suggested that more polar and less bulky group contribute positively at R2 position, whereas R1 position is unsubstituted or substituted by smaller group play an important role to its inhibitory activity. Docking study revealed that H-bond interaction plays significant role in the kinase inhibition activity. On the basis of docking analysis, compound 7f was found most potent compound as tyrosine-kinase inhibitors.

Acknowledgements

Author (NG) is grateful to Department of Science & Technology (DST) - Innovation in Science Pursuit for Inspired Research (INSPIRE) for providing DST-INSPIRE fellowship and also thankful to the Head, department of School of Pharmacy.

References:

[1] Trivedi GS & Srinivasan M (2004) Clin Biochem 37:618-635.

[2] Levitzki A (1999) Pharmacol Ther 82(2-3): 231-239.

[3] Levitzki A & Gazit A (1995) Science, 267: 1782-1788.

[4] Bari SB, Adhikari S & Surana SJ (2012) J Pharma Sci Tech 1(2): 36-45.

[5] Dengke L, Haofei Q, Ligong C, Bingni L & Xinran W (2014) Bioorg Med Chem Lett 24:1108.

PC-30

Pharmacological Assessment of Antidiabetic Potential of Hydroalcoholic Extract of *Cassia fistula* Linn. in Streptozotocin-induced Diabetic Rats

Sapna Malviya[1], Narendra Vyas[1], Ankur Joshi[1], Anil Kharia[1], Neelesh Malviya[2]
[1]Modern Institute of Pharmaceutical Sciences, Indore-453111 (M.P.)
[2]Smriti College of Pharmaceutical Education, Indore-452010 (M.P.)
E-mail address: smsapnamalviya@gmail.com

Abstract: Major public health problem "diabetes" is approachin*g* epidemic proportions globally and increasing at alarming rate all over the world. The Medicinal plants of India plays important role in the management of diabetes as they possess potent activity against diabetes. The present study protocol is aimed to evaluate antidiabetic activities of hydroalcoholic extract of *Cassia fistula* pod in streptozotocin-induced diabetic rats. Diabetes was induced in the rats by single intraperitoneal administration of Streptozotocin (60 mg/kg b.wt.). *C. fistula* pod extract at three different doses (100, 200 and 500 mg/kg b.wt./day) were administered orally with for 30 days to diabetic rats. The results were compared with standard drug glibenclamide (5 mg/kg b.wt./day) treated rats. The results showed that streptozotocin treated diabetic control rats suffered with decrease in the body weight and glycogen content in the liver as well as significant increase in the blood glucose and glycosylated hemoglobin (HbA1c) levels as compared to normal control rats. Oral administration of *C. fistula* pod extract or glibenclamide to diabetic animals for 30 days increased body weight and hepatic glycogen content and significant reduction in the blood glucose and HbA1c levels and as compared to diabetic control rats. The present results and study showed that *C. fistula* pod possess significant antidiabetic activity.

Introduction:

As per quoted in diabetic atlas of the International Diabetic Federation, 382 million people were affected by diabetes worldwide in the year 2013 and diabetes prevalence is expected to 592 million by the year 2035. According to World Health Organization projection of that diabetes will be the 7th leading cause of death in 2030. Diabetes mellitus (DM) is a chronic complicated metabolic disorder characterized by increased blood glucose level resulting from the defects in insulin secretion, insulin action, or both. *Cassia fistula* Linn. Caesalpiniaceae is an ornamental plant cultivated throughout India widely used for its medicinal properties. In traditional system of medicines pods of *C. fistula* has been recommended as anticancer, antifertility, antihyperlipidemic, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective and also used for the treatment and management of diabetes [1]. Henceforth the present study was designed to evaluate the antidiabetic efficacy of hydroalcoholic extract of *C. fistula* pod in streptozotocin induced diabetic rats.

Material and Methods:

Fresh pods of *C. fistula* were collected, dried powdered and subjected to soxhlet extraction at 60°C-70°C for 12 h. The extract was suspended in water before administering to experimental animals. The Adult, healthy, rats of Wistar strain weighing 170-200 g were used in the present study. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was approved by the Animal Ethical Committee of the Modern Institute of Pharmaceutical Sciences, Indore (1509/CPCSEA/2011).

Induction of Diabetes

Overnight fasted rats were induced diabetes by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight dissolved in citrate buffer (pH 4.5). To prevent initial drug induced hypoglycemic mortality STZ treated animals were given 2% glucose solution for 24 h after 5 h of STZ injection. The diabetic rats used in the study were having blood glucose level above 240 mg/dl.

Experimental design

The rats were divided into six groups, consisting of six animals each

Groups	**Treatment**
Group I	Normal rats- treated by vehicle (Positive Control)
Group II	Diabetic rats- treated by vehicle (Negative Control)
Group III	Diabetic rats- treated by extract 100 mg/kg b.w
Group IV	Diabetic rats- treated by 200 mg/kg b.w
Group V	Diabetic rats- treated by 500 mg/kg b.w
Group VI	Diabetic rats- treated by glibenclamide standard drug (5mg/kg b.wt./day)

Duration of study was 30 days and all treatments were given by oral route. After 24 hours of the last treatment, animals of different groups were weighed and autopsied under mild ether anesthesia. Blood was collected directly by cardiac puncture of which 2 ml was added to an anticoagulant vial for the estimation of parameters in blood [2]. The vital organs from each rat were dissected out, cleaned off from adherent fat and blood clot and weighed on a digital electronic balance. Blood glucose levels and body weights of experimental rats were determined at 6 day interval for a period of 30 days. Glycosylated hemoglobin (HbA1c) was estimated by glycohemoglobin reagent set (Accurex Biomedical Pvt. Ltd. Mumbai, India) and total hemoglobin (Hb) concentration by using Sahli's apparatus [3].

Results and Discussion:

As compared to initial body weight of experimental animals, the mean body weight of the rats of positive control group was significantly increased by 9.23% while the mean body weight of negative

control rats was significantly decreased. Diabetic rats treated with different doses of extract exhibited duration dependent significant increase in the mean body weight when compared to their initial body weight but it was comparatively less than that of positive control rats. Significant increase in body weight was observed in rats of group V after 10 days ($p \leq 0.05$, 4.37%), 20 days ($p \leq 0.05$, 10.96%) and 30 days ($p \leq 0.001$, 13.26%) as compared to their initial body weight (0 day). In group VI, the body weight gain was recorded as 9.51% ($p \leq 0.05$), 12.43% ($p \leq 0.01$) and 16.40% ($p \leq 0.001$) respectively after 10, 20 and 30 days of treatment period as compared with their initial body weight (0 day). Experimental animals of Group I showed sustained blood glucose level throughout the experimental period. In contrast to this, continuous increase in fasting blood glucose levels was recorded in negative control rats (group II) by 10.55% ($p \leq 0.05$), 14.72% ($p \leq 0.05$) and 17.30% ($p \leq 0.01$) respectively after 10, 20 and 30 days of experiment period as compared with their corresponding values on 0 day. The reduction in blood glucose level observed in the *C. fistula* extract (100, 200 and 500 mg/kg) treated rats on 30 days treatment duration was 40.52%, 45.79% and 52.31% respectively. In Group VI, the significant decrease in fasting blood glucose level ($p \leq 0.001$) by 54.46%, 62.40 and 63.48% respectively after 10, 20 and 30 days.

In Group II significant decrease in the levels of hepatic glycogen and total hemoglobin (Hb) with a concomitant significant ($p \leq 0.001$) increase in the percentage of glycosylated hemoglobin (HbA1c) in blood as compared to normal control rats (group I). The significant increase in the levels of hepatic glycogen [group III ($p \leq 0.05$), group IV, V and VI ($p \leq 0.001$)] and total Hb level in blood [group IV ($p \leq 0.01$), group V and VI ($p \leq 0.001$)] in diabetic rats treated with different doses of *C. fistula* pod extract or glibenclamide when compared to negative control rats. In contrast to this, the percentage of HbA1c in blood was significantly decreased [group III ($p \leq 0.05$), group IV, V and VI ($p \leq 0.001$)] as compared to negative control rats.

References:

[1] Rahmani AH (2015) Pharmacognosy Res 7:217–224.

[2] Malviya S & Rawat S (2012) Ethnopharmacol 3:169-173.

[3] Gandhi RG & Sasikumar P (2012) Asian Pac J Trop Biomed 2:281–286.

PC-49

Preliminary Phytochemical and Diuretic screening of Ethanolic and Aqueous Extract of *Zingiber officinale*

Manvendra Badore, Pallavi Mahajan, Prabhat Das, Narendra Badore, Sujit Pillai
GRY Institute of Pharmacy, Borawan, Khargone.
E-mail address: kumar_prabhat3027@yahoo.com

Abstract: The present study was to evaluate Diuretic activity of ethanolic and aqueous extracts of *Zingiber officinale* Rhizome in wistar rats. Ethanolic and aqueous extracts were administered to experimental rats orally at the doses of 500 mg/kg p.o. Furosemide (5 mg/kg) was used as positive control in the study. The diuretic effect of the extract was evaluated by measuring urine volume & sodium content. Urine volume was significantly increased by ethanolic extract in comparison to the aqueous and control group, while the excretion of sodium was also increased by extract. The ethanolic extract had the additional advantage over aqueous extract. We can conclude that ethanolic extract of *Zingiber officinale* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic furosemide.

Introduction:

Diuretics, also called water pills, are medications designed to increase the amount of water and salt expelled from the body as urine. There are three types of prescription diuretics. They're often prescribed to help treat high blood pressure. The drugs reduce the amount of fluid in your blood vessels, and this helps lower your blood pressure. Other conditions are also treated with diuretics. Congestive heart failure, for instance, keeps your heart from pumping blood effectively throughout your body. This leads to a buildup of fluids in your body, which is called edema. Diuretics can help reduce this fluid buildup. Ginger (*Zingiber officinale*) is one of the most widely used natural products consumed as a spice and medicine for treating nausea, dysentery, heartburn, flatulence, diarrhea, loss of appetite, infections, cough, and bronchitis. Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a popular spice used globally especially in most of the Asian countries. Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds. Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol (Figure 2). These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present. The aromatic constituents include zingiberene and bisabolene, while the pungent constituents are known as gingerols and shogaols. Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6- gingerdione and 10-gingerdione, 4- gingerdiol, 6-gingerdiol, 8-gingerdiol,

and 10-gingerdiol, and diarylheptanoids. The characteristic odor and flavor of ginger are due to a mixture of volatile oils like shogaols and gingerols. Ginger has been used as a spice as well as medicine in India and China since ancient times. It was also known in Europe from the 9th century and in England from the 10th century for its medicinal properties. Native Americans have also used wild ginger rhizome to regulate menstruation and heartbeat. Ginger is thought to act directly on the gastrointestinal system to reduce nausea. Therefore, it is used to prevent nausea resulting from chemotherapy, motion sickness, and surgery. Ginger is known as a popular remedy for nausea during pregnancy. Ginger is also used to treat various types of other GI problems like morning sickness, colic, upset stomach, gas, bloating, heartburn, flatulence, diarrhea, loss of appetite, and dyspepsia (discomfort after eating). According to Indian Ayurvedic medicinal system, ginger is recommended to enhance the digestion of food.

Besides these, ginger has been reported as a pain relief for arthritis, muscle soreness, chest pain, low back pain, stomach pain, and menstrual pain. It can be used for treating upper respiratory tract infections, cough, and bronchitis. As an anti-inflammatory agent, it is recommended for joint problems. Fresh juice of ginger has been shown to treat skin burns. Active component of ginger is used as a laxative and antacid medication. It is also used to warm the body for boosting the circulation and lowering high blood pressure. Because of its warming effect, ginger acts as antiviral for treatment of cold and flu. Ginger is also used as a flavoring agent in foods and beverages and as a fragrance in soaps and cosmetics. The present study was therefore aimed to explore the preliminary phytochemical screening and diuretic effects of ethanolic and aqueous extract of *Zingiber officinale* Rhizome.[1]

Material and Methods:

The Rhizome of *Zingiber officinale* was collected from local market and were authenticated by Dr. S. K. Mahajan, M. Sc, Ph. D, department of botany, Govt. P. G. Collage, Khargone, M. P. India and It has been identified and deposited.

Extract preparation

The Rhizome of *Zingiber officinale* were coarsely powdered and 1 kg of this powered plant material was extracted with the help of the soxhlet apparatus using ethanol as a solvent. The solvent from the ethanolic extract was removed under vacuum distillation; dried material was kept in a desiccators. Then the dried marc was again extracted with water.

Preliminary Phytochemical analysis

Zinger was analyzed for the various classes of phytoconstituents such as flavonoids, phenolic acids, anthocyanins, quinones, alkaloids, tannins, and saponins using standard phytochemical methods. Phytochemical tests were carried out following Shah and Quadry and Kokate [2].

Experimental animals

Male Wistar albino rats of body weight 150-200 g were obtained from the Institute Animal House. The rats were acclimatized in the department animal house at an ambient temperature of 25°C, under

a 12hour dark -12 hour light, cycle, for the whole period of the study. The animals were fed with a standard pellet diet and water *ad libitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental on Animals, New Delhi, India and the research protocol was approved by the Institute animal ethical committee (1151/ac/07/CPCSEA).

Experimental protocol [3,4]

Diuretic activity was determined by the following methods of Kau *et al.*, with minor modifications. The rats were randomly divided into four groups of six animals each as follows: (1) Control – given 25 ml/kg body weight of normal saline; (2) Furosemide (5 mg/kg) + normal saline (25 ml/kg) of body weight; (3) Ethanolic extract (500mg/kg) + normal saline (25 ml/kg) of body weight; (4) Aqueous extract (500mg/kg) + normal saline (25 ml/kg) of body weight. The animals were fasted overnight (18 h) prior to the test but with free access to tap water only and then were given an oral loading of normal saline (0.9%) of 0.05 ml per g body weight. Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 1 h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g b.w. Electrolyte (Na) concentrations estimated from the urine sample.

Measurement of Urine Output and Analysis of Electrolytes. Na concentrations were measured using digital flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na+.

Statistical Analysis. The results are expressed as mean values ± SD for pairs of rats. Statistical comparison was carried out by analysis of variance (ANOVA).

Results and Discussion:

Phytochemical Investigation

The result of phytochemical screening showed the presence of Alkaloids, Carbohydrates, Tannins, Volatile oil, saponins, Glycosides, Triterpenes, flavonoids.

Acute toxicity test

From the acute toxicity test we found the dose of 500mg/kg of both ethanolic and aqueous extract found safe dose for screening method.

Pharmacological estimation

The results of the evaluations carried out on the extracts are listed in Table 1 and Table 2. Table 2 shows the urinary volume (ml/100g/8h) while Table 1 shows the electrolyte (Na+) content (mequiv/100g/8h) of the urine of the animals.

Urine volume. Table 1 shows that the reference diuretic, furosemide, increased urine volume. The extracts were also showed their efficiency in comparison to standard. For the ethanolic extract, doses of 500 mg/kg body weight showed more potent effect than the aqueous group. Ethanolic extract of

Zingiber officinale shows significance increase in urine excretion. Thus, the diuretic effect of extract indicates an increase in both water excretion and excretion of sodium. Ethanolic extract (500 mg/kg) shows a significant result in excretion of water & sodium, which proves as a strong diuretic agent in compared to aqueous extract.

Table 1: Effect of oral administration of *Z officinale* and furosemide on sodium excretion

Treatment	Dose	Sodium (meq/100g/8 hr) $\times 10^{-2}$
Control (Normal Saline)	25 ml/kg	36.12
Standard (Furosemide)	5 mg/kg	92.50
Ethanolic Extract	500mg/kg	75.83
Aqueous Extract	500mg/kg	62.36

Table 2: Effect of oral administration of *Zingiber officinale* and furosemide on urine volume

Values are mean as ± SD

Treatment	Dose	Urine volume							
		1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr
Control (Normal Saline)	25 ml/kg	0.33 ± 0.51	0.5 ± 0.54	0.83 ± 0.40	1.0 ± 0.0	1.3 ± 0.51	1.83 ± 0.40	1.83 ± 0.40	2.0 ± 0.0
Standard (Furosemide)	5 mg/kg	1.0 ± 0.89	1.6 ± 0.51	2.5 ± 0.54	2.6 ± 0.51	3.16 ± 0.78	3.83 ± 0.40	4.5 ± 0.54	5.33 ± 0.81
Ethanolic Extract	500mg/kg	0.3 ± 0.51	0.6 ± 0.51	1.16 ± 0.75	1.8 ± 0.7	2.3 ± 0.8	3.0 ± 0.89	3.6 ± 0.81	4.16 ± 0.98
Aqueous Extract	500mg/kg	0.16 ± 0.40	0.6 ± 0.5	1.3 ± 0.51	1.5 ± 0.54	2.16 ± 0.40	2.5 ± 0.54	3.3 ± 0.51	3.83 ± 0.75

Conclusion:

The results obtained in this study provide a quantitative basis to explain the traditional use of *Zingiber officinale* as a diuretic agent.

Acknowledgement:

The authors are thankful to Mr. Prabhat Das, Mr. Narendra Badore and Dr. sujit Pillai for their support in providing research facility.

References:

[1] Prasad S and Tyagi AK (2015) Gastroenterology Res Practice 1-11.

[2] Kokate CK (2004), *Pharmacognosy*, Nirali Prakashan, Pune, pp. 1, 5, 57, 60.

[3] Kau ST, Keddi JR, Andrews D (1984) J Pharmacol Meth 11:67–75.

[4] Goyal RK Practical Pharmacology, 9th ed. B.S. shah Prakashan, Ahmedabad: (2009):143-144.

PC-51

Anti-Diabetic Potential of Aqueous, Methanolic and Saponin Extract of Leaves of *Ziziphus nummlaria* Linn.

Kushagra Dubey[1], Raghvendra Dubey[2], Revathi Gupta[2], Arun Gupta[2]
Smriti College of Pharmaceutical Education, Indore (M.P.)
Faculty of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore (M.P.)
E-mail address: kushu0129@gmail.com, raghuji22@gmail.com

Abstract: The aqueous and methanolic extract of the leaves of Zizyphus nummularia were obtained from successive solvent extraction. The methanolic extract was further solvent extracted with water saturated n-butanol solvent and organic layer was acidified with 1 N KOH to obtain the raw saponin extract. Different concentrations of extracts were treated with alpha amylase enzyme in phosphate buffer (pH 6.9), their spectroscopic estimation was done at 540 nm after stopping the reaction with DNS. All the extracts have produced significant enzyme inhibition and their IC50 value was observed to be 114.16 µg/ml ± 1.30 to 137.87 µg/ml ± 1.82.

Introduction:

Screening of the herbals for the safe and effective treatment of the disease is bottleneck in current research. Medicinal plants have always been integral to the traditional healthcare system all over the world to cure specific ailments. Plants of many medicinal values helps to overcome many chronic disorders and simultaneously the drawbacks of allopathic drugs such as severe side effects, cost effectiveness etc has boosted the use of herbal medicines to be an excellent remedy for diseases like cancer, diabetes, liver diseases and arthritis and due to the same the herbal drug extraction & isolation is of prime importance in present research. The specific phytochemical category based herbal extraction is widely used for the screening, identification and isolation of pharmacological active compound [1-2].

Diabetes mellitus is one of the very common chronic diseases across the world and exploring the therapeutic value of natural ingredients in such chronic disease by the researchers can be helpful in incorporating into everyday life of common people which may be an effective approach in the management of diabetic complications. This will also be helpful to decrease the socio economical burden on the middle class family of the society.

The present work is aimed for the successive extract of leaves of *Ziziphus nummularia*, family rhamnaceae which will be subjected to specific saponin extraction. The extracts were pharmacologically screened for antidiabetic potential using alpha-amylase inhibition assay.

Materials and Methods:

Plant Materials Leafs of *Ziziphus nummularia* were collected from Malwa region of Madhya Pradesh in the month of March-April, 2017 and were identified by the Department of Pharmacognosy,

College of Pharmacy, Dr. A.P.J.Abdul Kalam University, Indore (M.P.). The leaves were later air-dried, powdered and stored in an air-tight container for further use.

Shimadzu UV 1800 UV Visible spectrophotometer, Continue soxhlet extractor and Chemicals from Sdfine, Loba Chem and HiMedia Lab were used

Preparation of Extracts

Leaves were shattered and screened with 40 no. mesh. It was soxhlet extracted three times with petroleum ether for 4hr at 60^{o}C. After drying and levigation, one part of the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85^{o}C, then were filtrated and the other part was extracted with distilled water for 48hr under reflux condition. The alcohol solution (Filtrate) was evaporated to dryness and dissolved with water. After filtration and discarding the extraneous components, the solution was extracted by adding water-saturated n-butanol (1:1v/v), the n-butanol phase was then treated by 1M KOH, alkaline–water phase was removed. The n-butanol phase evaporated to dryness and the raw saponin was obtained. All extracts were screened for phytochemical analysis.

α- Amylase Inhibition Assay

Aqueous, methanolic and saponin extract of different concentrations from 80-160 µg/ml were prepared. About 0.5 ml extract was then treated with 0.5 ml of alpha amylase (0.5 mg/ml). The solution was then incubated at 25℃ for 10 minutes. About 0.5 ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 was added to all the tubes and was incubated at 25℃ for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes and cooled to room temperature. The solution was made up to 10 ml with distilled water and the absorbance was read in the UV- Visible Spectrophotometer at 540 nm against phosphate buffer as blank solution. Maltose is used as positive control.

Absorbance was calculated by using following formula

α-Amylase Inhibition Activity = (Ac+) – (Ac-) – (As-Ab) / (Ac+) – (Ac-) × 100

Where, Ac+, Ac-, As, Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme) respectively[2-5].

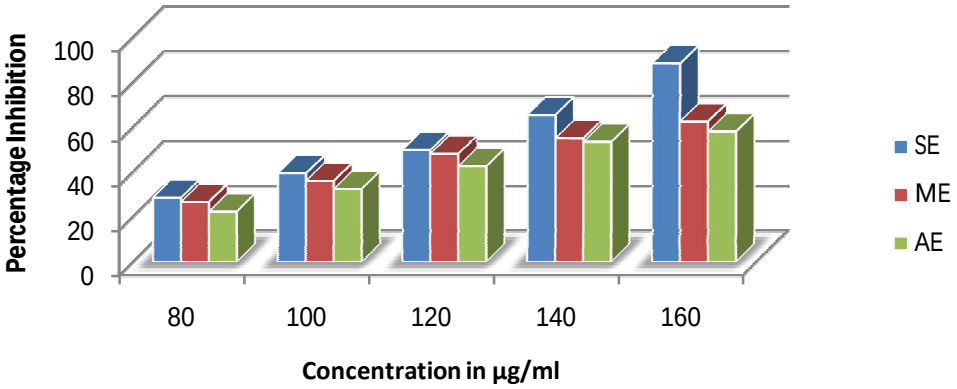
Results and Discussion:

The aqueous, methanolic & saponin extract of Zizyphus nummularia Linn leaves has confirmed the antidiabetic potential via alpha amylase inhibition assay. The inhibition percentage of aqueous, methanolic & saponin extract was observed to be 20.2 -58.3%, 26.66-62.53% and 28.53-88.38 % respectively as indicated in table Table 1& Figure 1. The maximum inhibition was showed by saponin extract which was observed to be 88.38 %. The result clearly indicated that the saponin extracts is more active than other extracts. The IC_{50} were observed in the range from 114.16 g/ml to 137.87µg/ml which indicates the concentration for 50% inhibition of enzyme activity.

Table 1: The Percentage Alpha Amylase Inhibition of different extract of *Zizyphus nummularia* Linn.

Extract of ZNL	Percentage Inhibition					IC_{50}
	80 µg/ml	100 µg/ml	120µg/ml	140 µg/ml	160µg/ml	
AE	22.3±1.2	32.4±1.05	42.5±1.71	53.4±0.31	58.03±0.47	137.87± 1.82
ME	26.6±1.6	36.6±1.80	48.2±0.55	55.06±0.75	62.5±0.80	129.65± 1.10
SE	28.53±1.02	39.4±1.31	49.8±1.86	65.04±0.11	88.38±1.20	114.16± 1.30

ZNL: Leaves of Zizyphus nummularia Linn, AE: Aqueous Extract, ME: Methanolic Extract, SE: Saponin Extract, IC50: 50% Inhibitory Concentration



SE: Saponin Extract, ME: Methanolic Extract, AE: Aqueous Extract

Figure 1: Graphical Plot of Alpha Amylase Inhibition of different extract of *Zizyphus nummularia* Linn.

Conclusion:

The result indicated that all the extracts of leaves of Zizyphus nummularia Linn are active towards alpha amylase inhibition activity. From the result it can be concluded that the extracts will be helpful in assistance the metabolism of carbohydrates and hence the above said extracts can effectively contribute for effective management of diabetes. In future the saponin extract can be subjected to isolation and the isolate can be pharmacologically evaluated which will provide an insight for the molecular mechanism by which the hypoglycemic action is obtained.

References:

[1] Mehta V, Sharma A,Tanwar S & Malairaman U (2016) Int J Pharm Pharm Sci 8(8): 357-362.

[2] Nickavar B, Abolhasani L & Izadpanah H (2008) Iran J Pharm Res 7(4): 297-303.

[3] Vijayalakshmi K, Selvaraj IC, Sindhu S & Arumugam P (2015). Int J Pharm Phyt Res 6(4): 856-861.

[4] Saraswathy M, Kalimuthu K, Chinnadurai V & Juliet SY (2017) Br J Pharm Res 15(2): 1-9.

[5] Kadali RM, Das CM, Vijayaraghavan R & Shanmukha I (2017) Natl J Physiol Pharm Pharmaco 7(5): 486-490.

PC-68

Hepatoprotective Activity of the Extract of *Crataeva nurvala* Bark Against CCl_4 Induced Hepatotoxicity in Rats

Reena Soni
Department of Pharmacy, Shri G.S. Institute of Technology & Science, Indore-03 (MP), India.
E-mail address: reenasoni019@gmail.com

Abstract: The present work is focused on investigation of hepatoprotective activity of bark of Crataeva nurvala. It's hepatoprotective activity was studied in the form of its aqueous and ethanolic extract and its isolated compound, against CCl4 induced hepatotoxicity in albino rats. The In-vitro study demonstrated the lowering of GPT and LDH level in isolated hepatocytes. Further more, an alteration in the level of biochemical markers, i.e., SGOT, SGPT, SALP and bilirubin were studied in-vivo on albino rats after CCl4 induced hepatic damage. Ethanolic extract (dose 250 mg/kg & 500 mg/kg) and isolated compound (dose 50 mg/kg) induced lowering of biochemical markers near to the normal levels in dose dependent manner, while there was no remarkable change with the aqueous extract (dose 250 mg/kg and 500mg/kg). Hence, the findings confirmed that ethanolic extract and isolated compound of C. nurvala bark possess hepatoprotective activity.

Introduction:

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. *Crataeva nurvala.* Buch. is a moderate sized evergreen tree with their rounded hard fruits, grows on the banks of canals and rivers throughout Bangladesh. Traditional uses of the investigated species are reported as contraceptive, oxitocic, urinary complaints, laxative, and litho tropic, febrifuge and as tonic [1].

Material and Methods:

Plant material

Stem bark of *C. nurvala* were collected from forest area of Sagar, M.P. and authenticated in Department of Botany, Dr. H. S. Gour University Sagar (M.P.). A voucher herbarium specimen number Bot/H/012010 was also preserved. The collected bark were dried under shade and powdered and stored in an airtight container.

Extraction and isolation

The powdered bark (1kg) was successively extracted in a soxhlet apparatus with petroleum ether (60-80°C), ethanol (95%) and finally with water [2].

Isolation and purification of compound

Isolation of triterpenoids from the ethanolic extract of bark was carried out on the basis of solubility [3]. Firstly ethyl acetate (150 ml) was added to the concentrated ethanolic extract (50 g) after about 20 minutes it gets separated in two fraction first ethyl acetate soluble fraction and ethyl acetate insoluble fraction. This ethyl acetate insoluble fraction was properly washed with methanol for 10 times and finally obtained creamish powder after drying, which is subjected to TLC studies which shown single spots with some impurities. Qualitative test for identification of triterpnoids was performed, which was found to be positive. For the separation of pure compound, it (creamish powder) was re-crystallized with 10 ml acetone [4].

Evaluation of hepatoprotective activity

The general principle involved in the evaluation of hepatoprotective activity is to induce toxicity with the help of hepatotoxins in experimental animals. The hepatotoxins are widely used to induce the diseased condition in experimental animals followed by an attempt to counteract their hepatotoxicities with the preparation under test. The magnitude of the protective activity is measured both *In Vitro* and *in-vivo* by estimating the two parameters viz. histopathological and biochemical parameter [5].

Results and Discussion:

A very important observation of *In Vitro* studies that ethanolic extract in higher dose is particularly very effective in decreasing the level of GPT, LDH and recovery is almost comparable to that of silymarin.

Table 1: Estimation of serum parameters and percentage restoration

Group	Conc.	GPT		LDH ▲ O.D./min./3 ml medium	
		Level (IU/L)	% Restoration	Level (IU/L)	% Restoration
Normal		4.4±0.21	0	3.41±1.31	0
CCl_4	10 mM	33.9±1.30	0	21.8±1.02	0
Ethanolic ext.	50 µg/ml	18.2±0.71	53.22	10.18±0.30	63.1
Ethanolic ext.	100 µg/ml	13.6±0.48	68.81	6.7±0.23	82.1
Aqueous ext.	50 µg/ml	28.3±1.21	18.98	18.1±0.60	20.1
Aqueous ext.	100 µg/ml	26.1±1.10	26.44	16.8±0.64	27.18
Isolated compound	50 µg/ml	20.8±1.01	44.4	13.4±0.47	45.67
Isolated compound	100 µg/ml	17.8±0.62	54.57	10.9±0.34	59.27
Silymarin	10 µg/ml	12.8±0.53	71.52	9.4±0.26	67.42
Silymarin	20 (µg/ml)	8.6±0.23	85.76	6.9±0.17	81.02

O.D.: Optical Density

A very important observation in the studies that ethanolic extract in higher dose is particularly very effective in decreasing the level of serum bilirubin. These rapid decreases in serum bilirubin is

suggest that ethanolic extract can be used in the acute condition of jaundice, and drug is effective in the maintenance of normal functional status of liver.

The histopathological studies also exhibit the efficacy of drug as liver protectant, simultaneous treatment of ethanolic extract with CCl_4 produces lesser degree of damage to the liver cells as compared to the animals treated with CCl_4 alone.

The biochemical functional and histopathological studies clearly show the hepatoprotective activity of *C. nurvala* Buch. justifies the use of this plant in folk medicine for jaundice.

Table 2: Percentage protection of animals by test samples (CCl_4 Model)

Groups	SGOT (AST) %	SGPT (ALT)%	SALP %	Direct Bilirubin%	Total Bilirubin%
Ethanolic Extract 250mg/kg	63.92	66.98	60.06	50.71	63.03
Ethanolic Extract 500mg/kg	78.12	78.33	73.16	62.14	70.29
Aqueous Extract 250mg/kg	9.39	6.29	3.89	12.14	21.78
Aqueous Extract 500mg/kg	18.25	8.23	4.6	22.14	27.06
Isolated Compound 50mg/kg	44.48	46.14	47.20	40	51.15
Standard Silymarin 25mg/kg	82.53	88.05	87.42	75.71	83.16

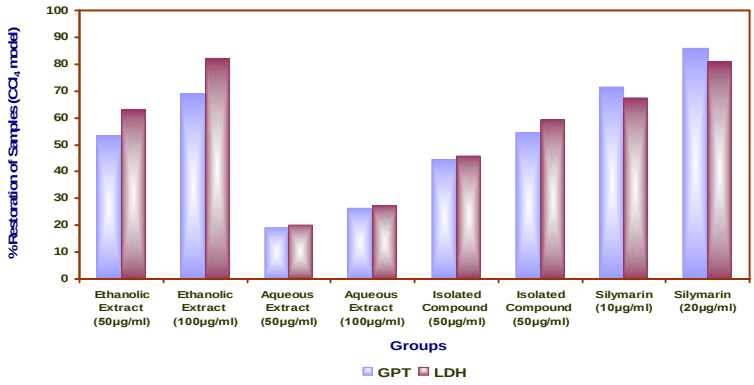


Figure 1: Percent restoration of isolated hepatocytes by test sample (CCl_4 Model)

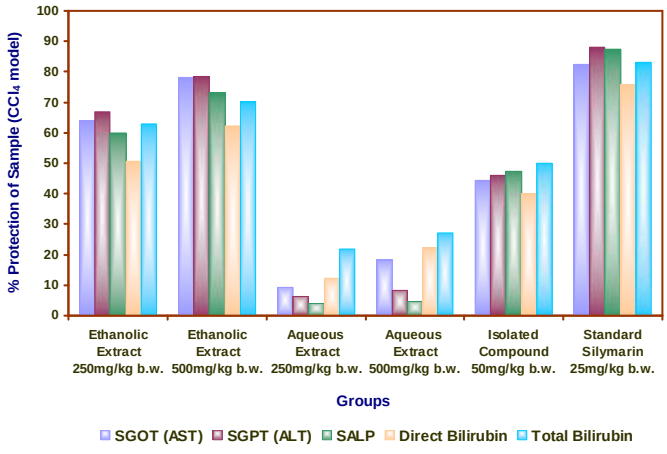


Figure 2: Percentage protection of animals by test samples (CCl_4 Model)

Conclusion:

Administration of ethanolic extract their isolated compound of *C. nurvala* stem bark showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. Ethanolic extract and their isolated compound has demonstrated significant better results as compared to aqueous extract when biochemical parameters are taken into consideration.

Acknowledgement:

The author is grateful to Regional research laboratory Jammu for conducting the *in-vitro* hepato-protective activity.

References:

[1] Sunitha S, Nagaraj M & Varalakshmi P (2001) Fitoterapia 72: 516-523.

[2] Bhaskar VH, Profulla MK, Balakrishnan BR, Balakrishnan N & Sangameswaran B (2009), Afr J Biotech, 8: 6453-6456.

[3] Alam MA, Haque ME, Shilpi JA & Daulla KA (2006), Bangl J Vet Med 4(1): 65-68.

[4] Bani S, Kaul A, Khan B, Ahmad SF, Suri K, Gupta BD, Satti NK & Qazi GN (2006) Phytother Res 20:279–287.

[5] Kumari A & Kakkar P (2008) Biomed Environ Sci, 21: 24-29.

PC-71

Formulation, Development and Evaluation of Herbal Garlic Lotion for Acne

Shikha Jaiswal, Shreesh Tiwari, Mohit Chaturvedi, Revathi Gupta
Dr. APJ Abdul Kalam University, Indore, (M.P.)
E-mail address: jaiswalshikha15@gmail.com

Abstract: Now a days synthetic chemicals are produces many harmful effects like cause irritation on skin, dryness, sometimes produce pimples etc. so that's why we selected herbal products for making herbal garlic lotion for the treatment of acne by using W/O emulsion method due to reducing side effects, cheap, better therapeutic effect, ease of available etc. present studied that all parameters passed and there is no cause irritation, redness, itches, etc. after administration or applied on skin its proved by skin irritation test.

Introduction:

Cosmetics

There are plenty of commercial acne treatments available, but they're often expensive and ineffective, and they come with unwanted side effects. Our aim and objective is to formulation, development and evaluation of lotion which can cure Vulgaris Acne. The product should be cheaper and effective. For formulation of Garlic lotion, preformulation studies have to be done.Lotions are applied to external skin use. While lotion may be used as a medicine delivery system, many lotions, especially face, hand lotions and body lotions are meant instead to simply smooth, moisturize and soften the skin. These may be used in anti-aging lotions, which can also be classified as a cosmetic in many cases, and may contain fragrances. Lotions can be used for the treatment of skin diseases such as: Antibiotics, Antiseptics, Antifungals, Corticosteroids, Anti-acne agents, Soothing, smoothing, Moisturizing or protective agents.

Materials and Methods:

Materials

Multani Mitti, Garlic paste, Honey, Cucumber, Turmeric, Aloe vera gel, Neem, sandalwood powder, lemon, rose water.

Method

W/O emulsion method.

Pre Formulation:

Preformulation study of powder extract.

Evaluation

Evaluation of Lotion The cream was evaluated for appearance, irritation test, viscosity, pH, total fatty substance, total residue, thermal stability and microbiological tests. The results are shown in

Accelerated stability study was also performed to check the changes during storage and is likely to influence quality, safety, and efficacy.

Table 1: Parameters of Powder Extract

S. No.	Parameters	Result
1.	Particle size	20-25um
2.	Angle of repose	32^{o}
3.	Bulk density	0.8gm/cc
4.	Tapped density	0.9gm/cc
5.	Carr's index	12
6.	Hausner ratio	1.13
7.	pH	7.2

Formulation of herbal garlic lotion:

Table 2: Formulation of herbal garlic lotion

S.No.	Ingredient	F1
1.	Multani Mitti	0.5
2.	Garlic paste	0.80
3.	Honey	1.0
4.	Cucumber	0.2
5.	Turmeric	0.2
6.	Aloe vera gel	1.0
7.	Neem powder/oil	0.5
8.	Sandalwood powder	0.5
9.	Lemon	0.3mg
10.	Fragrance (Rose Water)	q.s

Appearance

The appearance of the lotion was judged by its color, texture, roughness and its odour.

Irritation test

Apply this lotion for face mask on 10 minute.After a short time in irritation accuse does not but after which we do not have a irritation and 10 minute After wash on the face for lukewarm water.

Viscosity

Viscosity of formulated lotion was carried out with Brookfield Viscometer model LV-DV-II+, Helipath spindle type S-96.

pH

The pH meter was calibrated using standard buffer solution. About 5 ±0.01 g of the lotion was weighed in a 100 ml beaker and dissolved in 45.0 ml of distilled water and dispersed the lotion in it. pH of lotion was measured at 27ousing the pH meter.

Microbiological tests

Lotion was subjected for microbiological tests total viable count and gram negative pathogens like Salmonella, Pseudomonas aeruginosa, Escherchia coli.

Results and Discussion:

Lotion was evaluated following parameters like visual appearance, irritancy test, viscosity, pH, total fatty substances, total residue, thermal stability and microbiological analysis and preformulation studied was also performed after that observed there is giving better and excellent properties of powder flow. Appearance of lotion was good having smooth texture and easily spreadable property. Irritancy test was performed and there was no sign of redness and itching and safe for topical application. Viscosity of lotion was carried out using Brookfield viscometer and it was found in range between 19402cp - 19443cp. pH of lotion was found to be 7.28 which is nearer to the required pH of skin. Total fatty substances and total residue was found to be 23.01 % and 40.14 % respectively. lotion was thermally stable at 45˚C ± 1˚C for 48 hours when evaluated for the thermal stability. There was no phase separation.

Conclusion:

Herbal lotion is unique obtained from natural plants and some plant extract. It purpose to designed giving soothing effects and relieve from redness, pimples, inflammations and skin irritations and it provides better anti inflammatory, anti bacterial, anti septic and insect repellent activities. All the therapeutic nutrients give soothing qualities from plant oils such as Aloevera and Neem with the healing benefits inherent in oil.A complete acne treatment system is much more useful in treating acne from garlic. Mixture of all this ingredients made garlic lotion for best effect on the face &related skin problems.

References:

[1] Saraf S & Saraf S, ‘A Cosmetic manual Practical book’, second edition, page no.137.

[2] Lawson LDG (1998) Phytomedicines of Europe. Chemistry and Biological Activity. Series 691 American Chemistry Society, Washington, DC, 176-209.

[3] Yan X, Wang Z & Barlow P (1992) Food Chemistry, 45(2):135-139.

[4] Iberi B, Winkler G, Mullar B & Knobloch K (1990) Planta medica 56(3): 320-326.

[5] Bordia A, Bansal HC, Arora SK & Singh SV (1975) Atherosclerosis 21(1):15-19.

PC-77

Development and Validation of HPTLC Method for Estimation of Ellagic Acid in Antidiabetic Herbal Formulation

Satyendra Shrivastava[1], P.K. Dubey[1], B. Shrivastava[2], Pankaj Sharma[2]
[1]Swami Vivekanand College of Pharmacy, Khandwa Road, Indore (M.P.)
[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur
E-mail address: shrivastavapharma@gmail.com, skshrivastava@svcp.ac.in

Abstract: A simple, sensitive, precise, rapid and reliable HPTLC method for the estimation of ellagic acid in marketed herbal formulation, Glucomap tablet was developed. In this method, precoated Silica Gel F_{254} Plates were used as stationary phase and Toluene: ethyle acetate: formic acid: methanol (3:3:8:2 v/v) as mobile phase. Developed chromatogram was scanned at 280 nm, the wavelength of maximum absorption for ellagic acid. The aptness of developed HPTLC method for estimation of ellagic acid was established by validating it as per the ICH guidelines. The content of ellagic acid in crude drug *Terminalia arjuna* and polyherbal formulation was also studied. The developed method has been successfully applied for the determination of ellagic acid in polyherbal formulation.

Introduction:

Standardization is necessary to make sure the availability of a uniform product in all parts of the world [1]. Standardization assures a consistently stronger product with guaranteed constituents. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programs, in assessing safety and efficacy, in ensuring adequate supplies, and in the quality control of raw and processed materials [2].

High-performance thin-layer chromatography (HPTLC) is still increasingly finding its way in pharmaceutical analysis in some parts of the world. The technique achieves for given applications a precision and trueness comparable to high performance liquid chromatography (HPLC). It also allows simultaneous estimation of several samples utilising only a small quantity of a mobile phase, hence minimising the analysis time and cost [3]. On the basis of review of literature a few analytical techniques have been reported for the analysis of ellagic acid is available for its estimation in polyherbal formulations. The main aim of this study was to develop and validate an accurate and reproducible HPTLC method for estimation of ellagic acid in Glucomap tablet as an ingredient. The validation was done as per ICH guidelines [4].

Materials and Methods:

The fingerprint method for Glucomap tablet was developed by high-performance thin layer chromatography determination using ellagic acid as a standard. The fingerprinting method was developed for selected components of GT (*Terminalia arjuna*), laboratory batches (GT-I, GT-II, GT-III) & marketed formulation (GTM) via estimation of ellagic acid by using following experimental

techniques. All chemicals and reagents used were of analytical grade and were purchased from Hi-Media, India.

Table 1: Instrumentation and chromatographic conditions

Stationary phase	**Precoated Silica Gel F $_{254}$ Plates (Merck)**
Mobile phase	Toluene: ethyle acetate: formic acid: methanol (3: 3: 8: 2 v/v)
Saturation	40 mins
Temperature	25 ± 2 ºC
Development chamber	Glass twin trough development chamber
Applicator	CAMAG Linomat IV applicator
Scanner	CAMAG Scanner III Win Cats (4.06), Switzerland
Mode of scanning	Absorption (deuterium)
Detection wavelength	280 nm
Scanning Speed	20 mm/s

Calibration curve of ellagic acid

The stock solution of ellagic acid was prepared by dissolving 10 mg of ellagic acid in 100 ml of methanol. This solution was diluted as needed to prepare different concentrations of standard solutions. A stock solution of ellagic acid (100 µgmL^{-1}) was prepared in methanol. Different concentrations of stock solution were prepared & spotted on the TLC plate to obtain a 100 - 600 ng spot^{-1} of ellagic acid, respectively. The data of peak areas plotted against the corresponding concentrations were treated by least-square regression analysis method validation.

Method validation

The method was validated for precision, accuracy, limit of detection & limit of quantification, robustness, ruggedness & specificity of sample application.

Precision and accuracy

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng spot^{-1}for ellagic acid) was expressed in terms of percent relative standard deviation (%RSD). The intra- and inter-day variation for the determination of ellagic acid was carried at three different concentration levels of 100, 300, 600 ng spot^{-1}.

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD was determined based on the lowest concentration detected by instrument in the sample. LOQ was determined based on the lowest concentration quantified by the instrument in the sample (LOQ = 5 x LOD). In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were

experimentally verified by diluting the known concentrations of ellagic acid until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Robustness By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation and activation of pre-washed TLC plates with water; the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 600 ng spot−1 for ellagic acid and the % R.S.D of peak areas was calculated.

Ruggedness A solution of concentration 600 ng $spot^{-1}$ was prepared and analyzed on day 0 and after 6, 12, 24, 48 and 72 h. Data were treated for % RSD to assess ruggedness of the method for ellagic acid.

Specificity The specificity of the method was confirmed by analyzing the standard drugs and samples. The spot for ellagic acid in the sample was confirmed by comparing the Rf values and spectra of the spot with that of the standard. The peak purity of the ellagic acid was assessed by comparing the spectra at three different levels.

Recovery The recovery was determined by the standard addition technique. The pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard ellagic acid and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check for the recovery of the ellagic acid at different levels in the formulations.

Results and Discussion:

The method was validated for precision, accuracy, limit of detection & limit of quantification, robustness, ruggedness & specificity of sample application.

Precision and accuracy

The intra- and inter-day variation for the determination of ellagic acid was carried at three different concentration levels of 100, 300, 600 ng $spot^{-1}$ were calculated and depicted in table 1.

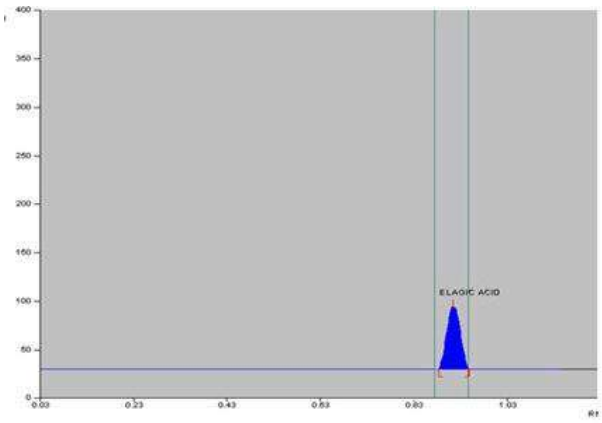


Figure 1: HPTLC chromatogram of ellagic acid

Table 2: Intra and inter-day precision of HPTLC method

Amount of ellagic acid	**Intra -day precision**		**Inter-day precision**	
(ng/spot)	Area ± SD	RSD %	Area ± SD	RSD %
100	985.65 ± 0.571	0.058	1148.42 ± 0.387	0.034
300	2052.88 ± 0.482	0.023	2275.17 ± 0.474	0.021
600	3984.26 ± 0.623	0.016	4159.34 ± 0.691	0.017
Mean		**0.032**		**0.024**

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD and LOQ were experimentally verified by diluting the known concentrations of ellagic acid and found to be 71.85 and 222.73 respectively (Table 2).

Robustness

The proposed method was found to be robust (Table 2).

Recovery

The recovery studies were done and determined by the standard addition technique. The %RSD was found to be 0.285.

Table 3: Summary of validation parameters of HPTLC (ellagic acid)

S. No.	**Parameters**	**Data of ellagic acid**
1	Retention Factor (Rf)	0.90
2	Beer's law limit (ng/spot)	100-600
3	Correlation coefficients (r^2)	0.996
4	LOD (ng/spot)	71.85
5	LOQ (ng/spot)	222.735
6	Precision (% RSD) Repeatability Intraday Interday	 0.325 0.032 0.024
7	Recovery Studies Accuracy(% RSD) SE	 0.285 0.236
8	Robustness	Robust
9	Specificity	Specific

(Mean value, n=6)

Estimation of ellagic acid in crude drug and formulations

The sample of selected components of GT (*Terminalia arjuna*), laboratory batches (GT-I, GT-II, GT-III) & marketed preparation (GTM) were prepared separately by weigh sample accurately 200 mg and extracted in methanol by heating and make up 100 ml volume with methanol. The appropriate aliquots from prepared above samples extract were withdrawn in 10 ml volumetric flask separately.

The sample solution was spotted on TLC plate & after that development & scanning of TLC plate. A single spot at Rf = 0.90 was observed in the sample chromatogram of the ellagic acid with some other components. No interference was found in analysis from the some other components present in the samples. The result of ellagic acid content was illustrated in table 3.

Table 4: Content of ellagic acid in crude drug and formulations

S. No.	Name		Ellagic acid content (% w/w)	Standard error
1	*Terminalia arjuna*		1.110 ± 0.135	0.104
2	Glucomap	GT-I	0.0255±0.134	0.116
		GT-II	0.0280±0.451	0.151
		GT-III	0.0310±0.362	0.120
		GTM	0.0220±0.235	0.148

Mean ± SD of 6 determinations

Conclusion:

The developed HPTLC technique is precise, accurate, and robust for the determination of ellagic acid in Glucomap tablet. Statistical analysis proves that the method is reproducible for the analysis of ellagic acid. The content of ellagic acid in marketed polyherbal formulation is comparable to laboratory formulations. Therefore, this method can be successfully used for the routine analysis of ellagic acid for standardization and quality control of pharmaceutical products containing ellagic acid as an ingredient.

Acknowledgement:

Authors gratefully acknowledge Research Laboratory of Institute of Pharmacy, Pt. Ravi Shankar Shukla University, Raipur, for assistance in HPTLC work.

References:

[1] Mukharjee PK (2002) 3rd ed. Business Horizons Pharmaceutical Publishers, New Delhi, 183-219.

[2] Rose EN, Nmedo KP & Samal PK (2008) J Pharm Tech 1: 310-312.

[3] Sethi PD (1996) CBS Publishers and Distributors, New Delhi, India, 11-17.

[4] ICH (2005) ICH-Q2 (R1), International Conference on Harmonization: Geneva.

PC-80

Effect of Ethanolic Extracts of *Abutilon indicum, Zea mays* and Combination on Calcium Oxalate Urolithiasis in Rat

Dishant Gupta[1], P.K. Dubey[2]
JJT University Jhunjhunu, Rajasthan
Principal, Swami Vivekanand College of Pharmacy, Indore
E-mail address: dishant2007@yahoo.co.in

Abstract: In the present study the effect of calcium oxalate urolithiasis urinary risk factor of ethanolic extract of *Abutilon indicum, Zea mays* and their combination have been studied in albino rats. From this study it is deduced that the possible effect of the ethanolic extract of *Abutilon indicum, Zea mays* and their combination can be assigned to be positive effect on the main urolithiasis risk factors.

Introduction:

Urinary stone disease continues to reside in an important place in daily urological practice. The average life time risk of stone formation has been reported in the range of 5-10 %. A predominance of men over women can be observed with an incidence peak between the fourth and fifth decade of life [1].Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–81% in males, and 47–60% in females. Occurrence of urolithiasis requires formation of a nidus, its retention and growth in the urinary tract which may cause obstruction of the ureter. *Abutilon indicum* species has been widely used as medicine in Ayurvedic system of medicine. *Abutilon indicum* (Malvaceae), commonly known as “Thuthi” is distributed throughout the hotter parts of India. *Abutilon indicum* commonly known as “Atibala” in Sanskrit gives excessive tonic strength. Phytoconstituents like β-Sitosterol (0.2%), tocopherol oil (0.3%) were isolated [2]. The word *zea mays* come from two languages. Zea comes from ancient Greek and is a generic name for cereal and grains. In traditional medicine, corn is used for relieving diarrhea, dysentery, urinary tract disorder, prostatitis, lithiasis, angina, hypertension and tumor [3].

Materials and Methods:

Description of plant

Abutilon indicum The botanical name of atibala is *Abutilon indicum* and it belongs to family Malvaceae.The plant grows throughout India and in Sri Lanka, at about an elevation of 1000-1, 500 metres. The perennial shrub grows 1.25-2 meters in height. Plant covered with minute hairs. Leaves are alternate, cordate and acute. The leaves are oblong, opposite, toothed, smooth and covered with fine white hair. The flowers are yellow, 2.5 cm in diameter. ***Zea mays*****:** The word *zea mays* come from two languages. Zea comes from ancient Greek and is a generic name for cereal and grains. In traditional medicine, corn is used for relieving diarrhea, dysentery, urinary tract disorder, prostatitis, lithiasis, angina, hypertension and tumor.

Pharmacological studies

Anti-urolithiatic activity [4]

Experimental design

Animal 6 groups and each group having 5 albino rats weighing 120-180 gm. were selected and housed under standard laboratory condition for a period of 14 days prior to the experiment. Experimental protocols were approved by our Institutional animal ethical committee, which follows guidelines of CPCSEA/ IAEC (Committee for the purpose of Control and Supervision of Experiments on Animals/Institutional Animal Ethics Committee).

Model Ethylene glycol induced model

Standard drug Cystone tablet 500 mg/kg body weight

Method 0.75% ethylene glycol induced kidney stone

Experimental group

Six groups contain 5 animals in each group were subjected to 0.75% ethylene glycol into drinking water for four weeks.

Group i: control group received only drinking water.

Group ii: model control group received drinking water + 0.75% ethylene glycol

Group iii: received drinking water +0.75% ethylene glycol + *abutilon indicum* extract 500 mg/kg

Group iv: received drinking water + 0.75 % ethylene glycol + *zea mays* extract 200mg/kg

Group v: received drinking water +0.75 % ethylene glycol + combination of both extract

Group vi: Received drinking water +0.75 % ethylene glycol + Standard drug 500 mg/kg

Statistical analysis

Standard evaluation was done using one-way analysis of variance (ANOVA) Statistical significance was set at $P< 0.001$. Results are presented as mean ± standard errors (S .E.).

Parameter

Total urinary volume Animals were placed in separate metabolic cages 24 hours before the surgery. Total urinary volume was measured, by using measuring cylinder, and reported in ml

Test for acidity Uric acid crystals were found to deposit most frequently in the concentrated acid urine. Thus the acidity of the urine was tested using pH meter**.**

Biochemical parameter of urine Urinary concentration of calcium, oxalate and cretinine were measured. Urinary oxalate was estimated according to the method described by Hodgkinson et al. 1 ml of urine was acidified by concentrated HNO_3 to solubelize crystals and then adjusted to pH 7 by NaOH in the presence of color indicator, the bromothymol blue. About 2 ml of saturated $CaSO_4$ and 14 ml of pure ethanol were added to precipitate oxalate overnight. The sample were centrifuged at 450 X g for 10 min and then filtered on filter paper. The precipitate obtained was solubelize in 10 ml of water acidified by 2 ml concentrated sulfuric acid. The sample were titrated by a solution of $KMnO_4$.Calcium analysis was performed by using a merck thermo spectronic U.V. double beam

spectrophotometer equipped with a Varian hollow cathode and a deuterium background corrector. Creatinine was estimated based on principle, the production of an orange colour by the interaction of Creatinine with alkaline sodium pirate. The colour produced was compared in a colorimeter, and the Creatinine content of the urine estimated by comparison with a Creatinine solution of known concentration [5].

Results and Discussion:

Percentage Yield of Extracts (%w/w)

Percentage yield (% w/w) of ethanolic extract of *Abutilon indicum* was found to be 12 % w/w. and ethanolic extract of *Zea mays* was found to be 10 %w/w.

Qualitative chemical evaluation

Ethanolic extract of *Abutilon indicum* showed the presence of amino acid, glucose, fructose, lutelion, carbohydrate glycoside, quercetion, Tannins, Pheniolic, and flavonoid.

Ethanolic extract of *Zea mays* showed the presence of alkaloids, glycoside, Carbohydrates, tannins, flavonoid, Saponin and steroid.

Anti-urolithiatic activity

The changes in the urine parameters in the experiment animals during the study are presented .The urine concentration of oxalate, calcium and Creatinine were increased significantly in animals administered with 0.75 percentage ethylene glycol. Four weeks treatment with ethanolic extract of *Abutilon indicum* significantly decreased urine concentration of oxalate (3.43 ±0.04), calcium (2.83±0.12) and creatinine (4.12 ± 0.06) as compared to model control (oxalate – 10.26 ± 0.09, calcium – 7.78± 0.19, creatinine – 6.79 ± 0.12) **.**The concentration of oxalate (3.55± 0.04), calcium (3.22±0.08**)** and creatinine (4.36 ± 0.08) of group IV treated with ethanolic extract of *Zea mays* were found to be significant as compared to model control (Group II). The concentration of oxalate (3.37 ± 0.13), calcium (2.85±0.04**)** and creatinine (4.15 ± 0.05) of group V treated with combination of ethanolic extract of *Abutilon indicum* and ethanolic extract of *Zea mays* were found to be more significant as compared to group II,group III,group IV**.**Moreover the group treated with combination of the two drugs (500 mg *Abutilon indicum* + 200 mg *Zea mays*) was found to be most significant from the entire group. The percentage reduction of all parameters of urine were found more in group V (500 mg *Abutilon indicum* + 200 mg *Zea mays*) and in group VI (standard). Urinary volume significantly decreased in the animals treated with the 0.75 % of ethylene glycol. Urinary volume were increased by 229 (Group III), 226 (Group IV), 274 (Group V), 281 (group VI) percentage compared to model control group . Urinary pH significantly increased in the animals treated with the 0.75 % of ethylene glycol. Urinary pH were decreased by 23.84 (Group III), 21.69 (Group IV), 25.58 (Group V), 24.97 (group VI) percentage with compared to model control group.

From the above results it was noted that the combination (ethanolic extract *Abutilon indicum* and ethanolic extract of *Zea mays*) were most significant.

In the present study of kidney stone, after the administration of ethanolic extract of *Abutilon indicum* and ethanolic extract of *Zea mays* and their combination to the group of rats urine analysis shows that the occurrence of stone was decreased when compared to the kidney stone control group and combination (ethanolic extract of *Abutilon indicum* and ethanolic extract of *Zea mays)* as effective as the standard group.

References:

[1] Abdul MM, Sarker AA, Saiful IM & Muniruddin A (2010) IJPPR 2: 1-4.

[2] Atmani F & Khan SR (2010) BJUI 85:621-625.

[3] Atmani F, Slimani Y, Mimouni M, Aziza M, Hacht B & Ziyyat A (2004) JE 95:87-93

[4] Atmani F, Slimani Y, Mimouni M & Hacht B (2003) BJU 92:137-140.

[5] Baheti DG & Kadam SS (2013) JAPER 3: 32-41.

PC-102

UV Spectrophotometric Determination of Piperine in Navasaya Churna: A Polyherbal Formulation

Nirmal Dongre[1], Pankaj Sharma[2], Birendra Shrivastava[2], PK Dubey[3]
[1]Indore Institute of Pharmacy, Indore.
[2]SOP, Jaipur National University, Jaipur.
[3]Swami Vivekananda College of Pharmacy, Indore.
E-mail address: dongrenirmal@gmail.com

Abstract: Navayasa Churna is an important ayurvedic formulation, is mentioned in Bhaishajyaratnavali in Pandu roga chikitsa[1] iscombination of Nine i.e. Amlaki, Bibhitaka, Haritaki, Marica Pippali, Sunth, Chitraka, Musta, Vidanga and Lauha bhasma. The formulation is dispensed for the treatment of Anemia (pandu), Hepatoprotective and Liver disorders. The method for spectrophotometric determination of piperine from the fruits of Piper longum, Piper nigrum and Navasaya Churna has been developed at absorption maxima 342.7nm. The concentration of piperine present in raw material was found to be 2.981 ± 0.38 % (w/w) in marica and 0.981 ± 0.047 % (w/w) in pippali, respectively and in three identical laboratory batch of Navasaya churna name NY-I, NY-II, and NY-III, was 0.223 ± 0.34, 0.219 ± 0.42, 0.215 ± 0.43 % (w/w), respectively with mean value 0.219 ± 0.903 % (w/w). The piperine content of all the three batches is found to be in close proximities with each other.

Introduction:

World health organization reported that 70% of the population in the developing countries relies on herbal or traditional medicines for their primary health care Ayurveda is our ancient system of medicine in India and various ayurvedic medicines are being used since vedic period. Most of the ayurvedic formulations are lacked in their defined quality control parameters and method of its evaluation [4]. World health organization has emphasized the need to ensure the quality and safety of medicinal plant products by using modern analytical and controlled techniques and applying suitable standards [2].

Navasaya Churna is well known ayurvedic formulation, comprised of the nine important medicinal plants *Embelica officinalis, Terminalia bellerica, Terminalia chebula, Piper nigrum, Piper longum, Zingiber officinalis, Plumbago zylenica, Cyperus rotundus, Embelia ribes and* Lauha bhasma. Navasaya churna comprised of fruits of *Piper nigrum* and *Piper longum* which contains piperine. Navasaya churna is used in the treatment of Anemeia (Pandu), Jaundice (kamala), Heart diseases (hrdroga), Piles (arsa) and Liver diseases [5]. Navasaya churna plays an essential role in the treatment of a wide variety of conditions. The present study was an attempt to develop the fingerprint method for Navasaya churna by UV spectrophotometric determination using piperine as a standard is an important and major content in formulation. The UV spectrophotometric analysis can be considered as one of the quality control methods for routine analysis.

Experimental:

The crude drugs were procured from local market of Indore, India and identified on the basis of morphological and microscopical characters and compared with standard Pharmacopoeial Monograph[3]. All the chemicals and solvents were used of AR Grade. Standard Piperine (98%) was procured from Sigma Aldrich.

Preparation of Navasaya churna

Navasaya churna, three batches name NY-I, NY-II, NY-III, were prepared in laboratory using method described in ayurvedic formulary. These three batches of Navasaya churna and powdered *Piper longum* and (Pippali), *Piper nigrum* (Marica), were estimated for their piperine contents against standard piperine solution on UV-Visible spectrophotometer (Shimadzu, UV-1700, Pharmaspec). As other ingredients not contain piperine are not included in present study.

Preparation of piperine extract of Navasaya churna

Reflux the powdered Navasaya churna (1 g) with 60 mL ethanol for 1 h. Filter the extract and reflux the marc left with 40 mL of ethanol for another 1 h. Filter and combine the filtrate. Concentrate the ethanol extract under vacuum till the semisolid mass is obtained. Dissolve the residue in 75 mL ethanol and filter through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 min, the supernatant was collected in 100 mL volumetric flask and volume was made with ethanol.

The same procedure was performed for each batch of Navasaya churna and separately powdered *Piper longum* (Pippali) and *Piper nigrum* (Marica) and solution (100 mL) of their piperine extract were prepared.

Preparation of standard solution of piperine

An accurately weighed piperine (100 mg) was dissolved in ethanol and volume was made up to 100 mL with ethanol in volumetric flask. 1mL of this solution was diluted with ethanol up to 100mL in volumetric flask to give 10 mg/mL piperine solution. Calibration curve from standard solution of piperine was prepared and with the help of this curve the piperine of Navasaya Churna was estimated. The method was validated for precision and accuracy.

Calibration curve of piperine

A series of calibrated 10 mL volumetric flask were taken and appropriate aliquots of the working standard solution of piperine were withdrawn and diluted up to 10 mL with ethanol. The absorbance was measured at absorption maxima 342.7 nm, against the reagent blank prepared in similar manner without the piperine. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted. The linear correlation between these concentrations (X-axis) and absorbance (Y-axis) were graphically presented and the slope (b), intercept (a) and correlation coefficient (r2) were calculated for the linear equation ($Y = bx + a$) by regression analysis using the method of the least square, (Table 1 and Figure 1).

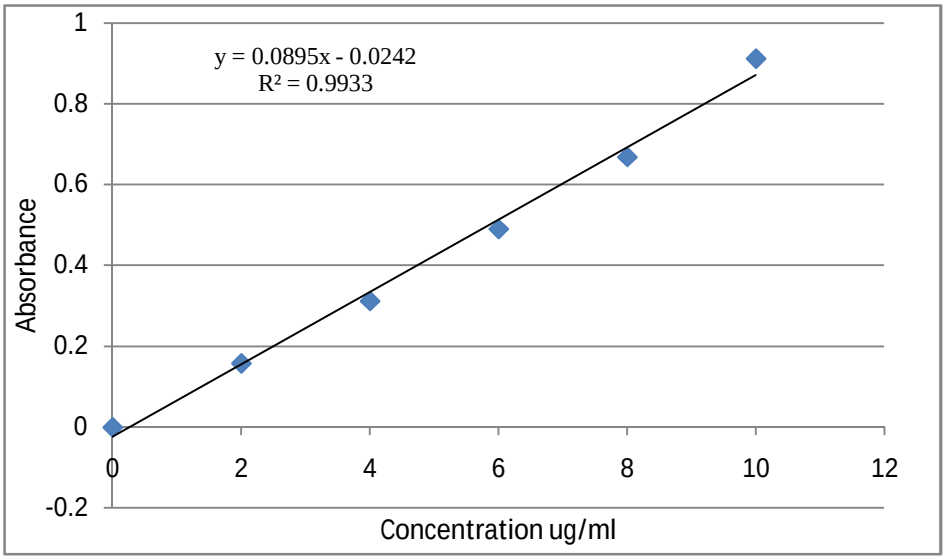


Figure 1: Calibration curve of piperine

Table 1: Optical characteristics, statistical regression data and validation parameter of piperine

S.No.	Parameter	Value
1	Absorption Maxima	342.7 nm
2	Beer's Law limit	2-10 ug /ml
3	Regression equation (y= bx+a)	y= 0.0895x -0.0242
4	Intercept (a)	0.0895
5	Slope (b)	0.0242
6	Correlation coefficients (r^2)	r^2 = 0.9933
7	Precision (n=6, % RSD)	0.351
8	Accuracy (%)	99.2

Estimation of piperine

The appropriate aliquots from piperine extract of each batch of Navasaya churna and separately *Piper longum* (Pippali) and *Piper nigrum* (Marica) were withdrawn in 10 mL volumetric flask separately. The absorbance for aliquots of each was noted at 342.7 nm. The Corresponding concentration of piperine against respective absorbance value was determines using the piperine calibration curve. The statistical analysis for checking uniformity in batches is also performed (Table-2)

Table 2: Estimation of piperine content in Navasaya Churna

S.no.	Name		Piperine content %w/w	Confidance level (95%)
1	Piper longum		0.981 ± 0.047	±0.494
2	Piper nigrum		2.89 ± 0.38	±0.268
3	Navasaya Churna	NY-I	0.223 ± 0.34	±0.327
		NY-II	0.219 ± 0.42	±0.425
		NY-III	0.215 ± 0.43	±0.223

Mean ± SD of six determinations, NY-I: Navasaya Batch I, NY- II: Navasaya Churna Batch II, NY-III: Navasaya Churna Batch III.

Precision and accuracy

The method was validated for precision and accuracy, by performing the recovery studies at two levels by adding known amount of piperine extract of Navasaya churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calculated (Table-3).

Table 3: Compilation data of recovery study

S.no.	Amount of piperine (µg/ml)			RSD%	SE	Recovery%
	Sample	Added	Estimated			
1	100	50	149.03 ± 0.62	0.409	0.25	99.24±0.25
2	100	100	198.22 ± 0.57	0.292	0. 237	99.13±0.23
Mean				**0.551**	**0. 248**	**99.18**

Mean ± SD of six determinations, **RSD** =Relative Standard Deviation, **SE =** Standard Error

Results and Discussion:

Piperine obeys Beer Lambert's law in concentration range 2-10 µg/mL at λmax 342.7 nm. The correlation coefficient (r2) was calculated where the r^2 value 0.9933 indicates the good linearity between the concentration and absorbance. The estimation of piperine content of Navasaya churna (three identical laboratory batch) and powdered *Piper longum* (Pippali) and *Piper nigrum* (Marica) was carried out seperately. The concentration of piperine present in raw material was found to be 2.89 ± 0.38 % (w/w) in marica and 0.981 ± 0.047 % (w/w) in pippali, respectively and in three identical laboratory batches of Navasaya Churna name NY-I, NY -II, NY -III, was 0.223 ± 0.34, 0.219 ± 0.42, 0.215 ± 0.43 % (w/w) (Table-2) respectively with mean value 0.219 ± 0.903 %(w/w).

In order to obtain precision and accuracy, the recovery study was performed at two levels by adding known amount of piperine with pre analyzed sample of piperine in Navasaya Churna. The experiment was repeated six times at both level (Table-3) and result shows 99.24 ± 0.25 and 99.13 ± 0.23 % recovery of piperine at both the level with mean value 99.18 ± 0.25 %, which prove reproducibility of the result. This shows significant precision of methods at 95 % confidence level. The relative standard deviation (RSD %) value was found to be 0.409 and 0.292 with mean 0.551 at both the level while the standard error was 0.25 and 0.237 with mean 0.248, respectively. From the data, it is obvious that the present method of spectrophotometric determination of piperine is simple, precise, accurate and suitable for routine analysis of piperine in Navasaya churna.

As Navasaya churna is a good source of piperine, these findings can be taken as one of the parameter, along with other parameters, for quality control of Navasaya Churna.

References:

[1] Bhaisajyaratnavali (1961) Chowkhamba Sanskrit Series Office, Varanasi, Sloke, ed^{n}2 35:19.

[2] World Health Organization (1998) Quality Control Methods for Medicinal Plants Materials, Geneva 1-15.

[3] The Ayurvedic Pharmacopoeia of India (1999) Part I Vol 2 1133.

[4] Jain V & Saraf SS (2007) Asian J Chem 19.5331-5335.

[5] Dongre N, Dubey PK & Shrivastava B (2017) IJPLS 11(8):44.

PC-103

Antioxidant and Antifungal Activity of Some Medicinal Plant Extracts

Sachin Kumar Jain, Praveen Sharma, Neelam Balekar, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Rajendra Nagar, AB Road, Indore (M.P.) India 452012
E-mail address: Sachin225819@rediffmail.com

Abstract: In this study, the antioxidant and antifungal activities of extracts of *Tabernaemontana alternifolia, Thuja occidantalis* and *Cajanus cajan* were recognized. Total yields, radical scavenging activity and the antioxidant property of the extracts were investigated. The free radical scavenging activities of extract was documented as 41.3 ± 2.1 to 76.9 ± 6.1%, likewise. The antioxidant capacity of extracts was recognized between 153.4 ± 10.1 to 336.7 ± 24.2 mg/g extract. This extract was explored for antifungal activity by paper disc method against 2 fungi by using flucanazole as a standard drug. The 0.5 % level of *Tabernaemontana alternifolia* extract exhibited 100% inhibition till the 7th day of incubation when compare to control (Ascorbic acid). Statistical variances within fungi were significant at $p<0.05$. The extract was to some extent effective against all the fungi used in experiment.

Introduction:

Recently, the use of spices and herbs as antioxidants and antimicrobial agents in foods is becoming of growing importance. Antioxidants have been extensively used as food additives to offer protection against oxidative degradation of foods [1]. Newly in different parts of the world, awareness has been paid to exploiting plant products as tale chemotherapeutant and preservatives in plant protection and food storage [2]. Fumigation is a handling measure which is intended to eradicate pests and microorganisms in fasting grains and other possessions as well with toxic chemicals or fumigants [3]. The antimicrobial property of spice and essential oils have been known for a long time, and a number of study of the antimicrobial effect of spices, essential oil and their components have been reported. Even though, most of the reports on natural products in agricultural areas are about insects, there are important reports enlightening that plant extracts and essential oil exhibit antimicrobial action against food and several storage fungi, leaf pathogens and soil borne.

Material and Methods:

Plant materials

Nag Kuda (*Tabernaemontana alternifolia*), Red cedar (*Thuja occidantalis*) and pigeon pea (*Cajanus cajan*) were obtained from a garden. Plants are stored in a dry, dark and cool room, and were grounded before use.

Extraction The spices were dried, grounded and extracted in 90% methanol + 9% water + 1% acetic acid mix. The extraction period was 24 h. After filtration; the filtrate was evaporated under vacuum,

less than 45 C^0.

Figure 1: *Tabernaemontana alternifolia*

Figure 2: *Thuja occidantalis*

Figure 3: *Cajanus cajan*

Antioxidant activity

Antioxidant activities in the latter samples were resolute *in vitro* via scavenging of the ABTS (2,2%-azino-bis-3- ethyl-benzthiazoine-6-sulphuric acid) radical, generated by a metmyoglobin: hydrogenperoxide system, as described earlier [4]. Samples were diluted 1/6 with the extraction solvent. Free radical scavenging activity were resolute by DPPH method and the results were articulated as IC_{50} (mg/ml), minimum extract required to inhibit the 50% of 1, 1-diphenyl-2-picrylhydrazyl [5].

Fungal strains

The fungi used in the assay *Aspergillus niger* and *Aspergillus parasiticus* were collected from the local market of Indore.

Antifungal assay

Filter paper of in diameter of 5 mm was soaked with 0.1, 0.3 and 0.5% of *Tabernaemontana alternifolia, Thuja occidantalis* and *Cajanus cajan* extract. Paper discs were placed on the cover of petri dishes. The consequence of extracts was tested alongside the mycelial growth of *A. niger* and *A. parasiticus* using Potato Dextrose Agar (PDA) *in vitro*. Medium (20 ml) was dispensed into each Petri plate and 5 mm diameter plugs of each species were excise from the margin of a 14-day-old culture grown on PDA. The colony diameter was calculated at several points and the percentage mycelia inhibition calculated.

$$I= [(C-T)/C] \times 100$$

Where I is inhibition (%), C is the colony diameter of control (mm), and T is the colony diameter of test (mm).

Statistical analyses

Outcome of the research were analyzed for statistical implication by analysis of discrepancy. This research was performed by three duplicates with a replicate.

Results and Discussion:

Total yield (%), antiradical activities (%) and antioxidant capacity (mg/g) of plant extracts are given

in Table 1. The yield of extract was established between 9.7 ± 0.7 % to16.3 ± 0.9. The antiradical activity of plant extracts was ranged between 36.5 ± 2.3 to 78.9 ± 7.1% (Table 2).

Table 2: Total yield, antiradical and antioxidant capacity of with several plants extracts (%)

Extract	Total yield	Antiradical activity (%)	Antioxidant capacity (m mol/100ppm extract)
Ascorbic acid (Standard)	-	39.4 ± 1.1	149.8 ± 12.4
Tabernaemontana alternifolia	13.4[a] ± 1.5[b]	41.3 ± 2.1	153.4 ± 10.1
Thuja occidantalis	11.3 ± 0.8	68.4 ± 4.5	278.2 ± 16.6
Cajanus cajan	10.7 ± 0.4	76.9 ± 6.1	336.7 ± 24.2

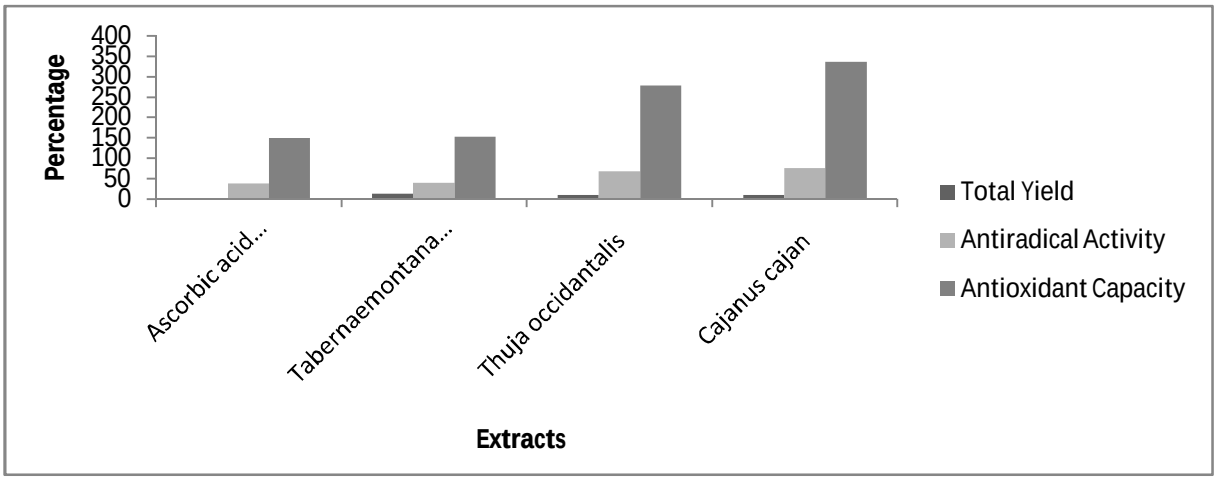
a Results are mean values of triplicate determinations; b Standard deviation

The activity of the extracts is qualified to their hydrogen donating capability. Comparison with BHT, these data obtained expose that the extracts are free radical inhibitors and primary antioxidants that react with the radicals. The antioxidant capacity 167.4 ± 12.3 to358.7 ± 17.6 mg/g extract (equivalent to ascorbic acid), correspondingly. Inhibition rates of all the extracts against *A. niger* decrease and even stimulated the mycelial growth of *A. parasiticus* during the end of incubation. *Tabernaemontana alternifolia* and *Thuja occidantalis* slightly inhibitory effect than *Cajanus cajan* extracts against *A. niger* (Table 1 & Fig. 4). Higher levels of *Cajanus cajan* extract showed greater fungi toxic action against *A. niger*. The 0.5% levels of *Cajanus cajan* showed inhibition with a steadily decreasing rate at the end of incubation.

Table 1: Percentage inhibition of some fungi mycelial growth with several plants extracts (%)

Fungi	Days	*Flucanazole* (Standard)			*Tabernaemontana alternifolia* (%)			*Thuja occidantalis* (%)			*Cajanus cajan* (%)		
		0.1	**0.2**	**0.3**	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
A. parasitius	3	8.2	8.5	8.9	10.3	12.4	14.4	13.2	17.3	19.3	14.2	20.3	23.7
	4	8.4	8.8	9.0	10.1	12.1	14.1	12.4	16.8	18.8	13.9	19.3	22.4
	5	8.8	9.2	9.4	9.9	10.2	13.5	12.1	15.9	17.2	12.6	17.3	21.5
	6	8.1	9.0	8.9	9.2	9.5	12.9	11.3	14.2	16.1	11.5	16.8	20.5
	7	9.1	8.7	9.5	8.5	8.9	11.3	9.3	8.9	15.3	10.5	15.2	19.4
A. niger	3	7.8	8.1	9.4	18.3	22.4	25.4	28.2	30.3	31.3	34.4	36.4	38.9
	4	7.5	8.4	9.0	17.1	21.3	24.1	27.4	26.9	30.8	33.5	29.6	37.8
	5	8.0	9.4	7.2	16.9	20.2	23.5	26.1	25.8	27.4	32.6	27.1	36.7
	6	8.2	9.2	8.4	15.2	19.5	22.9	25.3	24.1	26.4	31.5	26.6	35.2
	7	7.2	8.7	8.4	12.5	18.9	21.3	19.3	18.8	25.6	30.3	25.7	30.3

Figure 4: Total yield, antiradical and Antioxidant capacity of with several plants extracts (%)



Conclusion:

Fruits, vegetables and herbs are recommended at present as optimal sources of chemical constituents with antioxidant activity and supplementation of human diet with plants containing high amounts of compounds accomplished of deactivating free radicals may have valuable effects. Usually, the extent of the antioxidant and antibacterial property of the extracts could be accredited to their phenolic compositions.

It can be accomplished that extract obtained from these extracts can provide a good chance as an antioxidant and antifungal agent in food industry, if any Organoleptic effects are satisfactory. After these screening experiments, additional work will be performed to describe the anti-oxidative and antifungal activities in more detail. Supplementary research is needed on the determination of the association between the antioxidant capacity and the chemical composition of the plants.

References:

[1] Dorman HJD & Deans SG (2000) J Appl Microbiol 88:308-316.

[2] Hsieh PC Mau JL & Huang SH (2001) 18:35-43.

[3] Biavati B, Özcan M & Piccaglia R (2004) Food Microbiol 54(4):393-401.

[4] Ünver A, Arslan D, Özcan MM & Akbulut M (2009) World Appl Sci J 6(3): 373-377.

[5] Erkmen O & Özcan M (2004) J Essent Oil Bear Plants 7(3):279-287.

PC-107

Formulation, Development and Evaluation of Herbal Powder Shampoo

Ashutosh Chouhan[1], Shikha Jaiswal[1], Jitendra Malviya[1], Raghvendra Dubey[1], Sandeep Barman[2]
[1]Central India Institute of Pharmacy, Indore, (M.P.)
[2]BM College of Pharmaceutical Education And Research, Indore, (M.P.)
E-mail address: jaiswalshikha15@gmail.com

Abstract: Now a days synthetic chemicals are produces many harmful effects like irritation on skin, dryness, sometimes produce pimples etc. so that's why we selected herbal products for making herbal powder shampoo. In the present research work herbal powder shampoo by using methi seeds is good ingredient for smoothing hair, Amla used for strengthening hair or thickness of hair etc. Present study deals with the formulation, development and evaluation of herbal powder shampoo that was successfully prepared and evaluation parameter of this formulation was passed. This formulation is cheapest and there are no side effects, it is easily apply on the hair.

Introduction:

Cosmetics Cosmetics also known as make-up are substances or products used to enhance the appearance or fragrance of the body. Many cosmetics products are use to designed for applying onthe face, hair and other essential parts of body. In the 21st century, women generally use more cosmetics than men. They are generally mixtures of chemical compounds and some obtained from natural sources (such as coconutoil, etc.). Common cosmetics such as lipstick, mascara, eyeshadow, foundation, rouge, skin cleansers, skin lotions, shampoo, hairstyling products (gel, hair spray, etc.), perfumes etc.

Shampoo Shampoo is a care product of hair and used for cleaning hair. Shampooare used by applying it to wet hair, massaging product into the hair, and then rinsing it. Some users may follow a shampoo with the use of hair conditioner. The goal of using shampoo is to remove the unwanted dirt, dandruff on the hair without stripping out. Shampoo is generally made by combining a surfactant, most often sodium lauryl sulfate or sodium laureth sulfate, with a co-surfactant, most often cocamidopropyl betaine in water. Some special features of shampoos are available in market removing dandruff, color-treated hair, reduce gluten or wheat allergies, an interest in using an "all-natural", "organic", "botanical" or "plant-derived" product, for infants as well as young children ("baby shampoo" is less irritating). Shampoos are also use intended for animals that may contain insecticides or other medications to treat skin conditions or parasite infestations such as fleas.

Ideal characteristics of shampoo

- Should effectively and completely remove the dust, excessive sebum.
- Should effectively wash hair.
- Should produce a good amount of foam

- The shampoo should be easily removed by rinsing with water.
- Should leave the hair non dry, soft, lustrous with good, manageability.
- Should impart a pleasant fragrance to the hair.
- Should not make the hand rough and chapped.
- Should not have any side effects or cause irritation to skin or eye.

Materials and Methods:

Materials: Methi Seed Powder, Neem Powder, Amla Powder, Orange Powder, Reetha Powder, Ratanjot Powder, Peppermint.

Method: Sieving and Mixing.

Pre Formulation:

Formulation of herbal powder shampoo: After many trials and three formulations viz., F1, F2 & F3 was formulated. All different formulas prepared in different quantity as shown in table.

Table 1: Formulation of herbal powder shampoo

S.No.	Ingredient	F1	F2	F3
1	Methi Seeds Powder	0.80 gm	0.50 gm	0.30 gm
2	Neem Powder	0.80 gm	0.50 gm	0.5 gm
3	Amla Powder	2.2 gm	2.2 gm	1.2 gm
4	Orange Powder	1.5 gm	1.5 gm	1.0 gm
5	Reetha Powder	0.80 gm	0.50 gm	0.50 gm
6	Ratanjot Powder	1.7 gm	1.5 gm	1.0 gm
7	Peppermint	2.2 gm	3.3 gm	5.5 gm

Results and Discussion:

Now a days Medicinal plants used in the formulation of herbal shampoo were found in rich source of novel drugs. This ingredients are obtained from plantssuch as Methi, Reetha, Neem, Amla, Chinarose, Orange, Peppermint, Ratanjot and there are no cause side effects and has been reported for hair growth and conditioning. All quality control parameters were checked and parameters give a favourable result. The research work shows that the active ingredients of these drugs incorporated in shampoo give more stable and good aesthetic appeal. The pH of the shampoo has been shown to be important for improving and enhancing the qualities of hair, minimizing the irritation to the eyes and stabilizing the ecological balance of the scalp. The current trend to promote shampoo of lower pH is one of the minimizing damage of the hair. After completion of formulation all parameter done and result were reported. This formulation is cheapest and there are no cause side effects, it is easily apply on the hair and easily homemade formulation.

Table 1: Physical appearance

S.No.	Parameters	Result
1.	Colour	Yellowish green
2.	Odour	Slight pleasant
3.	Taste	Characteristics
4.	Texture	Fine smooth

Table 2: Different evaluation parameter

S.No.	Parameters	Result
1.	Particle size	20-25um
2.	Angle of repose	32^{o}
3.	Bulk density	0.8gm/cc
4.	Tapped density	0.9gm/cc
5.	Carr's index	12
6.	Hausner ratio	1.13
7.	pH	5.6
8.	Foaming property	Good Foam
9.	Nature of hair after wash	Soft and Smooth
10.	Dirt Dispersion	Moderate

Conclusion:

In the present research work the herbal powder shampoo by using methi seeds is good ingredient for smoothing hair. We formulated a herbal powder shampoo by using plant extracts which are commonly used traditionally lauded for their hair cleansing action.

Globalisation is the need of today and the world market will open for all by 2005. The world is also moving towards herbal medicines for health care, health foods and for cosmetic purposes including hair preparations. India is rich heritage for cultivation and production of herbal medicines due to its diversified climatic conditions. It is possible that herbal powder shampoo, although better in performance and safer than synthetic ones will be popular with the consumers.

After whole studies found that it is good for hair smoothing, strength, conditioning and shining also provided better effect for hair purpose.

References:

[1] Suriyprakash TNK, Kalaivani R, Lakshmana Prabu S & Sumathi A (2011) Elixir Pharmacy 39: 4639-4642.

[2] Saad AH & Kadhim RB (2011) IJRAP 2:1802-1806.

[3] Dubey S, Nema N & Nayak S (2004) Ancient Sci life 26: 38-44.

[4] Deshmukh S, Kaushal B & Ghode S (2012) Int J Pharm Biol Sci 3:638-645.

[5] Barel AO, Paye M & Maibach HI (2001) Handbook of Cosmetic Science and Technology 773-775.

PD-12

Quercetin Exerts Anti-convulsant Effects in Animal Model of Grand Mal Epilepsy: Modulation of GABA and Glycinergic Pathways

Gaurav Parihar, Bhubharti Dehariya, Santosh Ghule, Pankaj Dixit, Neelam Balekar
IPS Academy College of Pharmacy, Rajendranagar, A.B. Road, Indore, (M.P.) India – 452012
E-mail address: gauravpariharcop@gmail.com

Abstract: Quercetin is low molecular weight flavonoid having multiple neuropharmacological actions. Recently, its anticonvulsant effect was reported in rats using electrical kindling models. However, earlier cell culture studies have documented antagonistic action of quercetin on $GABA_A$, $GABA_C$ and Glycine channels, which is contrary to recent findings. Hence, the present study aimed at characterizing the effects of quercetin administration in various experimental models of epilepsy. Dose-dependent effects of quercetin on maximal-electroshock seizure (MES) were determined. Further, the effective dose was tested in pentylenetetrazol-induced seizures (PTZ), and strychnine-induced seizures to study the involvement of GABA and Glycinergic receptors. The results revealed a potent anticonvulsant effect of quercetin in MES induced seizures at a dose of 5 and 10 mg/kg. This effect was found to be retained in case of PTZ-induced convulsions and strychnine-induced convulsions (quercetin 10 mg/kg). The present finding warrants further substantiation along with their correlation to molecular mechanism of action.

Introduction:

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for antiepileptic drugs with novel structures and better safety and efficacy profiles. Quercetin reportedly has a wide range of biological activities. Recently quercetin (10 mg/kg) was reported to protect rats from kindling seizures after chronic administration of quercetin [40 mg/kg/day]. However an earlier work documented its proconvulsant potential by modulation of brain Hsp70 [70-kDal heat shock protein] in MES and NMDA-induced seizures [1]. Further, it was reported that quercetin is an antagonist at $GABA_A$ and $GABA_C$ receptors, Human glycine α1 receptor. The IC_{50} for brain glycine receptor is reported to be 10.7 µM and the mechanism is thought to be non-competitive in nature.The IC_{50} for $GABA_c$ and $GABA_A$ receptor was reported to be 4.4-4.8 µM. This evidence is contrary to the studies indicating anticonvulsant properties of quercetin. The aim of the present study was to systematically investigate the effect of quercetin administration on the incidence of convulsions in various experimental models of epilepsy [2, 3].

Materials and Methods:

Animals Adult male Albino Wistar Rats (150-200g) and Swiss Albino Mice (6-8 weeks) of either sex weighing about 18-20 g [Animal House of IPS Academy College of Pharmacy, India] were used for

the study. All experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee (IAEC) (Protocol no. CPCSEA/82/2011).

Drugs

Quercetin and pentylenetetrazole (PTZ) were purchased from (Sigma-Aldrich Co, St. Louis, MO). Strychnine (STR) was generous gift from Alpa Laboratories (Indore, M.P., India). Phenytoin injection (Dilantin, Pfizer, India), diazepam injection (Calmpose, Ranbaxy, India) were purchased locally. Quercetin was dissolved in propylene glycol. All drugs were prepared each day as fresh solutions or suspensions and administered intraperitoneally (i.p.) in a volume of 0.01 ml/g body weight.

Maximal electroshock convulsions

Electroconvulsions were produced by an alternating current (0.2 s stimulus duration, 50 Hz) delivered via standard auricular electrodes by a Hugo Sachs generator (Rodent Shocker, Type 221, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension [4].

Pentylenetetrazole (PTZ)-induced seizures

The PTZ-induced seizures in rodents are thought to be an experimental model of myoclonic convulsions in humans. Clonic convulsions were induced in mice by the s.c. administration of PTZ at the dose of 100 mg/kg. The clonic seizures were defined as clonus of the whole body lasting over 3 s with an accompanying loss of righting reflex [4].

Strychnine (STR)-induced convulsion

Swiss albino mice (18-25g) were divided into different groups each containing five animals and treated with either propylene glycol (5ml/kg) as control group, quercetin (1, 5, and 10 mg/kg, i.p.) as test group or glycine (750 mg/kg, i.p.) as standard group. Thirty minutes after drug/vehicle administration seizures were induced by intraperitoneal administration of 2 mg/kg strychnine nitrate. The time until occurrence of tonic extensor, convulsions and death was noted during 1h period [4].

Locomotor activity

Separate groups of mice were treated with quercetin (10 mg/kg, i.p.) or diazepam (2 mg/kg, i.p.) or respective control and were subjected to locomotor test session of 10 min in an actophotometer [5].

Muscle relaxant activity

Mice were selected for this test on their ability to remain on the revolving rod for at least 1 minute. The test compounds were administered intraperitoneally. Thirty minutes after intraperitoneal administration the mice were placed for 1 min on the rotating rod. The number of animals falling from the roller during this time is counted [5].

Statistical Analysis

Data were expressed as the mean±SD. wherever required the data were analyzed with one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparision test. $P<0.05$ was considered as significant.

Results and Discussion:

Maximal Electroshock Convulsion

A single maximal electroshock seizure produced an immediate tonic hind-limb extension of 5-10 s duration followed by clonic seizures, lasting up to 20 s. Following a maximal electroshock seizure, there was a significant ($P < 0.01$; ANOVA with post-hoc Tukey's test). A single dose administration of Quercetin (1, 5 and 10 mg/kg, i.p.) is significantly reduced hindlimb extension as compared to vehicle control after 4 h administration of Quercetin. On other hand 30 minutes after administration of Quercetin, only 10mg/kg is significantly reduced hindlimb extension.

Table 1: Effects of Quercetin (1mg, 5mg, 10m/kg) after 30 minutes and 4 h on maximal electroshock-induced seizures

Treatment	Dose	Duration of extensor in sec		% Recovery
		After 30 min	After 4hr	
Control	5ml/kg	15.80± 5.85	17.50±2.38	100
Phenytoin	25mg/kg	3.67±1.16[a]	4.40±1.40[b]	100
Quercetin	1mg/kg	13.40±3.36	7.60±0.89[b]	100
Quercetin	5mg/kg	13.40±4.22	8.80±2.17[b]	100
Quercetin	10mg/kg	3.75±0.96[a]	7,75±2.99[b]	100

Values are expressed as mean ± S.D. (n=5) [a]P<0.01, [b]P<0.001 when compared with vehicle-control by one way analysis followed by Tukey's test.

Strychnine induced convulsion

Quercetin at the dose of 10 mg/kg i.p. protected 100% and 80% of mice after 30 min and 4 h from tonic seizures respectively. The effect of glycine, 750 mg/kg (100% protection) 5mg and 10mg/kg 30 min. after administration of Quercetin significantly delayed the onset of the seizures.

Table 2: Effect of the Quercetin on STR-induced tonic seizures in mice

Treatment	Dose	Onset time of clonic convulsions		% Recovery	
		30 min	4 h	30 min	4h
Control	5ml/kg	472.6±141.9	472.6±141.9	0	0
Glycine	750 mg/kg	0.0±0.0 [c]	0.00 ±0.00[b]	100	100
Quercetin	1mg/kg	170.6±167.7[a]	283.2±234.1	0	20
Quercetin	5mg/kg	69.0±154.3[c]	292.2±183.7	80	20
Quercetin	10mg/kg	48.20±107.8[c]	99.40±138.1[a]	100	80

Values are expressed as mean ± S.D. (n=5). [a]P<0.05,[b]P<0.01, [c]P<0.001 when compared with vehicle-control by one way analysis followed by Tukey's test.

Pentylentetrazole induced convulsions

Quercetin (10mg/kg) after 30 min. administration at the tested doses did not significantly influence the latency period of duration of convulsions, and mortality. Whereas, diazepam (10 mg/kg, i.p.) treated animals failed to show any signs of convulsions and protected all the mice from PTZ-induced convulsions.

Table 3: Effect of the Quercetin on PTZ-induced seizures in mice

Treatment	Dose	Time of Clonic Convulsions in sec	% Recovery
Control	5ml/kg	230.4±27.5	00
Diazepam	25mg/kg	44.6±99.7[a]	80
Quercetin	10mg/kg	102.4±140.2	60

Values are expressed as mean ± S.D. (n=5). [a]P<0.01, when compared with vehicle-control by one way analysis followed by Tukey's test.

Locomotor activity

The average Actophotometer reading in the control group was after administration of Quercetin 10 mg/kg after 30 min did not reduced the locomotor activity. This shows that Quercetin did not have CNS depressant property.

Table 4: Effect of the Quercetin on locomotor activity in mice

Treatment	Dose	Photocell count within 30 min.
Control	5ml/kg	568.2±81.61
Quercetin	10mg/kg	505.4±107.2[ns]

Values are expressed as mean ± S.D. (n=5). [ns]P>0.05, when compared with vehicle-control by one way analysis followed by Tukey's test.

Rota Rod Method

Treatment with Quercetin (10 mg/kg), did not influence the rota rod performance in mice indicated the effects of these treatments were devoid of ataxic effects.

Table 5: Effect of the Quercetin on muscle relaxant activity in mice

Treatment	Dose	Rotating capacity of mice up to one min.
Control	5ml/kg	60.00 ± 0.00
Quercetin	10mg/kg	57.0 ± 2.8 [ns]

Values are expressed as mean ± S.D. (n=5). [ns]P>0.05, when compared with vehicle-control by one way analysis followed by Tukey's test.

Conclusion:

The results revealed a potent anticonvulsant effect of quercetin in MES induced seizures at a dose of 5 and 10 mg/kg and at higher dose 10 mg/kg STR-induced tonic seizures, PTZ-induced seizures. This effect was found to be mediated via actions on the GABAergic and Glycinergic ion channels. The present finding warrants further substantiation along with their correlation to molecular mechanism of action.

References:

[1] Kandratavicius L, Balista PA & Lopes-Aguiar C (2014) Neuropsychiatr Dis Treat 10:1693–05.

[2] Harvey RJ and Yee BK (2013) Nature Reviews Drug Disc 12: 866–885.

[3] Yang L, Shen H, Merlin LR & Smith SS (2016) Sci Rep 6: 31928.

[4] Cavarsana CF, Matsuob A, Blancoa MM & Mello LE (2015) Epi Behav 44: 90-95.

[5] Bhosale UA, Yegnanarayan R & Pophale PD (2011) Int J Appl Basic Med Res 1(2): 104–108.

PD-22

Memory Enhancing Activity of Hydroalcoholic Extract of *Terminalia catappa* Leaves

Ankur Joshi[1], Neelesh Malviya[2]
[1]Modern institute of Pharmaceutical Sciences, Indore
[2]Smriti College of Pharmaceutical Education, Indore
E-mail address: ankurpharmacology@gmail.com

Abstract: The present study was to evaluate the effect of *Terminalia catappa* on cognitive functions and cholinesterase (ChE) activity in scopolamine-induced amnesia in rats. The extract of *Terminalia catappa* was administered orally at three doses (100, 200 and 300 mg/kg) for 7 and 14 consecutive days to the respective groups of rats. Piracetam (120 mg/kg) was used as a standard nootropic agent. Learning and memory parameters were evaluated using elevated plus maze (EPM) and passive avoidance. Brain cholinesterase activity was evaluated. It was observed that *Terminalia catappa* at the above- mentioned doses after 7 and 14 days of administration in the respective groups significantly reversed scopolamine (1 mg/kg i.p.)- induced amnesia, as evidenced by a decrease in the transfer latency in the EPM task and step-down latency in the passive avoidance task. *Terminalia catappa* reduced the brain ChE activity in rats.

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that's slow in onset but, ultimately, results in dementia, uncommon behavior, personality change and, ultimately, death. Formation of memory is the most complex process and involves multiple neural pathways and neurotransmitters. It is documented that the cholinergic neural system plays a crucial role in learning and memory in humans and animals. Despite the great strides that have been made in the understanding and management of Alzheimer's disease (AD) and other neurodegenerative diseases, and disease related complications are increasing unabated. A variety of synthetic drug therapies are available for the treatment of Alzheimer but they associated with severe side effects and expensive. Therefore, there's an urgent need of exploring all the available options to address the menace of this disease. Plant medication and herbal formulations are frequently thought of to be less toxic and free from side effects than synthetic one. In spite of the presence of known synthetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease.

Material and Methods:

Experimental design

The experimental design was planned such that the effect of *Terminalia catappa* at doses of 100, 200 and 300 mg/kg could be evaluated after 7 and 14 days against scopolamine-induced amnesia. For this purpose, the rats were divided into two sets of eight groups each (16 groups). The treatment period for animals of set I and set II was 7 and 14 days, respectively. At the end of the treatment

period, all the animals were subjected to scopolamine (1 mg/kg i.p.) 60 min. The cognitive paradigms were evaluated 45 min after the scopolamine administration using the elevated plus maze (EPM) and passive avoidance models. Further, the animals were euthanized by cervical decapitation, and the brains were isolated to evaluate the anticholinesterase (ChE) activity of *Terminalia catappa*.

Exteroceptive behavioral models

EPM

The EPM served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in rats. The plus maze apparatus consisted of two open (50 cm × 10 cm × 40 cm) and two enclosed arms (50 cm × 10 cm × 40 cm), with an open roof, arranged such that the two open arms were opposite each other. The maze was elevated to a height of 50 cm from the ground to measure the anxiety index in rats.

Passive shock avoidance paradigm

Passive avoidance, based on negative reinforcement, was recorded to examine the long-term memory. The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3 mm stainless-steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock was delivered to the grid floor. The rats were initially trained and the step-down latency (SDL) was recorded. SDL is defined as the time taken by the rat to step down and place all four paws on the grid floor. Rats showing SDL in the range of 2-15 s during the training session were taken for the acquisition and the retention tasks. The acquisition task was carried out 90 min after the training session.

Biochemical Estimations

Estimation of ACh Levels in the Brain by Quantifying ChE Inhibition

After assessing the learning and memory paradigms in scopolamine- induced amnesia, rats from each group were euthanized by cervical decapitation. The whole brain was immediately removed and chilled in ice-cold phosphate buffer. After washing in ice-cold phosphate buffer, the brains were homogenized in 5 ml of phosphate buffer in a glass TEFLON homogenizer. The brain homogenate was then evaluated for enzyme activity using Augustinsson's method of analysis.

Results and Discussion:

Exteroceptive behavioral models: EPM: The effect of the vehicle, scopolamine control, *Terminalia catappa* (100, 200 and 300 mg/kg) and piracetam (120 mg/kg) were evaluated at the end of days 7 and 14. The scopolamine (1 mg/kg) control group showed a significant ($P < 0.01$) increase in TL values on the acquisition as well as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory. In the AT on day 7 for set I and on day 14 for set II, the *Terminalia catappa* at dose levels 100, 200 and 300 mg/kg demonstrated decrease in the TL as compared to the scopolamine control group. The results obtained were found to be statistically

significant ($P < 0.01$). In the RT on day 8 for set I and day 15 for set II, the *Terminalia catappa* at the dose levels 100, 200 and 300 mg/kg demonstrated a significant ($P < 0.01$) decrease in the TL as compared to the scopolamine control group. Piracetam (120 mg/kg p.o.) exhibited marked decrease ($P < 0.01$) in TL in comparison with the scopolamine control group. However, *Terminalia catappa* at the dose levels 200 and 300 mg/kg showed a decrease in the TL, which is comparable to that shown by piracetam ($P < 0.01$) [Table 1].

Table 1: Effect of the extract of *Terminalia catappa* on transfer latency (elevated plus maze paradigm) in scopolamine-induced amnesia in rats

Treatment Groups	TL on acquisition day (sec)		TL on retention day(sec)	
	7 day	14 day	7 day	14 day
Vehicle control	95.17±14.92	39.83±5.89	55.33±8.77	23.83±4.13
Scopolamine hydrobromide	143.67±17.2	118.8±11.03	128.33±19.53	118.83±26.13
Terminalia catappa (100)+ Scopolamine (1)	65.17±7.98	32.17±7.71	40.16±8.16	12.67±2.5
Terminalia catappa (200)+ Scopolamine (1)	55.5±4.75	17.33±4.73	23.83±3.69	10.17±2.33
Terminalia catappa (300)+ Scopolamine (1)	30.5±6.67	15.33±3.05	16.83±1.74	3.5±0.34
Piracetam(120)+ Scopolamine (1)	52.17±9.85	26.67±3.60	34.17±5.21	7.33±2.13

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

Passive shock avoidance paradigm: Scopolamine hydrobromide (1 mg/kg i.p.) decreased SDL on the AT and RT training, indicating impairment of memory. There is a slight increase in SDL after the administration of *Terminalia catappa* (100, 200 and 300 mg/kg p.o.) for 7 days as compared with the scopolamine control group on the AT and RT, indicating improvement in learning and memory of rats. However, in the AT, *Terminalia catappa* at the dose levels 100 and 200 mg/kg p.o. increased SLD, which is comparable to standard piracetam, but failed to exhibit a significant change when compared with the scopolamine control after 14 days of administration. However, *Terminalia catappa* (100, 200 and 300 mg/kg) was found to significantly ($P < 0.05$) decrease the SDL on RT when compared with the scopolamine control after 14 days of administration [Table 2]. *Terminalia catappa* 100, 200 and 300 mg/kg) after 14 days of administration showed a slight decrease in the SDL on acquisition day as compared to the groups that received the day 7 administration. However,

on the retention day, *Terminalia catappa* (100, 200 and 300 mg/kg) after 14 days of administration showed a marked increase in the SDL as compared to the day 7 administration groups [Table 2].

Table 2: Effect of the extract of *Terminalia catappa* on step-down latency (passive avoidance paradigm) in scopolamine-induced amnesia in rats

Treatment Groups	SDL on acquisition day (sec)		SDL on retention day(sec)	
	7 day	14 day	7 day	14 day
Vehicle control	2.67±0.67	1.83±0.47	3.16±0.79	2.16±0.60
Scopolamine hydrobromide	2.83±0.47	1.17±0.17	1.67±0.49	1.83±0.30
Terminalia catappa (100)+ Scopolamine (1)	3.67±1.41	3.00±0.58	4.00±0.85	6.00±1.51
Terminalia catappa (200)+ Scopolamine (1)	2.00±0.51	3.16±0.87	4.17±1.35	6.67±2.35
Terminalia catappa (300)+ Scopolamine (1)	2.00±0.76	3..33±0.72	5.00±1.49	5.67±1.89
Piracetam(120)+ Scopolamine (1)	2.5±0.76	3.5±0.72	5.83±1.25	7.00±2.13

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

Table 3: Effect of the extract of *Terminalia catappa* on AChE Inhibition Activity

Treatments (mg/kg)	AChE concentrated (μMol/minute/g of tissue)	Inhibition of AChE activity (%)
Control	6.742±0.18	
Scopolamine hydrobromide	10.39±0.35	35.11
Terminalia catappa (100)+Scopolamine (1)	5.683±0.28	45.30
Terminalia catappa (200)+Scopolamine (1)	4.907±0.31	50.12
Terminalia catappa (300)+Scopolamine (1)	4.967±0.31	52.19
Piracetam(120)+ Scopolamine hydrobromide(1)	3.968±0.19	61.8

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

Estimation of AChE activity in the brain:

In the standard group, the animals treated with piracetam (120 mg/kg p.o.) produced a significant reduction of AChE enzyme activity. In the treatment group, the animals treated with *Terminalia catappa* at 100, 200 and 300 mg/kg produced a significant reduction of AChE enzyme activity and as compared to positive control. Percentage inhibition of AChE activity of treatment group has shown a significant increase when compared to positive control [Table 3].

Results and Discussion:

The present study suggests that *Terminalia catappa* possesses memory enhancing activity in view of its facilitatory effect on the retention of spatial memory in scopolamine-induced amnesia. There is a decrease in the TL, i.e. rats were able to locate the dark zone immediately after exposure to the open arm in the EPM paradigm, which is an indicator of cognition improvement. In case of the passive avoidance paradigm, the SDL is increased on administration of *Terminalia catappa*. This suggests that the animal has the retention of memory of the shock once entered in the shock-free zone. The long-term administration of the *Terminalia catappa* extract (14-day administration) exhibited pronounced effect in the reversal of the scopolamine-induced amnesia in case of the passive avoidance paradigm as compared to the 7-days administration.

It is well known that cholinergic neuronal systems play an important role in the cognitive deficits associated with AD, ageing and neurodegenerative diseases.(24) In our study, Piracetam (120 mg/kg p.o.) and *Terminalia catappa* (100, 200 and 300 mg/kg p.o.) significantly lowered this activity. Hence, the memory- enhancing effect of the Terminalia catappa can be attributed to its anti-ChE activity. Therefore, the memory-improving activity of *Terminalia catappa* may be attributed to the anti- AChE, procholinergic, neuroprotective and nutritive properties of the *Terminalia catappa*. Hence, *Terminalia catappa* may be used in delaying the onset and reducing the severity of Alzheimer's disease. However, further investigations are warranted to explore the possible involvement of other neurotransmitters such as glutamate, Gamma aminobutyric acid (GABA) and catecholamines, responsible for the memory-improving property of *Terminalia catappa.*

References:

[1] Joshi H & Parle M (2006) Evid Based Complement Alternat Med 3:79–85.

[2] Vasudevan M & Parle M (2006) Phytomedicine 13:677–87.

[3] Vogel GH (2007) Drug discovery and evaluation, 3rd edition, Germany 565 -661.

[4] Joshi A, Soni P, Vyas N & Javed KP (2017) A European J Biomed Pharm Sci 4: 355-361.

[5] Joshi A, Soni P, Malviya S & Kharia A (2017) Int J Comp and Adv Pharmacol 2: 11-18.

PD-26

Anti-anaemic Activity of Hydro-Alcoholic Leaf Extract of *Luffa aegyptiaca* in Phenylhydrazine Induced Anemic Rats

Deepanshu Gupta, Ankur Joshi, Sapna Malviya, Anil Kharia
Modern institute of Pharmaceutical Sciences, Indore (M.P.), India, 453111
E-mail address: guptadeepanshu252@gmail.com

Abstract: The main aim of the current study is to determine the anti-anaemic activity in hydro-alcoholic leaf extract of *Luffa aegyptiaca* in phenylhydrazine induced anemic rats. Phenylhydrazine (60mg/kg) was administered intraperitoneally in rats for 2 days to induce anemia. The animals were divided into 5 groups containing 6 animals each. 1st group was served as normal control group, 2nd group was served as anaemic control administered with phenylhydrazine for two days, 3rd group was served as standard reference control administered with Vitamin B_{12} complex, 4th group was served as test control administered with 100 mg/kg of hydro-alcoholic leaf extract of *Luffa aegyptiaca* and 5th group was served as test control administered with 200mg/kg of hydro-alcoholic leaf extract of *Luffa aegyptica*. All the test drugs were given for 28 days daily through oral route. On 29th day blood was withdrawn, through tail puncture and subjected to the determination of RBC, Hb and percentage Haematocrit. Both the hydro-alcoholic leaf extract of *Luffa aegyptiaca*and Vit. B_{12} significantly increase the HB, RBC & % HCT level which indicates that *Luffa aegyptiaca* leaf exhibits' the anti-anaemic activity.

Introduction:

According to WHO anaemia is a condition that develops when blood lacks enough healthy red blood cells or haemoglobin. As per WHO anemia affects the lives of more than 2 billion people globally, accounting for over 30% of the world's population which is the most common public health problem particularly in developing countries occurring at all stages of the life cycle. Therefore, there is the need for proper management of micronutrient deficiencies most especially irons deficiency. Over the years, plant and plant products are being utilized as a source of medicine. In many developing countries, herbal medicines are assumed as greater importance in health care [1, 3].

Materials and Methods:

Plant material

The plant material is made up of the leaves of *Luffa aegyptiaca.*Theleaves was collected from medicinal garden of Modern institute of Pharmaceutical Sciences in August 2017. The sample of plant was identified and authenticated at Rajmata Vijayraje Scindia Krishi Vishwavidhalaya, College of Agriculture, Indore [2, 4, 5].

Preparation of Extract

The leaves were collected, shade dried and then converted into coarse powder. The powder was then filled in a Soxhlet apparatus for extraction by 70:30 hydro-alcoholic as a solvent. The Hydro-alcoholic extract was concentrated by vacuum distillation to dry. The collected extract was stored in suitable container and used for further pharmacological studies.

Animals

The healthy adult albino rats of Wistar strain of both sex, weighing about 150-200 g were obtained from the animal house of Modern Institute of Pharmaceutical Sciences, Indore. The rats of either sex were isolated and housed in separate cages during the course of experimental period and kept them at room temperature (24± 2°C) with a 12 : 12 h light/dark cycle. The animals were fed with standard pellet diet and provided water *adlibitum.* All the procedures and protocols were reviewedand approved by the Institutional Animal Ethics Committee of MIPS, Indore.

Anti-Anemic Activity

Induction of Anemia Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (60mg/kg) daily for 2 days.

Treatment of the animals

The anemic rats were randomly divided into 5 groups 6 animals each. Group I was served as normal control, received 1ml/kg of 0.1% Carboxy methyl cellulose solution. Group II was served as anemic control, received 1 ml/kg of 60 mg/kg of phenylhydrazine intraperitoneally for 2 days. Group III served as reference control, phenylhydrazine treated rats received1 ml/rat Vitamin B_{12} syrup through oral administration, by suspending in 1% CMC solution for 28 days. Group IV served as test control-I, phenylhydrazine treated rats received 100mg/kg of *Luffa aegyptiaca* hydro-alcoholic leaves extract through oral administration, bysuspending in 1% CMC solution for 28 days. Group V served as test control-II, phenylhydrazine treated rats received 200mg/kg of *Luffa aegyptiaca* hydro-alcoholic leaves extract through oral administration, bysuspending in 1% CMC solution for 28 days. On 29^{th} day, blood was collected in EDTA coated tubes, by tail puncture under phenobarbitone (45mg/kg, IP) anaesthesia. The following parameters like, Red Blood Cell count (RBC), Haemoglobin (Hb) and Haematocrit percentage (HCT) were evaluated in blood.

Results and Discussion:

Anti-anemic activity of *Luffa aegyptiaca* leaf extract on Phenylhydrazine inducedanemia in rats was studied and the results were shown on Table 1. The anti-anemic activity of *Tamarindus indica* leaf extract was assessed by determiningthe red blood cell count, haemoglobin and haematocrit percentage.

Table 1: Effect of *Luffa aegyptiaca* leaf extract on Phenylhydrazine induced anemia in rats

S. No.	Drug treatment	RBC (106 µL-1)	Hb (g dL-1)	HCT %
1.	Normal control (0.1% CMC)	8.91±0.61	13.52±0.55	47.88
2.	Anemic Control Phenylhydrazine (60mg/kg)	4.71±0.16	5.99±0.22	28.56
3.	Reference Control Vit B12 (1 ml/rat)	8.35±0.42***	13.13±0.73***	45.29**
4.	Test Control-I *Luffa aegyptiaca* (100 mg/kg)	8.14±0.59***	13.11±0.76***	43.81**
5.	Test Control-II *Luffa aegyptiaca* (200 mg/kg)	8.28±0.54***	13.25±0.71***	44.08**

Data were expressed as Mean ± SEM (n=6) *P<0.05, ** P<0.01 and *** P<0.001 Vs. Anemic Control.

Conclusion:

The ethanolic leaf extract of *Tamarindus indica* exhibits anti-anemic activity against phenylhydrazine induced anemia in rats. The anti-anemic effect produced by the *Tamarindus indica* leaf may be due to its high content of iron which is present in the plant.

Acknowledgement:

The authors are grateful to the President Modern group Mr. Arun Kharia, Chairman & Principal, Modern group Mr. Anil Kharia, Head of department Pharmacy Dr. Sapna Malviya and other faculty members for their support and guidance for this study.

References:

[1] Gerard T J & Bryan D (2015) Anatomy & physiology, 13th ed., Wiley India pvt. Ltd., New Delhi. 46-74.

[2] Khandelwal KR (2013) Practical Pharmacognosy, 22nd edition, Nirali prakashan, Pune, 20-24.

[3] http://www.who.int/topics/anaemia/en/ cited: 25-08-2017.

[4] Joshi A (2017). J Harmonized Res Applied Sci 5: 132-135.

[5] Kushwah H (2017) Int J Comp and Adv Pharmacol 2: 6-10.

PD-32

Design, Synthesis and Docking Study of Pyridine Derivative for Antidepressant Activity

Mangirish Deshpande[1], Mitali Naik[2], Risa Borges[2], Bheemangouda Biradar[1], Sunil Shingade[2]
[1]Department of Pharmacology, P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi ,Goa.
[2]Department of Pharmaceutical Chemistry, P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Goa.
E-mail address: mangirishd@gmail.com

Abstract: On the basis of literature survey compound 2-(1*H*-benzo[d]imidazole-1-yl)-*N*-(3-cyano-4,6-diphenylpyridin-2-yl) acetamide (6) was designed. Compound 6 and imipramine were virtually studied for their antidepressant activity by performing docking studies using Molegro Virtual Docker (MVD-2013, 6.0) on human MAO (PDB ID: 2BXS). Docking studies revealed its potential antidepressant effect as compared with imipramine. The result of docking study was validated by synthesizing and screening the compound for it's *in vivo* antidepressant activity following forced swim test and locomotor activity using actophotometer. Finally, we conclude that the docking result and experimental results are in good agreement.

Introduction:

Depression is a psychological disorder with a low mood, loss of interest, affecting sense of well being, anorexia, low energy, poor concentration and impaired sleep. This problem may become recurrent and even lead to suicide. According to WHOreport depression have become serious health problem affecting about 121 million people worldwide [1]. Pathophysiology of depression is not clearly understood, but some hypothesissuggests that depression and maniac disorder may be caused by lack of nor adrenaline and serotonin. Most of the synthetic drugs used as an antidepressant act on biogenic amines of the brain. Thus leading to increasing their concentration in respective part of brain [2]. MAO enzyme causes deactivation of biogenic amines. Thus by inhibiting the MAO intracellular concentration of endogenous amines may be increased, which seems to be the major cause of antidepressant effect. MAO inhibitors available in the market have serious side effects like hypertension, tremors, muscle rigidity and seizures Hence there is a need of potent antidepressant with less or no side effects [3].

Materials and Methods:

Chemicals

All the solvents and chemicals were purchased from SD Fine Chem Ltd, Kemphasol, Molychem, and Fisher Scientific Pvt.Ltd.

Animals

Male albino mice of 20-25g were obtained from animal house of PES's Rajaram and Tarabai Bandekar College of Pharmacy Farmagudi Ponda.

Molecular docking methodology

The molecular docking study was performed using Molegro Virtual Docker (MVD-2013, 6.0). The crystal structure of the monoamine oxidase - A co-crystallized with clorgyline inhibitor was downloaded from Protein Data Bank (PDB ID: 2BXS).Molecular docking study of the synthesized compound/ligand was performed in order to understand the various interactions between the ligand and enzyme active site in detail. The molecular docking study was performed for the target compound by using MVD-2013 (Version: 6.0).

Synthesis

Synthesis of 2-amino-4,6-diphenylpyridine-3-carbonitrile (4):

Synthesis of 2- chloro-*N*-(3-cyano-4,6-diphenylpyridin-2-yl)acetamide (5):

Synthesis of 2-(1*H*-benzo[d]imidazole-1-yl)-*N*-(3-cyano-4,6-diphenylpyridin-2-yl) acetamide (6):

2-Amino-4,6-diphenylpyridine-3-carbonitrile (**4**)

Yield: 50%, m. p.: 182^{o}C, IR (KBr, cm^{-1}): 3464.15 (NH), 3302.13, 3176.76 (C-H aromatic), 2206.57 (CN), 1573.91 (C=C); ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 7.20 (s, 1H, Ar-H), 7.45-7.49 (m, 3H, Ar-H),7.507.54 (m, 3H,Ar-H), 7.62-7.64 (m, 2H, Ar-H),7.988.00 (m, 2H, Ar-H), 5.41 (s, 2H, N-H).

2-Chloro-*N*-(3-cyano-4,6diphenylpyridin-2-yl)acetamide (**5**)

Yield: 64%, m. p.: 172^{o}C, IR (KBr, cm^{-1}): 3464.15 (NH), 3302.13, 3132.740 (C-H aromatic),2206.57 (CN),1639.49 (C=O),1604.77 (C=C), 754.17 (C-Cl); ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 7.21 (s, 1H, Ar-H), 7.45-7.48 (m, 3H, Ar-H),7.49-7.54 (m, 3H, Ar-H), 7.627.65 (m, 2H, Ar-H),7.99-8.01 (m, 2H, Ar-H), 5.36 (s, 1H, NH), 3.68 (s, 2H, CH_2).

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(3-cyano-4,6diphenylpyridin-2-yl)acetamide (**6**)

Yield: 66%, m. p.: 149^{o}C, IR (KBr, cm^{-1}): 3464.15 (N-H), 3176.76 (C-H aromatic), 2206.57 (CN), 1637.56 (C=O amide), 1573.91 (C=N); ^{1}HNMR (400 MHz, (DMSO- d6, δ ppm): 7.22 (s, 1H, Ar-H), 7.39-7.46 (m, 5H, Ar-H),7.47-7.51 (m, 3H, Ar-H), 7.53-7.59 (m, 5H, Ar-H),7.61-7.84 (m, 2H, Ar-H), 5.38 (s, 1H, NH), 3.69 (s, 2H, CH_2).

***In-vivo* studies:**

Animals

Male albino mice weighing between 20-25 gm were used. Animals were maintained under standard conditions in the animal house of PondaEduationSocietysRajaram and Tarabaibandekar College of Pharmacy, Ponda. The study was approved by Institutional Animal Ethics Committee with resolution number PESRTBCOP/IAEC; clear/2015-16/R-12

Acute toxicity studies

Acute oral toxicity studies of compound **6** was carried out on Swiss albino mice weighing between 25-30g according to OECD guideline No. 423.

Antidepressant activity

Antidepressant activity of compound **6** was evaluated by force swimming test and locomotor activity using actophotometer and was compared with reference standard imipramine [5].

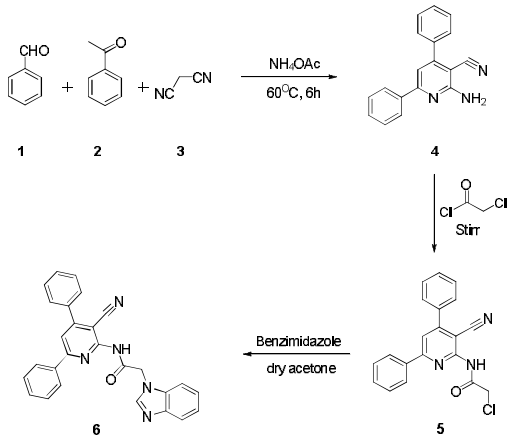


Figure 1: Synthesis of pyrimidine derivative

Treatment groups

Group 1: Normal control (saline)

Group 2: Compound **6** (200 mg/kg, p.o.)

Group 3: Imipramine (10 mg/kg, p.o.)[4]

Results and Discussion:

Docking study

Molecular docking study of compound **6** and imipramine was performed using Molegro Virtual Docker (MVD-2013, 6.0). The crystal structure of monoamine oxidase - A was obtained from the RCSB PDB website (PDB ID: 2BXS). The PDB structure 2BXS bound to the inhibitor clorgyline shows a true binding site for each of the subunits. An essential feature of the binding site is the conservation of hydrogen bondings and the aromatic π-π stacking interactions. The active pocket consists of amino acid residues such as Ala68, Ala409, Arg51, Asp64, Cys406, Glu436, Gly49, Gly67, Gly301, Gly405, Gly426, Gly447, Ile23, Lys305, Met300, Met445, Phe352, Ser223, Ser403, Thr52, Thr183, Thr435, Trp397, Tyr69, Tyr407, Val65 and Val182. Hence, to identify other residual interactions of the compound **6,** a grid box of 10.0 Å was constructed. Clorgyline being a known inhibitor, the center of this site was considered as the centerof search space for docking.

The docking results reveals that for compound **6,** the Mol-Dock score was -183.47, while for reference standard imipramine it is -112.101. The best orientation poses of the docked compound **6** and imipramine are shown in Figure 1. It was clearly observed that imipramine exhibited one hydrogen bond with Tyr407, while compound **6** displayed four hydrogen bonds with Tyr407, Tyr69, Ala68 and Met445.

Chemistry

The title compound was synthesised as outlined in the Scheme 1. The compound **4** was obtained by condensation and cycloaddition of benzaldehyde, acetophenone and malononitrile. Compound **4** was further treated with chloroacetyl chloride to afford compound **5**. Finally, compound **5** was reacted

with benzimidazole to afford the title compound **6**.The formation of all the synthesized compounds were ascertained by IR and ^{1}HNMR spectral data.

Table 1: Moldock scores and other energy calculations of compound 6 and imipramine by MVD-2013 against 2BXS

Compound	MolDockScore (Kcal/mol)	E- Inter (Protein-ligand)	H Bond No.		Heavy Atoms count	LE1	LE3	Docking Score (Kcal/mol)	Rerank Score (Kcal/mol)
Imipramine	-112.101	-134.749	1	-1.94795	21	-5.33816	-4.26125	-111.345	-89.4863
6	-183.47	-206.105	4	-7.39705	33	-5.55969	-3.82814	-180.712	-126.329

***In-vivo* activity**

Forced swim test The antidepresant activity of compound **6** was carried by force swim test and the results are depicted in Figure 2. In this test the reference standard imipramine at a dose level of 10mg/kg showed more significant decrease in the immobility time to 71.00±4.171 as compared to control (169.2±4.826). This synthesized pyridine derivative showed a significant decrease in the immobility time to 130.0±8.278 as compared to control.

Locomotor activity The locomotor activity of compound **6** was carried by using actophotometer and results are shown in Figure 3. The test results showed that the standard drug imipramine at a dose level of 20mg/kg produced incresed in the locomotor activity (621.8±18.13) compared to control (307.3±12.71). This synthesized pyridine derivative showed a significant increase in the locomotor activity to 585.8±24.88 as compared to control.

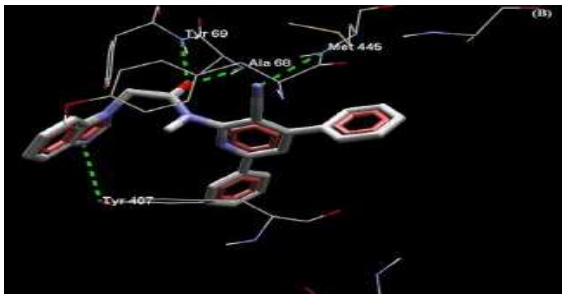


Figure 1: Showing the compounds docked in best of its conformation into the binding site of 2BXS (A) binding mode of imipramine forming 1H bond with Tyr407; (B) binding mode of compound 6 forming

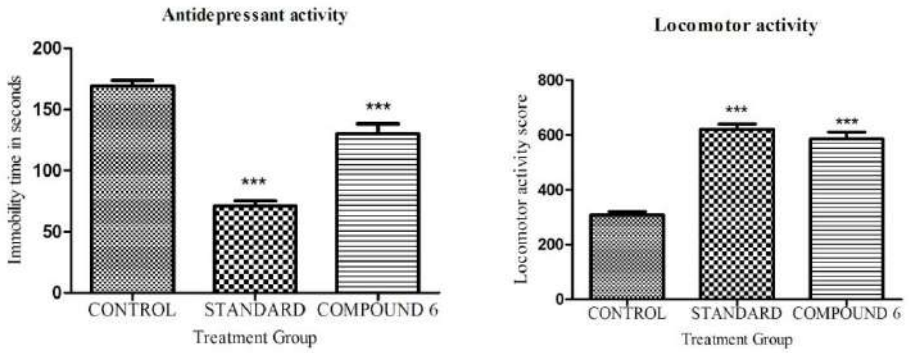


Figure 2 & 3: Antidepressant and Locomotor activity of pyridine derivative (Compound 6)

Values are expressed as mean ± SEM. Number of animals = 6. ***P<0.001 compared to respective vehicle treated control group. Result was analyzed by one-way ANOVA followedby Dunnett's multiple comparison test.

Conclusion:

Compound 6 exhibited better antidepressant potential and the docking study results are validated by *in vivo* activity.

Acknowledgement: Authors are thankful to the Principal, PES's Rajaram and TarabaiBandekar College of Pharmay, Farmagudi, Ponda, Goa for providing necessary research facilities.

References:

[1] http:www.who.int/mental_healt/management/depression/who_paper_depression_wfmh_2012 (accessed on 10.05.15)

[2] Brunton LL, Lazo JS & Parker LK (2006) Goodman and Gilman's The Pharmacological Basis of Therapeutics. Mcgraw Hill USA: 429-455.

[3] Eissa IH, El-Nagggar AM & El-Hashash MA (2016) Bio Med Chem 67:43-56.

[4] Dhingra D & Valecha R (2007) Ind J ExpBiol 45:610.

[5] Santosh P, Venugopal R, Nilakash AS, Kunjbiharis S & Mangala L (2011) Int J Pharm Pharm 3 :112-115.

PD-33

Evaluation of Anti Psoriatic Activity of Karanjin Oil

Thomson Alex, Shabnam Mansuri, Shaily Chaudhary, Neelesh Malviya
Smriti College of Pharmaceutical Education, Indore (Madhya Pradesh), India
E-mail address: thomsonlx07@gmail.com

Abstract: Psoriasis is defined as a type of severe inflammatory and autoimmune skin disease, caused due to genetic and natural/surrounding factors and the most successful way to tackle this disease is topical treatment. To examine potency of the herbal drug in the cure of psoriasis like lesions on mice skin is the core objective of this study. Imiquimod model of psoriasis was used in the evaluation of the drug and the qRT-PCR was done to check cytokines concentration in the blood serum of psoriasis induced mice. The drug karanjin oil was proved to have anti-psoriatic activity.

Introduction:

Psoriasis is non curable, non contagious, itchy and painful disease. Clinical features are scaling and redness. The cause is not known still but studies say that it is due to some genetic factor or immunological factors or biochemical process which regulates the proliferation and development of upper skin cells. Following factors aggravates psoriasis mental stress, more alcohol consumption, smoking etc.

Epidemiology

2-3% of world population is affected by Psoriasis. It occurs in both male and female. It is reported at birth as well as in old age and mainly seen in western countries. In India especially north india, males are more prone to psoriasis than female mainly in the age group between 30 yrs to 40 yrs of age.

Types of Psoriasis

1. Plaque psoriasis- Most general type of psoriasis characterized by swollen red scaly bumps covered with silvery white scales.
2. Guttate- Next common type of psoriasis after plaque psorisis, mainly seen in younger generations, characterized by small red spots.
3. Inverse- It is characterized by red non- scaly and shiny patch in the skin folds.
4. Pustular- It is a rare type of psoriasis. It mainly occurs in adults, characterized by raised pus filled bumps.
5. Erythrodemic- Rarest and most unsafe type of psoriasis characterized by red, crusty, pustular bumps all over the body.
6. Nail psoriasis- 50% of psoriasis patient have nail psoriasis, characterized by yellowish brown nails with gap between nail bed due to deposition of chalky material. Mainly seen in psoriatic arthritis patient.

7. Psoriatic arthritis- It is a type of disease in which patient is suffering from psoriasis as well as joint inflammation.

Pathophysiology

Being a T cell mediated disease that is characterized by uncontrolled scaling along with redness, pain and angiogenesis. The CD4+T cells stimulated the disease by complexing with antigen-presenting cells in the skin, which provokes the CD8+ T cells in the epidermis. This complex between CD4+ T cells, CD8+ T, dendritic cell and keratinocytes gives rise to cytokine soup which mainly consists of T helper cell type cytokins such as IL-1β, IL-12, IFN-γ and TNF-α. Thus the cytokines acts as biomarkers in the pathophysiology of psoriasis.

Treatment

Topical Therapy includes corticosteroids, anthralin etc, Systemic agents includes Cyclosporine etc, phototherapy includes PUVA, UVB; Herbal treatment and Homeopathic treatment.

Marketed Drugs

Zoratame (25 mg)**,** Silrox**,** Lobate-M**,** Graftin(25mg, 100mg)**,** Dosetil**,** Resol and Vit. D derivatives etc.

Models used in the study of Psoriasis

1. Spontaneous mutation
2. Genetically engineered rodents
3. Immunological reconstitution approach
4. Xenotransplantation
5. Imiquimod Model

qRT-PCR

The level of cytokinin was measured by PCR.

Application of Drug (Karanjin oil)

Once the physiological parameters confirmed the successful induction of psoriasis like lesions on the mice. The oil was applied on the lesions of the standard group and at the same time vanishing cream on the lesions of the test everyday for 1 week.

Cytokine measurement

After 8 days, blood samples from the lesions of mice of all the groups were taken and on them PCR was performed to check the concentration of cytokines(IL-1β, IL-12, IFN-γ and TNF-α) in the mice blood.

Results and Discussion:

PCR Test

Inflammatory mediators like cytokines act as Biomarker in the pathophysiology of Psoriasis. The levels of cytokines was checked in both controlled and standard. As shown in the Table, it was

observed that the levels of IL-1β, IL-12, IFN-γ and TNF-α was less and close to normal in the test and that of controlled and standard was very high.

Table 1: Cytokine level in standard, control and test group

Cytokine (pg/ml)	Standard group	Control group	Test group
IL-1β	305.02±11.67	312.32±9.65	130.20±17.00
IL-12	89.43±17.77	95.21±14.98	76.04±10.00
IFN-γ	32.87±9.01	35.88±8.29	25.07±2.05
TNF-α	934.66±155.35	978±145.33	578.22±107.66

All value represents the mean as well as S.D. (n = 6).

Conclusion:

During the study of Karanjin it was found that it gives the positive results and it was established that karanjin cured the psoriasis like lesions on mice skin which was induced by imiquimod.

References:

[1] Van der Fits L, Mourits S, VoermanJSA, Kant M, Boon L & Laman JD (2009) J Immunol 182:58365845.

[2] Gudjonsson J E, Johnston A, Dyson M, Valdimarsson H & Elder J T (2007) J Invest Dermatol 127: 1292-1308.

[3] Schon MP & Boehncke WH (2005) N Engl J Med 352:1899–1912.

[4] Lowes MA, Bowcock AM & Krueger JG (2007) Nature 445:866–873.

[5] Yadav PP, Ahmad GA & Maurya R (2004) Phytochem 65:429–442.

PD-36

Evaluation of Safety & Anti-Urolithiatic Property of Various Polyherbal Formulations using Ethylene Glycol-Ammonium Chloride & Potassium Oxonate Induced Urolithiasis in Rats & Mice Respectively

Rishabh Vaishnav[1], Smit Patel[1], Nilesh Patel[2]
[1]Department of Pharmacology,
[2]S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Vidhyanagar-384012.
E-mail address: dominatorvaishnav@gmail.com

Abstract: The aim of present work was to evaluate safety & anti- urolithiatic property of various polyherbal formulations using ethylene glycol-ammonium chloride & potassium oxonate induced urolithiasis in rats & mice and to evaluate and to evaluate effect of poly herbal formulation on oxidative stress, i.e. SOD, catalase, LPO and to evaluate of poly herbal formulation on physical parameter i.e. body weight, urine PH, urine volume

Introduction:

Kidney stone, also called as urolithiasis, when solid part or stone present in urinary track. When urine is supersaturated by salt, ions and minerals like calcium oxalate, Cystine, struvite and uric acid caused renal stone. Calcium have highest amount (60- 80 %) in stone. They have different size like small to large stone (staghorn stone). In some cases CaOx precipitation present in LOH membrane, its accumulate in renal papillae's sub epithelial space, it caused Randall's plaques. Symptoms of stone produces when it migrates down to urinary track or stay at same position. Various studies show that preliminary reason for stone formation is calcium phosphate cell which produces Nano bacteria. When low volume of urine, high amount of calcium excretion or both of this cause supersaturation of CaOx in urine lead to kidney stone. When absorption of oxalate in intestine rise (enteric hyperoxaluria), higher oxalate contain meal intake, oxalate synthesis altered by genetic abnormalities (primary hyperoxaluria-1 and 2) and dysfunction of transport mechanism in renal part. Although, main reason for urine supersaturation is genetic abnormalities bonded to calcium outcome and also pH, this phenomena is the main core reason of the urolithiasis [Coe *et al.*, 2005]. In stone formation, growth and crystal-membrane interaction, protein has main function in matrix and organic matrix of stone. [Govindaraj A *et al.*, 2002] [Sorokina EA *et al.*, 2004]. World's 10% of population are suffering from urolithiasis because of industrialized part effect. Kidney stones are common in industrial countries with a yearly frequency of 1 % to 2 %. Diet plan, Urine alkalinisation method, Analgesic drugs, Diuretics, Lithotripsy methods, Allopurinol and various Surgery basic steps for curing of CaOx and uric acid type stone. Commonest side effects of management are hypersensitivity, headache, GIT abnormalities and nausea. Acute trauma, bruising & damage to blood vessel of the kidney are side effect of ESWL.Herbal medicines are moderately safer compared to the allopathic medicines. In case

of Allopathic medicines the gap between ED50 & LD50 is very low while comparing with the herbal medicines. Key objectives of herbal medicine management are cure without any adverse or side effects. Ayurveda has listed herbal medicines to cure or prevention of kidney stone diseases (mutrashmari) example like, *Tribulus Terrestris* (Gokshrua), *Crataeva Nurvala* (Varuna Tree), *Boerhaavia Diffusa* (Punarnava), *Hordeum vulgare* (Barely), *Bergenia Ligulata* (Pashanbheda), *Asparagus racemosus* (Shatamull), *Crataeva religiosa* (Bidasi), *and Dolichos Biflorus* (Kulthi). Our primary objective of study is to determine anti- urolithiatic property of polyherbal formulation on rats & mice.

Materials and Methods:

Materials Ethylene glycol (EG) 0.75% (v/v), Ammonium chloride 1% (w/v) and Potassium oxonate

Method Acute oral toxicity study of polyherbal formulations was carried out according to OECD 425 guideline in mice. Two methods were used to check anti-urolithiatic effect of polyherbal formulations i.e. Ethylene glycol (EG) 0.75% (v/v) uric acid, oxalates. Ammonium chloride 1% (w/v) and Potassium oxonate induced urolithiasis in rats and mice. Ammonium chloride was given first seven days continuously with ethylene glycol to accelerate process of renal stone formation. EG was continued for 28 days in all groups except normal control group. 5 various polyherbal formulations were treated in groups 3-7 and group -8 received standard drug. Blood and urine were collected at 14^{th} & 28^{th} days in order to measure serum creatinine, uric acid, potassium, chloride, phosphorus, calcium, chloride and urine volume, pH, uric acid, oxalate, potassium, chloride, calcium, phosphate & oxalate. Second animal model employed to check anti-urolithiatic effect was Potassium oxonate induced hyperuricemia in mice. Potassium oxonate (250 mg/kg, p.o.) was given to all groups excluding normal control group. Polyherbal formulations were treated 60 minutes after potassium oxonate for consecutive 7 days in groups 4-7 and group-8 received standard drug. On 8^{th} day, blood and urine were collected in order to measure water & food intake, serum creatinine, uric acid, urine volume, pH,

Results and Discussions:

High level of serum calcium, potassium, chloride & acidic pH in DC group showed the production of renal stone and RTA. Whereas polyherbal formulations decreased serum calcium, potassium, chloride level nearer to normal group and made urine pH alkaline. Poly herbal formulations showed effect against kidney stone, RTA as well as oxidative stress.

Table 1: Urolithiasis induce by Ethylene Glycol a n d Ammonium Chloride in Rats

Result of Body Weight

	Normal	Diseases	Calcirex	Calcirex	Cistonil	Cistonil	Cistonil	Standard
0 week	173.8±8	173.0±8	172.8±7	174.3±5	172.3±9	177.7±9	178.0±8	172.7±6
1st week	175.8±7	163.8±6	173±8	142.7±10	135±7	149.3±7	176.5±6	168.8±6
2nd week	188.7±8	177.7±6	172±18	167.3±10	164.3±6	174±11	195.8±6	190.2±7
3rd week	209.5±9	209.5±4	170±29	199.2±9	199±9	210.2±12	213±36	213±5
4th week	228.3±10	223.16±5	238.3±9	230.2±9	228.6±11	257.2±13	250.±11	240.6±8

Toxicity study was performed according to OECD 425 guideline. Polyherbal formulations found safe up to dose of 2000mg/kg

Table 2: Effects on Serum Calcium (mg/dl)

Group	Serum Calcium (mg/dl)	
	14th Day	**28th Day**
Normal control	5±1	5.5±0.42
Disease control	16.5±0.5	18.5±0.7###
Calcirex capsule	8.5±0.5	9±0.36***
Calcirex syrup	15±1	15.1±0.6**
Cistonil capsule	15±11	15.6±0.4**
Cistonil syrup	13.5±0.5	15.8±0.8*
Cistonil tablet	10±2	8.3±0.5***
Standard	6.5±1.5	6.6±.33***

Table 3: Effects on Serum Creatinine

Group	Serum Creatinine (mg/dl)	
	14th	**28th Day**
Normal control	0.08±0.03	0.111±0.05
Disease control	1.431±0.11	1.49±0.13###
Calcirex capsule	0.669±0.13	0.402±0.05***
Calcirex syrup	0.628±0.09	0.49±0.08***
Cistonil capsule	1.11±0.24	0.802±0.1**
Cistonil syrup	1.237±0.03	1.039±0.12*
Cistonil tablet	0.562±0.07	0.462±0.10***
Standard	0.395±0.05	0.325±0.10***

Table 4: Effects on Serum Uric Acid

Group	Serum Uric acid (mg/dl)	
	14^{th}	28^{th}
Normal control	0.8±0.29	1.96±0.21
Disease control	6.7±0.53	$7.02±0.41^{###}$
Calcirex capsule	6.2±0.62	$5.11±0.67^{*}$
Calcirex syrup	6.2±0.24	$5.16±0.41^{*}$
Cistonil capsule	4.6±0.54	$3.99±0.67^{***}$
Cistonil syrup	5.9±0.26	$5.87±0.34^{ns}$
Cistonil tablet	3.9±0.51	$3.28±0.35^{***}$
Standard	3.69±0.43	$3.24±0.21^{***}$

Effects on various urine parameters:

Biochemical parameters (serum: calcium, creatinine, phosphate, potassium, chloride), urine analysis parameter (urine: pH, volume, calcium, phosphate, potassium, chloride, oxalate) found nearer to normal range in polyherbal formulation treated groups. So it shows polyherbal formulations having effectiveness against urolithiasis.

Discussion: Our primary motto was to check the effect of various poly herbal formulations' effect on urolithiasis on different *in-vitro* models. We employed two different animal models i.e. Ethylene glycol model and potassium oxonate induced urolithiasis. Both models showed significant urolithiasis and it was evident by acidic pH of urine in DC. So, it was affirmative signal for stone formation & also for kidney damage. On other side, treatment group showed basic or normal range pH value. So, it was proved treatment was worked properly. It can be used to treat renal stone (CaOx & Uric acid) & kidney damage.As per the acute toxicity study, poly herbal formulations were completely safe up to the dose of 2000 mg/kg. All animals were survived with purely healthy condition. There was no any abnormality or other side effect like condition observed.

Conclusion:

Toxicity study was performed according to OECD 425 guideline. Polyherbal formulations found safe up to dose of 2000mg/kg.

Biochemical parameters (serum: calcium, creatinine, phosphate, potassium, chloride), urine analysis parameter (urine: pH, volume, calcium, phosphate, potassium, chloride, oxalate) found nearer to normal range in polyherbal formulation treated groups. So it shows polyherbal formulations having effectiveness against urolithiasis.

Significant increased level in SOD & Catalase activity found & drop in LPO level was observed, which shows that polyherbal formulations have anti-oxidant activity.

As per the result, we concluded that various poly herbal formulations have potential to cure or treat renal stone like CaOx as well as uric acid stone & RTA. Cistonil tablet & Calcirex capsule were best poly herbal formulation among all to use in urolithiasis as treatm

Refrences:

[1] A.K. Sinha (1972) "Colorimetaric assay of catalase Anal. Biochem 47: 389-95.

[2] Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) J Biol Chem: 193: 265.

[3] Adhirai M & Selvam R (1993) Jpn J Med Sci Biol 50:9.

[4] Adhirai M & Selvam R (1997) J Nutr Biochem 8:32.

[5] Adhirai M & Selvam R (1998) J Pharm Pharmacol 50:501.

PD-39

Comparative Study of *Carica papaya* with Marketed Product for the Treatment of Wounds in Diabetic Rodents

Shaily Chaudhary[1], Akash Yadav[2]
[1]Smriti College of Pharmaceutical Education (SCOPE), 4/1 Pipliya Kumar Kakad, MR-11, Dewas Naka, Indore (Madhya Pradesh), India.
[2]IPS Academy College of Pharmacy, Knowledge Village, Rajendra Nagar, A.B. Road, Indore (Madhya Pradesh), India.
E-mail address: s.shailychaudhary@gmail.com

Abstract: Diabetes is an unending situation associated with peculiarly elevated levels of sugar (glucose) in the blood. This chronic illness is also related with the degenerative extended pathological disorders like retinopathies, nephropathies, atherosclerosis, wound healing problems and cataract. Tissue renovates and wound curing are complicated processes that engross inflammation, granulation and tissue remodeling. Wound curing is characterized by two stages via inflammation and proliferation. The therapeutic utilization of *Carica papaya* relies because of the existence of papain an active constituent. This whole study emphasize on the effect of diabetes (high blood glucose sugar level) on the wound curing rate of rodents.

Introduction:

The quote for diabetes is diabetes can hit anyone, from any walk of life and it does in numbers that are significantly increasing. It is a main cause of heart failure, blindness, amputations, amplified wound healing rate increased thirst, kidney failure, urination, increased hunger, fatigue, blurred vision, lack of sensation or prickling in the feet or hands sores that do not heal easily, inexplicable weight loss and stroke [1]. Wounds occur when the skin is broken down or scratched due to injury [2]. Streptozotocin (STZ) is a naturally occurring chemical, formerly known as an antibiotic, and later on studies found that it is mainly toxic to the insulin-producing beta cells of the pancreas [3].

Material and Methods:

Materials required

Streptozotocin, glucometer, accu chip, povidone iodine ointment, *Carica papaya* gel and wistar rats.

Induction of diabetes

Diabetes (DM) was induced chemically as mentioned by Wu *et al*. [1]. Briefly after 12 hour fast, rats received one intra peritoneal (i.p.) injection of streptozotocin (65mg/kg) freshly prepared in sodium citrate buffer of 0.1 M (pH 4.5). At 8^{th} day after streptozotocin injection blood glucose measurement was performed on tail vein blood by using a glucometer Accu-Check. Rats those fasting blood glucose level is more than 250 mg/dl were measured as diabetic. Water intake and weight was observed all the way throughout the study, and to confirm the diabetic status, fasting blood glucose measurement was repeated on the day of euthanasia.

After diabetes confirmed in animal

Wounding After diabetes was established in animals, with the help of punch biopsy machine (diameter of 6 mm; Accu-punch) make a full thickness around wound at the upper back of each rat in every group. Wound were traced on $1mm^2$ graph paper on the same day of wounding and consequently at a gap of 3 days up to 12th day then on alternating days until healing was absolute. Changes in wound area were calculated on continuous mode and the speed of wound healing measured as given in the formula below.

Histopathology of wounds and measurements of percentage of wound healing by excision wound model was done in the following groups on 0, 8^{th} and 12^{th} day

Group 1: Control [vehicle]

Group 2: Positive control [standard povidone iodine]

Group 3: Treated with *Carica papaya*

Borders of the wounds were marked on a transparent paper by a fine tip permanent marker. The portion (in square millimetres) inside the borders of each tracing was determined plan metrically. The wound area of each animal was measured on 0 day, prearranged time period initially at 3 h post wounding and successive measurement of wound area from both the three groups was taken on days 4, 8, and 12 post wounding. The grades of wound measurements on different days were articulated as percentage wound contraction. The values were articulated as per cent values of the 0 day capacity and were intended by Wilson's formula as follows:

$$\%\ \textbf{of Wound contraction} = \frac{\text{0 day wound area} - \text{unhealed wound}}{\text{0 day wound area}} x\ 100$$

Photographs of animals on 0, 8^{th} and 12^{th} days post wounding of every group were taken.

Results and Discussion:

Measurements of proportion of wound contraction on 0 day:

For Control Group 1, the % of wound reduction was found to be = 2%

For Group 2 Positive control, the % of wound reduction was found to be = 6%

For Group 3: Treated with Carica Papaya gel the % of wound reduction was found to be = 6%

Measurements of percentage of wound contraction on 4^{th} day:

For Control Group I, the % of wound contraction was found to be = 6%

For Group 2 Positive control, the % of wound contraction was found to be = 23%

For Group 3: Treated with Carica Papaya gel the % of wound contraction was found to be = 32%

Measurements of percentage of wound contraction on 8^{th} day

For Control Group 1, the % of wound narrowing was found to be = 13%

For Group 2 Positive control, the % of wound narrowing was found to be = 42%

For Group 3: Treated with Carica Papaya gel the % of wound narrowing was found to be = 57%

Measurements of percentage of wound contraction on 12th day

For Control Group 1, the % of wound reduction was found to be = 22%

For Group 2 Positive control, the % of wound reduction was found to be = 63%

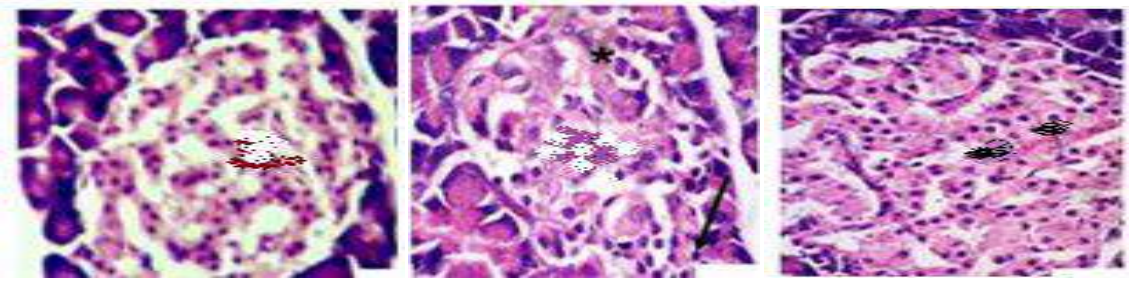
For Group 3: Treated with Carica Papaya gel the % of wound reduction was found to be = 67%

On understanding the data obtained by the analysis of % of wound narrowing on these 4 constitutive days, it was observe that the wound healing property of povidone iodine and Carica Papaya gel were very close and *Carica papaya* increase the wound healing improvement in diabetic rats.

Group1 Group 2 Group 3

Figure 1: Wound healing photographs of wounds in rat on 8th day after wound formation in group.

Group1(control wounded non-treated); group 2 (Positive control); and group 3 (treated with *Carica papaya*)

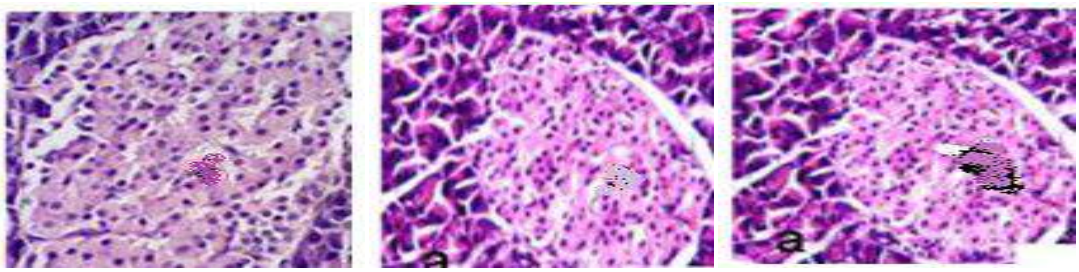


Group 1 Group 2 Group 3

Figure 2: Histological changes during the wound-healing process on the 0 day

Group 1(control wounded non-treated), group 2 (Positive control) and group 3 (treated with *Carica papaya*)

The histopathology of the wound curing slide represent that on day zero the scars and injury of tissue for all the three groups are approximately same.



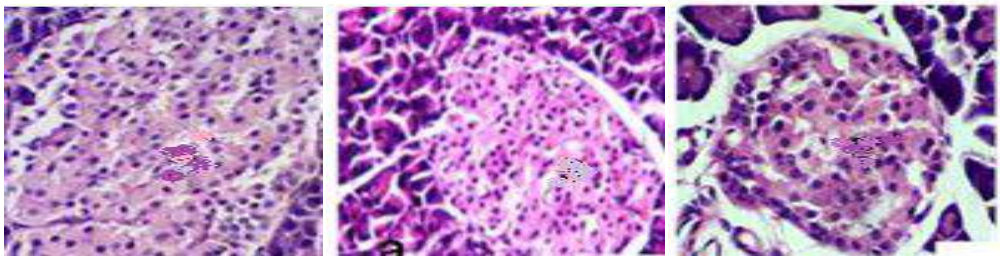
Group 1 Group 2 Group 3

Figure 3: Histological changes during the wound-healing process on the 8th day

Group 1 (control wounded non-treated), group 2 (Positive control) and group 3 (treated with *Carica papaya*)

The histopathology of the wound curing slide represent that on the eight day angiogenesis, collagen deposition, granulation, tissue formation, and wound narrowing take place more rapidly in group 2 and group three as compare to group 1. Even it was observed that collagen deposition and tissue development is greater in group 3 in the comparison of group 2.

Histopathological images of groups on 12th day:



Group 1 Group 2 Group 3

Figure 4: Histological changes during the wound-healing process on the 12th day
Group 1 (control wounded non-treated); group 2 (Positive control) and group 3 (treated with *Carica papaya*)

The histopathology of the wound curing slide represent that on day twelve the scars and injury of tissue for all the three groups are totally different. As group 2 and group 3, *Carica papaya* debrided wounds speedily, replacing sloughs with granulation tissue.

Conclusion:

The following research was emphasized on the development of a newest topical therapeutic agent *Carica Papaya*; increase the wound curing process in diabetic rats. With this expectation we examine the healing proficiency of papaya latex formulated as 1.0 and 2.5% hydrogels. We created a full width around wound at the upper back of every rat in every group in wistar rats separated into three groups as following; Group-I (negative control) received no treatment. Group-II was treated with standard drug povidone iodine hydrogel (positive control). Group-III received the *Carica Papaya* hydrogel. The efficacy of treatment was estimated on the base of wound narrowing rate, wound healing photographs in rats and histopathological report of animals of group 1, group 2 and group 3. It was bring into being that *Carica Papaya* was a superb therapeutic agent, which used to improve the wound curing rate extensively in diabetic rodents.

References:

[1] Wu KK & Huan Y (2008) Curr Protocol Pharmacol 40:1-14.

[2] Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD & Goel RK (2009) Ind J Exp Biol 47:32-40.

[3] Murthy S, Gautam MK, Goel S, Purohit V, Sharma H & Goel RK (2013) Bio Med Res Int 1-9.

PD-46

Wound Healing Activity of the Hydro-Alcoholic Extract of *Datura Stramonium Leaves* in Wistar Albino Rats

Priyanshu Shekhar, Ankur Joshi, Sapna Malviya, Anil Kharia
Modern Institute of Pharmaceutical Sciences, Indore (M.P.), India, 453111
E-mail address: priyanshupatna011@gmail.com

Abstract: *Datura Stramonium* has been known for many potential uses. Leaves of datura are used in herbal medicine especially in case of Ayurveda for asthma and bonesetting. Roasted leaves is applied over the area of relieve pain. Leaves and leaves are used in antiasthematic, antispasmodic, hypnotic & narcotic. The hydro-alcoholic extract of *Datura Stramonium* leaves were investigated for wound healing potential in rats. *Datura Stramonium* leaves were dried, crushed in coarse powder hydro-alcoholic extract was obtained and turned to 10% ointment form. In the course of this study, 18 male wistar albino rats weighing approximately 150- 180g were used in this research. Group 1 as control group, Group 2 as reference control were treated topically with Povidone-Iodine Ointment USP, Group3 as test control were treated with 10% *Datura Stramonium* ointment. Wound healing was monitored on days 4,8,12,16 and histopathological evaluation was carried out on the samples. Leaf extract of *Datura Stramonium* promotes wound healing via bactericidal activity.

Introduction:

Wound are in escapable events of life, which can be arises due to chemical, physical or microbial infections. Our knowledge about wound healing mechanism is still incomplete. Some valuable information has been obtained by comparing results from animal experiments with clinical observation. *Datura Stramonium* is a popular tropical shrubs and most commonly found in India. Datura is most commonly used in asthma, reduce fever, protect the heart, eliminate pain, increase fertility, boost hair health etc. Datura is a genus of nine species of poisonous vespertine flowering plants belonging to the family solanaceae.

Materials and Methods:

Plant material

Leaves of *Datura Stramonium* were obtained from medicinal garden of Modern Institute of Pharmaceutical Sciences, Indore and were identified at Department of Botany Holkar Science College,

Indore. A voucher specimen was submitted in our MIPS, Indore for further reference.

Preparation of extract

Datura Stramonium fresh leaves of this plant were cut and wash with distilled water and dried in oven 50^0 C for 5-7 days until fully dried. The dried leaves were grind and 50g of blended leaves powder was extracted by soxhlet extraction by hydroalcholic solution (70:30). The obtained extract was then

formulated in the form of 10% ointment. 1g of the leaf extract was mixed with 100 g of formula known as the ointment base whose standard constituents are: white bees wax (2g), hard paraffin wax (3g), propylene glycol (5g), cetostearyl alcohol (5g), and white soft paraffin (85g).

Experimental animals

During this experiment 18 wistar rats of either sex were issued from animal house of Modern Institute of Pharmaceutical Sciences, Indore. Wistar rats (150-180g) were divided into three groups of six rats. The animals were housed in standard environmental condition, 12 hrs. Light/dark cycle for two weeks. During the course of the experiment the rats were administered a standard pellet diet and water *ad-libitum.*

Anaesthesia

Using 1 ml syringe, a calculated dose per body of Thiopental sodium were administered subcutaneously via the ventral part of abdomen of the each rats. The process of anesthetizing the rats was done whenever the wounds were be the measured and dressed as well.

Excisional wound model

The dorsolateral aspect of the thoracic wall was saved and thereafter cleaned with methylated spirit. A 2 cm by 2 cm wound was created on the dorsolateral aspect of the thoracic wall under aseptic condition; achieving prior sedation with parenteral.

Treatments

After making the surgical wounds, all rats were randomly divided in three different groups.

Group I: Control

Group II: Povidone-Iodine Ointment USP

Group III: *Datura Stramonium* ointment (10%)

On day four, eight, twelve, and sixteen four animals were randomly selected for observation of percentage of healing of wound on the rats.

Wound size at day zero(0) – Wound size on the given day X 100

Wound size on day zero (0)

Table 1: Effect of Hydroalcholic extract ointment of Leaves of *Datura stramonium* % wound closure of excision wounds

Group	Treatment	4th Day	8th Day	12th Day	16th Day	Period of epithelization in days
Group I	Control (Simple ointment base B.P.)	15.8±0.6	27.2±1.0	48.2±1.8	68.5±2.6	26
Group II	Povidone-Iodine Ointment USP	35.2±0.1	76.8±0.1	89.8±0.5	97.1±0.4	18
Group III	Hydroalcholic extract (10%)	34.4±1.0	76.8±1.2	84.3±2.3	92.5±2.1	19*

Results and Discussion:

The effect of hydro alcoholic leaf extract ointment on excision wound model, the wound healing contracting ability in different contraction was significantly greater than that of control. The 10%w/w extract ointment treated groups showed significantly wound healing from fourth day onwards, which was comparable to that of the standard drug, Povidone-Iodine Ointment USP treated groups of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 10% w/w extract ointment treatment group. The result of present study revealed that hydro-alcoholic extract of *Datura Stramonium* leaf have significant wound healing activity in excision wound model.

Conclusion:

In this study, the effect of *Datura Stramonium* leaves was screened for excision wound healing activity on adult male wistar rats. This research has therefore showed that *Datura Stramonium* has agents to promote wound healing activity.

Reference:

[1] Choudhary N, Kaur M & Singh A (2014) Indian J Res Pharm Biotech 2(2): 1071-1081.

[2] Nayak BS, Ramdeen R, Adogwa A, Ramsubhag A & Marshall JR (2012) Int Wound J, 9(1): 650–655.

[3] Patil S, Ghodke DS & Magdum CS (2009) Int J Pharm Tech Res 1 : 500 – 501.

[4] Chitra P, Sajithal G B & Chanrakaran G (1998) Ind J Exp Biol 36: 896 – 901.

[5] Pandey M & Worlikar P S (2012) Int J Health Allied Sci 2012; 1: 59 – 63.

PD-62

Theileriosis in Buffalo: A Case Study

Rashmi Sharma, Nidhi Choudhary, Vivek Agrawal, H.K.Mehta, Anjali Singh, Kamlesh Choudhary
Department of Veterinary Medicine, College of Veterinary Sc. A.H., Mhow, (M.P.)
E-mail address: rashmi3818@gmail.com

Abstract: Bovine theileriosis is caused by the protozoan parasite *Theileria annulata* and *Theileria parva* and is transmitted through tick *Hyalomma* and *Rhipicephalus*, respectively leading to economic losses and causes devastating losses to the livestock worldwide. This paper deals with clinical signs, diagnosis and treatment of theileriosis in buffalo having 4years of age and clinical signs observed were; high fever (106.7^{o}F), anorexia, enlarge lymph nodes, dyspnoea and ocular discharge with pale mucous membrane. The peripheral blood smear confirmed the presence of Koch blue bodies. The animal was treated with a single dose of Buparvaquone, 2.5 mg/kg i.m. along with supportive therapy.

Introduction:

Theileriosis is a disease caused by an apicomplexan protozoan parasites belonging to the family of Theileriidae. The *Theileria* spp. infecting animals include *T. velifera, T.taurotragi, T. buffeli, T. mutans, T. annulata* and *T. parva*. Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic *Bovidae*. They are transmitted by ixodid ticks, and have complex life cycles in both vertebrate and invertebrate hosts [1].

Bovine theileriosis is caused by the protozoan parasite *Theileria annulata* and *Theileria parva*. The disease is considered one of the most destructive obstacles to livestock production. The incubation period varies from 4 to 14 days after attachment of the infected ticks to the host. The disease may last as little as three to four days in the acute form or may be prolonged for about 20 days [2]. Clinical signs of the infected buffaloes include, pyrexia (40.5–41.5 °C), anorexia, enlargement of superficial lymph nodes (parotid, prescapular and prefemoral), slight nasal and ocular discharges with congestion of conjunctiva and salivation. Constipation was recorded in some cases later turning to tarry diarrhoea, with pale mucous membranes, milky infiltration of the cornea and respiratory distress in the form of dyspnoea, coughing and evidence of pulmonary oedema and nervous manifestations in the form of hyperesthesia, head pressing, convulsions, tremors and paddling prior to death [3].

The diagnosis of theileriosis in acute cases is mainly based on clinical findings and microscopic examination of Giemsa's stained thin blood smears. In long standing carrier animals blood smears are negative on microscopy. The advent of the PCR coupled with the specificity of deoxyribonucleic acid (DNA) hybridization had led to the development of specific and sensitive molecular diagnostic tests to detect and characterize the organisms that cause theileriosis [4].

Haemoprotozoan parasites, which include *Babesia, Theileria* and *Trypanosoma*, often present a challenge to successful livestock farming and cause devastating losses to the livestock industry

throughout the world. Haemoprotozoan infections have a global distribution. This is due to the fact that their vectors; ticks and bloodsucking flies, also have a global distribution. The hot and humid climate is very conducive for the development and survival of potential vectors and is a constant source of infection to susceptible animal. Annual economic losses due to theileriosis alone have been estimated to the tune of US$ 800 million. The recovered animals from acute or primary theileriosis remain infected for a long period and even for the rest of their life, so acting as reservoir of infection for ticks and cause natural transmission of disease. Therefore detection of *Theileria* in asymptomatic carrier cattle and buffaloes is important for implementation of successful control programme.

History and Observations

Buffalo aging 4 years was presented at Teaching Veterinary Clinical Complex (TVCC) veterinary college Mhow with a history of high fever (106.7 ˚F), anorexia, and agalactia and tick infestation. On clinical examination buffalo was found severely dull and depressed with pale mucous membrane, enlarged lymph nodes, rough hair coat, dyspnoea and ocular discharge. Buffalo was suspected to heamoprotozoan diseases and for confirmation, blood smears were made from ear tip.

Figure 1: Buffalo showing pale mucous membrane.

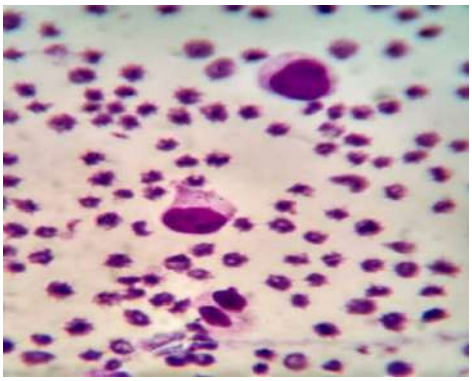


Figure 2: Pale mucous membrane in presence of κoch's blue bodies in blood smear

Therapeutic Management and Discussion:

The buffalo was diagnosed positive for *Theileria a*fter blood smear examination stained with Giemsa stain. Intra-erythrocytic piroplasm and Koch's blue bodies were found on microscopic examination (100X) of prepared smears. The buffalo was treated with inj Buparvoquone (Butalex) @ 2.5 mg/kg

b.wt injected deep IM in the neck region, inj OTC @ 10mg/kg b.wt along with Normal saline 4lit IV, inj Paracetamol @ 10 mg/kg b.wt IM, Avil 10ml IM, Tribivet 10ml IM. The buffalo responded after first day of treatment. Inj paracetamol, Avil, Tribivet and OTC were continued for next 4 days along with inj Normal Saline. Second day temperature came down to almost normal.

This is in accordance with (4) who used Buparvoquone along with supportive therapy for the successful treatment and the oxytetracycline treated animals produced a serological response and were immune to a 50-fold higher challenge with the immunizing stabilate, several animals in the buparvaquone did not show a serological response and were not immune to challenge.

References:

[1] Collins NE, Allsopp MT & Allsopp BA (2002) J Vet Med 50(6):309-310.

[2] Durrani Z, Ahmad M, Ashraf M, Khan MS, Khan JA, Kamal N & Mumtaz N (2008) J Anim Pl Sci 18(2-3):59.

[3] Islam ST, Dar RR, Sheikh AA, Dogra P, Gupta R, Patel P, Ganaie MY & Wani JM (2017) Int J Curr Microbiol App Sci 6(10): 1400-1404.

[4] Ngumi AS, Young D, Lampard SK, Mining SG, Ndungu AC, Lesan SM, Williamson A, Linyonyi DP & Kariuki K (1992) Vet Parasitol 43(1–2):15-24.

[5] Office of International Des Epizooties (2014) OIE Terrestrial Manual on Theileriosis. Chapter 2: 1-23.

PD-66

Comparative Study of Phenytoin Sodium and Ethanolic Extract of *Nardostachys Jatamansi* Augmented With Phenytoin Sodium to Evaluate Anti-Epileptic Activity of the Drug

Shabnam Mansuri, Thomson Alex, Shaily Chaudhary
Smriti College of Pharmaceutical Education, Indore (Madhya Pradesh), India
E-mail address: mansurishabnam866@gmail.com

Abstract: Epilepsy is a disorder of nervous system which leads to episodes of sensory interruption, loss of perception, or ***seizures,*** coupled with irregular electrical bustle in the brain. It is generally treated by drug therapy using anti-epileptic drugs. The core objective of this study is to comparatively study anti-epileptic activity of phenytoin sodium and ethanolic extract of jatamansi augmented with phenytoin sodium. Convulsions were induced in the mice by pentylenetetrazol (PTZ) ***seizure*** model and ***maximal electroshock seizure (mes)*** model. Jatamansi extract augmented with phenytoin sodium was the drug used for the comparative study the anti-epileptic activity of phenytoin sodium. And jatamansi extract augmented with phenytoin sodium gave better results.

Introduction:

Epilepsy is defined as a brain disorder with episodic seizures coupled with irregular electrical bustle in the brain. Seizures are of two types, partial seizure and generalized seizure. Partial seizures usually start in one section of brain and affect the part of the body associated with that section of brain. It is further classified into:

1. **Simple Partial seizure**: This affects muscle activity leading to jerking of body parts like face, arm, etc.
2. **Complex Partial seizure**: Doesn't cause seizure but impair perception, i.e. the patient will not response.

Generalized seizure occurs in entire brain and hence affects entire body. It is further classified into-

1. **Myoclinic seizure**: This is a short term seizure leading to muscle jerks which doesn't last for more than 3 seconds.
2. **Atonic seizure**: This leads to sudden muscle weakness causing patient to fall on head or face leading to injury. Last for maximum 16 secs.
3. **Tonic seizure**: This leads to sudden muscle stiffening usually occurs in sleep but may occur while awake which lead to fall and cause major head injuries. Last for 20 seconds.
4. **Tonic clonic seizures**: This leads to stiffening of the muscle along with rhythmic jerking and twitching of the body. Patient makes noise due to the contraction of chest muscle leading to flushing of air from the vocal cord; it also leads to wetting and soiling due to contraction bladder and bowel respectively. Patient may also bite the tongue which causes bleeding. It lasts for 5 mins.

5. **Absence seizure**: This only cause's loss of consciousness and the patient don't have memory of it.

Other seizure types include infantile spasm and psychogenic non-epileptic seizure.

Causes of epilepsy are brain injury, structural deformity in the brain growth, genetic and may the combination of the same. Seizers are triggered by number of factors like stress, climatic conditions, lack of sleep, fever, menstrual cycle, alcohol etc.

Epidemiology: Epilepsy is mainly seen in underdeveloped countries and in villages compare to developed countries and cities.

Diagnosed by EEG, Blood Test, PET scan and Spinal Tap.

Epilepsy can be **treated** by taking medication, without medication or by complementary medications.

Models of Epilepsy are genetic animal model, chemically induced model (PTZ) and electrically induced animal model (MES).

Materials and Method:

Pentylenetetrazone (PTZ)

It is also called pentamethylenetetrazol or metrazol. Chemical formula is $C_6H_{10}N_4$. It is used to induce seizures in the pre-clinical study of anti-epileptic drugs. It binds to the picrotoxin binding site of GABA-A receptor complex.

Phenytoin Sodium

It is diphenylhydantoin Sodium, having chemical formula C15H11N2NaO2. A hydantoin derivate and nonsedative antiepileptic agent with anticonvulsant activity. Phenytoin sodium promotes sodium efflux from neurons located in the motor cortex, thereby stabilizing the neuron and inhibiting synaptic transmission. This leads to a reduction in posttetanic potentiation at synapses, an inhibition of repetitive firing of action potentials and ultimately inhibits the spread of seizure activity.

Jatamansi

Botanical name is *Nardostachys jatamansi.* Its dried rhizome and roots are the main medicinal part. Its medical use is in epilepsy, schizophrenia, stress and anxiety.

Extraction

Extract of N. jatamansi root was prepared by extracting 100 grams of jatamansi powder in 90 % ethanol (1L) at 50 °C to 60 °C in a Soxhlet extractor for 72 hours. The cooled liquid extract was concentrated by evaporating its liquid contents in rotary evaporator, with an approximate yield of 20 %. The dried ethanol extract was suspended in distilled water. The drug, N. jatamansi root extract (NJE) was administered.

Electro Convulsometer It is used in the application of maximal electro-shock using corneal electrodes. It is used in the study of anti-convulsant activity using MES model. It includes Digital Voltmeter, Analog Type Ammeter, Analog Timer, Multiplier and Three pair of corneal Electrodes.

Animals

Male CD-1mice is albino coat color mice ideal for different multitasking models, safety and efficacy testing, surgical model etc.

Allotment of groups

Three groups were formed one of control, one of test and other of standard. Each group consisted of 6 mice fed with usual diet (Atromin pellets) and water ad libitum. All the experiments were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) of SPARC. Animals surviving after completion of the study were sent for their disposal under the IAEC approved protocol.

Administration drug

All drugs were freshly prepared before administration. The control group was administered with sterile 0.9% saline i.v. The standard group was administered by phenytoin sodium i.v. The test group was administered by jatamansi extract and phenytoin sodium, dose 50mg/kg orally and 75mg/kg i.v respectively.

PTZ Test

Mice were administered 85 mg/kg dose of PTZ subcutaneously into a loose fold of skin of the neck, between two shoulder blades. Animals were observed over the course of 60 min for appearance of clonus and TE.

MES Test

A drop of saline solution (0.9% saline) was placed in each eye of mice. Convulsions were induced by placing the corneal electrode in the mice cornea. Readings were noted.

Result:

The comparative results of tonic, clonic and stupor of controlled, standard and test are given in the below table:

Table 1: Comparative results of tonic, clonic and stupor

Groups	Tonic	Clonic	Stupor	Dead or Alive
Control	110	120	140	Dead
Standard	86	90	100	Alive
Test	98	95	80	Alive

Conclusion:

The test group showed positive and better result, proving jatamansi and phenytoin combination better than phenytoin sodium.

References:

[1] Kupferberg H (2001) Epilepsia 42:7-12.

[2] Loscher W, Honack D, Fassbender CP & Nolting B (1991) Epilepsy Res 8:171-89.

[3] Anand N (1990) Comprehensive Medical Chemistry, Pergamon press. Oxford, 1:113.

[4] Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P & Engel J (2005) Epilepsia 46:470.

PD-76

Studies on Combined Toxicity of Pendimethalin and Cadmium Chloride with Ameliorative Effect of Vitamin E-Selenium and Turmeric in Male Albino Rats

Supriya Shukla, Sushma Chakravarti, Anupam Chaturvedi, Sachin Verma, Aditya Sharma, Kuldeep Singh
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, MHOW (M.P.)-453446
E-mail address: s.shukla@gmail.com

Abstract: The present investigation was undertaken to study the pathology of pendimethalin and cadmium chloride and to evaluate the different concentration were used as ameliorative agents to reduce theoxidative damage caused by free redicals genreted by toxic pollutants at molecular levels. A total of 5 groups each of 12 rats were formed apart from control group. 2 groups of heavy metal and pesticide and 3 group of ameliorative oxidant in varying concentrations were formed Group T2 was given standard ration along with combination of cadmium chloride at 500 ppm with pendimethalin (38.7%) at 1000 ppm/kg feed, Group T3 was given cadmium chloride at 100ppm/kg feed and P pendimethalin (38.7%) at 1000 ppm/kg feed with standard ration, Group T4 was fed with the combination of cadmium chloride at 50 ppm/kg of feed with pendimethalin at 1000 ppm/kg of feed and alpha tocopherol-selenium at 18 ppm/kg of feed. Lastly group T5 and T6 were given turmeric at 0.5%and 1% concentrations respectively along with the combination of cadmium chloride at 50 ppm with pendimethalin (38.7%) at 1000ppm and alpha tocopherol-selenium at18ppm/kg of feed. During the toxicity studies observation like effect on CNS, effect on locomotors system recorded on the days 0, 7, 14, 21 and 28 of experiment.

Introduction:

Cadmium is a well known human carcinogens and a potent nephrotoxins. Higher concentrations are found in the kidneys of animal slaughtered for food, in wild mushrooms, and in sea food such as mussels and oysters [1]. Hepatic necrosis induced by cadmium chloride at 2.5mg/kg bwt s.c. in mice and was examined bio-chemically, haematologically and histopathlogically in order to study the time dependent effect [2]. Cadmium caused primarily renal tubular lesion and sometimes glomerular lesions in rabbits, rats, swine and monkeys [3]. The protective effects of melatonin alone and vitamin E with selenium combination against cadmium indused oxidative damage in rat liver 1 mg/kg body weight and concluded that vitamin E had protective effects against cadmium induced oxidative damage [4]. Curcumin has a free radical scavenger activity, especially on the hydroxyl radical, which explains its capacity to protect DNA from damage in human cell cultures exposed to radiation. It is more efficient than vitamin E as an anti-radical agent and as an inhibitor of lipid per-oxidation [5]. The objective of this study was to study the combined toxicity of Pendimethalin and Cadmium chloride with ameliorative effect of vitamin E-Selenium and turmeric in male albino rats.

Materials and Methods:

The study was conducted on male albino rat of 2 months age group, weighing around 80-150 gms. Before the start of the experiment, rats were kept in laboratory conditions for a period of 7 days for acclimatization. All the experimental animals were kept under constant observations during the entire period of study. The rats were maintained with good hygienic conditions and provided with standard feed and water *ad lib.* A total of 5 groups each of 12 rats were formed apart from control group. 2 groups of heavy metal and pesticide and 3 group of ameliorative oxidant in varying concentrations were formed Group T2 was given standard ration along with combination of cadmium chloride at 500 ppm with pendimethalin (38.7%) at 1000 ppm/kg feed, Group T3 was given cadmium chloride at 100 ppm/kg feed and P pendimethalin (38.7%) at 1000ppm/kg feed with standard ration, Group T4 was fed with the combination of cadmium chloride at 50ppm/kg of feed with pendimethalin at 1000 ppm/kg of feed and alpha tocopherol-selenium at 18ppm/kg of feed. Lastly group T5 and T6 were given turmeric at 0.5% and 1% concentrations respectively along with the combination of cadmium chloride at 50ppm with pendimethalin (38.7%) at 1000 ppm and alpha tocopherol-selenium at 18 ppm/kg of feed. During the toxicity studies observation like effect on CNS, effect on locomotors system recorded on the days 0, 7, 14, 21 and 28 of experiment. Haematological parameter and biochemical parameters were studied. At the end of the 21st and 42nd day of experiment, animals were sacrificed by cervical dislocation. The organs like lung, liver, kidneys, spleen, heart and thymus were examined for any gross abnormality. A portion of the tissues were fixed in 10 % formalin. The formalin fixed tissue pices were serially processed through acetone and benzene and embedded in paraffin.The micro sections were cut and stained in haematoxylene and (H&E) and examined for histopathological changes.

Results and Discussion:

Six groups of rats (Group T1 –T6) comparising 12 animals each was fed with cadmium choride and pendimethalin and vitamin E –Se along with Turmeric for 6 weeks and six animals from each group were sacrificed at the end of 21st and 42nd day of experiment. The details of the experiment are-

Toxicity study In present study the rats were slightly dull, dipressed, reduced feed intake and exhibited sluggish movements in T2, T3 and T4 group.

Body weight

In present study, there was a significant reduction in body weight in the cadmium chloride and pendimethalin fed groups as well as rats treated with alpha tocopherol as compared to the control.Supplementation of alpha- tocopherol along withcadmium chloride and pendimethalin caused marginal increase in body weight as compared to toxic group.

Haematological studies*Total erythrocyte count* The total erythrocyte count was found significantly reduced in the rats treated with cadmium chloride and pendimethalin (T2, T3, and T4) the values were

improved in T5 and T6 in 21st day of experimwent and almost analogous to control group at the terminal stage of study, when turmeric is given along with vitamin E at varying disease (T5 and T6).

Haemoglobin concentration Decrease in haemoglobin concentration in pendimethalin and cadmium chloride fed rat of group T2, T3 and T4 might be due to anemia as a result of depressed erythropoiesis and erythrolysis.

Total Leukocyte count There was a dose dependant reduction in the TLC values in T2, T3 and T4 groups and improvement in turmeric plus vitamin E treated group when compared with other groups at end of the experiment.

Differential Leucocyte Count The overall study of differential leukocyte count did not revealed much significant in monocyte, eosinophils and basophils. Minor variations were seen in neutrophils (toxic) and lymphocytes in treated groups as compared with control.

Biochemical studies

Aspartate amino transferase (SGOT) There was dose dependant significant increase in AST value in T2, T3 and T4 groups and slight improvement in the AST value in turmeric plus vitamin E group compared with respective toxic group at end of the experiment.

Alkaline phosphate (ALP) There was a dose dependent significant increase in ALP value in T2, T3 and T4groups and minimum improvement in turmeric plus vitamin E treated group when compared with toxicity groups.

Serum total protein There was a dose of dependent decrease in total protein value in T2, T3 and T4 groups and significant improvement in turmeric plus vitamin E treated group when compared with toxicity groups.

Blood urea nitrogen (BUN) There was a dose dependent increase in blood urea nitrogen values in T2, T3 and T4 groups and significant improvement in turmeric plus vitamin E treated group when compared with toxicity groups.

Creatinine There was a dose dependent increase in creatinine in T2, T3 and T4 groups and significant improvement in turmeric plus vitamin E treated group when compared with toxicity groups.

Gross and histopathology The rat of group T1 (control group) revealed normal morphological appearance in organs examined during the study i.e., liver, kidney, brain, spleen at the end of experiment. The path-morphological changes observed in pendimethalin and cadmium chloride fed along with turmeric and vitamin E treated groups have been recorded organ wise follow-

Liver Hepatomagaly with rounded border, congestion and hemorrages was noticed in T2, T3 and T4 toxic groups.

Kidney kidney revealed congestion and hemorrhage at the both sacrifice day (21st and 42nd) of experiment in both toxicity levels (group T2 and T3).

Lungs Area of congestion and hemorrhage were observed in the lungs of groups T2, T3 and T4 at the 21st and 42nd day of experiment.

Heart in the group T2 and T3 mild degenerative changes were observed in heart muscles at 21st day of experiment.

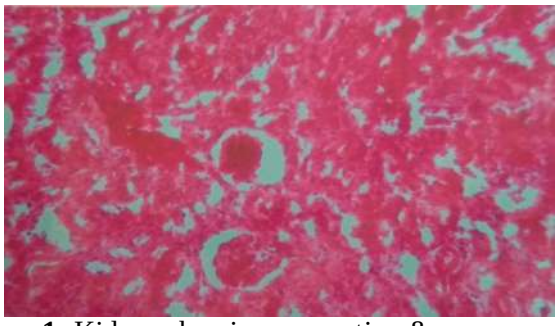


Figure 1: Kidney showing congestion & Swelling of both proximal and distal convulated tubules (H&E 400X)

Figure 2: Heart showing congestion and hemorrhage (H&E 400x)

Figure 3: Lacrimation and rough hair coat (group T2, T3, T4)

Figure 4: Ataxic gait and convultion (group T2, T3, T4)

Conclusion:

Ameliorative effect of turmeric was best observed in sub-acute toxicity of day 21 at 1% concentration but turmeric at 1% concentration did not show any corrective change in chronic toxicity of day 42.

References:

[1] Fried KW & KK Rozman (2008) John Wiley and Sons Ltd., 513-655.

[2] Karmarkar R, Bhattacharya R & Chatt M (2000) J Biometals: 231-239.

[3] Kara H, Aydin C, Konar V, Dayangac A & Servi K (2008) J Biol Trace Ele Res 120:205-211.

[4] Fatma ME, Demendash I, Fatma M, Kedwwany YS & Baghdadi HH (2004) Food Chem Toxicol 42:1563-1571.

[5] Prakash KC, Majeed AC, Hooper MJ & Porkras MA (2003) Environ Toxicol Chem 19: 1317-1323.

PD-91

Effect of *Parthenium hysterophorus* on Oxidative Stress in Pancreatic Tissue of Diabetic Rats

Vijay Patel, Arjun Patidar, Saurabh Pagaria
BM College of Pharmaceutical Education and Research, Indore, India
E-mail address: vijaysuman1@gmail.com

Abstract: Increased oxidative stress and decreased endogenous antioxidant defense has been shown to be a prominent and early features in diabetes mellitus. The present study focused on investigating the possible protective role of *Parthenium hysterophorus* against free radical mediated damage in pancreatic tissue of alloxan induced diabetic rats. Diabetes was induced in rats by injecting 150 mg/kg Alloxan monohydrate IP. The results revealed that administration of 50 mg/kg & 100 mg/kg of *Parthenium hysterophorus* extract significantly increased pancreatic glutathione, superoxide dismutase, catalase level ($p<0.01$) as well as significantly reduced pancreatic total nitrate/nitrite content and lipid peroxidation ($p<0.01$) after 72 hr. In conclusion, the study suggests that *Parthenium hysterophorus* is effective in significantly reducing the oxidative stress in pancreatic tissue of diabetes rats as evidenced by the increase in antioxidant enzymes, reduction of lipid peroxidation and total nitrate/nitrite level.

Introduction:

The role of oxidative stress in both type I & type II diabetes mellitus is currently under investigation [1]. It has been established that locally produced reactive oxygen species (ROS) & nitric oxide (NO) induced after cytokine stimulation are involved in β-cell destruction through the induction of apoptosis [2]. Pancreatic β-cells are particularly susceptible to the deleterious effects of ROS because of their low level of antioxidant enzyme genes as compared to other tissue [3]. Hence the cellular antioxidant status is an important determinant of its susceptibility to oxidative damage. Reduced glutathione (GSH) is an endogenous antioxidant that acts as a first line defense system against pro-oxidant status. Depleted GSH level has been repeatedly reported in several tissues of experimental diabetes animals, including eye, aorta, kidney as well as small intestine. Lipid peroxidation, a key marker of oxidative stress is the result of a chain reaction evoked by ROS & eventually leads to extensive membrane damage & dysfunction [4]. *Parthenium hysterophorus* L. compositae, also known as Congress grass, Chatak chandani (Hindi) is light green with branching stems reaching the height of 2 m in good weeks of germination. All parts of the plant are reported to be used as a bitter tonic, febrifuge, emmenagogue, antidyscentric etc. *Parthenium hysterophorus* is a combination of biologically active flavonoids including guercetagetin & 6-hydroxykaempferol which have marked spectrum against oxygen free radicals & thus holds the promise for the prevention & treatment of variety of human disorders caused by oxidative stress [5].

The present study was thus undertaken to access the protective effect of *Parthenium hysterophorus* on oxidative damage induced by alloxan in rat's pancreatic tissue. The result could serve as a step towards the development of a mechanism based therapeutic approach for the management of diabetes & provide the basis for the usefulness of the potent antioxidants.

Materials and Methods:

Chemicals

All the chemicals & reagents were of analytical grade & procured from E. Merck (India) Ltd.

Plant Extraction

Fresh plant of *Parthenium hysterophorus* was collected & authenticated at the plant anatomy research center, Chennai. The flowers were dried under sunlight & powdered. 20gm of the powdered drug was boiled with 100 ml of distilled water for four hours and evaporated to dryness. The yield of extract was about 2.5gm & was suspended in 5% Tween 80 & used for oral administration.

Animals

Adult albino wistar rats of either sex, weighing 150-200 gm were acclimated for a period of 10 days at room temperature & 50% relative humidity. They were housed in a standard cage & water ad libitum. All the experimental procedures were performed after prior approval from the institutional animal ethical committee and are in accordance with the CPCSEA, India.

Induction of diabetes

Alloxan monohydrate, 150 mg/kg body wt. dissolved in normal saline & injected IP in 18 hr previously fasted animals. After 72 hr, blood glucose level of each animal was measured by glucose oxidase method & rats having blood glucose level more than 200 mg/dl were selected for study.

Experimental design

Rats were divided in to 5 groups of 6 animals each & given following drug treatment orally in a single dose. At the end of 72 hr, all animals were fasted overnight and sacrificed by cervical decapitation. Dissected pancreatic tissue was washed with normal saline. 100 mg of pancreatic tissue was dissolved in 1 ml, 0.1 M Phosphate buffer solution (pH 7.0). The homogenate was centrifuged at 10000 rpm for 20 min at 4^0c and the clear supernatant was used for biochemical analysis.

Group-1 (Normal Control) : Normal rats received 5% Tween 80 suspension only

Group-2 (Diabetic control) : Diabetic rats received 5% Tween 80 suspension only

Group-3 (Standard) : Diabetic rats received 100 mg/kg Vitamin C (ascorbic acid)

Group-4 (Test-1) : Diabetic rats received aq. extract of *Parthenium hysterophorus* 50 mg/kg

Group-5 (Test-2) : Diabetic rats received aq. extract of *Parthenium hysterophorus* 100 mg/kg

Biochemical Analysis

Serum glucose was estimated by autoanalyser using a commercial assay kit (ERBA diagnostic GmbH, Germany), according to the method described by basu. The level of TBARS, malondialdehyde

(MDA) a commonly used marker for lipid peroxidation was measured spectrophotometrically by the method of uchiyama & mihara. Total glutathione (GSH) was evaluated by the method of Sedlak and Lindsay. Superoxide dismutase (SOD) was measured by using the method of Ellman. Catalase activity was evaluated using method of Claiborne. The total nitrate/nitrite content, an indicator of NO-production, was estimated in pancreatic tissue homogenate, according to the procedures of commercially available kit (R&D System UK).

Statistical analysis

The results were analyzed by one-way ANOVA test followed by Dennett's test. Graph pad prizm 3.0 software used. The results were expressed as mean ± S.E.M., n=6. P-value <0.05 were considered to be significant.

Result and Discussion:

As shown in table1, Alloxan produced a significant increase in pancreatic MDA level. The administration of *Parthenium hysterophorus* L. extract significantly reduced the pancreatic MDA level compared to diabetic control group at 72 hr ($p<0.01$).

Table 1: Effect of *Parthenium hysterophorus* Linn. on lipid peroxidation, Pancreatic nitrate/nitrite, and antioxidant's level in normal and diabetic rats

S. No.	Treatment	Lipid Peroxidation (MDA) (µM/g)	GSH (mM/g)	CAT (µM of H_2O_2 consumed /mg protein)	SOD (µM/mg protein)	Pancreatic nitrate/nitrite µM /l
1	Normal control	0.16±0.01**	0.110±0.01*	3.51±3.11**	4.91±0.28**	219.43±7.9**
2	Diabetic control	0.32±0.0	0.058±0.02	1.13±1.21	2.78±0.15	501.11±9.89
3	Vitamin C	0.15±0.03**	0.39±0.99**	4.79±2.71 **	4.92±0.27**	201.34±1.8**
4	*P. hysterophorus* Extract 50 mg/kg	0.24±0.01**	0.28±0.05**	1.17±1.07*	3.14±0.85*	199.38±9.5**
5	*P. hysterophorus* Extract 100 mg/kg	0.19±0.01**	0.31±0.01**	2.77±0.90**	4.10±0.14**	151.7±8.9**

The values are mean ± SEM. (n=6). *p<0.05 & **p<0.01; when compared with diabetic control group

Table 1 illustrate that alloxan treatment consistently reduced pancreatic GSH, CAT and SOD content as compared to control group. Administration of *Parthenium hysterophorus* L. extract significantly elevated the pancreatic antioxidant's level ($p<0.05$) and reached maximum level at 72 hr. Such effect was more obvious ($p<0.01$) with high dose of *Parthenium hysterophorus* i.e. 100 mg/kg.

Alloxan caused a significant increase in total nitrate/nitrite content. However Diabetic animal treated with *Parthenium hysterophorus* L. extract showed significant reduction in the pancreatic total nitrate/nitrite level as shown in table1. Such effect was obvious at both doses used following 72 hr.

Alloxan induced diabetes is a well documented model of experimental diabetes. This compound causes severe necrosis of pancreatic β-cells. The sensitivity of β-cells to oxidative stress has been attributed to their low levels of antioxidants compared with other tissue. Accordingly, maintenance of β-cell oxidant status and their protection against oxidative damage might delay the onset of diabetes as well as the development of its complications.

The current study revealed that alloxan significantly induced hyperglycemia. Such effect might be explained by the possible pancreatic damage caused by observed significant rise in lipid peroxidation as well as total nitrate/nitrite content. Interestingly *Parthenium hysterophorus* restored the oxidant status of pancreatic tissue; such result suggests a protective effect of *Parthenium hysterophorus* against alloxan action.The observed increase in the level of lipid peroxides in alloxan treated rats might be due to the increased generation of different radical species. These radicals have been documented to stimulate degradation of DNA, lipids, and carbohydrates leading to hyperglycemia and related glucose auto-oxidation. These results are in accordance with previous findings whereby alloxan treated rats showed marked increase in pancreatic cells lipid peroxidation.

Alloxan treated rats showed significant elevation of total pancreatic nitrate/nitrite levels. Such finding coincide with the previously published studies that proved the production of NO by β-cells in presence of alloxan, has been implicated in the development of diabetes. NO reacts with the superoxide radical to form the noxious peroxynitrite that contributes in the pathogenesis of diabetes complications. The data presented reveled marked protective effect of *Parthenium hysterophorus* against alloxan induced elevation of total nitrate/nitrite level in pancreatic tissue. Whereby, concurrent treatment with *Parthenium hysterophorus* normalized the pancreatic NO levels.

Alloxan treatment leads to depletion of pancreatic GSH content, which significantly affects the overall Redox potential of the cell. In the current study, the depletion of pancreatic GSH, CAT and SOD effect was reversed by the administration of *Parthenium hysterophorus.* A possible explanation of this effect is that these compound function as free radical scavengers and therefore increase the available free GSH which detoxify the reactive intermediary oxygen product of lipid peroxidation induced by alloxan. In summary, *Parthenium hysterophorus* possess potent protective effect on the induction of diabetes by alloxan. The data provided suggest that the mechanism underlying such protection is mediated via prevention and restoration of pancreatic antioxidant defense system.

Conclusion:

Based on the oxidative stress hypothesis of alloxan action it was considered as an adequate model for investigating the role of free radicals in the pathology of diabetes mellitus. The present study demonstrates that *Parthenium hysterophorus,* a potent antioxidant can exert anti-diabetic effect by preserving pancreatic β-cell function.

References

[1] Wolf S (2013) Br Med Bull 49:642-652.

[2] Jaeckel E, Manns M & Von Herrath M (2002) Ann NY Acad Sci 958:7-25.

[3] Lenzen S, Drinkgern J & Tiedge M (1996) Free Radic boil Med 20:463-464.

[4] Halliwell B & Gulteridge J (1999) Free Radic Biology and Med (1999) 3rd Ed. Oxford: Clarendon Press 291-299.

[5] Shen MC, Kathleen K & Mabry TJ (2002) The Cell Res 39:157-159.

PD-94

In-vivo Antioxidant Effect of *Abroma Augusta* in Diabetes Induced Oxidative Stress

Rekha Bisht
Indore Institute of Pharmacy, Indore (M.P.), India
E-mail address: rekha.al03@gmail.com

Abstract: The present study was aimed to investigate the *In-vivo* antioxidant activity of roots of *Abroma augusta* in streptozotocin-nicotinamide induced Type-II diabetes. The extraction (Hot continuous extraction process) was carried out with solvents of different polarity. Commercially available Vit-E (100 mg/kg b.w.) was used as a standard drug. *In-vivo* antioxidant activity of plant extracts (250 mg/kg b.w.) was assessed by measuring SOD, CAT and LPO in the blood of Type-II diabetic animals. The results of the study revealed the significant effect on SOD, CAT and LPO level in animals treated with petroleum ether extract ($p<0.0001$) followed by aqueous extract ($p<0.001$) of *A. augusta* significantly as compared to diabetic control. The results of the study suggested the antioxidant activity of plant extract which prevents from oxidative stress and provide protection to vital tissues like liver, kidney, heart etc.

Introduction:

Oxidative stress plays a significant role in the pathogenesis of diabetes. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Hyperglycaemia-induced glucose oxidation initiates membrane lipid peroxidation which is vital for the maintenance and integrity of cell function and initiates a non-enzymatic glycation of proteins, which in turn lead to enhanced production of ROS or result in decreased efficiency of inhibitory and scavenging system [1]. Increase in levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of various complications of diabetes mellitus [2].

A. augusta Linn (Sterculiaceae), also known as Ulatkambal (Bengali and Hindi), is a large spreading bushy shrub with fibrous barks and irritant hairs. It is widely distributed (native or collective) throughout the hotter parts of India, in U.P, Sikkim, Khasia Hills and Assam [3]. It is widely used in gynecological disorders and also used as abortifacient and anti-fertility agent. A study by Bhuyia *et al* [4], reported the *in-vitro* antioxidant effect of leaves of *A. augusta*, whereby our study is aimed to investigate the the *in-vivo* antioxidant effect of roots of *A. augusta* in diabetes induced oxidative stress.

Material and Methods:

Plant Material

The crude drugs of *Abroma augusta* Linn. (roots) were collected from the local herbal garden of Dehradun, Uttarakhand. The crude drug was authenticated at Forest Research Institute of India (FRI), Dehradun. The voucher specimen (No. 157029) of the plant was deposited in the Forest Research Institute herbarium. Soon after authentication collected parts of the plants were shade dried until they were free from moisture and were ground to coarse powder.

Chemical

Pyrogallol and hydrogen peroxide, phosphate buffer and Tris buffer were obtained from S.D. fine chemicals Ltd., India. Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), 5, 5'dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Sigma, USA. Vitamin E was procured from commercial sources.

Preparation of Extracts

The powdered plant material was extracted by Hot Continuous Extraction (Soxhletion) method. The extraction was carried out with solvents of different polarity in succession, starting with highly polar solvent (petroleum ether, benzene, chloroform, acetone, and ethanol). Aqueous extract was prepared separately using chloroform water I.P. by maceration process [5, 6].

Acute Toxicity Studies

Acute oral toxicity study for the test extracts of the plant was carried out using OECD/OCED guideline 425.

Limit Test at 2000 mg/kg

By performing Limit test at 2000 mg/kg of *A. augusta*, 250 mg/kg was found to be effective.

Antioxidant Activity

Assay Methods Used for Antioxidant Activity

Lipid Peroxidation (LPO)

To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2ml of 28% trichloroacetic acid was added and centrifuged. 1ml of 1% thiobarbituric acid was added to 4 ml of supernatant, than heated in boiling water for 60 min and cooled immediately. The absorbance was measured at 532 nm by spectrophotometric method. On the basis of the molar extinction coefficient of malonaldehyde (MDA) (1.56×10^5) lipid peroxidation was calculated, and expressed in terms of nanomoles of MDA/g Hb.

Following formula was used to calculate LPO: **A= abc**

A= Absorbance, **a** = Extinction coefficient, **b** = path length, **c** = Concentration

Superoxide Dismutase (SOD) It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50µl of lysate, 75mM of Tris-HCl buffer (pH 8.2), 30mM EDTA and 2mM of pyrogallol were added. Absorbance was recorded spectrophotometerically at 420 nm for 3 min. One

unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein.

Following formula was used to calculate SOD:

$$\% \text{ inhibition} = \frac{1 - (\Delta_{blank} - \Delta_{sample}) \times 100}{\Delta_{blank}}$$

Catalase (CAT)

Catalase activity was determined in erythrocyte lysate. 50µl of the lysate was added to a cuvette containing 2ml of phosphate buffer (pH 7) and 1ml of 30mM H_2O_2 (hydrogen peroxide). Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The catalase activity was determined on the basis of molar extinction coefficient of H_2O_2 (43.6 M cm^{-1}). One unit of activity is equal to one millimoles of H_2O_2 degraded per minute. It is expressed as units per milligram of protein.

Following formula was used to calculate CAT: **A = abc**

A = Absorbance, **a** = Extinction coefficient, **b** = path length, **c** = Concentration

Experimental Protocol for Antioxidant Activity

Induction of Type-II Diabetes

Diabetes was induced as per the method described by Ananda et al with slight modification (streptozotocin-nicotinamide induced Type-II diabetes). Animals showing fasting blood glucose higher than 250 mg/dl were considered as diabetic and used for the further study. Nine groups of animal having six rats in each group were used and all groups of animal received treatment for 7 days. **Group-1:** Normal control, **Group-2:** Diabetic control, **Group-3:** Diabetic animal+Vitamin E (standard drug) **Group-4:** Diabetic animal+Petroleum ether extract of *A. augusta*, **Group-5:** Diabetic animal+Benzene extract of *A. augusta*, **Group-6:** Diabetic animal+Chloroform extract of *A. augusta*, **Group-7:** Diabetic animal+Acetone extract of *A. augusta*, **Group-8:** Diabetic animal+Ethanol extract of *A. augusta*, **Group-9:** Diabetic animal+Aqueous extract of *A. augusta*.

***Collection of Blood Sample and* Evaluation of Antioxidant Activity**

Blood sample was withdrawn on 7th day of the study from the retro-orbital plexus. After collecting the blood sample, above mentioned assay methods were used for evaluation of antioxidant activity of various extract of *A. augusta*.

Statistical Analysis: All the results were expressed as the mean ± Standard error mean (SEM). Data was analyzed by using two way ANOVA followed by tukey's multiple comparison as post-hoc test. The limit of statistical significance was set at $P<0.05$.

Results and Discussion:

Effect of *A. augusta* in Diabetes Induced Oxidative Stress: In the present investigation various extracts of *A. augusta* were screened for antioxidant activity. *In-vivo* antioxidant activity was assessed by measuring SOD, CAT and LPO in the blood of diabetic animals **(Table-1).**

Table 1: Effect of *A. augusta* on Oxidative Stress in Diabetic Rats

S. No.	Groups	Superoxide Dismutase (Units/mg protein)	Catalase (Units/mg protein)	Lipid Peroxidation (nmMDA/g Hb)
1	Normal	47.26± 7.56	297.20±6.56	84.00±4.34
2	Dia. Control	16.19± 4.35^{a*}	189.50±4.67^{a*}	189.5±5.89^{a*}
3	Dia. control + Vit. E	45.97± 2.14^{b*}	301.0±1.98^{b*}	96.05±2.89^{a‡b*}
4	Dia. control + Pet.ether extract	37.01 ±3.56^{a‡b*}	201.26±5.56$^{a* b*}$	134.53±7.35^{a*b*c*}
5	Dia. control +Benzene extract	18.13± 3.67^{a*c*}	161.17±5.70^{a*c*}	189.82±4.79^{a*c*}
6	Dia. control + Chloroform extract	17.41 ± 4.98^{a*c*}	188.45±3.44^{a*c*}	185.41±3.68 ^{a*c*}
7	Dia. control + Acetone extract	19.14±9.12^{a*c*}	188.22±7.27^{a*c*}	182.36±9.45^{a*c*}
8	Dia.control + Ethanol extract	21.27±9.67^{a*c*}	187.47±2.45^{a*c*}	186.91±4.75^{a*c*}
9	Dia. control + Aqueous extract	29.80± 3.76$^{a*b\#c*}$	190.41±2.87^{a*b†c*}	174.74±7.56^{a*b†c*}

a= Vs Normal control, b=Vs. Diabetic control, c=Vs. Active control; *= p<0.0001, ≠ = p< 0.001, †= p< 0.01, ‡= p<0 .05

Effect on SOD and CAT level

SOD and CAT are two major antioxidant defense system of the body which protect the cell membrane and other cellular constituents against oxidative damage by free radical species and plays an important role in protecting the cell against the potentially deleterious effect of reactive oxygen species. In diabetic animals, serum concentration of SOD and CAT reduces, which may results in number of deleterious effects due to accumulation of superoxide radicals (O^{2-}) and hydrogen peroxide. SOD and CAT level came close to normal level in animals treated with petroleum ether extract and aqueous extract of *A. augusta* in diabetic animals significantly as compared to diabetic control. The positive effect of extracts on antioxidant enzyme level clearly indicates the free radical scavenging activity of *A. augusta*, which could exert a beneficial effect against pathological changes caused by free radicals.

Effect on LPO: Increased free radical generation induces Lipid Peroxidation, refers to oxidative degeneration of lipids that impairs cell membrane functions resulting into cell damage and leading to

severe diseases. The most significant reduction on LPO was observed with petroleum ether extract followed by aqueous extract of *A. augusta* as shown in Table-1 suggests the antioxidant activity of plant extract which prevents from oxidative stress and provide protection to vital tissues like liver, kidney, heart etc.

Conclusion:

The results of the present study concluded that the petroleum ether extract of *A. augusta* possesses potent antioxidant and lipid peroxidation activity and can be employed in protecting tissue from the oxidative stress, which may be responsible for its hypoglycemic property. Further studies to isolate the active components of *A. augusta* are needed to explore the research on abovementioned plant usage in different oxidative stress induced diseases.

References:

[1] Dallak M & Bin-Jaliah I (2010) Pak J Physiol 6:1-5.

[2] Maritim AC, Sanders RA & Watkins JB (2003) J Biochem ***Mol*** Toxicol 17:24-38.

[3] Khan T, Ahmad W, Bashir S, Iqbal Z, Ahmad B & Ahmad M (2003) Pak J Biol Sci 6:1142-1144.

[4] Bhuiya AM (2013) The Experiment 14:888-893.

[5] Harborne JB (1984) Phytochemical method-a guide to modern techniques of plant analysis. 2nd ed. New York: Chapman and Hall.

PD-106

Evaluation of Antidiarrheal Activity of Methanolic Extract of *Daemia extensa* R. Br. Seeds

Praveen Sharma, Sachin Kumar Jain, Neelam Balekar
IPS Academy College of Pharmacy, Rajendra Nagar, AB Road, Indore, MP, India, 452012
E-mail address: praveensharmacop@gmail.com

Abstract: Diarrhea is one of the most general causes for thousands of deaths each year. Consequently, identification of new source of antidiarrheal drugs becomes one of the most prominent focuses in modern research. Our aim was to investigate the antidiarrheal activity of methanolic extract of *Daemia extensa* R. Br. (MEDE) leaves in rats. Antidiarrheal effect was evaluated by using castor oil-induced diarrhea at 200 mg/kg and 400 mg/kg body weight in rats where the extract showed considerable antidiarrheal effect by inhibiting 49.47% and 62.85% of diarrheal period at the doses of 200 and 400mg/kg, correspondingly These observed effects are comparable to that of standard drug loperamide (5mg/kg). So these results indicate that bioactive compounds are present in methanolic extract of *Daemia extensa* leaves including significant anti-diarrheal activity and could be accounted for pharmacological effects.

Introduction:

Due to unhygienic livelihood situation, peoples of the third world counties are very prone to numerous common diseases including diarrhea. According to the World Health Organization (WHO), diarrhea is the second foremost reason of death of children less than five years of age [1]. During diarrhea, the normal bowel association becomes changed, which results in an increase in water content, volume, or frequency of the stools [2]. Despite the efforts of international organizations to control this disease, still the incidence of diarrhea is very high [3, 4]. Some antibiotics are used as antidiarrheal drug, but these drugs sometimes show some adverse effects and microorganisms are tend to enlarge resistance towards them [5]. Consequently the investigation for safe and more successful agents from plant origin has continuous to be an important area of active research. Many plant species have been screened for substances with therapeutic activity. For the treatment of diarrhea, medicinal plants are a potential source of antidiarrheal drugs.

Materials and Methods:

Collection of plant materials

Fresh seeds of *Daemia extensa* were collected locally from the Indore district of Madhya Pradesh and identified by Department of Botany, Government Degree College Indore and submitted to department. The seeds were shade dried and were crush to moderately coarse powder.

Preparation of extract

The freshly collected seed were dried under shade, sliced into small pieces, pulverized using a mechanical grinder and passed through 40 mach sieve, and preserved in air tight container for further use. The powdered seed were extracted with 95% methanol. After exhaustive extraction, the extract was concentrated by distillation procedure. A brownish black colored residue was obtained (yield 19.8% w/w), which was kept in a desiccators. This methanolic extract of *Daemia extensa* seed (DES) was used in further experiments.

Experimental Animals

Albino Wistar rats of both sex weighing between 150-250 g were used. The experimental etiquette was permitted from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were specified diet and water *ad libitum.*

Acute toxicity studies

Acute toxicity was carried out as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Organization for Economic Cooperation and Development (OECD). Group of three rats weighing between 22-30 g were selected and kept for 3-4 hrs fasting with free access to water. Doses were calculated according to body weight and seed extracts were dissolved in rice bran oil and administered orally at a starting dose of 2000 mg/kg and were observed for 24 hours.

Castor Oil-Induced Diarrhea in Rats

Rats of both sexes (95–100 g) were fasted for 18 hours. The selected rats for castor oil-induced diarrheal test were separated into four groups (= 5). Group I was given normal saline (2 mL/kg) orally as control group and Group II received loperamide (5 mg/kg) as standard group. Groups III-IV received MEDE (200 and 400 mg/kg b. wt. i.p.). After 1 h, all groups received castor oil 1mL each orally. Then they were positioned in cages lined with adsorbent papers and pragmatic for 4h for the presence of characteristic diarrheal compost. The 100% was measured as the total number of feces of control group. The activity was articulated as % inhibition of diarrhea. The percent (%) inhibition of defecation was measured.

Gastrointestinal Motility Test

This test was done according to the method of Mascolo et al. and Rahman *et al.* For this test, selected rats were divided into four groups of six rats in each. At first, 1mL castor oil was given orally in every rat of each group to produce diarrhea. After 1 h, Group I (control group) received saline (2 ml/kg) orally. Group II received standard drug (loperamide 5mg/kg b. wt. i.p) and Groups III-IV (the rest of the two groups) received MEDE (200 and 400mg/kg b. wt. i.p. resp.). After 1 h, all animals received 1mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour after following the charcoal meal administration, all animals were sacrificed and the distance covered by the charcoal

meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of distance moved.

Statistical Analysis

The data are characterized as mean ± S.E.M, and statistical significance was carried out retaining one way analysis of variance (ANOVA) followed by Dunnett t-test where $p<0.05$ was measured statistically momentous using Graph pad 5 software.

Results and Discussion:

Castor Oil-Induced Diarrhea

In case of castor oil induced diarrheal test, the methanol extract of *Daemia extensa* showed a marked antidiarrheal effect in the rats (Table 1). In both doses, 200 mg/kg and 400 mg/kg, extract produced momentous ($p < 0.01$) defecation. The seed extract doses of 200 mg/kg and 400 mg/kg decrease the total amount of wet feces produced upon administration of castor oil (6.33 ± 0.93 and 5.79 ± 0.52 g) at doses 200 mg/kg and 400 mg/kg compared to the control group (5.00 ± 0.33 g) at the dose of 5 mg/kg.

Table 1: Anti diarrheal activity of ethanolic extract of *Daemia extensa* on castor oil- induced Diarrhea in rats

Treatment	**Dose**	**Total number of feces**	**% inhibition of defecation**	**Total number of diarrheal feces**	**% inhibition of diarrhea**
Castor oil + saline	2ml/kgp.o.	19.07±3.14	--	10.5±0.34	1.7±0.08
Castor oil + Loperamide	5 mg/kg i.p	8.05±0.74[a]	60.60	4.98±0.12	4.8±0.07
Castor oil + seed extract	200 mg/kg i.p	12.56±1.25 [a]	40.95	7.98±0.16	49.67±0.15
Castor oil + seed extract	400 mg/kg i.p	9.68±0.76 [a]	52.04	5.05±0.18	62.85±0.08

Mean ± SEM (n = 6). Significant at [a] $p<0.01$ compared to control group.

Gastrointestinal Motility Test

The methanolic extract of *Daemia extensa* lessened gastrointestinal distance (101 ± 2.82 cm to 57.2 ± 1.41 cm) traveled by the charcoal meal in the rats noticeably compared with the control group. Loperamide (5mg/kg) produced a marked (46.53%) decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 2).

Table 2: Effect of MEDE leaves on small intestinal transition in rats

Group	Treatment	Total length of intestine	Distance travel by marker	% Inhibition of gut motility
I	Castor oil + Saline (2 mL/kg p.o)	107.8 ± 2.36	100 ± 2.82	50.56
II	Castor oil + Loperamide (5mg/kg i.p)	101.28 ± 1.66	42 ± 0.17 [a]	45.53
II	Castor oil + leaves extract (200mg/kg i.p)	100.18 ± 3.08 [a]	63.6 ± 1.11[a]	32.00
IV	Castor oil + Leaves extract (400mg/kg i.p)	91.7 ± 2.81[a]	54.2 ± 1.21[a]	41.36

Values were expressed as mean ± SEM. (n = 6). [a]$p < 0.01$ when compared with control group

Conclusion:

The conclusion of the present study offer persuasive evidence that methanolic extract of *Daemia extensa* (MEDE) seeds possesses significant antidiarrheal activity. Antidiarrheal effect is rapid, long lasting, and statistically important at both 200 and 400 mg/kg doses. Nevertheless, additional chemical and pharmacological studies are requisite to isolate the bioactive compounds and explicate the specific mechanisms responsible for the observed pharmacological activities of this plant.

References:

[1] Andrade SFD, Lemos M, Comunello E, Noldin VF, Filho VC & Niero R (2007) J Ethnopharmacol 113:252-257.

[2] Begum N, Mayuren C, Balaji N, Chinnapa RY & Aravind K (2008). Adv Pharmacol Toxicol 9(3): 33-36.

[3] Malairajan P, Gopala Krishnan G, Narasimhan S & Jessika-laveni K (2008). Indian J Pharmacol 40:126-128.

[4] Boyd WA (1970). Text Book of Pathology Structure and Function in Disease. (2nded.). United Kingdom: Lea & Febiger.

[5] Jude EO & Paul A (2009 J Pharm Sci 22: 384-390.

PD-111

Wound Healing potential of Grandiflorenic Acid Isolated from *Wedelia trilobata* Linn.

Aayushi Kavachale, Taru Shrivastava, Neelam Balekar
IPS Academy College of Pharmacy, Indore (M.P.) 452012
E-mail address: neelambalekar@gmail.com

Abstract: The ethyl acetate fraction from ethanolic extract of *W. trilobata* leaves displayed antibacterial and fibroblast stimulatory activities thereby suggesting potential wound healing properties. Ethyl acetate fraction was further subjected to bioassay guided fractionation, which afforded isolation of grandiflorenic acid (GA). GA exhibited potential *in vitro* wound healing activity due to fibroblast stimulation and inhibiting inflammatory phase of wound healing, evident by reduced levels of inflammatory cytokines from macrophage RAW 264.7 cells. The aim of the present study was to evaluate wound healing activity of GA formulated in ointment base (0.5% and 1.0% w/w) using excision, incision and dead space wound models in experimental rats. Treatment of wound with isolated grandiflorenic acid 1.0% w/w topically exhibited significant ($p<0.01$) wound healing activity in all three models as compared to control groups. High rate of wound contraction, decrease in period of epithelialisation, high tensile strength, increase in dry granulation tissue weight.

Introduction:

Wound care is often complex, frequently time-consuming, sometimes confusing and nearly always expensive. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered. Medicinal plants have been used since immemorial time for the treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. This revival of interest in plant derived drugs is mainly due to the current widespread belief that “green medicine” is safe, and clinically effective, better tolerated by patients, less expensive and globally competitive.

Wedelia is seen at its best in tropical climate, where heat and humidity combine to help it produce great sheet of foliage starred with golden daisy flowers. *Wedelia trilobata* contains diterpene (kaurenoic acid), eudesmanolide lactones and luteolin (in leaves and stems). Kaurenoic acid has antibacterial, larvicidal and trypanocidal activity; it is also a potent stimulator of uterine contractions[1]. The ethyl acetate fraction from ethanolic extract of *W. trilobata* leaves displayed antibacterial and fibroblast stimulatory activities thereby suggesting potential wound healing properties [2]. The ethyl acetate fraction was further subjected to bioassay guided fractionation which afforded isolation of GA [3]. GA exhibited potential *in vitro* wound healing activity due to combination of fibroblast stimulation and inhibiting prolonging inflammatory phase of wound healing evident by reduced levels of inflammatory cytokines from macrophage RAW

264.7 cells [4]. The aim of the present study was to evaluate wound healing activity of GA formulated in ointment base (0.5% and 1.0% w/w) using excision, incision and dead space wound models in experimental rats.

Material and Methods:

GA was isolated from leaves of *W. trilobata* in the previous studies [2]. The ointment of grandiflorenic acid (0.5 and 1% w/w) was prepared (Table 1) and stored in airtight container in 4˚C.

Table 1: Formulation of grandiflorenic acid in excision wound model

S.No.	Ingredient	Percent (w/w)	Percent (w/w)
1.	Mineral oil	10	10
2.	White petrolatum	30	30
3.	Glyceryl monosterate	10	10
4.	Cetyl alcohol	5	5
5.	Glycerin	5	5
6.	Potassium sorbate	0.1	0.1
7.	Grandiflorenic acid	0.5	1
8.	Purified water	39.4	38.9

Evaluation of Grandiflorenic acid ointment

Physical parameters of cream formulation such as pH, consistency, spreadability and extrudability were determined. Stability studies were carried out following ICH guidelines, to ensure that formulation is stable during different storage conditions.

Animal grouping and treatment

The Wistar rats of either sex weighing 150-200 g were used. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). Wistar rats were divided into four groups consisting of six animals. Group I: Negative control (ointment without GA); Group II: Positive control (povidone iodine ointment); Group III: GA Ointment (0.5%); Group IV: GA Ointment (1%)

Excision wound

A full thickness of the excision wound of circular area (approximately 600 mm^2) was made on the shaved back of the anesthetized rats. The treatment was started as per given treatment schedule. The wounds were monitored and the area of wound was measured on 0, 3, 6, 9, 12 and 15^{th} post-wounding days. The period of epithelialization was calculated as the number of days required for falling of the dead tissue without any residual raw wound.

Incision wound

An incision wound of about 6 cm in length and about 2 mm in depth were made with sterile scalpel on the shaved back of the anesthetized rats. The parted skin was stitched with sterilized needle at 0.5 cm intervals. The wounds of animals in the different groups were treated as per treatment schedule for the period of 10 days. When wounds were cured thoroughly, the sutures were removed on the post-wounding day and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 11th day. Thereafter, the animals were euthanized and the tissues were processed for histopathological examination.

Dead space wound model

Dead space wounds were created by subcutaneous implantation of sterilized cotton piths (10 mg) on the right side groin and axilla on the ventral surface of each rat. The granulation tissues formed on the cotton piths were excised carefully on the 10th post wounding day under light ether anesthesia. The tissue was dried overnight at 60°C and the dried granulation tissue weight was recorded on the 11th day. The granulation tissue so harvested was subjected to hydroxyproline estimation.

Statistical analysis

Data are expressed as a mean ± s.d. Statistical evaluation was carried out using one-way ANOVA followed by Tukey's test. The values of $p < 0.05$ were considered to be statistically significant.

Excision wound study

The wound healing contracting ability of animals treated with GA 0.5% and 1.0% topically was found to be significantly higher ($p < 0.05$) on day 12 and 15 as compared to the control (Table 2). The epithelialization period (complete healing) was also found to be 22.3 ± 1.2 and 20.3 ± 0.9 days in case of animals treated with GA topically, 0.5% and 1.0% respectively.

Table 2: Effect of grandiflorenic acid in excision wound model

Treatment & Doses	**Percentage wound contraction (mean ± SEM)**					**Period of epithelization (days)**
	Day 3	**Day 6**	**Day 9**	**Day 12**	**Day 15**	
NC	25.2 ±1.6	24.0±1.5	21.7±1.2	19.3±0.9	16.2±1.0	26.2±1.8
PC (Povidone iodine)	22.5 ± 0.8	18.8±0.9	13.8±0.7	9.0 ±0.5[a]	5.2 ±0.6[a]	19.0±1.7[a]
GA (0.5% w/w)	24.2 ±1.2	21.8±1.0	19.0±0.9	15.3±0.8	10.3±0.9[a]	22.3±1.2[a]
GA (1% w/w)	23.2 ±1.0	19.8±0.9	16.2±0.9	10.7±0.7[a]	5.3±0.6[a]	20.3 ±0.9[a]

NC: Negative control; PC: Positive control, Values are mean ± s.d.,(n=6) . Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. [a]p<0.05 with negative control

Incision wound study

GA 1.0% topical, significantly increased ($p<0.05$) the tensile strength on 10^{th} post wounding day (501 ± 3.4 g respectively when compared to control (245.5 ± 2.9 g) (Table 3).

Dead space wound study

The groups treated with GA 1.0% w/w topical, significantly increased weight of granuloma by 67.4 ± 0.9 mg/100g, respectively compared to control 30.1 ± 0.9 mg/100g (Table 3).

Table 3 Effect of grandiflorenic acid in incision and dead space wound model

Treatment	Incision wound	Dead space wound	
	Tensile strength on 10^{th} day (g)	**Granuloma wt. (mg/100 g)**	**Hydroxyproline content (µg/mL)**
NC	245.5 ± 2.9	30.1 ± 0.9	1.5 ± 0.1
PC (Povidone-iodine)	612.5 ± 4.1[a]	74.5 ± 0.8[a]	5.1 ± 0.2[a]
GA (0.5% w/w)	463.9 ± 3.2[a]	54.5 ± 1.1[a]	2.9 ± 0.2[a]
GA (1.0% w/w/)	501 ± 3.4[a]	67.4 ± 0.9[a]	4.3 ± 0.2[a]

Values are mean ± s.d., (n=6). Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.

[a]$p<0.05$ with negative control

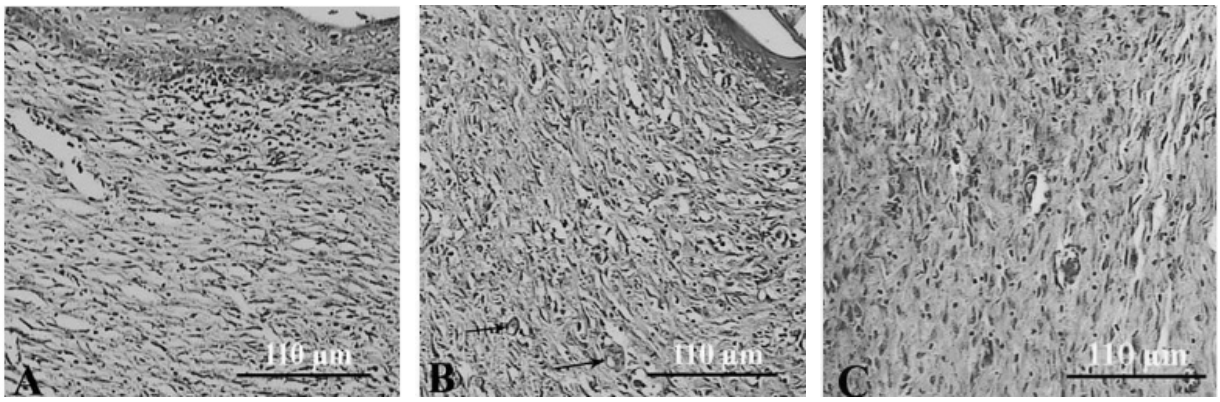


Figure 1: Histological examination (H & E stains, 40X) of the healed wound section of the

A Negative Control: Granulation tissue contains less collagen, fibroblasts, and blood capillaries and more inflammatory cells.

B Positive Control: Granulation tissue contains lower number of lymphocytes and macrophages. Collagen fibers are organized and the tissue is aligned.

C GA ointment (1%): Granulation tissue contains more collagen and fibroblasts with absence of inflammatory cells.

Discussion and Conclusion:

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of wound. It depends upon the repetitive abilities of tissue, type and extent of damage and general state of health of tissues (Jain et al., 2006). The undifferentiated mesenchymal cells of the wound margin modulate themselves into

fibroblasts, which start migrating into the wound gap along with the fibrin stands. The collagen composed of amino acid (hydroxyproline) is the major component of extracellular tissue, which gives strength and supports. Breakdown of collagen liberates free hydroxyproline could be used as an index for collagen turnover. Hence, in this study the model were used to access effect of grandiflorenic acid. The result of present study showed that grandiflorenic acid possesses a definite pro-healing action. In excision wound model ointment of leaves of grandiflorenic acid 1 % w/w topically showed better percentage wound closure effect against control and other treated groups on 15th day by enhanced epithelialization.

References

[1] Li SF, Ding JY, Li YT, Hao XJ, Li SL (2016) Molecules 21: 457.

[2] Balekar N, Katkam NG, Nakpheng T, Jehtae K, & Srichana T (2012) J Ethnopharmacol 141:817-824.

[3] Balekar N, Nakpheng T, Katkam NG, & Srichana T (2012) Phytomedicine 19:1178-1184.

[4] Balekar N, Jain DK, & Upadhyay RK (2013). Thai J Pharm Sci 37:125-133.

PD-112

Wound Repair and Regenerating Effect of Eugenol Isolated from Ethyl Acetate Soluble Fraction of Ethanolic Extract of *Cinnamomum tamala* Leaves in STZ Diabetic Rats

Rupesh Soni[1], D.N. Srivastava[2]
[1]DDM College of Pharmacy, Gondpur Banehra (Upper)-177213, Tehsil Amb, Distt. Una (H.P.)
[2]B. R. Nahata College of Pharmacy, Mandsaur-458001 (M.P.)
E-mail address: rupeshsoni77@gmail.com

Abstract: Diabetes is a chronic hyperglycaemic disorder; leads to developed several complications including delayed wound healing after any injury. These non-healing wound ends up to organ or limb salvage. The available modern medications are not capable to fully control over these complications. There are several evidences that these complications can easily treated by using herbal of folklore medicines. The leaves of *Cinnamomum tamala* used by traditional peoples in the treatment of diabetes and associated wound healing. In our previous study we had found that the ethanolic extract of leaves of *Cinnamomum tamala* is most active in treatment of wound healing in diabetic rats. The aim of our study was to find the active isolated eugenol from ethyl acetate soluble fraction of ethanolic extract of *C. tamala* leaves responsible for wound healing activity in diabetic rats. The Wistar albino rats were made diabetic by single i.p. injection of Streptozotocin (60 mg/kg). The excision, incision and dead space wound were created on back side of rats. The eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* was applied topically in excision wound model while in incision and dead space wound model the eugenol isolated from ethyl acetate soluble fraction (100 mg/kg) was give orally for 16 days. In the excision wound model the wound area and day of epithelisation both were significantly decreased eugenol isolated from ethyl acetate soluble fraction treated rats. In incision wound model the significantly higher tensile strength was observed in rats treaded orally with eugenol from ethyl acetate soluble fraction.There were significant increase in weight of wet & dry granulation tissue with increased amount of hydroxyproline, collagen and elastin was observed in eugenol treated rats by ethyl acetate soluble fraction. The results suggested that the eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* can be beneficial in treatment of wound healing in diabetic rats.

Introduction:

Modern world is facing a critical health problem that is diabetes. The number of patients with diabetes and its complications increasing day by day and reached up to 220 million in this year [1]. Diabetes is a group of disorders characterized by hyperglycaemia resulting due to abnormalities in glucose metabolism. Diabetes is associated with glycation of essential proteins and hormones, due to presence of high blood sugar level. In diabetic patient decrease in collagen content of skin can generates impaired and non healing abnormalities in wound or injured area [3]. Diabetic wounds are slow, non-healing wound that can persist for weeks despite adequate and appropriate care. Such wounds are

difficult and tough to manage. The wound healing process is the sequence of repaiment of connective tissue including migration, inflammation, proliferation and differenciation of cells [4]. As per WHO the effective treatment of diabetes and its complications can be possible by using herbal or traditional medicines [5]. In our previous study we had found that the ethanolic extract of *Cinnamomum tamala* leaves has beneficial effect in healing of wounds in diabetic rats. The Ethyl acetate fraction of *Cinnamomum tamala* leaves showed presence of tannins and phenolic compounds which are having potent antioxidant activity. The oxidative stress is responsible for induction of diabetic complication. Hence in present study eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves was used to investigate wound repair and regeneration activity in diabetic rats.

Materials and Methods:

The eugenol is isolated as active constituent from ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala.* Wistar albino rats of either sex weighed between 120-150 gm were used for the wound healing activity. Induction of Diabetes: Rats were made diabetic by a single injection of Streptozotocin (60 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5) after overnight fasting. Blood was drawn from the tail vein 24 h after the injection and the glucose level was estimated by glucose oxidase method by using Accu-Chek Glucometer before and 72 hrs after STZ injection. Animals showed blood glucose level more than 250 mg/dl were selected for further cutaneous wound healing activity in diabetic animals. The eugenol isolated from ethyl acetate fraction of ethanolic extract (10 % w/w) of the dried leaves of *Cinnamomum tamala* well triturated in pastel mortar with steric acid ointment base and used further in excision cutaneous wound healing model in diabetic rats.

Excision wounds sized 300 mm^2 and 2 mm^2 depth were made by cutting out piece of skin from the shaven area. Wound areas were measured on days 0, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker. Recording of wound areas were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation.

Excision wound model:

Group I (NC): Normal Control; Normal rats topically treated with Plane steric acid ointment.

Group II (DC): Diabetic Control; Diabetic rats topically treated with Plane steric acid ointment.

Group III (DT): Diabetes Treated; Diabetic rats topically treated with ointment of eugenol of ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* (100 mg/kg).

A longitudinal paravertebral incision of six centimeters in length was made through the skin and cutaneous muscle on the back in anesthetized rats. After the incision, surgical sutures were applied at intervals of one centimeter. The wounds were left undressed (day 0). The sutures were removed on

the 8th post wound day and the application of extract was continued. The skin-breaking strength was measured on the 11th day by tensiometer.

Incision wound model:

Group I (NC): Normal Control; Normal rats treated with plane vehicle of 0.5 % w/v sodium CMCorally.

Group II (DC): Diabetic Control; Diabetic rats treated with vehicle of 0.5 % w/v sodium CMC orally.

Group III (DT): Diabetes Treated; Diabetic rats treated with 100 mg/kg of eugenol of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* suspended in 0.5 % w/v sodium CMCsuspension orally.

Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on left side in the groin and axilla on the ventral surface of each rat. On the 11^{th} post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the weight of the granulation tissue, the tissue was dried at 60ºC for 12 hr, and the dry granulation tissue weight was recorded. This dried tissue was further used to estimate hydroxyproline, collagenand elastinlevel in skin of normal and diabetic rats.

Dead space wound model:

Group I (NC): Normal Control; Normal rats treated with plane vehicle of 0.5 % w/v sodium CMC orally.

Group II (DC): Diabetic Control; Diabetic rats treated with vehicle of 0.5 % w/v sodium CMC orally.

Group III (CtPii-EAC): Diabetic rats treated with 100 mg/kg of eugenol of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* suspended in 0.5 % w/v sodium CMC suspension orally.

Biochemical analysis: At the end of experiments the wound area, % wound closure and day of epithelisation was recorded in excision wound model. In incision wound model the tensile strength was measured. In dead space wound model the weight of wet & dry granulation tissue, amount of hydroxy- proline, collagen and elastin were measured.

Statistical analysis: The data were expressed in Mean±SEM and statistically analyzed by oneway analysis of variance followed by dunnett's test. $P<0.05$ considered as significant.

Results and Discussion:

There was significant increase in wound healing parameters during treatment with eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* as compared to control groups of normal and diabetic rats. Effect on wound parameters of excision and incision wound model as shown in Table 1, the effect of eugenol of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves on wound area; % wound closure and day of epithelialization in excision wound model and tensile strength & blood glucose level in incision wound model in diabetic rats. The eugenol of ethyl acetate fraction treated rats showed significant

increase in % wound closure and decrease in wound area on 16th day of treatment. The day of scar falling i.e. epithelisation was decreased with decrease in blood glucose level. In incision wound model the tensile strength of euegnol of ethyl acetate fraction treated rats was found increased along with decrease in blood glucose level with comparison to diabetic control rats.

Effect on wound parameters of excision and incision wound model: As shown in Table 2, the effect of eugenol of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves on wet & dry weight of granulation tissue, amount of hydroxyproline, collagen and elastin. In dead space wound model the weight of wet & dry granulation tissue was significantly increased with significant increase in level of hydroxyproline, % collagen and % elastin in the eugenol of ethyl acetate fraction treated rats with comparison to diabetic control rats.

In present study photochemical screening showed the presence of high amount of phenolics and tannin compounds in ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves. The Phenolics and tannins are the potent antioxidants reported in literature. Sharma et al, and Kar et al reported that ethanolic extract of *Cinnamomum tamala* leaves exhibits antihyperglycemic activity The high blood glucose level is responsible for delayed wound healing and eugenol isolated from ethyl acetate fraction treated rats showed significant decrease in blood glucose level during wound healing process.

Table 1: Effect of eugenol isolated from ethyl acetate fraction of ethanolic extract of Cinnamomum *tamala* treatment in excision and incision wound model.

S. No.	Groups	Wound Area (mm^2)	% Wound Closure	Day of Epithelisation	Tensile Strength (gm/mm^2)	Blood Glucose Level (mg/dl)
1.	Normal Control (NC)	54.83±0.94	82.23±0.38	24.67±1.05	233.0±3.92	74.00±3.04
2.	Diabetic Control (DC)	126.2±1.86*	59.32±0.62*	44.00±1.41*	157.8±2.11*	402.3±8.87*
3.	Diabetic Treated (DT)	5.55±0.47*	98.21±0.14*	10.83±0.40*	335.0±1.76*	70.33±2.95*

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and diabetic treated groups were compared with DC. P value considered as $P<0.001$ Significant (*)

Table 2: Effect of eugenol isolated from ethyl acetate fraction of ethanolic extract of *Cinnaomum tamala* treatment in dead space wound model.

S. No.	Groups	Wet Granulation Tissue Wt. (mg)	Dry Granulation Tissue Wt. (mg)	Hydroxyproline (µg/ml)	% Collagen	% Elastin
1.	Normal Control (NC)	223.5±3.45	54.33±1.22	6.00±0.25	44.81±1.92	260.7±1.12
2.	Diabetic Control (DC)	223.5±3.45	35.50±1.23*	3.63±0.12*	27.10±0.89*	157.7±5.21*
3.	Diabetic Treated (DT)	223.5±3.45	124.5±2.39*	9.35±0.18*	69.76±1.39*	405.9±8.09*

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and diabetic treated groups were compared with DC. P value considered as P<0.001 Significant (*)

Conclusion:

The isolated eugenol from ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves was evaluated for wound healing activity in diabetic rats. The all four phases (hemostasis, inflammation, granulation and remodelling) of wound healing studied by excision, incision and dead space wound models. The high blood glucose level is the root cause of delayed wound healing in patients of diabetes. The treatment of eugenol isolated from ethyl acetate soluble fraction promotes wound healing by decrease in blood glucose level, faster contraction of wound and increased granulation of tissue with increased tensile strength. This action may be due to anti diabetic, antioxidant and antimicrobial activities of phytoconstituents like phenolics and tannins which present in ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves. Further studies are needed to identify active faster wound healing activity with detailed mechanism of action.

References:

[1] Goodson WH & Hunt TK (1979) Surg Gynecol Obstet 149:600.

[2] Nayak BS, Anderson M & Periara LM (2007) Fitoterapia 78:540-544.

[3] Nayak BS, Pereira L & Muhraj D (2007) Indian J Exp Biol 45:739-743.

[4] Prasad V, Jain V, Girish D & Dorle AK (2006) J Herb Pharmacother 6(3-4):105-15.

[5] Reddy KG & Enwemeka SC (1996) Clin Biochem 29:225-229.

PD-113

Consumer Adverse Drug Reactions (ADRs) Reporting in Malaysia: A Retrospective Analysis of Spontaneous Reports from the National Pharmacovigilance Database from 2008 to 2015

Vidhya Hariraj, Zoriah Aziz
Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
E-mail address: zoriah@um.edu.my

Abstract: Many countries are incorporating direct patient reporting of adverse drug reactions (ADRs) into their pharmacovigilance systems as patients provide a different insight into drug safety compared to healthcare professionals. In Malaysia, consumer reporting of ADRs and issues with product qualitiesbegan in 2007.The aim of this study was to examine consumer reports in terms of ADR categoriesby System Organ Class (SOC), suspected products and the seriousness of the ADRs.

Methods:

The Malaysian Pharmacovigilance database was retrospectively searched from 2008 to 2015 to identify consumer reports. We excluded reports of adverse events following immunisation, and descriptively analysed eligible reports using SPSS version 20. Chi-squared test with significance level of$p < 0.05$ was used to evaluate the association of various categorical variables with serious ADRs.

Findings:

Out of the 101,957 ADR reports available in the National ADR database for the period 2008 to 2015, only 81 (0.08%) reports were by consumers. The majority (64%) of the consumer reports used the consumer reporting form while a small proportion used ADRs form designed for healthcare professionals. Almost half of these 81 reports involved complementary and alternative medicines (CAM)while other major classes of products involved were prescription medicines (19%) and cosmetics (11%). Of the total ADRs reports, the three main SOC involved were skin and appendages disorders (26%), body as a whole (25%), and central and peripheral nervous system disorders (9%). The Malaysian Pharmacovigilance Centre obtained and tested 45samples, of which 19(42%) were CAMs products found to be adulterated with prescription drugs while 4 cosmetic productsexceeded the permitted limit for hydroquinone or mercury.More than half of the reports involved unregistered products or unapproved cosmetics. Two factors found to be significantly associated with serious ADRs were status of product registration and presence of product adulteration.

Conclusion:

Our preliminary findings show that consumers have the potential to provide valuable feedbackparticularly for serious ADR reports. The National Centre should allocate specific resources for the assessment of consumer reports to strengthen pharmacovigilance.

Review Articles

PA-16

Automated Robotic Dispensing System-A Review

Nimish Ajmera, Kuldeep Vinchurkar, Neelam Balekar
IPS Academy College of Pharmacy, Indore-452012 (M.P.) India
E-mail address: nimishajmera9@gmail.com

Abstract: Pharmaceutical automation involves the mechanical processes of handling and distributing medications. Technology improvements soon resulted in a more compact and steady model.The old way of pharmacist dispensing prescriptions was in tablet or capsule form with a simple tray and spatula. Pharmacy automation has the potential to revolutionizepharmacy dispensing and stock management processes,achieve increased safety and quality in medicine use,and reduce cost, Many other technical automation systems were used like automated dispensing cabinet (ADC), were computerized system is mainly done for controlling and tracking of drug dispensing. They also are called unit-based cabinets (UBCs), automated dispensing devices (ADDs), automated distribution cabinets or automated dispensing machines (ADMs), or Robotic Process Automation (RPA) is an emerging form of clerical process automation technology based on the notion of software robots or artificial intelligence (AI).

Introduction:

Any pharmacy task may be involved, including counting small objects (e.g. Tablets, capsules); measuring and mixing powders and liquids for compounding; tracking and updating customer information in databases (e.g., personally identifiable information(PII), medical history, drug interaction risk detection); and inventory management. Automated dispensing systems offer sophisticated dispensing capabilities, more accurate placement, and greater consistency, while remaining simple and intuitive to program. Hospital pharmacies have traditionally provided medications for patients by filling patient-specific cassettes of unit-dose medications that were then delivered to the nursing unit and stored in medication cabinets or carts. ADCs, which are designed to replace non-automated floor stock storage, were introduced in hospitals in the 1980s and have facilitated the transition to alternative delivery models and more decentralized medication distribution systems. While no one method for medication distribution works for every facility, implementing automated dispensing cabinets as part of a decentralized or hybrid medication distribution system can improve patient safety and the accountability of the inventory, streamline certain billing processes, and ultimately, lead to increased nursing and patient satisfaction.

Furthermore, by restricting individual drugs – such as high-risk medications and controlled substances – to unique drawers within the cabinet, overall inventory management, patient safety, and medication security can be improved. Automated dispensing cabinets allow the pharmacy department to profile physician orders before they are dispensed.

Table 1: Sales volume breakdown of all pharmacies compared with "robotic dispensing machine" pharmacies.

Sales volume of all pharmacies compared with "*robotic dispensing machine*" pharmacies		
Sales volume (Net annual sales in Million euros)	Fraction of all pharmacies in Germany in this sales volume category	Fraction of pharmacies with ROWA robotic dispensing machine in the respective sales volume category (n=70)
<1.5	43.4%	11.4%
1.5-2.0	39.4%	27.1%
2.1-2.5	11.6%	24.3%
>2.5	5.6%	37.1%

Automated cabinets can also enable providers to record medication charges upon dispensing; reducing the billing paperwork pharmacy is responsible for. In addition, nurses can note returned medications using the cabinets' computers, enabling direct credits to patients' accounts. Since automated cabinets can be located on the nursing unit floor, nursing have speedier access to a patient's medications.

Role of automated dispensing in healthcare system:

Dispensing medications in a community pharmacy before the 1970s was a time-consuming operation. A typical community pharmacist was working longer hours and often forced to hire staff to handle increased workloads which resulted in less time to focus on safety issues. These additional factors led to use of a machine to count medications. Automated dispensing is a pharmacy practice in which a device dispenses medications and fills prescriptions. Automated dispensing cabinets, which can potentially handle hundreds of different medications, are available from a number of manufacturers. Though members of the pharmacy community have been utilizing automation technology since the 1980s, companies are constantly refining and improving ADCs to meet changing needs and health standards in the industry. Wrong drug and wrong dose errors are the most common errors associated with ADC use. Look-alike drug names and drug packages are common variables that lead to selection errors. For example, morphine and hydromorphone are two different opioid analgesics that frequently get confused.

The studies in the United States evaluated the impact of robots on the machine packaging of drugs in unit doses. Unit dose means the delivery of a single, packaged, clearly identifiable drug to a specific patient. This form of drug dispensing is receiving more attention from both the qualitative and the safety perspective.

Current state of the industry:

A tablet counter has become a standard in more than 30,000 sites in 35 countries (as of 2010) (including many non-pharmacy sites, such as manufacturing facilities that use a counting machine as a check for small items). During the 1990s through 2012, numerous new pharmacy automation products

came to market. During this timeframe, counting technologies, robotics, workflow management software, and interactive voice recognition (IVR) systems for retail (both chain and independent), outpatient, government, and closed-door pharmacies (mail order and central fill) were all introduced. Additionally, the concept of scalability - of migrating from an entry-level product to the next level of automation (e.g., counting technology to robotics) - was introduced and subsequently launched a new product line in 1997. Based on the counting technology employed in preceding models, later machines included the ability to help the pharmacy operate more effectively. Equipped with a new computer interface to a pharmacy management system, with workflow and inventory software.Various companies are currently developing a range of remote tablet counters, verification systems and pharmacy automation components to improve the accuracy, safety, speed and efficiency of medication dispensing.

Benefits to modern pharmaceutical dispensing:

Benefits of robotics to pharmacy Dispensing & to support the following:

1. Safety & quality

These analyses consistently show reductions inerror rates ranging from a modest 16% decreaseto a 65% decrease at hospital, London.A number of hospitals in the UK (which use asimilar dispensing model to Australia, i.e. full-pack dispensing) have shown that the use of roboticdispensing has enabled hospitals to releasepharmacists to clinical pharmacy activitiesbecause pharmacists spent less time in thedispensary.

2. Financial

The use of pharmacy robotics has led to areduction (20%-30%) in the amount and value ofinventory required to be held in pharmaciesresulting in:

• Savings in interest costs associated withholding inventory; and

• A reduction of stock holding in the order of2.25% of annual turnover.

• Shifting the skill mix towards lower paid staffwithout increasing error rates;

• Reducing overtime and the use of agency staffdespite increases in dispensing activity; and

• Dealing with increased dispensing activitywithout additional staff.

3. Process efficiencies

Implementation evaluations have observeddecreases in prescription turnaround time's andincreases of up to 20% in the number of itemsdispensed per hour. These efficiencies were achieved by stock being delivered directly to thestaff dispensing the medicine thereby reducingwalking and picking time.While the primary function of pharmacyrobots is individual patient dispensing, imprestorder picking and restocking can also be doneusing pharmacy robots. Dispensing solutions also have the ability to use QR codes to call up dispense programs for different parts. The feature makes these solutions extremely easy for operators to use, thus lowering the learning curve barrier for semi-automated equipment.

References:

[1] Lin A, Huang Y, Punches G & Chen Y (2007) Am J Health-Syst Pharm 64:1832–9.

[2] Franklin B, O'Grady K, Voncina L, Popoola J & Jacklin A (2008) Int J Pharm Pract 16:47–53.

[3] Pedersen C, Schneider P & Scheckelhoff D J (2006) Am J Health-Syst Pharm. 63:327–45.

PA-25

Herbal Cosmeceuticals in Hair Growth Promotion

Anindya Goswami[1], Neelesh Malviya[2]
[1]Department of Pharmacy, Mandsaur University, Mandsaur
[2]Smriti College of Pharmaceutical Education, Indore
E-mail address: anindya06@redifffmail.com

Abstract: Hairs are defending appendages on the body. They remain on one or other part of body from the time of birth to death. People who bear plentiful and lustrous hair are regarded as young and beautiful. Any provocative or disparaging disease of the skin on scalp may demolish hair follicles in its wake. Hair loss is a symptom that is exceptionally difficult to quantify and to evaluate. Very few synthetic drugs have been found to be fruitful in generating new hair in individuals that too with serious side effects. Thus it is very important to develop new therapeutic materials to stop hair loss and to enhance hair growth. Cosmeceuticals is one interesting area, which is getting more popular. Although it has not yet been incorporated into the mainstream of medical care because of limited scientific evidences and lack of mechanistic understanding, cosmeceuticals comprising of herbal constituent is becoming an increasingly attractive approach all over the world.

Introduction:

Hair is one of the external barometers of internal body conditions. It is an important part of human body. Hair is the receiver of an invariable series of environmental assaults termed as weathering. Such potentially destructive influences as rain, air pollutants, wind, sea water, chemicals contribute to the ecological process known to cause structural and chemical squalor to hairs. Hair loss is a universal problem; having affected both sexes of all races to diverse extents for as long as mankind has existed. Cosmeceuticals are the fastest-growing segment of the natural personal care industry. The use of cosmeceuticals has severely risen in recent years. This appreciably increases the armamentarium of the clinician in humanizing the treatment of skin, hair, and other conditions. Traditionally we have a rich source of botanicals which has been used for improving the conditions of hair or in hair re-growth. The goal of the present article is to provide an overview of the various herbal principles which can be used as a cosmeceutical for providing an alternate therapy for hair loss.

Hair and disorders of hair

There are approximately 5 million total body hair follicles, of which 100,000 to 150,000 are scalp follicles. In adults, 90% of the hair follicles are in the growing (anagen) stage and the remainder is in the resting stage (telogen) stage [1].

Hair growth is the increasing, bodily outcome of coordinated progression of cellular proliferation and differentiation within a hair follicle. The stem cells, which consign to the outcome of a hair follicle, come into a stage of immense propagation those consequences in the formation of mature hair follicle.

The main problems associated with hairs are pigmentation (fading), dandruff and falling of hairs (shedding) and balding. Alopecia is dermatological disarray that has been known for more than 2000 years and a frequent dilemma in cosmetics as well as most important health care practice. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors.

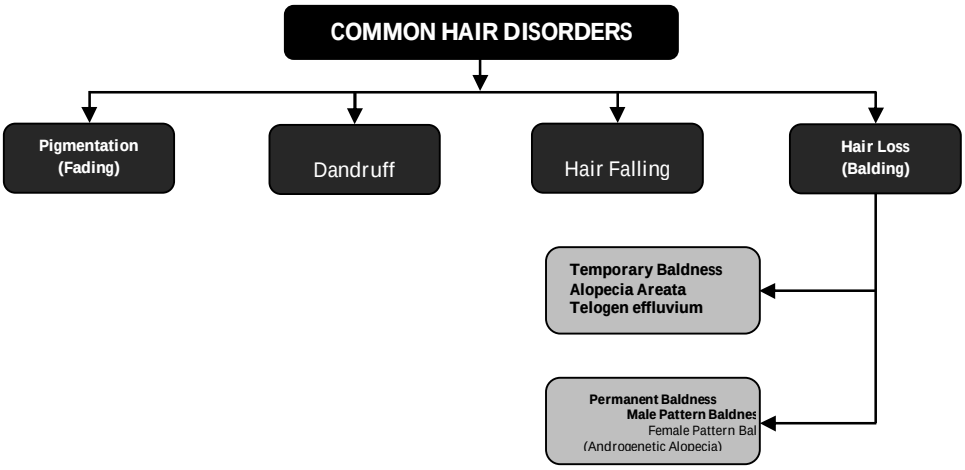


Figure 1: Common Hair Problems

Management of hair problems

Cosmeceuticals are becoming an increasingly attractive approach all over the world. A number of herbal components have been acclaimed with hair growth promoting activity and formulating them into suitable cosmetic formulation can be well received as far as the patient compliance is concerned. The usage of hair care cosmeceuticals has drastically hiked in recent years, which in turn has increased the spectrum of the concerns to broaden their range of products to enhance the comeliness of the patients associated with hair fall problems and alopecia in particular. For the cure of hair, cosmetics are applied topically to the scalp and hair.

A hair cosmeceuticals product comprises of conditioning agents, special care ingredients and hair growth stimulants.Conditioning agents are proposed to convey softness and gloss, to reduce distribute and to augment disentangling facility. These shampoos are designed around one or more specific ingredients particular for their clinical usefulness in these circumstances. Hence, current antidandruff ingredients are practically all-effective antifungal agents - zinc pyrithione, octopirox, and ketoconazole. A minoxidil-related compound (2, 4-diamino-pyrimidine-3-oxide) is a cosmetic agent with claim of acting as a topical hair growth stimulant. Its mode of action has been projected to be the prevention of inflammation and perifollicular fibrosis. Some amount of efficacy of 2, 4-diamino-pyrimidine-3-oxide has been investigated in the prevention of seasonal alopecia.

Herbs in hair growth promotion Medicinal plants have been used for the supervision of hair diseases since the remote past but, herbal systems of cosmetics have become ever more popular in

recent years. India has the well recognized and well knowledge on traditional herbs and their formulations. Herbal cosmeceuticals are of furthermost reputation because they are purely made up of active principles from herbs and shrubs.

Table 1: The list of some plant having hair growth promoting activity according to Ayurvedic Pharmacopoeia of India [2]

Traditional name	Parts used	Biological name	Family
Asana	Heartwood Stem bark	*Pterocarpus marsupium* Roxb.	Leguminosae
Bibhitaka	Fruits	*Terntinalia bellerica* Roxb.	Combretaceae
Gunja	Seed	*Arbus precatorius* Linn	Leguminosae
Ketaki	Roots	*Pandanus tectorius* Soland	Pandanaceae
Bharngaraja	Whole plant	*Eclipta alba* Hassak	Asteraceae
Gambhari	Fruits	*Gmelina arborea* Roxb.	Verbenaceae
Nili	Root Whole plant, Leaf	*Indigofera tentoria* Linn.	Papilionaceae
Kadali	Rhizome	*Mussa paradisiacal* Linn.	Musaceae
Nirgundi Leaf	Root Leaf	*Vitex negunda* Linn.	Verbenaceae
Sahacara	Whole plant	*Barleria prionitis* Linn.	Acanthaceae
Utpala	Flower	*Nymphae stellata* Willd	Nymphaceae
Karnasphota Seeds	Roots , Seeds	*Cardiospermom halicacabom* Linn.	Sapindaceae
Nilajhinti	Roots	*Barleria strigosa* Willd.	Acanthaceae

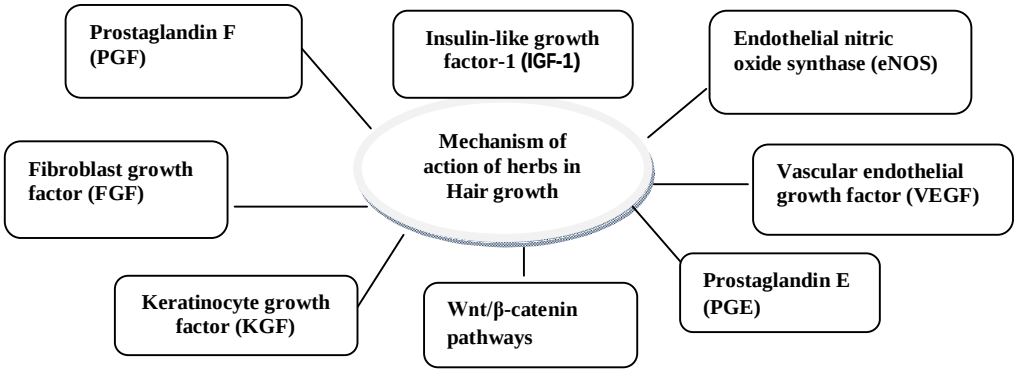


Figure 2: Different mechanisms of herbs in hair growth

Plants mentioned in Charak Samhita and Shandrdhar Samhita Grantha in treatment of alopecia includes Bhringraj, Tulsi, Mulethi, Kaner, Dudhi, Devdaru, Makoy, Harad, Bahede, Manjistha (Indian Madder), Lodh, Bhilawa, Mallika, Babchi, Sahchar, Kumbher, Arjuna, Patol, Kateri,and Dakh.
Natural drug treatments are for hair problems and reason a priceless as well as a valuable present from nature.

Table 2: Different Polyherbal Formulations with Promising Hair Growth Promoting Activities [3-5]

Plants in Herbal Formulation	Formulation
Citrus limonis Osbek, *Cuscuta reflexa* Roxb.; *Emblica officinalis* L., *Centella asiatica* (L.) Urban, *Allium cepa* L., *Lawsonia inermis* L., *Azadirachta indica* (L) *Adleb., Eclipta alba* (L.) Hassak, *Ocimum sanctum* Linn. *And Eugenia caryophyllus* Thunb.	Herbal hair oil
Hibiscus rosa- sinensis L.,*Tridax procumbens* L.	Herbal hair oil
Eclipta alba (L.) Hassak, *Hibiscus rosa sinensis* Linn, *Nardostachys jatamansi* DC	Herbal hair oil
Glycyrrhizae radix, Persicae Semen, Salviae radix, *Angelicae gigantis* radix, *Zanthoxyl fructus, Ginseng radixAlba, Cnidii rhizoma,* and *Carthami flos*	Herbal hair oil
Emblica officinalis, Centella asiatica(L.) Urban, *Aloe vera (L.) Burm.f., Ocimum sanctum* Linn., *Eclipta alba (L.)* Hassak	Polyherbal Ointment
Cuscuta reflexa Roxb., *Citrullus colocynthis* Schrad., and *Eclipta alba* Hassk.	Herbal cream
*Trigonella foenum-graecum*Linn., *Semecarpus anacardium* L.F., *Trigonella corniculata* (L.)	Herbal gel
Poria cocos, Thuja orientalis, Espinosilla, Lycium chinense Mill, Coix lacryma-jobi and *Polygonum multiflorum*	Cubosomal Suspension
Trigonella foenum-graecum Linn. And Butea monosperma	Herbal hair Ointment
Sophora flavescens, Pleuropterus multiflorus, Fructus rubi, Semen glycine,, Rehmanniae radix	Hair Lotion
Arnica Montana L., *Aloe socotrina* Linn. , *Emblica officinalis* Gaertn, *Terminalia chebula* Retz, *Nyctanthes arbortristis* L., *Pilocarpus jaborandi* Vahl	Herbal Cream
Tridax procumbens Linn., *Hibiscus rosa sinensis* Linn., *Trigonella foenum graecum* Linn., *Embilica officinalis* Linn.	Herbal hair oil
Embelica officinalis Linn, *Hibiscus rosa sinensis* Linn, *Bacopa monnieri* L., *Trigonella foenum graecum* Linn	Herbal hair oil
Cicer arietinum Linn and *Cyperus rotundus* Linn. *Ocimum sanctum* Linn.	Herbal hair creams
Emblica officinali, Centella asiatica, Aloe vera, Ocimum sanctum Linn. Eclipta alba.	Herbal hair Oint.
Cyperus rotundus	Hair oil & cream
Allium sativum	Herbal shampoo
Eclipta alba, Lippia nodiflora	Herbal gel
Semecarpus anacardium, Trigonella foenum graecum and *Azadirachta indica*	Herbal hair oil
Emblica officinalis Hibiscus rosea, Accacia concina), ginger Zingiber officinalis, Eclipta alba	Herbal shampoo Powder
Murraya koeniigi, Bacopa monnieri, Trigonella foenumgraecum Murraya koenigii	Herbal hair oil
Azadirachta indica , Semecarpus anacardium, Trigonella foenum graecum , Cocos nucifera	Hair Oil
Nephelium lappaceum Linn	Herbal shampoo

Conclusion:

Hair, one of the vital parts of the body consequent from ectoderm of skin, is defensive appendages on the body and considered accomplice structure of the integument along with sebaceous glands, sweat glands and nails. Hair fall was in the earlier days recognized as a sign of aging and was a cause for a great deal of awkwardness. Hair growth is divided into three phases: anagen, catagen and telogen. Contrasting hair follicles of other animals, the hair follicles of humans are not in the same rotation at the same time; each follicle has its individual program. Management of hair fall is extremely multifaceted. The long-established system of medicine in India acclaims a number of herbal drugs for hair growth endorsement. Natural products are very well-liked and well acknowledged in the cosmetic

and hair care industries and about 1000 plant extracts have been examined for hair care treatment. There are many products available in the market, which are prepared by combination of one or more herbal drugs and find acceptability as hair growth promoter. Herbal Cosmeceuticals provide a new revolution for hair growth.

References:

[1] Lubitz E & J DK (1981) Cell Tiss Res 220:787-795.

[2] The Ayurvedic Formulary of India (2003) Part-I, Second edition, Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homoeopathy 1-756.

[3] Patel S, Sharma V, Chauhan NS, Thakur M & Dixit V K (2015) Curr Drug Disc Tech 12:21-42.

[4] Saraf S, Hargude SM, Kaur CD & Saraf S (2011) Res J Top Cos Sci 2:18-20.

[5] Regupathi T, Chitra K, Ruckmani K, Lalitha KG & Kumar M (2017) J Pharmacol Clin Res 2: ID.555581.

PA-67

Regulatory Guidance on OOS/OOT Investigations

Ankita Vishwakarma, Mousumi Kar, Neelam Balekar, D. K. Jain
IPS Academy College of Pharmacy, Indore, Madhya Pradesh
E-mail address: anki.vish2408@gmailcom

Abstract: In this paper an attempt is made to explain the general introduction and outline of out of specification and out of trend results. Out of specification results are those results that fall outside the established acceptance criteria present in the official compendia. Out of trend results are the results that fall outside the prediction interval and does not follow expected trend. Various guidelines are like US-FDA, MHRA, ICH guidelines are used investigate the oos and oot results. CAPA should be implemented as it come Quality Management System to correct and prevent the non conformities.

Introduction:

OOS means the test results that falls outside the specifications or acceptance criteria which has been specified in the official compendia monographs or the finished product specifications in registration dossiers. These test results does not comply with predetermined acceptance criteria.

OOT results is generally a stability results that does not follow the expected trend, either in comparison with other stability batches or with respect to previous batches result collected during a stability study procedures to identify OOT depend on available data that define the norm.OOT results are not necessarily OOS.

OOS / OOT results may be observed during the analysis of

- stability study
- finished API
- intermediate procedures
- in process
- raw material
- packaging material

Reasons:

1. Laboratory error
2. Process error
3. Sample homogeneity1. Laboratory error involves error in method of analysis, error in calculation , analyst error
4. 2. Process error include operator error , equipment failure , quality of raw material used.
5. 3. Sample homogeneity involves sampling error, error in handling of sampling, pooling of sampling.

For OOT results identification two methods are involved

1. Qualitative method: In this method with the help of graphical representation oot results are identified

2. Quantitative method: In this method statistical data is used to determine the oot results.

Various guidelines are used to investigate the oos results

1. US-FDA (US – Food and Drug Administration)
2. MHRA (Medicine and Healthcare products Regulatory Agency)
3. CDER (Centre for Drug Evaluation and Research)
4. ICH (International Conference on Harmonisation)

Procedures to investigate oos results according to USFDA. It involves two phases

1. Phase 1: Laboratory Investigations
2. Phase 2: Manufacturing investigations

Phase 1: Laboratory Investigations

It involve the rechecking of document with same analyst and re testing with different analyst with original sample .If the laboratory investigations identifies an error that justifies invalidating the original result then this should be documented and the original analysis repeated exactly. If on the other hand the laboratory investigation is inconclusive then the investigation must proceed outside.

Phase 2: Manufacturing Investigations

It involves re sampling and reanalysis or retesting. Re sampling that is taking sample from the original batch or analyzing the specimen from any additional unit as apart of original sample. Retesting should be performed on the portion of original sample by the same analyst if there is laboratory error, if not retest should be performed by different analyst and retest should not be done more than 3 times.

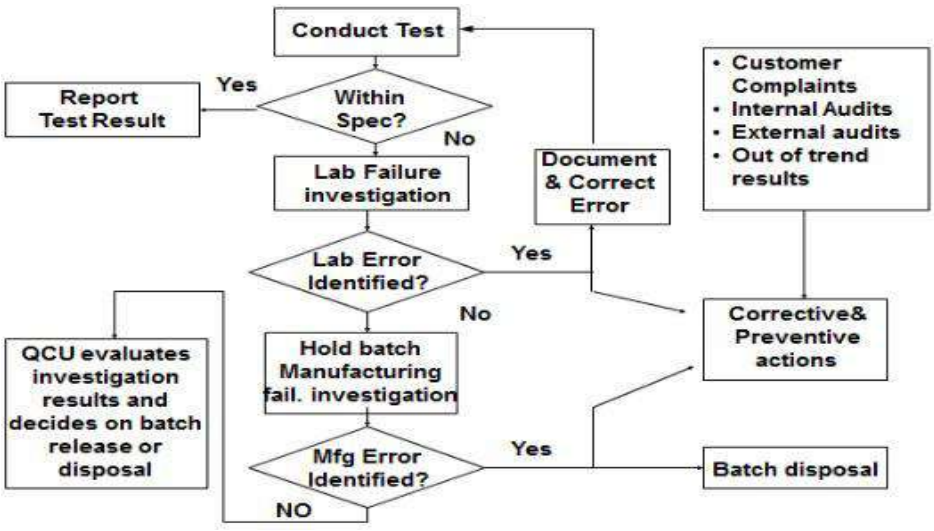


Figure 1: OOS investigation flow chart

OOT result investigation methodology

Statistical approach i.e 3 sigma approach

1. A minimum of 25 – 30 batches data should be compiled for fixing trend range.
2. Results that shall be obtained from 25 batches tabulated, average value, minimum and maximum values are noted.
3. Standard deviation shall be calculated for these 25 batches.
4. Standard deviation should be multiplied by 3 to get 3 sigmavalue.
5. Maximum limit is obtained by adding 3 sigma value to average value of 25 batches.
6. Minimum limit is obtained by substracting the 3 sigma value by average value of 25 batches.
7. The minimum and maximum values shall be taken as the trend range for upper and lower limits.
8. Any value shall be out of this range shall be considered as out of trend value.

Limitations of 3 sigma approach: the products with limited data, the appropriate limits may be difficult to determine. This can lead to wrongly centered, too narrow too wide OOT limits.

For OOS results Batch Disposition

- IF OOS IS CONFIRMED that is the batch does not meet established specifications and standards then the batch is REJECTED.
- FOR INCONCLUSIVE RESULTS in case oos results can not be concluded then the results should be further considered by Technical Management Team.

Both European Regulators and FDA expect pharmaceutical products manufacturers to have in place an effective system for CAPA to correct process deficiencies and maximise process improvement.
CAPA refers to Corrective and Preventive Action: (CAPA, also called corrective action / preventive action, or simply corrective action) are improvements to an organization's processes taken to eliminate causes of non-conformities or other undesirable situations. CAPA is a concept within good manufacturing practice (GMP)
Corrective Action: Its action to eliminate the detected non conformity or undesirable situation.
Preventive Action: Its action to eliminate the cause of a potential non conformity or other undesirable potential situation.
CAPA procedures is accomplished in six basic steps

1. Identification
2. Evaluation
3. Investigation
4. Analysiss
5. Action plan
6. Evaluation of action

Conclusion:

All the OOS/OOT investigations should be thorough, timely, unbiased, well documented and scientifically proved. The guidelines such as USFDA, MHRA serve as a valuable guidance to company seeking to deal with OOS, OOT results. CAPA should be implemented in pharmaceutical companies to correct and prevent the non conformities.

References:

[1] https://www.fda.gov/downloads/drugs/guidances/ucm070287.pdf (Accessed on oct 2017)

[2] https://www.gov.uk/government/publications/out-of-specification-investigations (Accessed on nov 2017).

PA-75

A New Approach in Contraception: Nasal Drug Delivery

Nayany Sharma, Neha Kamalpuria, Sanjay Jain
Indore Institute of Pharmacy, Indore
E-mail address: nananysharma@gmail.com

Abstract: The pulmonary route is very useful way to administer drugs to systemic circulation due to presence of highly vascularised sub epithelial layer. This route also bypasses the first pass metabolism reducing the dose and dosing frequency. Contraceptive steroids like 17β-ethinyl estradiol, 17α-ethinyl estradiol, have been reported having satisfactory bioavailability when administered through nasal route. Emergency contraceptives when given through oral route involve many side effects due to frequent dosing as there are many physiological barriers. Novel drug delivery system provides the improved patient compliance due to ease in administration or application and also having fewer side effects. Currently there are limited clinical data to prove that the nasal route is efficient to use for emergency contraception. There is need to design a delivery system which provides the economical and improved bioavailability to achieve the intended contraceptive effect. The aim of the present review is to study various contraceptives that can be administered through nasal/ pulmonary drug delivery system in both male and female.

Introduction:

Nasal route for drug administration is used as an alternative route for the drugs that are restricted for intravenous administration. A large surface area available for drug absorption makes the nasal route a promising route for the drug absorption, numerous microvilli and highly vascularised epithelium allow the drug to pass directly in the systemic circulation and avoid first pass metabolism. Due to direct passing of drug in systemic circulation it offers quick onset of action and rapid therapeutic blood levels and due to this dose and dosing frequency of the drug is also reduced, having fewer side effects. Drugs that can be administered by nasal route include peptides and protein drugs for their rapid systemic absorption.

Oral Contraceptives

Estrogen and progestrogen are widely used combination for the oral contraceptives which act by interfering with the reproductive cycle of the female. Ethinyl estradiol and levonorgestrel are other synthetic drugs which are used in combination as contraceptives but the disrupt they fertilization of egg and inhibiting the implantation of egg in uterus, so these drugs can be taken after unprotected sex to avoid pregnancy [1].

Various side effects of oral contraceptives due to long term effect are Irregular periods, Menorrhagia (heavy periods), Dysmenorrhea (painful periods), Endometriosis, Premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD), Acne, hirsutism (excess hair growth) and alopecia. Besides

side effects, several drug interactions to anticoagulants, antibiotics, or anticonvulsant drugs are at risk due to hepatic enzyme induction. In term use contraceptive steroids also interfere in synthesis of hepatic protein synthesis and fibrinolytic proteins and also leads to fatty liver [2].

The researchers studied that contraceptive steroid ethinyl estradiol extensively metabolize when given orally in solution to some animal species. They found that the drug not only got metabolized in liver but also in the gut walls and is not negligible percentage [2].

Nasal Route for Delivery of Contraceptives: As discussed earlier nasal route is very efficient in delivering drugs especially hormones as it has highly vascularized sub epithelial layer it also bypasses the first pass metabolism and deliver the drug direct to systemic circulation and have a quick pharmacological action and reduced dose[3].

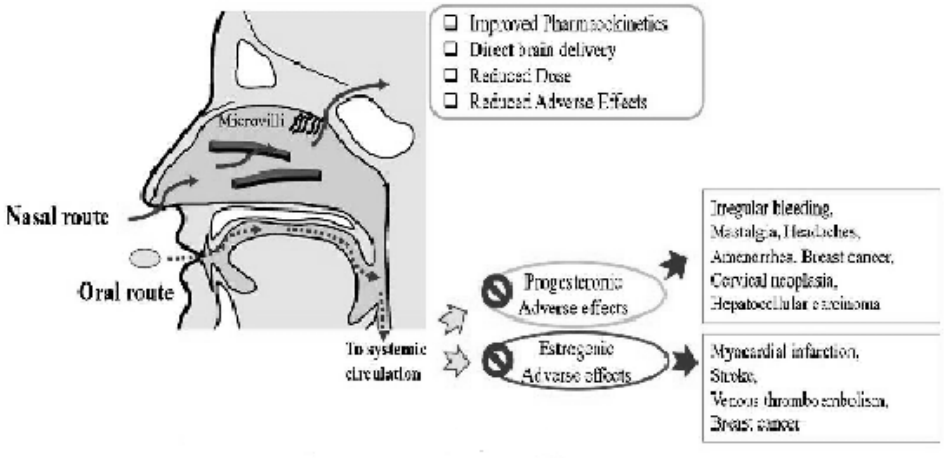


Figure 1: Nasal vs oral route

Factors affecting nasal absorption

There are few barriers for the nasal delivery for any drug like physicochemical properties of the drug, nasal mucociliary clearance and they can be overcome by use of various agents like mucoadhesives to increase retention time etc. One of the important factors in nasal drug delivery is inadequate nasal drug absorption. Several absorption enhancers are used to achieve the required drug absorption.

Physicochemical properties of the drug

Partition coefficient of the drug, the pKa, the molecular weight of the drug, perfusion rate and perfusate volume, and solution pH and drug concentration are some of the physicochemical properties of the drug that influence the nasal absorption. It is having a direct relation between the log of the proportion of the dose absorbed and the log of the molecular weight

Mucociliary clearance

It is very important, non specific and defence mechanism in nasal drug delivery as due to this effect the drug is effectively cleared from the nasal tract. To overcome this problem mucoadhesives agent can be use. Mucociliary transit time in humans has been reported to be 12 to 15 min.

Intra nasal dosage forms

Microspheres Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm.When different microspheres have been evaluated for nasal drug delivery the Microsperes of different materials have been evaluated in vivo as nasal drug delivery systems. Microspheres of albumin, starch and DEAE-dextran absorbed water and formed a gel-like layer, which was cleared slowly from the nasal cavity.

Liposomes Liposomes are phospholipid vesicles composed of lipid bilayers enclosing one or more aqueous compartments Liposomes provide an efficient delivery system because they are biocompatible, biodegradable, and relatively nontoxic. As a drug-delivery system, liposomes can significantly alter the pharmacokinetics and pharmacodynamics of entrapped drugs, like by enhancing drug uptake, delaying rapid drug clearance.and reducing drug'toxicity.

Liposomes are attracting considerable interest for drug delivery to the nasal mucosa in fact, they are known to sustain the release of the entrapped drugs owing to their surface viscosity Their action on nasal mucosa is related to the incorporation of phospholipids in the membrane, opening “new pores” in the paracellular tight junction [5].

Gels Chitin and chitosan have been suggested for use as vehicles for the sustained release of drugs in nasal delivery. It was reported that chitin was able to control the release of the drugs in gel formulation.

Future prospects As nasal delivery has provided many benefits in absorption continuous studies are going on in delivery of drugs through nasal route like nasal vaccines and nasal contraceptives for emergency contraception. As it will provide many benefits like needle free administration which reduced associated problems with needle stick injuries and its disposal. Increased acceptance of nasal vaccine products in both adults and children make it more acceptable. Key challenges and opportunities for the future will include translating in vivo data to clinical outcomes. Particular focus should be brought to designing delivery strategies that take into account the broad range of diseases, populations and healthcare delivery settings that stand to benefit from this unique mucosal route.

Conclusion:

The study relates to the novel emergency contraceptives method therapeutically available for having ease of administration/ application and at the same time devoid of side effects, so as to improve patient compliance. Since most of the therapeutics employed in emergency contraceptive are hormones that are prone to a wide range of physiological barriers post oral administration requiring frequent administration doses and thus increasing the chances of side effects. There is a need for designing delivery system offering ease of administration with economically available and improves compliance for the intended purpose of achieving contraceptive effect. The nasal route can be regarded as a convenient and efficacious alternative to the currently employed administration routes

for availing therapeutics benefits of contraceptive agents. However, currently there is a limited data to substantiate the clinical usability of nasal route for delivery of contraceptive drugs. This requires that randomized controlled clinical trials of sufficient size be taken to statistically prove the efficacy of nasal route for emergency contraception. The present review discusses about improvement in a method of contraception, hormone replacement administering intranasal an contraceptives.

References:

[1] Misra A (2016) J Pharm Ana In 12:2471-8122

[2] Türker S, Onur E & Ózer Y (2004) Pharm World Sci 26: 137-142.

[3] Bawarshi-Nassar RN, Hussain AA & Crooks PA (1989) Drug Metab Dispos 17:248-254.

[4] Hussain AA, Hirai S & Bawarshi R (1983) Nasal dosage forms containing natural female sex hormones. Google Patents US 4383993 A.

[5] Shahiwala A & Misra A (2004) Fertil Steril 81: 893-898.

PB-14

Designer Drugs – A Review

Kirti Upadhyay, Ankit Jain, Neelam Balekar
IPS Academy College of Pharmacy, Indore-452012 (M.P.) India
E-mail address: kirtiupadhyay7@gmail.com

Abstract: Designer drugs also known as "legal highs," include synthetic cannabinoids, Synthetic stimulants and Synthetic hallucinogens. Compound has evolved rapidly to evade legal regulation and detection by routine drug testing. Synthetic drugs with hallucinogenic properties have become very popular among recreational drug users. They mainly cause acute toxicity, psychiatric and medical effects, which may be severe and multiple deaths have been reported. These drugs may have similar effects to other known drugs, but have completely dissimilar chemical structures (e.g. JWH-018 vs THC). There are still so many researches going on to detect and treat adverse effect caused by synthetic analogues.

Introduction:

There is growing international concern about the syntheticanalogues of controlled substances being manufacturedand distributed to circumvent drug laws and evadeinterdiction. These compounds are referred to as "designerdrugs" or "legal highs" (Table 1). They are substanceswith psychotropic effects that are intentionallymarketed and distributed for recreational use by exploitinginadequacies of existing controlled substance legislation. Non-chemists can easily synthesize the compoundswith readily available raw materials, or they can obtain the synthetic compounds directly. The chemicals are oftenpackaged with labels that inaccurately describe productcontents, which may vary substantially regarding chemical content and concentration. Labels often include thephrase, "not for human consumption," in an attempt toavoid legal risk. Designer drug use has expanded in thepast decadeespecially among young adults leading to significant problems for some users.Although the emerging designer drug trend was initiallyrecognized by increasing calls to US poison controlcentres, the incidence of designer drug problems inemergency departments (EDs), hospitals, and other medicalsettings is largely unknown. Only a small percentageof those using designer drugs will come into contactwith the health-care system, but consequences of usecan be severe. Familiarity with designer drugs can helpclinicians recognize common adverse reactions and life-threateningconsequences. This article will focus on threenewer designer drugs: substituted cathinones (commonlyreferred to as "bath salts"), synthetic cannabinoids (SCs; e.g., "Spice"), and synthetic hallucinogens (e.g., "N-bomb") [1].

Table 1: Categories of designer drugs [2]

Drug class	Chemical name	Chemical origin	Slang names
Stimulant	Mephedrone	Cathinone	Bath salts (Ivory Wave, Vanilla Sky), meow-meow, M-Cat
	Methylone		Sextacy
	Methylenedioxypyrovalerone (MDPV)		NRG-1
	Naphyrone		
Cannabinoid	JWH-018; JWH-073; JWH-250	Laboratory of J.W. Huffman	Spice, K2, K9, Aroma, herbal highs, Scooby Snax.
	CP 47,497; CP 47,497-C8; CP 59,540; cannabicyclohexanol	Pfizer laboratory	
	HU-210	Hebrew University laboratory	
	UR-144	CB2 receptor agonist	
	Oleamide	Fatty acid	
Hallucinogen	25I-NBOMe	Free University of Berlin	N-bomb, Solaris, Smiles, Cimbi-5
	25B-NBOMe		
	25C-NBOMe		

Epidemiology of emerging designer drugs

Designer drugs are most prevalent among teens and adults upto age of 40 years. Designer drugs are sold without age restriction, which also makes them attractive to younger individuals. Widespread availability, including purchase via the internet, has contributed to expanded use. Marketing designer drug products as “legal high” alternatives may contribute to the perception of greater safety or purity compared to traditional illicit drugs, which could promote increased consumption. Risk factors for adolescent experimentation with and problems resulting from designer drug use include parents with substance use disorders (SUDs), poor family relationships, poor discipline, or high family conflict; adolescents involved with foster care or the criminal justice system are also at risk. In response to rising designer drug use and consequences, a series of state and federal

initiatives have been enacted during the past several years prohibiting the manufacture, sale, and possession of many designer compounds [3].

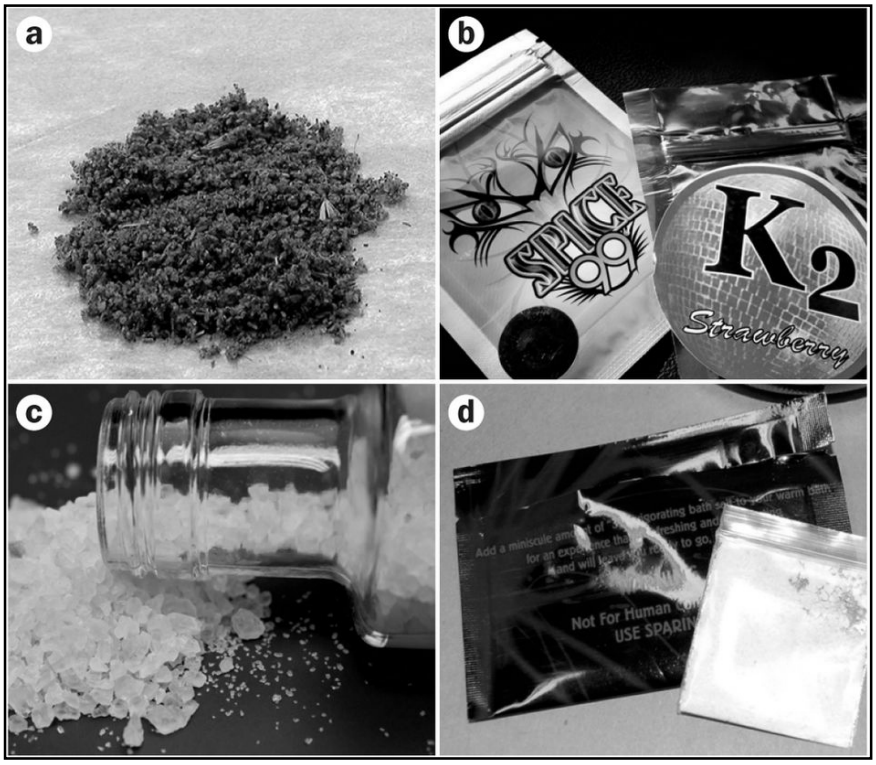


Figure 1: Showing various types of drugs a) Cannabis, b) Spice K2, c) Bath salt type, d) Marijuana compound

Global perspective

United Nations Office on Drugs and Crime estimates that about 230 million use an illegal drug at least once a year and the use of designer drugs is rapidly increasing. North America remains the biggest illicit drug market in the world, as well as the region reporting the highest drug-related mortality rate. According to figures approximately 1 in every 20 deaths among persons aged 15-64 in North America is related to drug abuse.In Europe, the number of notifications of new designer drugs received by the European Monitoring Centre for Drugs and Drug Addiction center (EMCDDA) averaged five per year from 2000 to 2005. This had increased to 49 by 2011, indicating that a new psychoactive substance was introduced in the European market almost every week.Asia and Africa are emerging as centers for the production and trafficking of illicit substances including newer designer drugs that substitute the illicit drugs banned in developed countries. Codeine-based cough syrups, dextropropoxyphene, benzodiazepine, buprenorphine and pethidine are the preparations most commonly diverted for the manufacture of new designer drugs. In 2007, India was named one of the 20 major hubs of illegal drug traffic along with a number of its neighboring countries, Pakistan, Afghanistan and Myanmar in the East. At the last national survey in 2000-01 there were 70 million drug users in India. Among those treated for drug problems in India in 2010, 66 % abused opioids (33 % heroin, 14 % opium and 19 % prescription opioids), 22 % abused

cannabis and 12 per cent other substances which includes the newer designer drugs. The use of synthetic drugs grew to account for 15% of reported users in recent times.

The Effects of Designer Drugs

Depending upon the drug taken, a person may experience feelings of exhilaration, prolonged periods of wakefulness, decreased appetite, extreme relaxation, amnesia and feelings of detachment. Unwanted effects might include hallucinations, panic attacks, aggressive behaviour or feelings of paranoia. In addition, there may be physical effects like nausea, significant changes in blood pressure, seizures, slurred speech and blackouts. These drugs can even cause coma and death.In the case of designer drugs, many of the signs of abuse are similar to the signs of addiction to alcohol or street drugs:Changes in behaviour: isolation from family; defensive about drug use, Unexplained weight loss or gain, Changes in hygiene or personal appearance, Confused or disoriented behaviour, Paranoia, Problems with sleeping: insomnia, and restlessness [4].

Treatment for designer drug Addiction

Hospitalization for the adverse effects of designer drugs affords an excellent opportunity (teachable moment) for advising patients to decrease their substance use and for engaging them in treatment. Although prospective treatment data are limited, once a designer drug use disorder diagnosis is made, acute and long-term treatment is likely necessary. Recovery from SUD in general is possible, and those who are treated have less disability than those who remain untreated. Long-term treatment of designer drug use disorders likely involves similar components to that of other types of addiction treatment, including behavioral components, such as individual and group counseling with cognitive-behavioral therapy, motivational enhancement therapy, and 12-Step self-help group facilitation. Unfortunately, pharmacologic treatment data to guide management of those with designer drug use disorders are unavailable.

Common designer drugs in recent years include:

- “Spice” (synthetic marijuana)
- Ecstasy (“Molly”—synthetic psychoactive drug similar to amphetamines and mescaline)
- Bath salts (a lot of substance variability, but often contains one or more synthetic chemicals related to cathinone)
- Methylenedioxypyrovalerone (MDPV) (one of the chemicals found in bath salts)
- Mephedrone (commonly found in bath salts)
- Methylone (commonly found in bath salts)
- 2C family (synthetic hallucinogens)
- Krokodil (a less-expensive heroin substitute; like heroin, it is a synthetic morphine derivative)

Detection and Analysis

Since these drugs are developed by modifying chemical structures of banned drugs, it is difficult to detect these drugs using existing methods for drug analysis. Methods commonly used for drugs of abuse include colorimetric detections, gas chromatographic (GC)–mass spectrometric, and liquid

chromatographic (LC)–mass spectrometric. However, newer drugs are constantly developed in order to evade the current detection techniques. It is necessary to predict the modifications possible in the existing drugs and develop techniques to detect such modified compounds and potential new psychoactive substances [5].

Conclusion:

Designer drugs have come to play an increasing role as drugs of abuse. The coming years will without a doubt see more planner drugs develop on the illegal medication scene. Therefore, the legal, clinical, and law requirement groups must know about the presence of these drugs. When the clinical symptoms resemble a known class of drugs such as opioids and benzodiazepines but no positive detection by standard toxicology testing is present, designer drugs should be considered as a possibility.To this end, contextual analyses and research on the toxicology and clinical manifestations of designer drugs are important to distribute where proper.

References:

[1] King LA & Kicman AT (2011) AJC, 3: 401–403.

[2] Johnson LA, Johnson RL & Portier RB (2013) J Emerg Med 44:1108–1015.

[3] Wiegand TJ, Wax PM, Schwartz T, Finkelstein Y, Gorodetsky R & Brent J (2012) J Med Toxicol; 8:360–377.

[4] Gunderson EW, Haughey HM, Ait-Daoud N, Joshi AS & Hart CL (2014) 35:184–189.

[5] Brewer TL & Collins M. (2014) J Spec Pediatr Nurs 19:119–126.

PB-18

Continuous Process Verification as per USFDA Guidelines on Process Validation

Priti Kour Sandhu, Jayanti Mukherjee, Vimukta Sharma
BM College of Pharmaceutical Education and Research, Khandwa road, Gokanya, Madhya Pradesh-452020
E-mail address: priti302@gmail.com

Abstract: FDA process validation guidance defines three stages as process design, process qualification and continuous process verification. This paper reveals real time promotes control of the process for wastage reduction and product quality improvement. Continued process verification is ongoing assurance is gained during routine production that process remains in a state of control. This project report provide the data generated during the compression & coating validation and continuous process verification of the same by executing a series of batches to conclude the quality of subsequent batches can be predicted with a higher degree of assurance to meet the desired quality attribute.

Introduction:

As per US Food and Drug Administration (1987): Process validation is establishing documented evidence which provides a high degree of assurance that a specified process will consistently produce a product meeting its pre-determined specifications and quality characteristics. Process validation involves a series of activities which takes place over the lifecycle of the product and process. Validation guidance describes process validation activities in three stages.

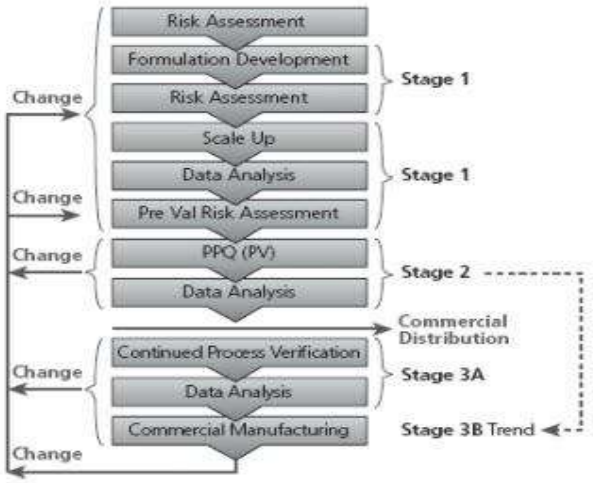


Figure 1: Process Validation Lifecycle (Stage 1, 2 & 3)

The purpose behind the continuous process verification is to provide documented evidence that the process of manufacturing is capable to produce the batches within the specification limit and there is no significant variation between results of different batches.

The objective of continuous process verification report is to assure that the process remains in state of control (validated state) during routine commercial manufacturing.

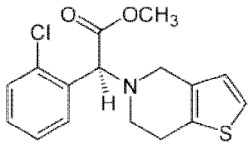
Material:

Generic Name: Clopidogrel

Chemical Name: 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl-benzamide

Formula: $C_{16}H_{16}ClN_3O_3S$

Structure:



Classification: Antiplatelet Drug

Dosage Form: Oral Tablet: 75 mg and 300 mg

Solubility: It is practically insoluble in water at neutral pH but freely soluble at pH=1. It also dissolves freely in methanol, dissolves sparingly in methylene chloride, and is practically insoluble in ethyl ether.

Mechanism of Action: Clopidogrel is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. The drug works by irreversibly inhibiting a receptor called P2Y12, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes.

Table 1: Results of study

Sr. No.	Parameter	Specification	Observed	
			Min	Max
1	Weight of 10 tablets (g)	6.370 – 6.630	6.461	6.587
2	Weight variation (mg)	(6.500 ± 2.0%)	633	674
		618 – 683		
3	Thickness (mm)	(650 ± 5.0%)	4.8	5.0
		4.70 – 5.20 mm		
4	Hardness (Kp)	08 – 22 Kp	13	20
		(80 – 220 N)		
5	Disintegration time (Minutes)	NMT 15 minutes	03	06
6	Friability (%w/w)	NMT 0.8 %w/w	0.0	0.0

All the results were found within the control limit

Adverse effects include hemorrhage, severe neutropenia, and thrombotic thrombocytopenic purpura (TTP).

Method:

Continuous process verification (CPV) of Clopidogrel Tablet 75mg by using "Minitab" Software for statistical process control (SPC). Statistical Process Control uses statistical tools to observe the performance of the production process in order to predict significant deviations that may later result in rejected product. "Minitab" is software which helps to analyze the data. It provides a simple, effective way to input the statistical data, manipulate that data, identify trends and patterns, and then extrapolate answers to the current issues.

Discussion:

Above project work outcome shows that continuous process verification is a framework for gathering and analyzing data of final product quality and process consistency. All project results were completely based on the statistical process control for astringent the process and quality parameters limits.

CPV = TRUST BUT TO VERIFY CONTINUOUSLY

Conclusion:

Based on the complied data of Clopidogrel Tablets 75 mg it is concluded that all the result of critical process parameters and critical quality attributes are within the specification limit and as per the trend analysis by Minitab there were no variation observed between the commercial batches.

References:

[1] A WHO guide to good manufacturing practice (GMP) requirements (1997) Global Training Network, World Health Organization, 105–108.

[2] Federal Food Drug and Cosmetic Act (1978) Code of Federal Regulations, Title 21, Parts 210 & 211, 43.

[3] Pankaj K & Shankar J (2009) Text book of statistical process control and process capability, 2: 11.

[4] Kieffer RG (1995) J Pharm Sci Technol 49(5): 249–252.

PB-61

Reversible Covalent Kinase Inhibition: A Rational Approach to Drug Design

Priyanka Soni [1, 2], Ankur Joshi [3], Neelesh Malviya [2], Jitendra Sainy [1], Rajesh Sharma [1]
[1]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore.
[2]Smriti College of Pharmaceutical Education, Indore.
[3]Modern Institute of Pharmaceutical Sciences, Indore.
E-mail address: soni.rgpv@gmail.com

Abstract: Drugs that form a covalent bond with a protein target often show enhanced selectivity, potency and utility for biological studies. Pharmaceutical companies are reluctant to investigate covalent kinase inhibitors, along with safety concerns, which are known to interact with amino acid residues and react reversibly that can produce long lasting effect without detrimental effects. In this review the focus is on the designing of reversible inhibitor that includes interaction of these drugs with their target via a reversible covalent bonding and may retain the specificity advantages gained through the covalent binding of a certain amino acid residue while decreasing the potential negative outcome of irreversible drug activity.

Introduction:

Drug discovery processes have sought to modify molecules to achieve maximum thermodynamic affinity from the history dates back. Protein-protein interaction is a critical aspect of all the cellular processes. Protein kinases are key regulators of cell function that constitute one of the largest and most functionally diverse gene families. A protein kinase is the enzyme that regulates other proteins by phosphorylation. Kinases turned on or off by phosphorylation and dephosphorylation processes, by binding of activator proteins or inhibitor proteins, or small molecules, or by controlling their location in the cell relative to their substrates. Protein kinases are entangled with many human diseases; most notably cancer and thus kinases are one of the most focused protein families for small-molecule inhibition.

Covalent inhibition of protein kinases:

The inhibition of Protein kinase involves bonding of small molecule by either reversible or covalent inhibition. When drug molecules interact with their biological targets under equilibrium binding conditions, the desired drug–protein interaction is a rapid and reversible process and which is said to be the conventional strategy. But in advanced drug discovery process the focus is on the covalent targeting of the protein target since, many of the drugs which are discovered by serendipity (e.g. Acetylsalicylic acid, Penicillin, and Omeprazole) are actually the covalent inhibitors and till today these are leading the market. Covalent drugs have proved to be successful therapies for various indications, with nearly 30% of drugs on the market acting via a covalent mechanism of action.

Covalent targeting mainly include irreversible pathway which actually determines the specificity and the residence time of the drug. It generally results in long lasting effect till the time body cells produce

a new enzyme. A covalent drug proves to reduce the risk of development of resistances which is the main case with many other drugs that binds reversibly and follows chemical equilibrium kinetics.

Irreversible covalent inhibition verses reversible covalent inhibition

Drugs with prolonged on-target residence times often show superior efficacy. In case of irreversible covalent inhibition there are potential benefits like high potency and extended duration of action because of an electrophile on the ligand and a nucleophilic center in the protein results into first reversible association and then the formation of covalent bond between the two. But, the above said inhibition also provided with toxicological effects like protein haptenization that have hindered the designing of this concept. This suggests that off-target binding of these inhibitors leads to a greater risk of toxicity. While the potential risks of covalent inhibition are known, the sustained duration of inhibition offers several advantages:

- Enhanced biochemical efficiency.
- Less frequent dosing reducing the burden on the patient.
- Dissociation of pharmacokinetics from Pharmacodynamics.

In addition, success stories have been reported where previously considered as “difficult” or even “undruggable” proteins have been targeted by covalent inhibitors3. The Selectivity can be accomplished through:

1. Optimization of the initial non-covalent association.
2. Application of bioinformatics techniques to optimize placement of an appropriately tempered warhead on the inhibitor in relation to an accessible, non-conserved nucleophile on the protein.

Here are a no. examples that applied covalent reversible targeting and gained great success like in chronic lymphocytic leukemia, Ibrutinib to inhibit BTK, in EGFR-cell lung cancer afatinib and AZD9291 (Osimertinib). In reversible covalent inhibition there is a two step approach involves, first the fast initial binding step, followed by second, a slower reversible inactivation step as reported by Devkota A. K. et al.

Most of the covalent inhibitors developed against protein kinases target a non-conserved cysteine residue located at the active site of the kinase. Through bioinformatics analysis of the human kinome, Zhang et al. have shown that almost 200 different protein kinases contain a cysteine residue in the vicinity of the nucleotide binding pocket. Devkota A. K. et al. Molecular docking performed by them, suggests that a nitrile group binds within 4.5 _ of the active site Cys146 to form a reversible thioimidate adduct. Also an investigation by Miller et al, suggests fragment base design and reversible covalent targeting of non-catalytic cysteines to develop potent and selective kinase inhibitors. Study by Taunton and colleagues report the use of electron-deficient Michael acceptors for reversible addition/ elimination reactions with thiols under physiological conditions. They apply 2-

cyanoacrylates and –acrylamides to convert a known irreversible kinase inhibitor into a reversible covalent inhibitor.

Advantages over irreversible covalent targeting:

Such an approach might lead to the improved affinity and selectivity often associated with protein inhibition through the covalent capture of non-conserved residues without suffering from toxicological risks associated with the irreversible modification of proteins.

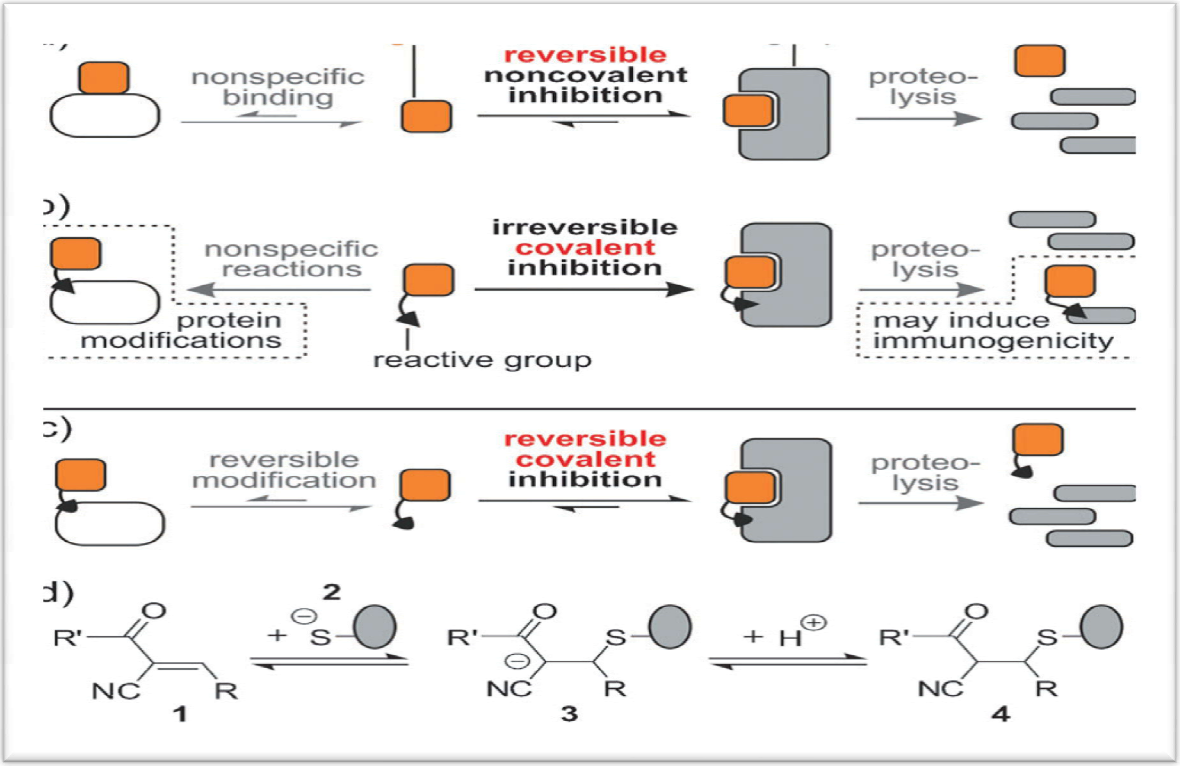


Figure 1: Mechanism of inhibition: a) reversible non covalent inhibition, b) irreversible covalent inhibition, c) reversible covalent inhibition

In principle, this strategy should be applicable to a large number of disease relevant target proteins. For example, it was estimated that approximately 20% of all kinases might be accessible for cysteine-based targeting [5]. Inhibitors that form a reversible covalent bond with a noncatalytic cysteine of the target may prove widely applicable for obtaining prolonged residence times. Reversible covalent drugs have at least two theoretical advantages over their irreversible counterparts. First, relative to reversible covalent drugs, drugs that rely on intrinsically irreversible chemistry (for example, acrylamides) are more likely to form permanent covalent adducts with off-target proteins, including both closely related targets (for example, off-target kinases with a homologous cysteine) and unrelated targets with hyper-reactive cysteines. Second, and equally important, reversible cysteine engagement may enable fine tuning of the inhibitor residence time, a feature that would facilitate the use of such inhibitors not only in therapeutic applications requiring sustained target engagement, but also in applications where more rapid target disengagement is preferred.

Conclusion:

Covalent inhibitors have attracted significant attention from drug developers in recent years.. Covalent kinase inhibitors offer several advantages over first generation reversible ATP-competitive inhibitors. First, they can achieve better potency against millimolar concentrations of ATP inside the cells because the inhibition is time-dependent. Second, with two-step inhibition mechanisms, they offer better selectivity. Third, they are less affected by kinase mutations than reversible ATP competitive inhibitors

As many drug targets, including kinases, have an accessible cysteine in their binding site, there is broad opportunity to discover reversible covalent inhibitors for use across many therapeutic areas. This chemistry, when deployed against a poorly conserved noncatalytic cysteine, provides enhanced selectivity and potency, while avoiding the formation of irreversible covalent adducts. Given the potential such an approach could offer in drug discovery, it is essential to further investigate the applicability and toxicity of the pharmacophores.

References:

[1] Serafimova IM, Pufall MA, Krishnan S, Duda K, Cohen MS, Maglathlin RL, McFarland JM, Miller RM, Frödin M & Taunton J (2012) Nature Chem Biol 8:471-476.

[2] Lee CU & Grossmann TN (2012) Angew Chem Int Ed 51:8699-8700.

[3] Singh J, Petter RC, Baillie TA & Whitty A (2011) Nature Rev Drug Dis 10:307-317.

[4] Smith AJT, Zhang X, Leach AG & Houk KN (2008) J Med Chem 52: 225–233.

[5] Schwartz PA, Kuzmic P, Solowiej P, Bergqvist S, Bolanos B, Almaden C, Nagata A, Ryan K, Feng J, Dalvie D, Kath JC, Xu M, Wani R & Murray BW (2017) Proc Natl Acad Sci 111:173–178.

PC-27

A Review on: Elicitor Technique "An Approach to Preserve and Enhance Plant Secondary Metabolites Production"

Rajiv Saxena, Neelesh Malviya
Smriti College of Pharmaceutical Education, MR-11, Indore-452010 (M.P.)
Mandsaur University, Mandsaur
E-mail address: rajivbiotech@rediffmail.com

Abstract: Plants are the major source of drug in most of the developing countries. Factors like climate change are putting extra burden on existence of rich medicinal plant due to which many rare and common plant species are on the erg of extinction. Biotechnological approaches can contribute to preserve the beneficial effects of valuable plants. Elicitor technology uses various biotic and abiotic materials that enhance the production of various secondary metabolites of rare and endangered species. Elicitor application stimulates the plant immune responses that can lead to the generation of defensive molecules including phyoalexine that can have new or improved pharmacological activities.
Key Words: Climate change, secondary metabolites, elicitors, phytoalexins.

Introduction:

Herbs are very popular as source of medicines in most of the developing countries and are now becoming extremely appreciable in developed countries and people are using it to strive good health in the face of chronic stress and to treat illness. As the use of herbal medicines is increasing tremendously since the last decade because of their large therapeutic advantages and lesser side effects, many Phyto-pharma Industries are coming up in India and contributing a major share in Indian drug Industry. Since ever changing climatic conditions and factors like climate change, global warming etc. are adversely affecting the rich flora and fauna. Very less attention is given on identifying the adverse effects of climate change and warming on secondary metabolite production by medicinal plants. This aspect is very much essential to study and scientifically consider especially in developing countries, as a larger proportion of population relies on medicines which are obtained from plants for treating existing diseases [1]. Today various tissue culture techniques are used to enhance yield of secondary metabolites. This can be achieved by trigger stress response like using elicitors, precursors and biotransformation, change in environment conditions, change in medium constituents etc. Elicitors can be used as enhancer of plant secondary-metabolites production and can play an essential role in biosynthetic pathways to enhanced production of many commercially important compounds [2].

Table 1: Classification of elicitors

ELICITOR				
Physical elicitor	Injury			
Chemical Elicitors	Abiotic	Metal ions like lantharium, europium, calcium, silver etc.		
	Biotic	Complex composition	Yeast cell wall, fungal spores etc.	
		Defined composition	Carbohydrates	Chitosan, guargum, pectin, alginate
			Proteins	Cellulose, glutathione
			Lipids	Lipopolysaccharides
			Volatiles	C-6 –C-10

Mechanism of action:

Elicitors are compounds stimulating any type of plant defence [3]. Plants can activate separate defence pathways depending on the type of pathogen encountered [4]. The better understanding of plant signalling pathways has led to the discovery of natural and synthetic compounds called elicitors that induce similar defence responses in plants as induced by the pathogen infection. Different types of elicitors have been characterized (as shown the table 1) like carbohydrate, protein, lipid, yeast cell wall, glycoproteins etc. After the application of elicitor the activation of signal transduction pathways occurs that lead to the generation of active oxygen species (AOS), phytoalexin, pathogen related proteins, reinforcement of plant cell wall associated, , synthesis of defence enzymes, callose accumulation etc. These defensive molecules play an important role in protecting the plant from pathogenic damages [5].

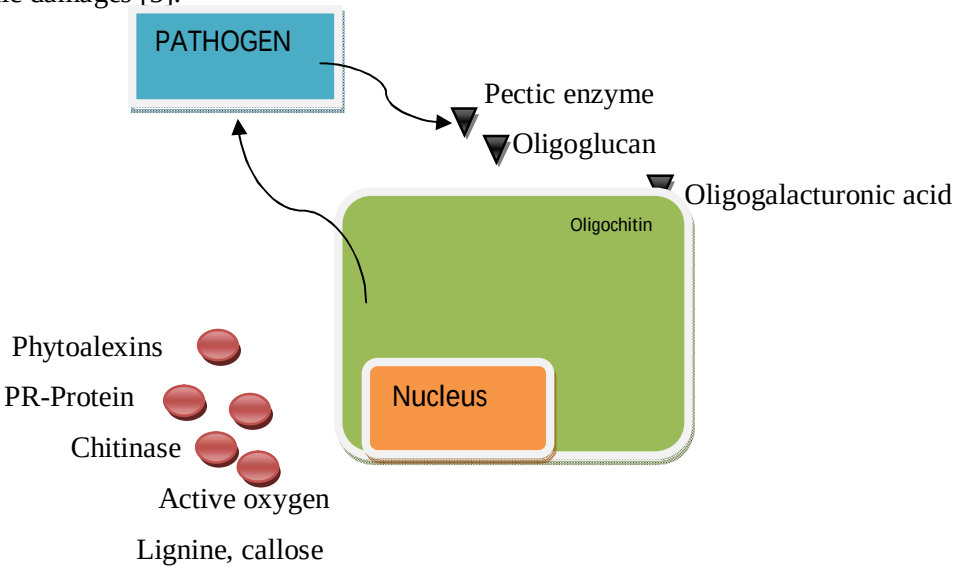


Figure 1: Mechanism of elicitor action

Method of elicitors application and uses:

There are various methods by which researchers are using the elicitor technology. One such novel method is use of elicitor under cell suspension condition. A systematic representation of the technology can given as-

- Ex-plant collection and authentication. The plant is selected on the basis of its applications, status of its extinction or its endangered condition in particular geographical location.
- Optimization of callus culturing media of selected plant.
- Generation of cell suspension of callus and also optimize different elicitor concentrations in cell suspension condition that will lead to the generations of enhanced secondary metabolites.
- Evaluation of generated and identified secondary metabolite quantitatively and qualitatively in the extracted solvent from cell suspension, after their chromatographic separation.
- Pharmacological evaluation of the generated plant secondary metabolites from cell suspension culture for various activities like anti-microbial and anti-oxidant.

Many new and enhanced plant secondary metabolites are generated by using cell suspension culture. It also provides an excellent environment for detailed investigation of biochemical and metabolic pathways. The *In-vitro* studies specially plant tissues culture and suspension cultures have wider application in diverse field for the production of commercially important secondary metabolites. Many researchers have successfully produced secondary metabolites such as solasodine production from calli of *Solanum eleagnifolium,* pyrrolizidine alkaloids production from root cultures of *Senecio,* cephaelin and emetine from cultures of *Cephaelis ipecacuanha*, quinoline alkaloids from cell suspension cultures of *Cinchona ledgeriana*. Few examples of elicitors and their activity in increasing secondary metabolite production-

Table 2: Elicitors examples and uses

Elicitor	Plant	Product
Methyl jasmonate	Taxus Sp	Paclitaxel, taxanes,diterpenes
Methyl jasmonate	Oryza sativa leaves	Putrescine
Copper sulphate	Hyoscymus albus	Phytoalexins
Silver nitrate	Solanum tuberosum	Free and conjugated polyamines
Vanadium sulphate	Catharanthus roseus	Catharanthine, ajmalicine

Conclusion:

Medicinal plants are abundantly available throughout the country. They are more focused and studied than before because they have the ability to producing many benefits to mankind specially in the line of medicine. Medicinal plants and herbs are known to ayurveda since long time. Medicinal plants

have vital applications in the field of Pharmaceuticals, cosmetics, agriculture and food industries. Climate change, pollution, soil fertility etc. are adversely affecting the productivity of our plants especially medicinal plants where secondary metabolites production in terms of quality and quantity is major concern. Many researchers are working on protecting the beneficial effects of these rare and endangered plants. Elicitor technology is proving its potential in protecting and enhancing the level of secondary metabolites. When the elicitors are combined with tissue culture technique a new area of research is developed in which enhanced plant metabolite production can be achieved and the plant under biological stress can also be protected. Elicitor here triggers and activate plant defence system as a result of it, new defensive molecule like pytoalexins, pathogen related proteins, active oxygen species etc is generated that can have variety of new and improved pharmacological action. So, In-vitro plant propagation coupled with elicitor technology can be have wider applications in terms of preservation of plants and generation of new and improved secondary metabolites with novel pharmacological actions.

References:

[1] Gairola S (2010) J Med Plan Res 4(18):1825-1829.

[2] Patel H & Krishnamurthy R (2013) J Pharmacog Phytochem 2:61-65.

[3] Radman R, Sacz T, Bucke C & Keshvartz T (2003) Biotechnol Appl Biochem 37:91-10.

[4] Garcia-Brugger O (2006) Mol Plant-Microbe Int 7:711–724.

[5] Thakur M & Sohal B (2013) ISRN Biochem 1-10.

PD-45

Multidisciplinary Approaches for Prevention and Understanding Pathogenesis of Metabolic Syndrome

Vikas Kumar Jain[1], Gajendra Choudhary[2], Gajanan Darwhekar[1]
[1]Acropolis Institute of Pharmaceutical Education and Research, Indore
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore
E-mail address: vikaspharma2209@yahoo.com

Abstract: The Metabolic Syndrome is a global health issue that affects roughly 25% of adults over the age of 20 and up to 45% over age 50 in our society. Extrapolation from the present epidemiological studies predicts that up to 40% of the population could be affected in the year 2030. An understanding of the cause of the metabolic derangement that occurs obesity and features of obesity/overweight and insulin resistance also provided a significant risk for developing type 2 diabetes therefore, an understanding of the pathogenesis and through it, a multidisciplinary approach to allow effective treatment and prevention strategies to be formulated. Primary treatment of the metabolic syndrome is lifestyle therapy-weight loss, increased physical activity, diet and also involves patient-education and intervention at various levels. But as the condition progresses, drug therapies and natural remedies directed toward the individual risk factors might be required. Ultimately, it might be possible to develop drugs that will simultaneously modify all of the risk factors. Further this review sought to investigate the relationship between various markers of metabolic syndrome and therapeutic strategy in light of their emerging role in future.

Introduction:

The metabolic syndrome (MS), also known as Syndrome X, is characterized by obesity, altered glucose homeostasis with insulin resistance (IR), Reaven's syndrome, dyslipidemia, hypertension, and prothrombotic and proinflammatory state, increases the risk of cardiovascular morbidity and mortality. Rapid globalization & industrialization occurring in developing countries has resulted in considerable increase in life style related diseases. Indians are known to have a higher probability of suffering from increased central adiposity, an imbalanced lipid profile and hyperinsulinemia. Lipotoxicity of insulin –dependent tissue and ectopic fat depots are emerging as fundamental processes in the pathogenesis of MS. Lifestyle intervention, such as increased physical activity, show great promise as agents for disrupting the disease progression and may act via direct or in direct mechanisms on the underlying pathology of MS. Evidence from several studies suggests that there is an inverse association between prevalent metabolic syndrome and the following: household income/wealth, educational level/achievement and socio-economic status [1].

Diagnostic criteria for MS

Many international organizations and expert groups have attempted to incorporate all the different parameters used to define MS as shown in Table [2].

WHO	NCEP:ATPI II	IDF	AHA/NHLBI	Consensus definition
Impaired glucose tolerance, T2DM, or IR	Fasting plasma glucose110-125 mg/dl	Fasting plasma glucose 100 mg/dl or greater.	Fasting plasma glucose 100 mg/dl or greater.	Fasting plasma glucose 100 mg/dl or greater
BMI>30 ,waist hip ratio >0.90 (men), 0.85 (women)	Waist circumferene >40 (men), >35 in (women)	Waist circumferene if BMI > 30 kg/m2	Waist circumference 102 cm or greater in men, 88 cm or greater in women.	Elevated waist circumference
TG>150 mg/dl, HDL<35 (men), <39(women)	TG>150 mg/dl, HDL<40 (men), <50(women)	TG>150 mg/dl, HDL<40 (men), <50(women)	TG>150 mg/dl HDL<40 (men), <50 (women)	TG>150 mg/dl HDL<40 (men), <50 (women)
Hypertension: medication or BP >160/90 mm Hg Microalbuminuria>20µg/min	Blood pressure >130/85 mm Hg	Blood pressure >130/85 mm Hg	Blood pressure >130/85 mmHg	Blood pressure >130/85 mm Hg

Pathophysiology of MS

Most studies show that the MS is associated with an approximate doubling of cardiovascular disease risk and a 5-fold increased risk for incident T2DM. The pathogenesis of MS is multi-factorial that major underlying causes were obesity and IR. Atherogenic dyslipidemia includes elevation of lipoproteins containing apolipoprotein mB, elevated triglycerides, and increased small particles of low density lipoprotein (LDL) and low levels of high density lipoproteins (HDL). Several other factors exacerbate the syndrome: physical inactivity, advancing age, endocrinal dysfunction and genetic aberrations affecting individual risk factors [3].

Genetic basis of MS

The etiology of MS involves environmental influence, genetic predisposition and the genetic interaction among various genes. A proper understanding of the interplay between genetic factors and

biochemical/environmental factors is required in order to prevent or reverse the different components of MS. Two major approaches have been used in studies investigating genetic factors and MS, namely, the genome wide approach and the candidate gene association approaches.

Clinical features of MS

MS is a cluster of diseases which develops in various stages of disease. The MS is characterized by a proinflammatory, IR, dyslipidemic proatherogenic state that arises from interaction of overeating, sedentary life styles a genetic predisposition to visceral adiposity.

Correlation among the risk factors of MS

As MS is a combination of disorder so it is very important to understand the relationship among these factors, which are discussed here [4].

Link between obesity and dyslipidemia

The changes in lipid metabolism seen with abdominal fat accumulation have been well characterized and include hypertriglyceridemia, reduced HDL cholesterol, and increased numbers of small, dense LDL particles. The hypertriglyceridemia seen with abdominal obesity and insulin resistance is related to the over secretion of triglyceride-rich very low density lipoprotein (VLDL) particles. An increased rate of hepatic FFA uptake stimulates the secretion of apo B-100, leading to increased numbers of apo B-containing particles and possibly hypertriglyceridemia.

Link between obesity and hypertension

Obesity is a major risk factor for the development of atherosclerotic cardiovascular disease. More recently, it has become evident that obesity is invariably accompanied by a significant decrease in plasma adiponectin levels and that adiponectin has many defensive properties against obesity-related diseases, such as hypertension. The effect of obesity on vascular function may be mediated by the hormone leptin has been shown to have angiogenic activity, increase oxidative stress in endothelial cells, and to promote vascular cell calcification and smooth muscle cell proliferation and migration.

Link between obesity and IR

IR is strongly associated with obesity and physical inactivity, and several mechanisms mediating this interaction have been identified. A number of circulating hormones, cytokines, and metabolic fuels, such as non-esterified FFAs originate in the adipocyte and modulate insulin action. An increased mass of stored triglyceride, especially in visceral or deep subcutaneous adipose depots, leads to large adipocytes that are themselves resistant to the ability of insulin to suppress lipolysis.

Link between obesity and inflammation

Interleukin 6, approximately 30% of which is produced by adipose tissue, not only predisposes to insulin resistance but enhances the hepatic production of acute phase proteins, such as C-reactive protein or fibrinogen. These factors in turn may directly contribute to atherosclerotic plaque progression and rupture. Therefore, inflammation within white adipose tissue may be a crucial step contributing to the emergence of many of the pathologic features that characterize the MS and result in diabetes and atherosclerosis.

Preventions and treatments of MS

There are a number of treatment options including drug therapy, psychotherapy, surgery, lifestyle changes and herbal and homeopathic remedies and it is important that you weigh up each option before making a decision [5].

Pharmacological therapies

The idea of reducing multiple risk factors with a single drug or a combination is attractive and needed. At present, the only drugs approved for treatment are those that target the individual risk factors. New pharmacological strategies include dual PPAR alpha/gamma agonists, drugs with pleiotropic effects or combination therapies. Diets high in fats tend to promote obesity; hence inhibition of digestion and absorption of dietary fats is a logical remedy in treating obesity. A proliferation of high-cost, anti-obesity products is in the market. However, they exhibit side effects, such as gastrointestinal and kidney problems and only orlistat and sibutramine can be used long-term, in spite of issues regarding weight loss and tolerance.

Surgeries

Another method becoming more common is bariatric surgical treatment, or gastric bypass. This surgery should be restricted to those patients with a body mass index (BMI) ≥40 or ≥35 combined with significant co-morbidities. This extreme method of weight loss has the greatest long term success, with at least 80% of patients maintaining a body. Although bariatric surgery can be used safely in appropriately selected individuals, the procedure contributes to higher morbidity and mortality in the elderly, and not all elderly individuals qualify for or desire surgical treatment.

Natural therapies

With more and more people rejecting chemical drugs due to the fear of side effects, natural-based products are fast gaining popularity, thus justifying extensive research in this field. Natural ingredients and medicinal plant preparations may enhance satiety, boost metabolism, and speed up weight loss. Including Despite the global market for satiety, fat burning, dietary supplements and other weight management remedies, patient awareness of these products is insufficient.

Therapeutic life style changes

Following lifestyle changes will reduce your risk of heart disease, diabetes and stroke: Lifestyle therapies will dampen the syndrome and slow its progression at every stage but particularly in its early phases. Other non-pharmacologic methods for losing weight include hypnosis, acupuncture and support groups like Overeaters Anonymous.

Hypnotherapy

In the past 30 years, several investigations have piloted the addition of hypnotherapy to cognitive behavioral therapy for weight loss. A meta-analysis revealed an average weight loss of six pounds with the addition of hypnotherapy and an increased effect over time.

Acupuncture therapy

More studies have been performed on acupuncture as a treatment for obesity than any other therapy. Design flaws, however, prevent determination of acupuncture's efficacy for weight loss.

Conclusion:

The multiplicity of effectors operative in the pathophysiology of MS has shaped pharmacological strategies that are representative of the new way of pharmacological management of polygenic disease, therapeutics should be based on global risk assessment and should follow current treatment guidelines for each of the risk factors. This review of the studies on accessible multidisciplinary approaches for the treatment of obesity is provided, which attempts to improving knowledge on the use of herbal preparations, and encouraging obese patients to consume them along with an enhanced exercise regimen and a healthy diet should be continued.Finally, the MS is an evolving concept that continues to be data driven and evidence based with revisions forthcoming.

References:

[1] Despres J (2008) Arterioscler Thromb Vasc Biol 28(6):1039-49

[2] Ford ES (2010) Diabetes 2(3):180-93.

[3] Reilly PM (2004) Disease Mechanisms 1(2):187-94.

[4] Srikanthan K (2016) Int. J. Med. Sci. 13: 1-5.

[5] Christodoulos SF (2005) TRENDS Endocrinol Metabol 16(6): 254-60.

PD-50

Current Scenario of Pharmacovigilance

Arzoo Khan, Shiv Hardenia, Neelam Balekar, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Indore (M.P)
E-mail address: vishzoozoo@gmail.com

Abstract: Present article focuses on strategies and current scenario of pharmacovigilance sector in India. It comprises main extract from such sector is adverse drug reactions which describes harm associated with the use of given medications at normal dose.India is the fourth largest producer of pharmaceuticals in the world. It is emerging as an important Clinical trial hub in the world. Many new drugs are being introduced in our country. Hence present article gives explanation about need for a vibrant pharmacovigilance system in the country to protect the population from the potential harm that may be caused by some of new drugs. Now days in India, pharmacovigilance situation has been progressing step by step as what it was in the past. The office of the Drugs Controller General of India has been attempted to implement a pharmacovigilance program in India along with its training modules. Most of pharmaceutical companies stab to regulate and implement an effective system of reporting adverse events of drugs introduced in the Indian market with newly beginning of dedicated parmacovigilance department. This review is aimed to offer a study about necessity of implementation of pharmacovigilance for solving current problems and strategies for upliftment in standards up to the level of developed countries.

Introduction:

The word pharmacovigilance has derived from the Greek word pharmacon means “drug” and the Latin word vigilare means “to keep awake or alert, to keep watch.” Pharmacovigilance is the pharmacological science relating to the detection, assessment, understanding and prevention of adverse effects, particularly long term and short term side effects of medicines. Recently, the concerns of pharmacovigilance have been widened to include herbal, traditional and complementary medicines, blood products, biological, medical devices and vaccines. Generally speaking, pharmacovigilance is the science of collecting, monitoring, researching, assessing and evaluating information from healthcare providers and patients on the adverse effects of medications, biological products, herbalism and traditional medicines with a view to identifying new information about hazards associated with medicines and preventing harm to patients .Therefore pharmacovigilance deals with not only adverse effect of drug but also it deals with polypharmacy, iatrogenesis, paradoxical reaction and serious adverse event of a drug. Substandard medicines, medication errors, lack of efficacy, use of medicines for indication that are not approved and for which there is inadequate scientific basis, case reports of acute and chronic poisoning, assessment of medicine related mortality abuse and misuse of medicines, and adverse interaction of medicines with chemicals, other medicines and foods and drinks. Pharmacovigilance is an important and integral part of clinical research. Both clinical trials

safety and post marketing pharmacovigilance are critical throughout the product life cycle. With a number of recent high-profile drug withdrawals, the pharmaceutical industry and regulatory agencies have raised the bar. The review will give insight on this important issue to the decision maker for marketing of new drug in India i.e. post marketing surveillance studies and proper precautions for that, and as well as it might be used for the educational material to the teacher and student who would like to know details about this important topic[1].

Organizations of Pharmacovigilance

1. The world health organization (WHO)
2. The International Council for Harmonisation (ICH)
3. The Council for International Organizations of Medical Science (CIOMS)
4. The International Society of Pharmacovigilance (ISoP)
5. The Central Drugs Standard Control Organization:

Present scenario of pharmacovigilance The position of the pharmacist within the health care system has continually been subject to discussion and change. The pharmacist‟s primary mission traditionally has been to dispense drugs as prescribed by a physician and to ensure that these drugs meet the required standards. Nowadays the pharmacist also frequently acts as a consultant on pharmacotherapy. In the United Kingdom and United States, pharmacists are, to a degree, also authorized to write out prescriptions, which incidentally has been a long-standing practice in many countries where doctors are in short supply.Whereas initially the pharmacist‟s role focused on the chemical aspects and raw materials of drugs, the local production of medicines, and the dispensing role, today it has shifted more toward reducing the prevalence of ADRs and drug-drug interactions, and providing information and instruction about appropriate drug use. The pharmacist has become a consultant on drug therapy, both for physicians and patients. In the Netherland a legislative bill is being prepared to award the pharmacist the official status of co-consultant, thus making him jointly responsible for pharmacotherapy. The term pharmaceutical care is often used to describe the more comprehensive interpretation of the occupation, although the term is given different meanings and often thought to be too vague . The role pharmacists play or are given to play also depends on the circumstances in which they exercise their profession. Nevertheless, the fundamental role of the pharmacist will always be to ensure that medicines are used safely. The literature mentions several other ways pharmacists can contribute to the safe use of drugs. In addition to their responsibilities relating to drug dispensing and compliance, pharmacists can play a prominent role in areas such as record keeping, education, and monitoring over the counter products and alternative therapies and because computerized dispensing drug systems are becoming more prevalent, the pharmacist‟s role is becoming more important, both as a user and in his/her capacity of system manager. The reporting of ADRs is one of the roles pharmacists could have [2].

Table 1: Some Pharmacovigilance companies in India [3]

S.No	Location	Company name	Company website
1	Hyderabad	Paraxel	https://www.parexel.com/
		Vimta labs	http://www.vimta.com/
		Sristek	http://www.sristek.com/
		Srikrishna pharma	http://www.srikrishnapharma.com/
2	Delhi	Mankind	https://www.mankindpharma.com/
		Wipro	http://www.wipro.com/documents/pharmcovigilance-at-wipro.pdf
		Synogen	http://www.synogen.com/
		Apcer, delhi	http://www.apcerpharma.com/
3	Ahmedabad	Inc research	https://www.incresearch.com/
		Lambda	http://www.lambda-cro.com/
4	Bengaluru	Biocad	http://biocadglobal.com/contacts/?Country=india
		Accenture	http://www.accenture.com/

Table 2: Some Training Institutes for Pharmacovigilance in India [4]

S.No.	Location	Training Institutes	contact	Website
1.	Ahemdabad	Epsilon Electronics	9879626181	www.epsilonelectronics.in
2.	Banglore	ClipLab	080-67475801 9886523338	http://cliplab.co.in/
		CSIR IIT	08048142752 9035536222	www.csiriit.com
3.	Chandigarh	Academy of clinical intelligence (ACI)	01724663037 9988756469	http://www.acitrainings.com
4.	Delhi	Kapman life science academy	9205337935 9729637935	www.kapmanacademy.co.in
		MGITI	01127393756	http://www.mgiti.org
5.	Pune	Clip lab	02067090270	www.cliplab.co.in
		MC Square Education Pvt.Ltd.	02032405933 9767360330	www.mc2academy.com
6.	Jaipur	Panacea Biosciences	09268467206	www.panaceabiosciences.com
		VPM Classes	9001252666	www.vpmclasses.com

Conclusion:

The PV in India has become an important public health issue as regulators, drug manufacturers, consumers, and healthcare professionals are faced with a number of challenges. The PV in India

continues to grow, evolve, and improve. India is the largest producer of pharmaceuticals and now emerging as an important clinical trial hub in the world. Apparently, the requirements for professional specialization, a combined view on PGx and clinical requirements are needed. That helps to identify factors that increase the risk of unwanted outcomes from drug therapy and prior to commencing drug treatment and in tailoring drug treatment for individual patients. The PV has also involved in Data Mining Technology in spontaneous reports submit to the national surveillance systems. Furthermore, it is responsible in India of entire campaign to improve PV knowledge and increase the number of ADRs reports up to the gold standard level established by the WHO [5].

References:

[1] Pirmohamed M, James S, Meakin S, Green C, Scott AK & Walley TJ (2004) BMJ 329:15–9.

[2] Pharmawisdom (2017). http://www.pharmawisdom.com/Basic-Pharmacovogilance.htm.

[3] http://pharmapathway.com/list of-pharmacovigilance-companies-in-india.

[4] http://www.yet5.com/institutes/courses/1103/1/pharmacovigilance-training-centres.html.

[5] WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems, World Health Organization, Geneva, 2004.

PD-57

Rare Diseases and its Diagnosis

Etisha Parikh, Kuldeep Vinchurkar, Neelam Balekar,
IPS Academy College of Pharmacy, Indore (452001)
E-mail address: etishaparikh@gmail.com

Abstract: Rare diseases are a group of conditions which affect very small numbers of people. Definitions vary although generally a disease is considered rare if it affects fewer than 1-5 individuals per 10,000 in the general population. Most people suffering from rare diseases do not have any treatment options available for their condition. Yet many countries do not have plans, policies or legislation to help lead research on rare diseases and improve the management of these many conditions. Awareness and understanding of rare diseases is often low, and many patients struggle to find adequate information about their condition. As a result, upon diagnosis, patients may feel isolated, overwhelmed and unsupported. Traditionally, research and development of improvement in our understanding of treatments for rare diseases has been neglected in favour of more common diseases. However, in the last twenty years, concerted public policy efforts have led to and availability of effective treatment options.

Introduction:

Terminology concerning, orphan, rare and neglected diseases has evolved when the previous background report was published. In the 1980s and 1990s particularly in the USA, the term “orphan diseases” was commonly used to designate diseases that because they were only affecting a small population, saw no investments to find a diagnosis and a cure. Recognizing these facts led to ground breaking legislation [1]. In the late 1990s the term “neglected diseases” was promoted which referred to tropical infectious diseases that existed in substantial numbers in remote poor areas of low income countries and hence suffered from the same lack of investments. These diseases are the subject of another background paper and are not addressed in this background paper. Rarity is the key concept on which the orphan diseases definition rests, rarity either in terms of absolute numbers of patients in the USA or in rates of prevalence in Europe and other countries [2].

Examples of rare diseases

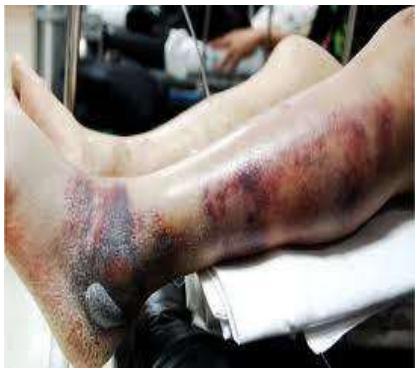
Some rare diseases are actually known to the general public. Examples of the best known rare diseases may be

- Cystic fibrosis,
- Sarcoidosis,
- Haemophilia,
- Phenylketonuria (PKU)

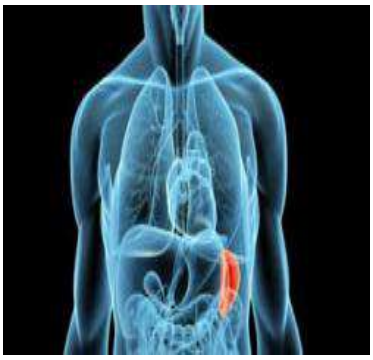
- Severe acute respiratory syndrome (SARS).

Mowat-Wilson syndrome

Necrotizing fasciitis

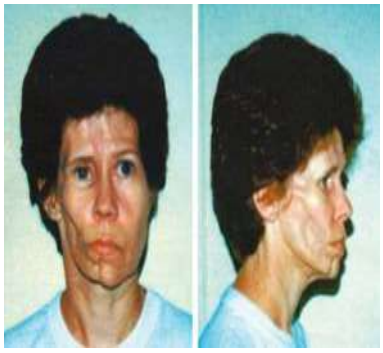


Mowat-Wilson syndrome



Wolman disease

Necrotizing fasciitis



Parry –Romberg syndrome

Alien- hand syndrome

Argyria

Figure 1: Some rare diseases

List of 10 rare diseases affecting children

- Batten disease
- Duchenne muscular dystrophy
- Candle syndrome
- Childhood interstitial lumd disease
- Ehlers-danlos syndrome
- Elis van creveld syndrome

- Gaucher disease
- Krabbe disesase
- Neuroblastoma

Causes of rare disease

Individuals and families struggle with these questions as they try their best to grasp the meaning and impact of a rare disease diagnosis. In the past two decades, epidemiologic, molecular, and other research that takes advantage of scientific and technological advances in the biological sciences has greatly increased the number of rare diseases that have an identified causes usually, although not invariably, genetic. The Orphan Drug Act, the Rare Diseases Act, and other policy initiatives discussed in this report have contributed to this knowledge by focusing attention, resources, and incentives on the study of rare conditions and products to treat them [3].

Genetic Causes: Notwithstanding the imprecision in the count of rare diseases and the difficulty of characterizing thousands of conditions, experts on rare diseases generally agree that the great majority of rare diseases perhaps 80 percent or more are genetic in origin. Many if not most are caused by defects in a single gene, for example, alpha1-antitrypsin deficiency (which may cause serious lung or liver disease) and Friedreich's ataxia (a neurological disorder that may also be accompanied by cardiac and other problems).

Infectious Agents: A number of rare diseases have infectious causes. Despite their rarity, some infections such as rabies, botulism, and Rocky Mountain spotted fever are relatively well publicized and feared. Others are truly obscure, for example, Naegleria fowleri. Newspapers and medical journals occasionally highlight cases of extremely rare infections such as Lemierre's syndrome, an often lethal disease (caused by Fusobacterium necrophorum) that was so nearly eliminated by the advent of antibiotics that it has been termed the “forgotten diseases [4].

Toxic Agents: Some rare diseases or conditions result from exposure to natural or manufactured toxic substances, including substances that appear as product contaminants. In the United States, examples include arsenic and mercury poisoning, mesothelioma (a cancer caused by exposure to asbestos), and eosinophilia-myalgia syndrome, which is associated with contaminated (or overused) tryptophan, a dietary supplement.

Other Causes: Rare conditions may have a variety of other causes. Examples include conditions caused by nutritional deficiencies (e.g., beriberi, which results from thiamine deficiency and is rare in the United States and injuries (e.g., commotio cordis, in which ventricular fibrillation and sudden death is associated with a nonpenetrating blow to the chest) [5].

Diagnosis: People with rare diseases can face an uphill battle to reach a diagnosis. In one survey conducted by Eurodis, a rare diseases organization in Europe, 25% of patients with 1 of 8 surveyed

rare diseases had to wait 5 to 30 years from the time of earliest symptoms to a confirmed diagnosis. Further, the first diagnosis received by 40% of patients turned out to be wrong. Speak up and ask questions- If you experience new symptoms that defy explanation, do not hesitate to bring up your concerns to your doctor. Even if your family doctor cannot determine a diagnosis, you may be referred to a specialist who can narrow down – or broaden – the search for answers [3].

Be organized- Your description of symptoms is only the beginning. Once a doctor has an idea of what might be the cause of your symptoms, diagnostic tests will likely be done. Ask for and keep copies of any diagnostic test that is done. With Gaucher disease, for instance, simple blood tests and genetic analysis may be all it takes to make the diagnosis. Same goes for any other paperwork or prescriptions you receive. Start a file for all materials related to your pursuit of a diagnosis

Be aware- Keep track of not just your paperwork, but also how your condition progresses. Record each symptom you note, including the time and date when it happens, a ranking from 1 to 10 of how severe it seems, as well as any recent treatments, medications, or other potentially relevant information (e.g., stress level, life changes) [1].

Search smart-Use your symptoms as search terms, including those symptoms that may seem unrelated to one another. The internet is a powerful tool overflowing with information not all of it perfect or credible, but it could take you a step in the right direction if you know where to look. The National Organization for Rare Disorders (NORD) and the Canadian Organization for Rare Disorders (CORD) are great places to start.

Accept support-A trusted friend or family member who escorts you to doctor visits can provide moral and emotional support and can also help you to remember and understand all of the new information you might be receiving [5].

Conclusion:

The most important ones that should continue to be supported are:

- Networks of excellence that focus on research infrastructure (e.g. registries) as well as provision of disease-related information at beyond (guidelines, diagnosis, patient experience)
- Initiatives that focus on rare disease classification
- Fundamental research into the disease process to increase rare disease understanding.
- Incentives for development of therapeutics (e.g. clinical trial-funding programmes).
- Assessment methods adapated to small and very small patient populations (e.g. marketing authorisation and reimbursement).

In addition, more support is needed for:

- Translational research to increase translation of disease understanding into drug development or healthcare innovation (e.g. NIH bench to bed grants).

- Innovative diagnostic methods of rare diseases to enable early intervention.
- Research, infrastructure as well as implementing guidelines for medical and psychosocial care for rare diseases. This would be especially beneficial for those patients for whom underlying treatment is not yet available.
- Incentives for development of preventive strategies and validated diagnostic Techniques.
- Incentives to leverage existing knowledge and optimize the use of existing drugs (innovative drug delivery systems and drug repurposing).

Reference:

[1] Rare Diseases. Siope. Eu. 2009-06-09. Archived from the original on 3 December 2012. Retrieved 2012-09-24.

[2] Rare List. Global Genes. April 15, 2016. Retrieved April 15, 2016.

[3] Rare Disease Act of 2002

[4] Rare diseases: what are we talking about?

[5] Useful Information on Rare Diseases from an EU Perspective (PDF). European Commission. Retrieved 19 May 2009.

PD-65

LASA DRUGS

Vikas Patel, Rashmi Arora, Shubhrika Chandwani
Sri Aurobindo Institute of Pharmacy, Ujjain Road, Indore.
E-mail address: vikaspatel4182@gmail.com

Abstract: The existence of look-alike/sound-alike drug names is one of the most common causes of medication error and is of concern worldwide. As more medicines and new brands are being marketed in addition to the thousands already available, medication names may look or sound alike. Thus, the potential for error due to confusing drug names is very high. When patients take multiple prescription medications & receive care from different health care providers, medication history information may be less reliable and more difficult to verify. The problem of Look-Alike/Sound-Alike drug names has become a significant challenge to pharmacists, pharmacy technicians & patients.

Introduction:

Looks alike & sounds alike drugs can be easily confused, particularly if you are in a hurry and the prescription is badly written. No drug name is without problems. Any name can be written or spoken poorly enough so that it can be mistaken for another. Getting it wrong could mean that your patient's do not receive the treatment they need, and even that they develop problems that they did not have before. Being a Pharmacist it's our own duty to resolve this type of problems.

Overview of Literature

The existence of confusing drug names is one of the most common causes of medication error and is of concern worldwide. With tens of thousands of drugs currently on the market, the potential for error due to confusing drug names is significant. This includes brand or trademarked names. Many drug names look or sound like other drug names. Contributing to this confusion are illegible handwriting, incomplete knowledge of drug names, newly available products, similar packaging or labeling, similar clinical use, similar strengths dosage forms, frequency of administration, and the failure of manufacturers and regulatory authorities to recognize the potential for error and to conduct rigorous risk assessments, both for trademarked and brand names, prior to approving new product names.

More than 33,000 trademarked and 8,000 brands medication names were reported in the United States of America in 2004. The Institute for Safe Medication Practices (ISMP) has posted a page listing of medication name pairs actually involved in medication errors. There are many other look-alike, sound-alike (LASA) combinations that could potentially result in medication errors.

Medication error

Medication errors are errors in the process of ordering or delivering a medication, regardless of whether an injury occurred or the potential for injury was present. Some medication errors result in

adverse drug events. A medication error is any preventable event that may cause or lead to inappropriate medication use or harm to a patient.

Definition of medication error: "... any preventable event that may cause or lead to inappropriate medication use or patient harm, while the medication is in the control of the health care professional, patient, or consumer. Suchevents may be related to professional practice, health care products, procedures, and systems including:-prescribing order, communication, product labeling, packaging, dispensing and use".

Medication errors associated with look-alikeand sound-alike drugs

Since 2000 FDA has received more than 95,000 reports of medication errors. Approximately 25% of errors reported to national medication error reporting programs result from confusion with drug names that look or sound alike. Many of these errors were connected with LASA drug names.

Detailing evaluations made between January 1, 2003 and December 31, 2006 of more than 26,000 records from more than 670 healthcare facilities. Among LASA drug errors, 384 (1.4%) had led to harmful patient outcomes. Of these, 64.4% originated at the dispensary, with pharmacy technicians committing the initial error in 39% of cases and pharmacists in 24% of cases.

Strategies to prevent medication errors related to LASA drugs

Many strategies that may help prevent medication errors due to confusion between drug names can be implemented. Identifying look-alike and sound-alike drug pairs used in our facility that are most often involved in errors can be a helpful first step. Then incorporating the following strategies to reduce the risk of errors with those medications may be considered:

Writing orders and prescriptions

A 1979 study estimated that one-third of physicians' handwriting was illegible. Presumably little has changed over the years. To ensure that orders and prescriptions are legible, these may be printed instead of handwriting, may be written in sitting position rather than standing and should be written in a quiet area for writing what safety experts describe as a "sterile cockpit".

Problematic abbreviations

The FDA and ISMP in July 2006 embarked on a joint campaign to eliminate the use of potentially confusing abbreviations, symbols and dose designations in various forms of medical communications. These abbreviations, symbols anddose designations have been proven to be a barrier to effective communication and have resulted in significant harm to patients. For example, instead of writing "QD" which is often misread as QID, it is recommended that health care professionals spell out the word "daily."

Similar drug names

Handwritten medication prescriptions can be difficult to interpret particularly if these involve medications that have similar names such as Isordil – Plendil, Celebrex – Cerebyx, Lamictal – Lamisil, and Zyprexa – Zyrtec – Zantac. Many, if not all, of these drugs with similar names carry different indications for use; therefore, including the indication with the medication can reduce

confusion. Using bold print to clearly distinguish letters which differ on product and storage bins labels with look-alike drug names. This strategy is commonly referred to as "tall manlettering," (e.g., chlor PROMAZINE and chlor PROPAMIDE).

Including the indication

Including a drug's indication on the prescription is a simple safety measure. The indication, whether handwritten or communicated via check boxes, helps pharmacists and others avoid confusion between look-alike drug names. For example, if it is unclear whether a prescription says Celebrex or Cerebyx, a check mark in the "musculoskeletal" box would suggest that Celebrex is the desired drug.

Use of electronic systems

Electronic prescribing systems can produce computer generated prescriptions or can electronically transmit the prescription directly to the pharmacy. These systems (e.g., iScribe, MEDeMORPHUS, Touch Script) not only eliminate illegible handwriting but also can automate screening for allergies, drug-drug interactions, duplication of therapy, etc.22

Labeling and storage

We should follow the safe practices mentioned below for storage and usage of any medications.

Result & Discussion:

In the results of lasa drugs we find that the different –different drugs are with similar brand name, similar in look wise but differ in their concentration.

Table 1: Types of lasa drugs pairs

Preparations	Similar spelling / pronunciation	Similar packaging / labeling	Similar tablets / capsules
Tablets/Capsules	2745	868	1143
Injections	519	540	0
Liquid dosage forms	110	207	0
External use preparations	233	170	0
Chemotherapeutic agent	8	6	1

Figure 1: Similar in looks wise but having different concentration of different-different drugs of different brands and companies

The table of given drugs name having some similarities in name and pronunciation is almost similar but the durgs and concentration are different is given below:

Table 2: Sounds alike drugs.

Generic names	Trade names
Ciprofloxacin Erythromycin	Maprocin Macrocin
Amoxycillin Magaldrate	Mumox Marlox
Azithromycin Ranitidine	Ranzith Rantec
Omeprazole Losertan	Losectil Losardil
Omeprazole Atenolol	Cosec Carsec
Omeprazole Metoprodol	Prolock Preloc

Conclusion:

A cross-sectional survey was developed to study look-alike, sound-alike (LASA) drugs in hospitals in Thailand. This LASA report could be developed and integrated to suitable programs used in hospitals in order to identify and prevent medication errors in the future.

References:

[1] African Journal of Pharmacy and Pharmacology Vol. 7(6), pp. 227-239.

[2] Proceedings Am J Health-Syst Pharm 2367-2379.

[3] Chadwick M. Look-alike sound-alike health product names. Health Canada Workshop, 2003.

[4] Kaufman MB Formulary Watch 1, 2009.

[5] Darker IT, Gerret D, Filik R, Purdy KJ & Gale AG (2009) 7: 2008-2010.

PD-79

Cancer Stem Cells: A Newer Technique to Combating Cancer

Durgesh Nandan Shukla, Ashish Khasdev, Surbhi Jain, V. V. Dhote, Rajesh Pawar
VNS Group of Institution, Faculty of Pharmacy, Bhopal-462044 (M. P.)
E-mail address: dnshukla20@gmail.com

Abstract: A stem cell transplant aims to try and cure some type of blood cancer such as leukemia lymphoma and myeloma. It is also called a peripheral blood stem cell transplant. Providing innovative adult stem cell therapies for neurodegenerative disorders is developing a new technology that uses the patient's own bone marrow to treat diseases such as Parkinson's disease, Amyotrophic Lateral Sclerosis and Spinal Cord Injury. Despite these, use of stem cells in immune-modulation or reconstitution is one of the method used for decades in cancer therapy stem cells have self renewal with high replicative potential in multineage differentiation capacity. Cancer stem cells (CSCs) are cancer cells (tumor or hematological cancer) that possess characteristics associated with normal stem cell type, specifically the ability to give rise to all cell type. CSCs generate tumor through the stem cell that self-renew and differentiate into multiple cell type, such cells are proposed to persist in tumors as a distinct population and cause relapses and metastasis by giving rise into new tumor. The cancer stem cells are now considered as the back bone in the development of the cancer. Their role in carcinogenesis and its implications would bring us a step forward in the development of possible new cancer treatment option in future with NDDS approach.

Introduction:

A cell that has the ability to continuously divide and differentiate (develop) into various other kind(s) of cells/tissues. Cancer arises from the uncontrolled growth and division of cells, and is caused by the cooperation of mutations in DNA that activate genes that push cell division, and suppress natural anticancer mechanisms. Currently in the UK, around 275,000 people per year are diagnosed with cancer and, of these, around 150,000 die. For the 400 people who die each day from the disease, treatment is largely ineffective and offers at best an extension of life, rather than a cure.

Table 1: Types of stem cells

Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells

Kinds of stem cell

Figure 1: Stem cell

Embryonic stem cells

- five to six-day-old embryo
- Tabula rasa

Embryonic germ cells

- derived from the part of a human embryo or fetus that will ultimately produce eggs or sperm (gametes).

Adult stem cells

- undifferentiated cells found among specialized or differentiated cells in a tissue or organ after birth
- appear to have a more restricted ability to produce different cell types and to self-renew.

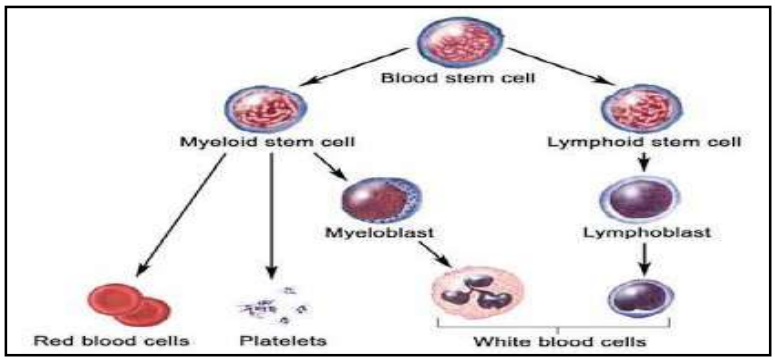


Figure 2: Blood stem cell

Umbilical cord stem cell

1. Also Known as Wharton's Jelly
2. Adult stem cells of infant origin
3. Less invasive than bone marrow
4. Greater compatibility
5. Less expensive

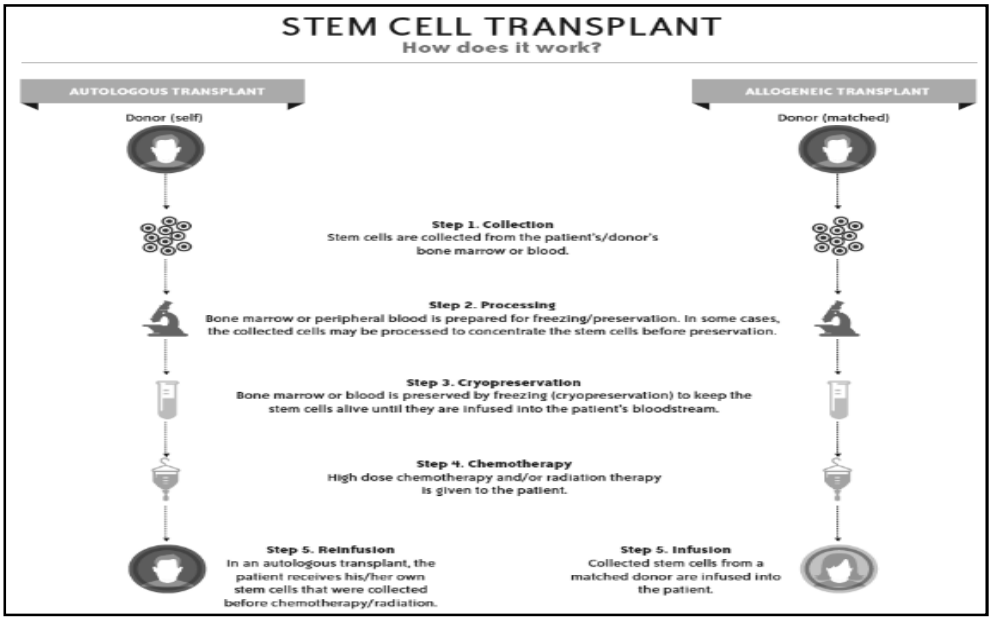
Figure 3: Umbilical cord stem cell

Three important functions:

1. Plasticity: Potential to change into other cell types like nerve cells
2. Homing: To travel to the site of tissue damage
3. Engraftment**:** To unite with other tissues

Stem cell charcterstics

1. 'Blank cells' (unspecialized) Capable of dividing and renewing themselves for long periods of time (proliferation and renewal)
2. Have the potential to give rise to specialized cell types (differentiation)



Stem cell Apllication

1. Stem cells can replace diseased or damaged cells.
2. Stem cells allow us to study development and genetics.
3. Tissue repair & regenerate spinal cord, nerve, heart, muscle, organ, skin
4. Cancers
5. Autoimmune diseases - diabetes, rheumatoid arthritis.

Conclusion:

In conclusion, stem cells and cancer cells share a lot of commonality. However, stem cells are proven be more primitive as compared to cancer and cancer stem cells. Under normal circumstances, stem cells maintain a homeostasis and replenish the adult cell pool while deregulation or imbalances of stem cells can give rise to cancer stem cells and eventually full blown cancer. It is, also important to maintain a well-balanced biology of pluripotent and adult stem cells, which are used for regenerative medicine, from the safety double umbilical cord blood transplantion.

References:

[1] British Society of blood and Marrow transplantation indication for BMT, 2013.

[2] UK Stem Cell Strategic Forum Report NHS Blood and transplant, 2010.

PD-92

NMDA Receptor: The Role in Psychotic Disorders

Priya Jain[1], Qazi Neelam[2]
[1]Swami Vivekanand College of Pharmacy, Indore
[2]Dr. APJ Abdul Kalam University, Indore
E-mail address: itzpriya14@gmail.com

Abstract: In recent year mental health has posed a serious threat all over the world. The neurotransmitter basis for many of the mental disorders has been extensively documented and putative pathways have been identified. In this study we have considered about the role of *N*-methyl-*D*-aspartate (NMDA) receptor involved in neurodegenerative disorders. Neuroreceptor research has focused on the development of several sites of NMDA receptor antagonists, a great diversity of diseases showing a disturbed glutamate neurotransmission has been linked to the NMDA receptor. NMDA-receptor hypofunction (NRH) is also beneficial for the treatment of psychic disorder regarding to NMDA receptor. The study illustrated the role of NMDA receptor blockers which are suggesting a role of sub site selectivity in tapping the therapeutic potential of NMDARs, controlling the neurodegenerative processes as well as their psychotic action are therapeutically beneficial for different CNS disorders.

Introduction:

In recent year mental health has posed a serious threat all over the world. Mental disorders range from mild anxiety states to behavioral changes and thought abnormalities. The causes of mental disorders are varied and complex in nature, and vary from condition to condition. The neurotransmitter basis for many of the mental disorders has been extensively documented and putative pathways have been identified. It plays very important role in the psychiatric disorders. The search for the pathophysiological substrates of mental disorders remains an important challenge for psychiatric research [1]. But majority of work has been done in the field of neurotransmitter. Among the amino acids, L-Glutamate is the major excitatory neurotransmitter in the mammalian CNS, and plays main role in the neuronal communication to neuropathology. It mediates fast excitatory signaling and running the processing in development, plasticity, learning and memory. Due to excessive ionotropic glutamate activation, arises destructive pathways including osmotic and calcium associated neuronal damage [2]. The mediated pathology of glutamate referred to as excitoxicity to and over activation of its receptors has been implicated as contributing mechanism in both acute and chronic CNS injury (e.g. stroke, trauma, hypoglycemia, ischemia, stroke, head trauma, spinal cord injury, and epilepsy has been implicated as contributing mechanism in both acute and

cronic CNS injury [3]. NMDA receptors mediate fast synaptic transmission via their associated calcium channels. The activation of these receptors are blocked by some drugs which are expected to lower the detrimental effects of ischemia by minimizing the extent of glutamate induced brain damage [4].

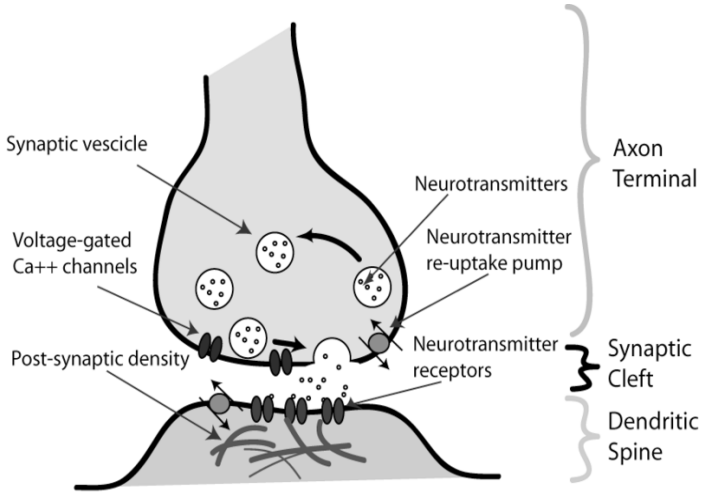


Figure 1: Major elements in a prototypical synapse (synapses allow nerve cells to communicate with one another through axons and dendrites, converting electrical impulses into chemical signals) Courtesy: www.mcatpearls.com/master/node121.html.accessed on 08/06/2007

Most of the known channel blockers as well as the antagonists of the glutamate-binding site provoke strong adverse side effects. These side effects are expected to be less pronounced for modulatory glycine-site antagonists making them a promising target.

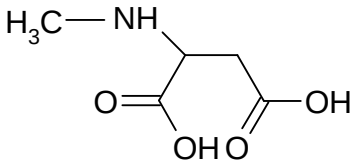


Figure 2: *N*-methyl-*D*-aspartic acid (NMDA)

The NMDA receptor (NMDAR) belonging to a class of postsynaptic ionotropic glutamate receptors performs important functions in the central nervous system. It is involved in neuronal signaling processes, memory consolidation, and synaptic plasticity.A number of different agonists and antagonists have been synthesized for the glutamate binding site. An important feature of the NMDA channel, which is particularly relevant to synaptic plasticity, is its high permeability to calcium, which is a central messenger molecule, and responses that collectively elicit synaptic modification.

Potential therapeutic uses of Glycine site ligands

The role of the glycine site of the NMDA receptor in pain states is very clearly proved by the fact that the concentration of its endogenous antagonist kynurenate is increased by NSAIDs thus mediating a prostaglandin independent antinociceptive effect. Acute nociception is thought to be mediated

principally in the spinal cord. However, they do play a key role in chronic pain states and hyperalgesia, both conditions in which thalamic neurons are involved [5].

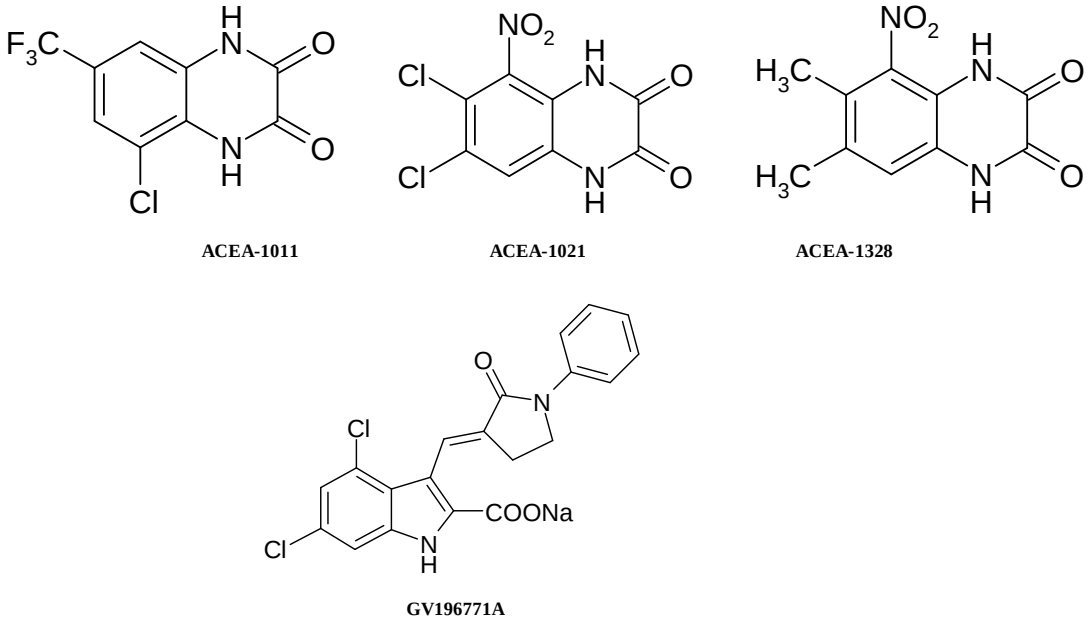
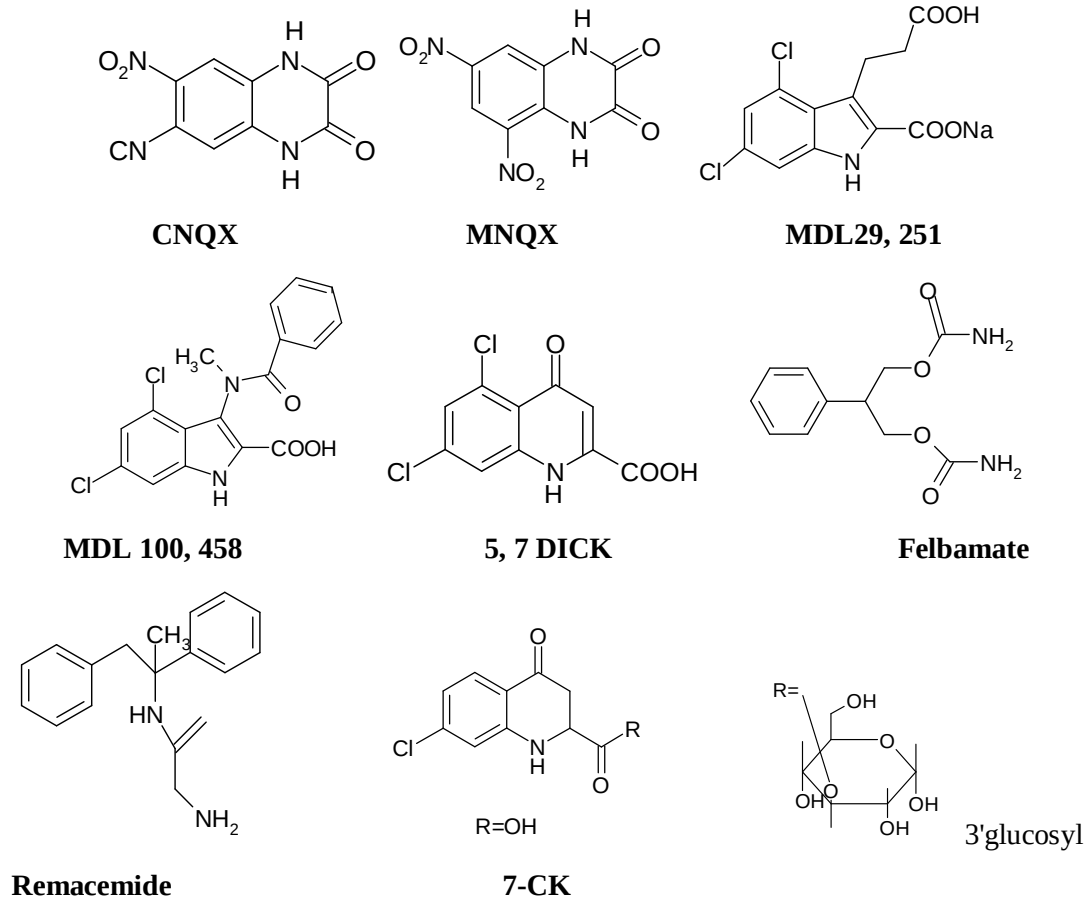
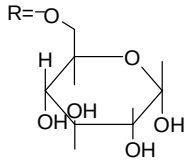


Figure 3: Glycine antagonists evaluated for pain treatment.

Epilepsy is a very common disorder of the CNS, which affects up to 1% of the world population. The disease is chronic and often progressive. The NMDA-receptor is deeply involved in the initiation or spread of epileptic neuronal hyperactivity.

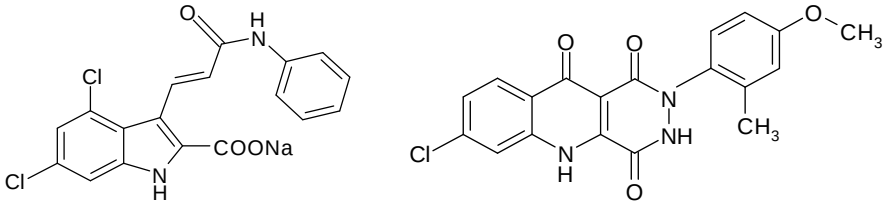




6'glucosyl

Figure 4: NMDA Antagonists investigated as anticonvulsants

Stroke means rupture or clogging of a blood vessel, which supplies the brain with nutrients and oxygen. Hypoglycemia and hypoxia produce an energy failure, which leads to excessive stimulation of the excitatory glutamatergic system. Consequently, NMDA-receptors become highly activated, so that the result is a massive calcium influx into the cells.



GV150526 **ZD9376**

Figure 5: Glycine site antagonists tested in stroke treatment

Conclusion:

N-Methyl-D-aspartate (NMDA) receptor channels play important roles in various physiological functions such as synaptic plasticity and synapse formation underlying memory, learning and formation of neural networks during development. They are also important for a variety of pathological states including acute and chronic neurological disorders, psychiatric disorders, and neuropathic pain syndromes resulting from acute excitotoxic insults (eg, ischaemic stroke, traumatic brain injury), diseases due to chronic neurodegeneration (eg, Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis), disorders arising from sensitization of neurons (eg, epilepsy, neuropathic pain), and neuro developmental disorders associated with NMDAR hypofunction (eg, schizophrenia). NMDA receptor antagonists have therapeutic potential in numerous CNS disorders ranging from acute neurodegeneration (e.g. stroke and trauma), chronic neurodegeneration (e.g. Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, ALS) to symptomatic treatment.

References:

[1] Peter JU (2015) World Psychiatr 14:116–118.

[2] Choi DW (1995) TINS, 18:58-60.

[3] Leeson PD & Iversen LL (1994) J Med Chem 37: 4053-4067.

[4] Paoletti P & Neyton J (2007) Curr Opin Phamacol 7:39-47.

[5] Guilbaud G, Nei A, Benoist JM, Kayser T & Gautron M (1987) Exp Brain Res 68:311-318.

PD-93

Plants Potentials for Anti Arthritic and Immuno modulators: A Review

Rupesh Pandey[1], Shiv Shankar Shukla[2]
[1]Pacific University, Udaipur
[2]Columbia Institute of Pharmacy, Raipur
E-mail address: rupeshpandey@svcp.ac.in

Abstract: Many allopathic drugs reported to be used for the treatment of inflammatory disorders are of least interest now a days due to their potential side effects and serious adverse effects and as they are found to be highly unsafe for human assistance. Herbals containing anti-inflammatory activity (AIA) are topics of immense interest due to the absence of several problems in them, which are associated with synthetic preparations. The primary objective of this review is to provide a deep overview of the recently explored anti-inflammatory agents belonging to various classes of phytoconstituents like alkaloids, glycosides, terpenoids, steroids, polyphenolic compounds, and also the compounds isolated from plants of marine origin, algae and fungi. Also, it enlists a distended view on potential interactions between herbals and synthetic preparations, related adverse effects and clinical trials done on herbals for exploring their AIA.

Introduction:

Arthritis is inflammation of one or more of your joints. The main symptoms of arthritis are joint pain and stiffness, which typically worsen with age. The most common types of arthritis are osteoarthritis and rheumatoid arthritis. The major complaint by individuals who have arthritis is joint pain. Pain is often a constant and may be localized to the joint affected. The pain from arthritis is due to inflammation that occurs around the joint, damage to the joint from disease, daily wear and tear of joint, muscle strains caused by forceful movements against stiff painful joints and fatigue.

Allopathic medications have been prescribed to alleviate symptoms of this disease which results into associated side effects like heart attack, stroke, stomach ulcers, bleeding from the digestive tract, and kidney damage etc [1]. Hence the use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. The plant, as one of the important sources, still maintains its original place in the treatment of various diseases, including arthritis, with minimum side effects. Considerable studies have been carried out on ethno medicinal plants; however, only few indigenous medicinal plants have attracted the interest of scientists, to investigate them as a remedy for arthritis.

Role of Immunostimulants or immunomodulators

Drugs which enhance immune response can be used for the prevention or cure of some infective conditions and also in the management of arthritis [2].

Immune Mechanism Basically there are two different types of lymphoid cells, T and B cells which mediate ‘cellular’ and ‘serologic’ or ‘humoral’ immunity, respectively. Both these types of cells are present in the circulating blood and in peripheral lymphoid tissues [3]. The recognition of the antigen

by the T cells leads to proliferation of these cells, infiltration of immune cells at the site of action and cellular immunity.These reactions may be manifested as Delayed type Hypersensitivity, tissue graft rejection [3]. The other limb of immune system involving B cells is responsible for the genesis of specific antibodies immunoglobulins (IgA, IgD, IgE, and IgM). The recognition of antigen (Ag) by the B cells leads to proliferation of these cells, conversion to plasma cells and generation of specific antibodies (Ab). The specific Ab binds with the specific antigen leading to its inactivation or even phagocytosis. Phagocytic activity by nitroblue tetrazolium dye reduction assay, lymphocyte proliferation by mitogen.

Table 1: List of herbal sources of anti-arthritic activity

Sr. No	Botanical name Family Part used	Family	Parts and Extract used	Chemical constituent	Model used
1.	*Abrus precatorius Linn*	*Leguminosae*	Red and White Seeds Ethanol	flavones luteolin, abrectorin, orientin, isoorientin and desmethoxycentaureidin 7-0-rutinoside	FCA
2.	*Asystasia dalzelliana*	*Acanthaceae*	Leaves Ethanolic	Steroids, flavanoid, alkaloids, tannins	FCA
3.	*Aristolochia bracteata*	*Aristolochiaceae*	Whole plant Petroleum ether, chloroform and methanol	Aristrolochic acid, alkaloids, flavanoids.	FCA
4.	*Butea monosperma. L*	*Fabaceae*	Whole plant, Petroleum ether	Gallic acid, pyrocatechin	FCA
5.	*Bauhinia variegate*	*Caesalpiniaceae*	Stem Ethanol	flavanoid, alkaloids	CFA
6.	*Borassus flabellifer L*	*Arecaceae*	Male flowers (inflorescences) Ethanolic	Steroids, saponins, borassosides.	FCA
7.	*Capparis spinosa L*	*Capparidaceae*	Fruit Ethanol:Water	Flazin, Guanosine, Capparine,	AIA
8.	*Capparis erythrocarpos*	*Capparaceae*	Roots Ethanolic	Proteins, poly phenols	FCA
9.	*Cassia uniflora Mill*	*Caesalpiniaceae*	Leaves Petroleum ether, ethyl acetate, methanolic	Proteins, poly phenols, alpha galactosidase	CFA
10.	*Cissampelos*	*Menispermaceae*	Roots Aqueous	Alkaloids, flavanoids	CFA

	pareira		ethanolic		
11.	*Cleome gynandra L*	*Capparidaceae*	Leaves Ethanolic	Alkaloids, carotinoids, flavanoids, phytates, saponins,tannins	AIA
12.	*Cocculus hirsutus*	*Menispermaceae*	Leaves Ethanolic	Alkaloids, carotinoids, flavanoids, phytates, saponins,tannins	FCA, FIA
13.	*Costus speciosus*	*Costaceae*	Aerial part Methanolic	Diosgenin, succinic acid	FCA
14.	*Elaeocarpus sphaericus*	*Elaeocarpaceae*	Fruit Ethanolic	Alkaloids, carbohydrates, glycosides	FCA
15.	*Ficus bengalensis*	*Moraceae*	Stem bark Methanolic	Alkaloids, glycosides	FCA, FIA, AIA
16.	*Glycosmis pentaphylla*	*Rutaceae*	Stem bark Ethanolic	Alkaloids, flavanoids	FCA
17.	*Glycyrrhiza glabra*	*Leguminosae*	Rhizomes Methanolic	Alkaloids, glycosides	CFA
18.	*Harpagophytum procumbens*	*Pedaliaceae*	Roots Ethanol	Alkaloids, glycosides	FCA
19.	*Hybanthus enneaspermus*	*Violaceae*	Whole plant Alcoholic and aqueous	Alkaloids, carbohydrates, glycosides,	FCA
20.	*Justicia gendarussa Burm F*	*Acanthaceae*	Leaves Ethanolic	Alkaloids, glycosides	FCA, CIA
21.	*Lawsonia innermis*	*Lythraceae*	Leaves Hydroalcoholic	Alkaloids, carbohydrates, glycosides, phytosterols, Saponins, tannins, proteins, flavanoids	FCA, FIA
22.	*Merremia emarginata Burm.F*	*Convolvulaceae*	Whole plant Ethanolic	Alkaloids, carbohydrates, glycosides,	FCA

FCA- Freund's Complete Adjuvant induced arthritis, AIA- Adjuvant induced arthritis, CIA- Collagen induced arthritis, FIA- Formalin induced arthritis, AIA- Agar induced arthritis, CFA- Complete Freund's Adjuvant induced arthritis

Chemical Constituent Wide ranges of phytoconstituents were responsible for anti-arthritic activity includes alkaloids, glycosides, tannins, phenolics, anthocyanins, sterols, triterpenoids etc. These

phytoconstituents present in plant exert desired pharmacological effect on body and thus act as natural anti-arthritic agents.

Conclusion:

The proposed research work is thought to be a step towards providing directions for further research for better treatment with least toxicities for arthritis disease.

References:

[1] Baranwal VK, Irchhaiya R & Alok S (2012) Res J Pharmacog Phytochem 1: 1-5.

[2] Saux Nicole Le & Canadian Paediatric Society, Infectious Diseases and Immunization committee biologic response modifiers to decrease inflammation: Focus on infection risks Paediatr child health. 2012.

[3] Nawale R & Poojari S (2013) Res J Pharmaceutical Biol Chem Sci

[4] Sudaroli M & Chatterjee TK (2007) J Med Plants 3:1-7.

[5] Kumar S & Kumar VR (2011) Asian J Pharm Clin Res 1:1-6.

PD-104

PAMPA (Parallel Artificial Membrane Permeability Assay): A Review

Abhishek Naugriaya, Anu Hardenia, Vikas Patel
Sri Aurobindo Insrtitute of Pharmacy, Sanwer road Indore.
E-mail address: abhi7497naugriaya@gmail.com

Abstract: The passive transfer and permeation of drugs across a membrane are important determinants of ADME processes, and are related to the physicochemical properties of drugs. Recently, it has become possible to assess the passive membrane permeation-utilizing artificial membrane technologies that mimic real biological membrane. Biological membranes have a large variation among each organ. The intestinal brush border membrane and the blood–brain barrier membrane are mainly composed of phospholipids. The skin permeation barrier is mainly composed of ceramides, cholesterol, and free fatty acids. These lipids organize a lipophilic permeation barrier for hydrophilic molecules. The Parallel Artificial Membrane Permeability Assay (PAMPA) aims at predicting the passive membrane properties of drugs.

Introduction:

PAMPA is an extension of the black lipid membrane (BLM), in which a planer membrane is constructed by brushing a lipid–organic solvent mixture over a small hole (0.5mm diameter). A serious drawback of BLM for use in drug discovery is that the membrane is extremely fragile**.** PAMPA is a high-throughput methodology, based on a lipid artificial membrane (formed by mixtures of lecithins and an organic solvents), useful to predict passive oral absorption. A multi well micro liter plate is used for the donor and a membrane acceptor compartment is placed on top. The main objective of this assay is to estimate the permeability of compounds passively absorbed by trans cellular transport. This assay allows the throughput of hundreds of compounds per day. One drawback is that it is a system that only allows a simple classification of solutes: for example, low, medium, and high probability of absorption. PAMPA models have demonstrated a high degree of correlation with permeation across a variety of barriers, including Caco-2 cultures and skin.

The PAMPA membrane can be instantly prepared at the time of use, while Caco-2 requires a 3–21 day culture before use. PAMPA enables studies of passive trans cellular permeation without intervention by paracellular and active transports. This simplicity is advantageous for quantitative structure–permeability relationship (QSPR) studies. PAMPA has a synergistic effect when combined with other tools. For example, combination with the in silico paracellular model was found to increase in vivo predictability. Several reports suggested that the combination of PAMPA and Caco-2 could diagnose the participation of active transporters. For this use, PAMPA permeability can be corrected by an in silico paracellular pathway model for the Caco-2 tight junction. If Caco-2 permeability is different to that estimated by PAMPA permeability, the compound undergoes active transport. This diagnosis can trigger further studies to confirm the permeation mechanism. A combination of

PAMPA and a high-throughput solubility assay enables biopharmaceutical classification in early drug discovery. These kinds of in combo approaches are synergistically effective in various drug discovery situations [2].

Principle of the method and expectations

The PAMPA model (Parallel Artificial Membrane Permeability Assay) is an artificial barrier of phospholipids dissolved in an organic solvent. This model allows evaluation of the apparent permeability (Papp) of an NCE through a lipid bilayer.

Contrary to the Caco-2 model, the PAMPA model does not take into account the contribution of active or passive transcellular transport (mechanisms of influx / efflux) via specific transporters and the paracellular transport, occurring when crossing cell membranes and epithelia.

PAMPA allows permeability assessment of a NCE on a wide pH range and therefore a better understanding of its site of absorption along the gastrointestinal tract (stomach, small intestine).

The PAMPA model allows assessment of:

- Contribution of passive diffusion in the absorption of a compound;
- Influence of the pH on the NCE permeability;
- Screening and selection of compounds of interest;
- Comparison of various pharmaceutical formulations (oral absorption efficiency);
- Determination of absorption kinetic parameters.

Material and Methods:

Propranolol, Warfarin and Furosemide, 1% (w/v) lecithin /dodecane, PBS buffer containing 1% DMSO, ACN / MeOH (1:1, v/v) containing 25 ng/ml terfenadine and 50 ng/ml tolbutamide, 0.1% formic acid.

Working solutions of each compound are prepared from 10 mM stock solution in DMSO diluted to a final concentration of 10 µM in PBS buffer (pH 7.4, 1% DMSO).

1% (w/v) lecithin/dodecane is added to the donor side of the Multi Screen Filter Plate, then 10 µM controls or test compound working solution is added. The receiver side of the Multi Screen Filter Plates is filled with PBS buffer containing 1% DMSO. The plates are kept at room temperature for 24 h. Samples are collected from the donor and receiver sides. The donor side's samples are diluted 20-fold with PBS (1% DMSO). All receiver and diluted donor side samples are mixed with ACN/ MeOH (1:1, v/v) containing 25 ng/ml terfenadine and 50 ng/ml tolbutamide as internal standards. Samples are vortexed and then centrifuged at 4 °C. An aliquot of the supernatant is transferred to a 0.65 ml tube for LC-MS/MS analysis.

Each compound is analyzed by reversed phase HPLC using a Kinetex 2.6µ C18 100Å column (3.0 mm X 30 mm, Phenomenex). Mobile phase – Solvent A: water with 0.1% formic acid, solvent B: ACN with 0.1% formic acid.

Data Analysis:

The amount of compound is determined on the basis of the peak area ratio (compound area to IS area) for the two sides.

LogPe is determined using the following equations:

LogPe = Log {C*-Ln(1-[drug]$_{acceptor}$/[drug]$_{equilibrium}$)}

C = V_D*V_A /{(V_D+V_A)*Area*time}

Recovery is calculated as follows:

%Recovery = (Total compound mass in donor and receiver compartments at the end of the incubation / Initial compound mass in the donor compartment) x 100

Chemicals and reagents

Furosemide, ranitidine, carbamazepine, naproxen, Isoformononetin, prunetin, glycitein. equol and nobiletin, Pre-Coated PAMPA Plate System.

The PAMPA plate system warmed for 30 minutes at room temperature, prior to use. In summary, the 96-well filter plate, pre-coated with lipids, was used as permeation acceptor and a 96-welI receiver plate was used as permeation donor. Compound solutions were prepared by diluting 10 mM DMSO stock solutions in ammonium acetate buffer (10 mM, pH 4 & 7), The compounds solutions were added to the wells (300 ^L/well) of the receiver plate and ammonium acetate buffer (10 mM, pH 7) was added to the wells (200 |lL/well) of the pre-coated filter plate. Then the filter plate was coupled with the receiver plate and the plate couple was incubated without agitation at room temperature for 4 hours. IFN permeability was assessed in triplicate at pH 4.0 and 7.4 whereas; the permeability of markers compound was assessed in triplicate at pH 7.4. Naproxen was taken as highly permeability markers and furosemide as low permeability markers. At the end of the incubation, the plates were separated and 150 jjL solutions from each well of both the filter plate and the receiver plate was transferred to glass vial. The concentrations of permeability markers (naproxen and furosemide) were determined in donor and acceptor samples by high-performance liquid chromatography (JJPLC) method whereas the donor and acceptor samples for other compounds were quantified by LC-MS/MS and permeability was calculated using following formula:

Pe (cm/s) = {-ln[l-CA(t)/Ceq]}/[A*(Wp+ 1/Va)*!]

Standardization of Permeability (PAMPA and In-situ) Models Where,

P« = permeability in cm per second

A = filter area (0.3 cm")

Vd = donor well volume (0.3 ml)

Va = acceptor well volume (0.2 ml)

t = incubation time (seconds)

CaI) = Concenti'ation in acceptor well at time t

CD(t) - Concentration in donor well at time t

And Ceq = [CD(t)=^Vo + CA(t)=*=VA]/(VD+VA)

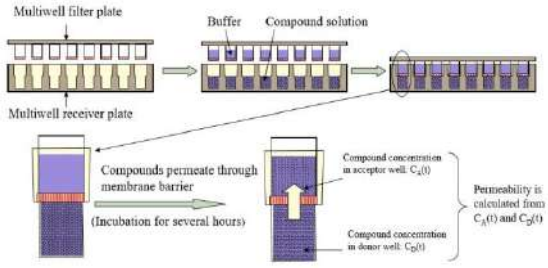


Figure 1: Experimental setup for PAMPA

Furosemide and ranitidine were found to be within acceptable range for low permeability compounds (value less than I.O x 10'* cm/s) while carbamazepine and naproxen was found to be within acceptable range for high permeability compounds (value higher than 1.0 x lO'*'' cm/s). These values prove the reliability of the PAMPA set. All other tested compounds showed high permeability at both the point as shown in Table 1 [4].

Table 1: Permeability of several compounds at different pH

Compound	Permeability (P^ X 10'*) (cm/s)	
	At pH 4.0	At pH 7.0
Carbamazepine	0.1	0.01
Furosemide	0.0	0.0
Ranitidine	5.2	4.1
Naproxen	4.6	5.5
Tamoxifen	6.0	6.1
Centchroman	6.2	13.0
Fonnononetin	11.3	16.5
Isoformononetin	30.05	37.85
Genistein	11.72	10.18
Biochanin A	27.10	42.51
Equol	17.97	15.95
Prunetin	24.77	27.82
Glycitein	23.04	11.83
Nobiletin	17.7	19.5

Conclusion:

This assay was found to be high throughput, low cost, stable and reproducible. PAMPA is potentially useful tool in evaluating the transcellular membrane permeability of a compound. The PAMPA model was standardized proving reproducibility and reliability of the PAMPA plate which can be used later to study permeability of NCEs or marketed drugs. The standardized model can further be used to study permeability of NCEs or drug compounds. PAMPA is often used at various pH values in order to measure the permeability pH profiles, as the permeability of ionizable compounds depends heavily

on the pH of the buffer. Since the pH range of the intestinal tract varies between pH 6 and 8, this is the range of pH values most often used. Kerns recommended to measure in a range from pH 4 to pH 7.4, in order to predict both bases and acids correctly [5].

Reference

[1] Sinko B, Kokosi, J & Avdeef A (2009) Chem Biodivers 6: 1867-1874.

[2] Faller B (2008) Curr Drug Metab 9: 886-92.

[3] Prestes L (2010) Mini Rev Med Chem 10: 1071-6.

[4] Standardization of Permeability (PAMPA and In-situ) Models shobhganga section 7.1.

[5] Bermejo M (2004) Pharm Sci 21**:** 429-441.

PD-105

Review Article on Organ-on-a-Chip with Spatial Heterogeneity Using a 3D-Bioprinting Technology

Shubhrika Chandwani, Rashmi Arora, Abhishek Naugriaya
Sri Aurobindo Insrtitute of Pharmacy, Sanwer road Indore
E-mail address: shubhrika.321@gmail.com

Abstract: Organ-on-a-chip engineering aims to create artificial living organs that mimic the complex and physiological responses of real organs, in order to test drugs by precisely manipulating the cells and their microenvironments. The fabrication methods currently available not only need a secondary cell-seeding process and result in severe protein absorption due to the material used, but also have difficulties in providing various cell types and extracellular matrix (ECM) environments for spatial heterogeneity in the organs-on-chips. Heterotypic cell types and biomaterials were successfully used and positioned at the desired position for various organ-on-a-chip applications, which will promote full mimicry of the natural conditions of the organs [1].

Introduction:

Organs-on-chips have been mainly prepared using several micro engineering methods, such as soft lithography, replica molding, and the micro contact printing technique. These methods also have several drawbacks in the aspect of biological structure 3preparation. For example, poor selectivity of various cell types for spatial heterogeneity and the difficulty of providing multiple types of extracellular matrix (ECM) environments for cell–ECM interactions are the main drawbacks regarding the biological structure preparation. Furthermore, living organisms have complex and organized 2D-to-3D micro scale structures composed of multi-layers, cell types, ECMs, and many other elements which could make the fabrication of organs-on-chips difficult using the current methods. Thus, establishing a novel micro engineering method that can overcome the aforementioned drawbacks is very important [2].

These days, 3D printing is used to produce functional devices in diverse fields, such as tissue scaffolding, prototyping, electronics, sensors and micro fluidic research, because of its capabilities in producing designed, complex micro-architectures. 3D bioprinting is an advanced 3D printing technology that uses cells and biomaterials as printing materials. The most promising advancement of 3D bioprinting is that biocompatible polymers, ECM-based hydrogels, and multiple cell types can be delivered simultaneously and positioned as intended to fabricate complex 3D biological constructs. There have been interesting studies that applied 3D bioprinting of a micro-organ on pre-prepared microfluidic platforms for organ-on-a-chip applications; however, there have been no reports describing the use of this technology for whole organ-on-a-chip fabrication including a microfluidic system with multiple cell types and biomaterials for optimal biomimicry. Until recently, we have

actively developed 3D bioprinting systems and methods using multiple cell types and biomaterials for complex-shaped heterogeneous tissue models [3].

Materials and Methods:

As a platform material for an organ-on-a-chip, poly (ε-caprolactone) for the preparation of the hydrogels, 3% w/v gelatin, hydrogels were prepared by dissolving gelatin in serum-free M199 medium, and 2% w/v collagen type 1 hydrogels were prepared by dissolving a lyophilized collagen sponge into 0.5 M of acetic acid and neutralized by 10 N sodium hydroxide. 10-times concentrated Dulbecco's modified Eagle's culture medium was added to the pH-adjusted collagen hydrogels (1/10th of the volume) to supply the medium to the cells during the printing process. The printing conditions were adjusted depending on each hydrogels property.

Design of an organ-on-a-chip platform

A 3D bioprinting code was generated for the printing system, and the organ-on-a-chip platforms were printed, which is an easy approach to fabrication of an organ-on-a-chip platform with a complex design via 3D bioprinting. A fluidic channel with internal dimensions of 1.5 mm × 1.5 mm × 15 mm was prepared for multiple applications: for various organ-on-a-chip platforms, protein absorption testing, and liver-on-a-chip application. ECM-based hydrogels for the 3D micro-environment were printed with a 400 μm thickness for various organ-on-a-chip platforms and the liver-on-a-chip. For the dye absorption test, a channel with the same internal size as the one above was prepared by the 3D printing method and polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Corporation, USA) replica molding.

Measurement of the contact angle and protein absorption

Distilled water (5 μl) was dropped onto each of the PCL and PDMS platforms, and each contact angle was measured by a droplet analysis device To compare the dye absorption of the PCL-printed and PDMS replica-molded devices, a solution of Rhodamine B (1 μm in phosphate-buffered saline) was pumped through the devices. To perfuse through the devices, the dye was ejected at a flow rate of 5 μL min^{-1} from the perfusion pump. After perfusion for 12 hours, each channel was cut through the vertical section, and the absorption depth was visualized by using a co focal microscope

Scanning electron microscopy

All printed constructs were dried under vacuum at room temperature, then coated with platinum in a sputter coater operating at 15 kV, was used to examine the hydrogel position within the printed platform and to analyze the printed microfluidic channels of the organ-on-a-chip platform.

Cell preparation, labeling, and encapsulation into hydrogels

Human hepatocellular carcinoma (HepG2) cell lines and human umbilical vein endothelial cells (HUVEC). HepG2 was cultured in Dulbecco's modified Eagle's medium with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. HUVEC was cultured in a complete endothelium medium under the same conditions as the HepG2 cell culture.

To label the cells, the HepG2 cells were incubated at 37 °C in a 5% CO_2 incubator for 15 min with DiI (red) or DiO (green) fluorescent dyes. These HepG2-DiI-labeled cells and HepG2-DiO-labeled cells were then used as two different representative cell types. These green and red-labeled cells were encapsulated in collagen type 1 or gelatin hydrogels and used in preparing various organ-on-a-chip platforms. The concentration of cells used was from 1 to 5 × 10^6 cells ml^{-1}. A co focal microscope was used for visualization.

Printing of various organ-on-a-chip platforms

An empty cavity was first prepared, and cell types and ECM-based hydrogels for the micro-environment were printed in the prepared empty cavity. Secondly, with several layer-by-layer processes, the side walls of the fluidic channel were printed. The fluidic channel was also covered and sealed by printing the housing material in a manner wherein it is crossing above the pre-printed side walls of the fluidic channel to avoid fluid leakage. Lastly, the tube connection part for dynamic stimulation was printed. In the process of printing the tube connection, an optimal design was selected to avoid leakage. In the described process, 2D, 3D, 3D/3D vertical, 3D/3D horizontal and 3D/2D models for various organ-on-a-chip platforms were prepared using the 3D bioprinting technology.

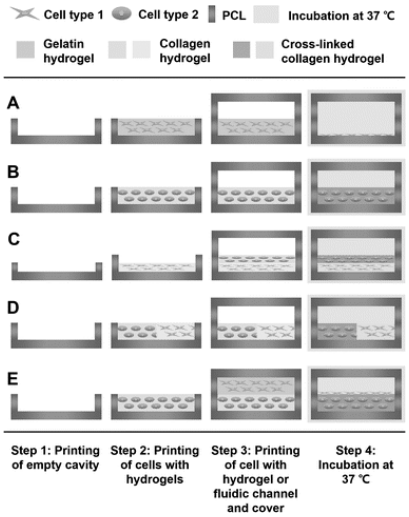


Figure 1: 3-D Printing Process of Various Organs on Chip Platform

3D bioprinting of an organ-on-a-chip platform

Among multiple candidates of biocompatible polymers for 3D bioprinting, PCL was chosen as a housing material for the organ-on-a-chip platforms. PCL is not only non-toxic, but also has a relatively low melting point of 60 °C compared with other biocompatible thermoplastics, resulting in high cell viability during the printing process.

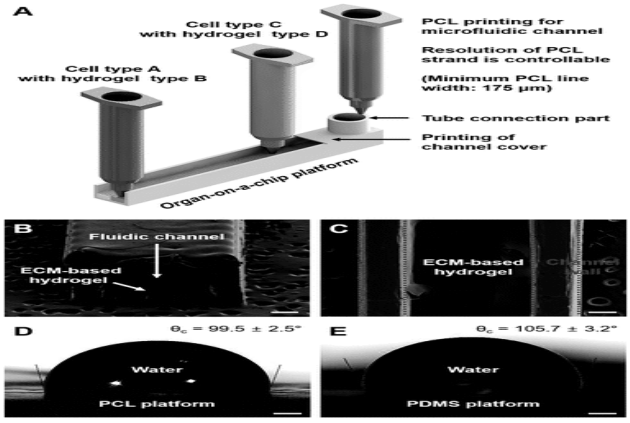


Figure 2: Schematic Illustration of the 3D Bioprinting Technology for the Organ on a Chip

Evaluation of protein absorption

Protein absorption in a cell culture device is very important for the accurate measurement of cell metabolism and drug sensitivity. As mentioned before, most organs-on-chips have been fabricated by soft lithography and a PDMS replica molding process. However, it is generally known that for a non-specific/hydrophobic protein, less than approximately 500 da of its molecular mass can be absorbed into PDMS. This protein absorption can cause major concern regarding the cell culture in the organ-on-a-chip platform, since the culture medium contains numerous proteins and growth factors. For example, non-specific protein absorption onto PDMS can change the protein level within the medium, which may severely affect the cell culture conditions and functions. Nevertheless, this issue has been ignored in many studies because of the lack of an alternative micro engineering technique. To compare the protein absorption into the channel wall in our 3D bioprinted organ-on-a-chip platform with that in the PDMS platform, two microfluidic channels with the same internal dimensions of 1.5 mm × 1.5 mm × 15 mm were prepared with PCL and PD.

Printing of various organ-on-a-chip platforms

Gelatin hydrogels have unique thermo-sensitive properties. These materials are in the gel state at low temperature; however, they turn into liquid form at 37 °C. Using these gelatin hydrogels, gelatin hydrogels with cells were first printed. After the incubation process, only gelatin material in the liquid state was removed, and only the cell components remained. Thus, the use of gelatin hydrogels as a printing material followed by an incubation process at 37 °C made it possible to deliver the cells only in the 2D state in the printed microfluidic system. It was possible to make the 3D micro-environment for the cells with encapsulation in the collagen hydrogels. By comparing the 2D and 3D models, the morphological cell differences were observed. The cells were observed with stretched and scattered shapes in the 2D model; however, the morphology of the cells in the 3D model was rounded in shape due to the 3D micro-environment caused by the collagen hydrogel the day after the 3D bioprinting process. In addition to the 2D or 3D model with a single cell type, the 3D/3D vertical and 3D/3D horizontal models with heterotypic cell types were fabricated. These heterotypic models could be engineered due to the advancement of the 3D bioprinting technology. Lastly, by applying the gelatin

and collagen hydrogels to the 2D and 3D models, respectively, the 3D/2D model was successfully prepared. The cells in the 2D and 3D parts of the 3D/2D model showed similar cell morphology to the individual 2D and 3D models. Thus, the results indicate that appropriate delivery of cells and ECM-based hydrogels to the desired positions in channels and effective preparation of heterotypic dimensional models are possible with a 3D bioprinting technology, which will lead to optimal biomimicry of native organisms with organs-on-chips. We have shown that printing of 2D cell patterns, 3D cell patterns, dividing 3D cell patterns, and stacking cell patterns in the microfluidic channel are possible. Therefore, not only the various organ-on-a-chip platforms suggested, but also much more complex platforms can be designed and printed through a 3D bioprinting technology.

Conclusion:

Although bioengineered 3D microsystems and organ-on-chip technologies are relatively new and still require further validation and characterization, their potential to predict clinical responses in humans could have profound effects on drug discovery and environmental toxicology testing. The scale-up of these complex technologies, together with systems integration of the engineering into easy to use, scalable, reproducible and user-friendly systems will be key to their future success. Equally critical will be for the field to move from lab-based prototyping to commercial manufacturing of these chips in materials suitable for drug discovery applications. This is crucial because despite the many desirable properties of PDMS most commonly used in microfluidic systems, PDMS has poor chemical resistance to organic solvents and it can absorb small hydrophobic molecules, thus compromising rigorous chemical testing of potential therapeutic agents. In addition, it will be important to ensure that appropriate biomarkers and assays are developed for use with these microsystems, and to validate that *In Vitro* results can be extrapolated to the human situation.

References:

[1] Hyoja D & Nam G (2016) Pohang Gyeongbuk, First published on 15th June South Korea: 790-784.

[2] Bhatia SN & Ingber DE (2014) Nat Biotechnol 32: 760–772.

[3] Rompay Van KK (2010) Antiviral Res 85: 159–175.

[4] Nge PN, Rogers CI & Woolley AT (2013) Chem Rev 113: 2550–2583.

[5] Xi'an (2017) Biomicrofluidics 9:11

PE-41

An Overview on International Educational Funding Organizations

Alifiya Rounak, Akash Yadav, Neelam Balekar, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Knowledge Village, Rajendra Nagar, A.B. Road, Indore (Madhya Pradesh), India
E-mail address: alifiya27899@yahoo.com

Abstract: Funding is the act of providing financial resources, usually in the form of money, or other values such as effort or time, to finance a need, program, and project, usually by an organisation or government. There are various methods of funding such as general fundraising, project funding, programme funding and core financing. In this review paper various organizations are shown who are funding various projects, research works, provides grants for travelling to attend seminar and also provide funds for organizing conferences, symposia and seminars. Such organization are International Education Research Foundation (IERF), Indo French Centre for the Promotion of Advance Research (IFCPAR), The Royal Society UK, The World Academy of Sciences (TWAS), International Centre for Genetic Engineering and Biotechnology (ICGEB),Department of Biotechnology (DBT) India, etc.

Introduction:

Research funding is a term generally covering any funding for scientific research, in the areas of both "hard" science and technology and social science. The term often connotes funding obtained through a competitive process, in which potential research projects are evaluated and only the most promising receive funding. Such processes, which are run by government, corporations or foundations, allocate scarce funds. Most research funding comes from two major sources, corporations (through research and development departments) and government (primarily carried out through universities and specialized government agencies; often known as research councils). Some small amounts of scientific research are carried out (or funded) by charitable foundations, especially in relation to developing cures for diseases such as cancer, malaria and AIDS.

Often scientists apply for research funding which a granting agency may (or may not) approve to financially support. These grants require a lengthy process as the granting agency can inquire about the researcher(s)'s background, the facilities used, the equipment needed, the time involved, and the overall potential of the scientific outcome. The process of grant writing and grant proposing is a somewhat delicate process for both the grantor and the grantee: the grantors want to choose the research that best fits their scientific principles, and the individual grantees want to apply for research in which they have the best chances but also in which they can build a body of work towards future scientific endeavors [1].

Grants are non-repayable funds or products disbursed or gifted by one party (grant makers), often a government department, corporation, foundation or trust, to a recipient, often (but not always) a non-profit entity, educational institution, business or an individual. In order to receive a grant, some form

of "Grant Writing" often referred to as either a proposal or an application is required. Most grants are made to fund a specific project and require some level of compliance and reporting. The grant writing process involves an applicant submitting a proposal (or submission) to a potential funder, either on the applicant's own initiative or in response to a request for proposal from the funder [2].

Various funding organizations:

1. International Education Research Foundation (IERF)

The research grant program is named in honour of Inez and LudwigSepmeyer because of their combined involvement in international education and their commitment to research and publications on world educational systems.

Name of Scheme: Sepmeyer Research Grant Program

Objective

The IERF's mission is to conduct research and disseminate information on world educational systems and to facilitate the integration of individuals educated outside the United States into the U.S. educational environment and work force.

Criteria

Each research grant proposal is evaluated on how well its project design and expected outcomes address the following criteria:

- supporting research for the evaluation of foreign academic credentials
- promoting the integration of students, scholars and professionals holding non-US academic credentials into the United States
- encouraging the exchange of information about the structure of educational systems and their corresponding credentials

Tenure: up to $1000 US

Application period: February 15th or August 15th

2. Indo French Centre for the Promotion of Advance Research (IFCPAR)

Indo-French Centre for the Promotion of Advanced Research (CEFIPRA) is India's first and France's only bi-national organisation committed to promote collaboration between the scientific communities of the two countries across the knowledge innovation chain. The Centre was established in 1987, with

the support from the Department of Science & Technology, Government of India and the Ministry of Foreign Affairs & International Development, Government of France.

Name of Scheme: IFCPAR/CEFIPRA

Objective: CEFIPRA supports high quality research groups through collaborative research projects in advanced areas of basic and applied science to nurture scientific competency through Collaborative Scientific Research Programme.

Criteria:

- The submission date is within the prescribed deadline
- Submitted both concept proposal and full proposal
- Joint Proposal Submission Agreement signed by both the Principal Collaborators submitted
- The proposal received is within the identified fields of Science & Technology, coordinated by CEFIPRA
- Investigators are from academic/research institutions in India/ France
- The collaborators have permanent position in an Indian or French University/R&D Institution
- Submitted quotations for the equipment applied (offline to CEFIPRA)

Tenure: upto 200 Euros

Application period: 31st March, 30th June, 30th September and 31st December[3]

3. The Royal Society UK

The Royal Society's motto 'Nullius in verba' is taken to mean 'take nobody's word for it'. It is an expression of the determination of Fellows to withstand the domination of authority and to verify all statements by an appeal to facts determined by experiment.

Name of Scheme: The Royal Society

Objective: The agency provides grants to support high quality UK science and foster collaboration between UK-based and overseas scientists. About 1,500 researchers are currently funded by the Royal Society, including approximately 700 Research Fellows. The agency's funding sources include a grant from the Department for Business, Innovation and Skills (BIS) as well as donations from individuals and organisations.

Criteria:

- All applications require the support of the Head of Department and approval from the university Research Office.
- Some applications also require the submission of a statement from a Head of Department or nominated referee.
- Applicants are responsible for ensuring that their nominated referee completes their statement by the deadline, which is usually one week after the submission deadline.

e. Tenure: Approx. 15,000 Euros

f. Application period: September [4]

4. The World Academy of Sciences (TWAS)

The World Academy of Sciences (TWAS) is a merit-based science academy uniting 1,000 scientists in some 70 countries. Its principal aim is to promote scientific capacity and excellence for sustainable development in the South. It was named "Third World Academy of Sciences" until 2004 and "TWAS, the academy of sciences for the developing world" before September 2012.

Objective: it is to reinforce and promote scientific research in basic sciences in developing countries; **s**trengthen developing countries' endogenous capacity in science; **r**educe the exodus of scientific talents from the South; **b**uild and sustain units of scientific excellence in S&TLC over a longer period to help them achieve a critical mass of highly qualified and innovative scientists capable of addressing real-life problems facing their countries.

Criteria:

- Individual applicants must be nationals of developing countries. They must hold a PhD, be at the beginning of their careers, but already have some research experience. They must hold a

position at a university or research institution in one of the S&TLCs and be under 45 years of age.

- Applications from women scientists and those working in Least Developed Countries are especially encouraged.
- Individual scientists who submit a satisfactory final report on a previous grant may apply for a renewal.

Tenure: USD 30000

Application period: 11 May 2017 [5]

Conclusion:

Different countries spend vastly different amounts on research, in both absolute and relative terms. For instance, South Korea and Israel spend more than 4% of their GDP on research while many Arabic countries spend less than 1% (e.g. Saudi Arabia 0.25%). USA spent $456.1 billion for research and development (R&D) in 2013, the most recent year for which such figures are available, according to the National Science Foundation. The private sector accounted for $322.5 billion, or 71%, of total national expenditures, with universities and colleges spending $64.7 billion, or 14%, in second place while Switzerland spent CHF 22 billion for R&D in 2015 with an increase of 10.5% compared with 2012 when the last survey was conducted. In relative terms, this represents 3.4% of the country's GDP. R&D activities are carried out by nearly 125,000 individuals, mostly in the private sector (71%) and higher education institutions (27%). There are N number of organizations that grants funds for various purposes such as for carrying out research work or for travelling to different parts of the world for attending various conference/ semina / symposium or for organizing such conference / seminar/ symposium in one’s own nation.

References:

[1] https://en.wikipedia.org/wiki/Funding [Accessed 9 Oct. 2017].

[2] https://en.wikipedia.org/wiki/Grant_%28money%29 [Accessed 10 Oct. 2017].

[3] http://www.cefipra.org/Collaborative_Research.aspx [Accessed 10 Oct. 2017].

[4] https://royalsociety.org/grants-schemes-awards/grants/about-grants/ [Accessed 11 Oct. 2017].

[5] https://twas.org/opportunities/research-grants [Accessed 13 Oct. 2017].

PE-43

Indian Educational Funding Organizations

Arvinder Kaur Sangha, Akash Yadav, Neelam Balekar, Dinesh Kumar Jain
IPS Academy College of Pharmacy, Knowledge Village, Rajendra Nagar, A.B. Road, Indore (Madhya Pradesh), India
E-mail address: amneet.kour@gmail.com

Abstract: Funding agencies are organizations that provide grants, scholarships, or other forms of support to programs, projects, and individuals in a specific area. Funding agencies may be non-profit organizations, private foundations, or government offices. The goal of a funding agency is usually to promote excellence or encourage interest in a particular subject, such as alternative energy, the environment, social works, or medical innovations. Finding appropriate funding agencies for a particular project is usually easily done through a simple Internet search; many websites provide extensive lists of funding possibilities by subject. Such organisations are University of Grants Commission (UGC), All India Council for Technical Education (AICTE), Council of Scientific and Industrial Research (CSIR), Department of Biotechnology, Department of Science and Technology, Indian Council of Medical Research, Department of Education etc.

Introduction:

Funding agencies may also be geared toward promoting study, innovation, or excellence in a particular area. Grants are often provided to groups or individuals that can demonstrate a need for funding for a project in line with the goals of the particular funding group. A foundation dedicated to gang prevention and awareness, for instance, might provide a grant to a local group that wants to set up after-school programs for at-risk middle school students.

Various funding organizations:

1. University Grant Commission (UGC)

University Grant Commission has the unique distinction of being the only grant-giving agency in the country which has been vested with two responsibilities: that of providing funds and that of coordination, determination and maintenance of standards in institutions of higher education.

University Grant Commission

Name of Scheme: Organising conferences, workshops, seminars in colleges.

Objective: The basic objective of the scheme is to bring together academicians and experts from different parts of the country and abroad to exchange knowledge and ideas. This will provide an in-depth analysis of subjects and update the knowledge of the participants from academic/research institutions.

Application period: Before 3-4 months of conferences, workshops, seminars.

Tenure: State Level seminar/Conference/ Workshop: Rs.1.00 lac, National Level seminar/Conference/ Workshop: Rs.1.50 lac, International Seminar/Conference/ Workshop: Rs.2.00 lac

Eligibility: Financial assistance under the scheme is available to all Colleges, which come under the purview of Section 2(f) and are fit to receive central assistance under Section 12B of the UGC Act, 1956. It shall be mandatory for every college to get accredited by the Accreditation Agency, after passing out of two batches or six years, whichever is earlier.

Name of Scheme: Emeritus Fellowship

O**bjective**: To provide an opportunity to superannuated teachers to pursue active research in their respective field of specialization in Indian universities/ institutions/ colleges given below:

• To universities/ institutions/ colleges under section 2(f) and 12 (b) of UGC Act, 1956.

• Deemed to be universities under section 3 of the UGC ct, 1956 which are eligible to receive grant in aid form from UGC.

• Universities/ Institutions/ Colleges funded by central State Govt. Institutes of National Importance.

Application period: June

Tenure: The number of slots available under the scheme is 100 for Science streams and 100 for Humanities, Social Sciences and Languages (total 200 slots) at any one given time basis. The assistance available under the scheme is:

• Honorarium of Rs. 31,000/- p.m. for two years (non-extendable)

• Contingency grant (non-lapsable) of Rs. 50,000/- p.a. towards secretarial assistance, travel within the country connected with the research project, stationery, postage, consumables, books and journals and equipments. However, books, journals and equipments etc. procured out of the contingency grant will be the property of the university/college/institute.

• Foreign visit in connection with the approved research work of the awardee will be limited to once in a year with the prior approval/no-objection of the Institution/University where the project work is being undertaken along with that of UGC. This will be further subject to no financial liability on the part of UGC.

• The expenditure would be accounted for audit by the university department concerned like any other departmental expenditure. The Emeritus Fellow would be entitled to such other privileges including medical facilities as available to the university faculty members, but not housing.

Eligibility: The eligibility will be based on the quality of research and published work contributed by the teacher in his/her service career. The awardee (superannuated) can work under this scheme with a well defined time bound action plan upto the age of 70 years or upto two years (non-extendable) of the award whichever is earlier. No extension under the scheme is admissible and hence the proposal should be well defined with a time bound action plan so that it is completed within the prescribed tenure.

2. All India Council for Technical Education (AICTE)

All India Council for Technical Education was set up in November 1945 as a national-level apex advisory body to conduct a survey on the facilities available for technical education and to promote development in the country in a coordinated and integrated manner.

All India Council for Technical Education

Name of Scheme: Research promotion scheme (RPS)

Objective: RPS Promotes Research in identified thrust areas of in Technical Education. RPS is aimed to create research ambience in the institutes by promoting research in engineering sciences and innovations in established and newer technologies. The objective of this scheme is to create and update the general research capabilities of the faculty members of the various Technical Institutes.

Application period: September

Tenure: Total funding of Rs.25 Lac. Recurring 15% and Non-Recurring 85% of the total grant.

Eligibility: AICTE approved Technical Institutions / University Departments with relevant PG programme. Full time regular faculty with research experience and publications. The preliminary research facilities should be available in the institute. One proposal from one PI (Principal Investigator) will be sanctioned at any point of time within the duration of project. Duration of project will be three year from the date of receipt of funds in the institute's account.

3. Council of Scientific & Industrial Research (CSIR)

CSIR covers a wide spectrum of science and technology – from radio and space physics, oceanography, geophysics, chemicals, drugs, genomics, biotechnology and nanotechnology to mining, aeronautics, instrumentation, environmental engineering and information technology.

Council of Scientific & Industrial Research

It provides significant technological intervention in many areas with regard to societal efforts which include environment, health, drinking water, food, housing, energy, farm and non-farm sectors.

Name of Scheme: CSIR senior research fellowship (SRF)

Objective: To provide award each year directly to young research workers who have shown promise in original research and propose to pursue research work in science, engineering, medicine or technology on specific projects.

Application period: October-December Tenure: The total tenure as SRF will not exceed 5 years. The total period of tenure as Research Fellow/ Associate will include any other fellowship of equivalent status from any other agency/institute and is computed after deducting the tenure of fellowship/associateship already availed. A Research Fellow/Associate who joins on the first day of the month, his/her tenure will be completed on the last day of the previous month. In other cases the tenure will be completed on the last day of the same month of joining the Fellowship. A SRF in a science/engineering/medical & pharmaceutical, veterinary discipline is entitled to a stipend of Rs.28, 000/- pm for the entire tenure of fellowship.

Eligibility: B. Pharm./B.VSc./B.Sc.(Ag) or equivalent degree with at least 55% marks and one publication in SCI Journal and should have completed at least three years research experience M. Pharm./M.VSc./MSc(Ag) or equivalent degree with at least 55% marks and one publication in SCI Journal and should have at least one year research experience.

4. Department of Biotechnology

Department of Biotechnology's vision is to attain new heights in biotechnology research, shaping biotechnology into a premier precision tool of the future for creation of wealth and ensuring social justice especially for the welfare of the poor.

Name of Scheme: Research Resources, Service Facilities and Platforms' Programme under Biotech Facility Scheme

Objective: The overall aim of this programme is to establish the new or up-gradation of existing research resources/service facilities and platforms in Indian Institutions/Universities engaged in

cutting edge research in frontier areas of Life sciences/Biotechnology and also to establish the infrastructure for various services in agriculture, medicine, environment and industry in public interest.

Application period: It is not fixed.

Tenure: A public and deemed university (other than supported under DBT-BUILDER Programme) may be supported to establish basic infrastructure for R&D, Teaching and short term training (1-2months) to students of the affiliated colleges up to maximum support of Rs.10.0Crore. In a year ONLY three universities will be supported. The support may also include the faculties and trainees under manpower. The private universities are NOT eligible for this support. An established R&D institutions and university may put up a proposal with following conditions: for establishing/upgrading a shared facility in which an equipment/Facility costing Rs.3.0crore or above will ONLY be supported.

Eligibility: Research Resource Grant and Platform – Public and deemed institution/University or private UGC recognized institution/University certified for doing R&D activities by Department of Scientific and Industrial Research (DSIR). Service Facilities – Public and deemed institution/university or private UGC recognized institution/University (including deemed universities) or any Private Indian industry with no foreign stake and certified for doing R&D activities by Department of Scientific and Industrial Research (DSIR).

Conclusion:

Online surveys are useful for determining knowledge translation training needs of researchers, research users and ultimately organizations Funders can play a role in developing new training opportunities as part of a broad effort, with partners, to build capacity for the use of health research evidence. Survey results would ideally be complemented with an objective needs assessment based on core competencies, and should be acted on in a way that acknowledges the complexity of knowledge translation in healthcare, existing training activities, and the expertise stakeholders already have but may not refer to as knowledge translation. The goal of a funding agency is usually to promote excellence or encourage interest in a particular subject, such as alternative energy, the environment, social works, or medical innovations.

References:

[1] https://www.ugc.ac.in/pdfnews/8246165_Guideline_for_ Devlop_grant_to_colleges. [Accessed 04 Oct. 2017].

[2] https://www.ugc.ac.in/pdfnews/7605981_revised-final-Emeritus-Fellowship.pdf [Accessed 6 Oct. 2017].

[3] http://www.aicte-india.org/downloads/handbook.pdf [Accessed 7 Oct. 2017].

[4] http://www.csa.iisc.ernet.in/academics/studforms/travelfunding /CSIR.pdf [Accessed 7 Oct. 2017].

[5] http://www.dbtindia.nic.in/wp-content/uploads/Guidelines_ RRSFPT2017f-_1_.pdf [Accessed 9 Oct. 2017].

PE-52

Academia-industry Collaboration: A driving Force for Accelerating Pharma Research

Neelesh Malviya, Ankit Mangal
Smriti College of Pharmaceutical Education, Indore (M.P.)
E-mail address: drneeleshmalviya@gmail.com

Abstract: Academia-industry collaboration is a persistent matter of debate for both academia and industries. A high-quality academic research can assist industry to produce economical product for the society. In recent years, it's apparent and obvious that there is great need of sharing knowledge between research institutions and industries. Current Pharma Market urgently requires collaboration of both the parties. Such Collaboration will translate research ideas into industrial products; in addition institutes will be more reachable and permissible by the industries. Currently, certain research institutions have faculty's fraternities that actively pursue links with industry, but they lack the interactions amongst themselves and with industries. Though academicians in best institutes engaged in research, but on higher end maximum of their research is limited to publications or patentability, very few are transformed into marketed or commercialized products. Our systematic review highlighted the current status of academia-industry collaborations in the field of pharma at national and international level.

Introduction:

The modern epoch of collaborations between innovative ideas and industrial facilities put forward a new platform to attend the global challenges and for the betterment of society [1]. Collaboration between academics and industries (Figure 1) is very essential for skills development, modernization, entrepreneurs, start-ups, enhancing research, troubleshooting and innovations [2]. In developing countries academia-industry collaboration fosters the commercialize research through mutual tie-ups, partnerships, research scheme, agreements and patents [3].

Academia-Industry collaborations

The quality of education is degraded day by day and very few government schemes are available for education organizations due to that research facilities of academics are not up to the mark. In that situation academicians are not cable to focus on innovative research, consequently industries not attracted towards academia-industry linkages [4]. The challenge for both is to make connectivity as per individual needs. Academicians need motivations in the form of finance and position to accelerate their research as per the need of industries, while industries need to understand the potential of academicians and provided them financial support to continue their research till commercial output drawn [5]. In that circumstances research works of academicians funded by industries it will help to them to work without any limitations this will possible if academics and work for each other through

partnership. Collaborations in this area are necessitated academicians work research as per the requirement of industries and industries provide research funding to the academicians.

Admirable academia-industry collaborations in pharmaceuticals

Recently it was observed that pharmaceutical research, nowadays, is growing through multifaceted dome. The research expenditures limit the drug discovery and development process due to that the cost of newer products are increases. In that situation if research institutions and pharmaceutical industries work together efficiently that can easily reduces the cost of the product and time taken for drug discovery and development. India is also emerging as a preferred destination for clinical and drug discovery research. Indian companies have expertise in process research and developing new drug delivery systems, and are strengthening pipelines in these areas. For India to become a global destination and innovation hub in pharmaceutical sector, quality and safety standards need to be improved, and patent protection and regulatory framework need to be further strengthened. Public and private sector need to work collaboratively and proactively to develop competent facilities and workforce in science and technology for India to lead new drug development and pharmaceutical R&D at forefront.

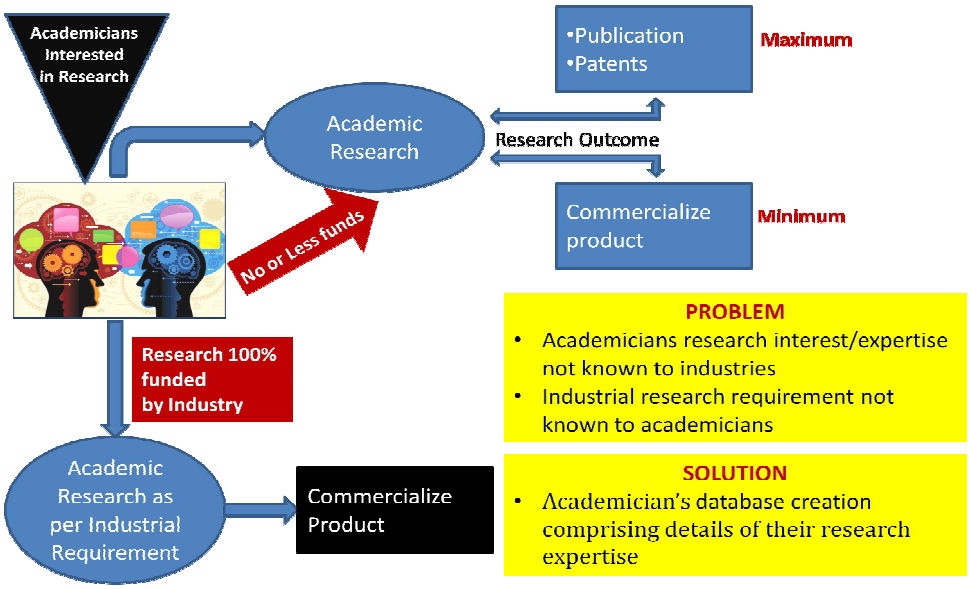


Figure 1: Outcomes of academic research vs. Academia-industry collaborations outcomes

Table 1: Pharma Industries collaborations with foreign Institutes

Pharma industry	Pharma academic institute	Objective of collaboration
GlaxoSmithKline	Crick Institute	Worked together and both contributed their resources, including lab space and staff, with the aim to start number of projects are in the upcoming years 2016 with the goal of discovering better targets for new medicines. They decided that research findings from the collaboration will be shared with the broader scientific community via joint publication in journals.
Sun Pharmaceutical Industries Ltd	Technion - Israel Institute of Technology	Worldwide research and license agreement for cancer treatment drug
Pfizer	Global Medical Excellence Cluster, a constituted group of Cambridge University, Imperial College London, King's College London, Queen Mary University London and Oxford Universit	To discover and develop new drugs for rare diseases, a range of around 6,000 disorders which each affect fewer than one in 2,000 people on the genetic basis of these diseases
GSK and AstraZeneca	Manchester Collaborative Centre for Inflammation Research (MCCIR)	To facilitate the development of innovative biomedical research and its successful translation into a medically usable drug.
Sanofi	Stanford University	To provide funding for five research programmes in a year and aid the development of new molecules
Sanofi	AVIESAN, the French Life Sciences and Healthcare Alliance	For research of various disease areas
Pfizer	Eight research institutions in Boston	Molecule development and for the first clinical studies in humans
Eisai**,** Japan	John Hopkins Brain Science Institute	To develop small-molecule glutamate carboxypeptidase II (GCPII) inhibitors, using Eisai's technology
Panacea Biotec ltd India	Vaccine Institute of Netherlands	For research on Inactivated Polio Vaccine; NRDC- India for Foot & Mouth Disease vaccine for veterinary use and Bio Farma-Indonesia for Measles vaccine

Table 2: Pharma Industries collaborations with Indian Institutes

Cadila Pharmaceuticals Ltd and Cadila Healthcare Ltd, also known as Zydus Cadila	NIPER, Ahmedabad	To exchange scientific personnel for education, training and research collaboration
Johnson & Johnson (J&J), and Sahjanand Technologies	NIPER, Ahmedabad	Development of curriculum as per industrial atmosphere orientations
Cipla	Serum Institute, Pune	Marketing and selling of vaccines products in abroad. Cipla will market Serum's products in Africa and Europe
Lupin Research Park, the R&D wing of Lupin	Birla Institute of Technology and Sciences – Pilani, Manipal University, Karnataka and Pune University	Established PhD center for Lupin employees to pursue their doctorate degree on industry specific research.
Panacea Biotec ltd India	National Institute of Immunology, India	To develop, manufacture and marketing of Anthrax vaccine, worldwide
Panacea Biotec ltd India	National Institute of Health, USA	For use of a peptide based formulations for generation of hair follicles and hair growth
Eris Lifesciences Pvt. Ltd	Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, Kerala	Joint development of certain unique drug delivery formulations using nanotechnology
Apogen Remedies Pvt. Ltd Andhra Pradesh, India	Bapatla College of Pharmacy, Guntur, Andhra Pradesh	To establish and make use of Formulation Development Center at Bapatla College of Pharmacy, Bapatla for the formulations research and development activities
Lupin Limited	Smt. Kishoritai Bhoyar (SKB) College of Pharmacy, Kamptee Maharashtra	Encouragement, exchange and live research projects for top performing students at the college

Conclusion:

It is quite clear that what really dominates at present the scene of collaborations is that the top pharmaceutical industries have preferred to collaborate with foreign institutes and research centers (Table1) than the Indian universities (Table 2). The rationale of very modest figure of collaboration to be recognized by pharma companies with Indian universities is not because there are too few pharma academic research centers or academicians engaged in research activity. Somewhat, besides many, but problem is the connectivity between industries and academic researchers is not properly established. Today, industries are searching for innovative solutions from the academic world to facilitate their requirements of advanced output and lesser expenditure. To accomplish this objective, practically

bridging between academia and industries is the solitary solution; industries must encourage and sponsored researches at academic level to translate their innovations into customer products.

Acknowledgement:

This manuscript is a part of project funded by National Science & Technology Management Information System (NSTMIS) a division of Department of Science and Technology (DST). The authors acknowledge the generous funding by DST-NSTMIS, New Delhi for this work.

References:

[1] Scientific Relations between Academia and Industry: Building on a New Era of Interactions for the Benefit of Society Report from an International Workshop on Academia –Industry Relations Sigtuna, Sweden, 22-25 November 2011.

[2]https://www.innovationpolicyplatform.org/sites/default/files/rdf_imported_documents/PromotingUniversityIndustryCollaborationInDevelopingCountries.pdf.

[3] Markus P., Valentina T., & McKelvey M (2013) Res Pol. 42:2;423–442.

[4] 2nd OECD Conference of ministers responsible for small and medium-sized enterprises (SMEs) promoting entrepreneurship and innovative SMEs in a Global Economy: towards a more responsible and inclusive globalisation Istanbul, turkey 3-5 June 2004.

[5] http://ec.europa.eu/invest-in-research/pdf/download_en/knowledge_transfe_ 07.pdf.

Courses

Contents

School of Architecture
- B.Arch (Affiliated to RGPV)

College of Commerce
- B.Com (Hons)
- B.Com (Plain/Comp. Appl./Foreign Trade)

School of Computers and Electronics
- BCA, MCA, MBA

School of Education
- B.Ed. (after graduation)

Institute of Economic and Research
- BBA (Foreign Trade)

Institute of Engineering and Science
- B.E. Comp Science/Elex. & Comm./Fire Tech. & Safety / Electrical & Electronics/Civil/Chemical/Mechanical
- M.E. (CS/EC/Power Elex./Chemical/Construction & Planning Management)
- M.Tech (Structural Engg./Industrial Safety) MBA, MCA

School of Fine Arts and Music
- B.F.A (4yrs)
- M.F.A. in Painting
- Concept designing course for Animation, Film & Games (2 yrs), 1 year certificate course in digital and matte painting and fine arts, B. Music/B. Dance/Geetanjali

College of Law
- B.A. L.L.B. 5 yrs after 12th
- L.L.B. (Hons) 3 yrs after graduation

College of Pharmacy
- B.Pharm
- M.Pharm. (Pharmaceutics/Pharmacology/Q. A.)
- Ph.D.

Institute of Fire & Safety
- Certificate course in Fireman Ship

Institute of Fashion Technology
- PG Diploma in Fashion Designing & Marketing (after graduation)
- Fashion Designing with CAD, Jewelry Designing, Modeling, Workshop

Mashal School of Hotel Management
- BHM (4 yrs)
- BBA (Hotel Management)
- BA (Int. Hospitality Admin.)

Institute of Business Management & Research
- BBA
- MBA (Full Time/Part Time)
- MBA (International Business)
- MBA (Business Economics)
- PGDM

Institute of Science, Laboratory Education and Library Science
- B.Sc (Biotech/Comp.Science/Elex./Life Science)
- M.Sc. (Biotechnology/Chemistry/Physics/Maths /Microbiology)
- B. Lib & Information Science (after graduation)
- Certificate of Library Automation
- Training for Library Automation

School of Arts, Tourism, Journalism Law and Education
- B.A. (Travel & Tourism Mgmt.)
- B. Journalism (1 yr and 3 yrs)
- L.L.B. (3yrs after graduation/5years after 12th)

Radio Jockey Courses
- Diploma in Radio Jockeying (DRJ)
- Radio Programming & Mgmt (DRPM)
- Radio Mgmt.
- PG Diploma in radio Programming and Mgmt. (PGDRM)
- Certificate course in Radio Jockeying (CRJ), Announcing, Broadcasting, Comparing & Dubbing (ABCD)

www.ipsacademy.org, principal.coph@ipsacademy.org

+91-731-4014701,02,03