Contents

Message from the Chief Patron

Dear Delegates,

It's a pleasure to welcome you all to the Ramanbhai Foundation 6th International Symposium.

This year's RBF symposium will focus on Advances in New Drug Discovery Technologies and Translational Research through state-of-the-art lectures, cutting-edge poster presentations, panel discussions and informal scientific networking.

Our overarching aim through the RBF Symposium has been to create a platform for knowledge-sharing where researchers, scientists, academicians and the industry can converge, discuss and share new trends in Drug Discovery and Development. We made a beginning with this endeavor in 2003 and today the biennial symposium has become an important event amongst the scientific community.

This year we begin with a focus on new technologies used in Drug Discovery and Development in our pre-symposium workshop sessions. Over the next two days, our panel of distinguished speakers will be sharing their knowledge and experience. As always, the interactive panel discussions and the poster presentations will open up new vistas of learning.

Your support and involvement has played an important role in making this knowledge-sharing platform a great learning endeavour. We are confident that you will find the Ramanbhai Foundation 6th International Symposium, an enriching experience.

With warm regards,

Pankaj R. Patel
Chief Patron

About Ramanbhai Foundation

A first-generation entrepreneur, Mr. Ramanbhai Patel was one of the stalwarts of the Indian Pharmaceutical Industry. At a time when the newly independent nation was heavily dependent on imports of drugs and pharmaceuticals, he had set out to prove that an indigenous company could provide innovative, research-based quality medicines.

Born at Kathor in South Gujarat on the 19th of August 1925, he began his career as an academician at the L.M. College of Pharmacy, a premier institute in Ahmedabad. This short stint in academics formed a lasting imprint on his mind and the resolve to contribute to the cause of research and education grew stronger over the years.

In 1952, Mr. Ramanbhai Patel turned a pharma entrepreneur. Armed with a sound business acumen, he laid a strong foundation for Cadila and contributed to the growth of the Indian Pharmaceutical Industry. Zydus Cadila today enjoys the coveted distinction of being one of the leading pharma groups in the country and a global healthcare provider.

Mr. Ramanbhai Patel had published several outstanding research papers and had taken a keen interest in research activities of the group. Today, Zydus Cadila is amongst the top investors in research. Mr. Ramanbhai Patel's contributions in the field of pharmaceutical education were equally noteworthy. Gujarat which earlier had only one pharmacy college now has several reputed pharmacy colleges. More importantly, Ramanbhai was instrumental in taking pharmaceutical education to the rural heartland of Gujarat, making professional courses more accessible to students in smaller townships.

In recognition of his services, Mr. Ramanbhai Patel had been bestowed with several prestigious awards: President of India's Import Substitution Award in 1973. Prof. M.L. Shroff Memorial National Award in 1987, The Glory of India Award in 1991 at Washington, Grahak Suraksha Award in 1992, Acharya Prafulla Chandra Ray Memorial Gold Medal in 1993 and the Eminent Pharmacist Award in 1994.

In a fitting tribute to his outstanding contributions to the growth of the pharma industry in India, he was conferred the Gujarat Businessman of Year Award in the year 2000. He was also honoured by Express Pharma Pulse with the 'Lifetime Contribution Award' for his contributions to the Indian pharma industry.

On the occasion of Gujarat's Pharma Centenary Celebrations in January 2008, Mr. Ramanbhai B. Patel was posthumously awarded a special plaque in recognition for his efforts in laying a firm foundation for Gujarat's pharmaceutical industry in the Post Independence era.

With a firm belief that new avenues would surely emerge, if one has the will to discover it, he dedicated his life to the quest for knowledge, as an academician, entrepreneur and a researcher.

The Zydus Research Centre, a state-of-the-art facility which was set up during his lifetime, spearheads the research initiatives of the Zydus group and supports the quest for innovations and excellence in the field of research.

The Ramanbhai Foundation today continues to spearhead programmes in the field of pharmaceutical research, education and healthcare – areas close to the Late Founder Chairman's heart.

The Ramanbhai Foundation is committed to a number of special initiatives in the field of education. The Zydus School for Excellence which was a dream nurtured by Mr. Ramanbhai B. Patel has been set up to provide a rich academic environment where children can seek creative expressions for their endeavours.

The Ramanbhai Patel - AMA Centre for Excellence in Education has also been set up to raise the bars of excellence in the field of education through progressive learning programmes for academicians, knowledge sharing forums and by studying successful models of education and creating a platform for sharing these experiences.

The Ramanbhai Foundation alongwith the Indian Pharmaceutical Association has set up the IPA-Shri Ramanbhai B. Patel Foundation (IRF) to recognise and honour lifetime achievements of senior pharmacists who have contributed to the growth of the profession of pharmacy in India. The IRF also awards merit scholarships to deserving students in the field of Pharmaceutical Sciences.

The Ramanbhai Patel International Symposium held every two years is devoted to the discussion on the current trends and developments in Pharmaceutical Sciences. Through the symposia, the Foundation aims to bridge the research endeavors taking place across the world and create a platform for knowledge sharing, tracing the development of new molecules from the laboratory to the market.

About Zydus

Zydus Cadila is an innovative global pharmaceutical company that discovers, develops, manufactures and markets a broad range of healthcare products. With a core competence in the field of healthcare, Zydus Cadila provides total healthcare solutions ranging from formulations, active pharmaceutical ingredients and animal healthcare products to wellness products. Headquartered in the city of Ahmedabad in India, the group has global operations in four continents spread across USA, Europe, Japan, Brazil, South Africa and 25 other emerging markets. The group aims to be a leading global healthcare provider with a robust product pipeline; achieve sales of over $3 bn by 2015 and be a research-based pharmaceutical company by 2020.

In its mission to create healthier communities globally, Zydus Cadila delivers wide ranging healthcare solutions and value to its customers. With over 15,000 employees worldwide, a world-class research and development centre dedicated to discovery research and eight state-of-the-art manufacturing plants, the group is dedicated to improving people's lives.

Zydus has a team of over 1000 scientists engaged in Research & Development. Our focus is on finding innovative therapies for diseases affecting mankind through continuous research and development. The major areas of research includes:

- **NCE research**
  - Cardio-Metabolic diseases
  - Inflammation & pain
  - Oncology
- **Biologics**
  - Biosimilar Therapeutic proteins
  - Biosimilar Monoclonal antibodies
  - Biobetters and Novel biologics
- **Vaccines**
  - Infectious diseases

About Zydus Research Centre

The Zydus Research Centre is the dedicated research arm of the Zydus Group. With its team of over 400 research professionals, ZRC spearheads the group's quest of creating healthier and happier communities globally. Spread over an area of over 4,75,000 sq ft, ZRC is working on cutting edge technologies in 14 different scientific disciplines to discover novel therapeutic agents. The center has capabilities to conduct drug discovery & development from concept to IND enabling preclinical and clinical studies.

About Vaccine Technology Centre

Vaccine Technology Centre (VTC) is the Vaccine division of the Zydus Group. VTC has two state-of-the-art R & D Centers, one located in in Catania, Italy; and the other in Ahmedabad, in the western part of India.

Zydus Vaccine division has indigenously developed, manufactured and launched India's first vaccine against H1N1 (Vaxiflu-S). The Vaccine Division's Rabies Vaccine Manufacturing facility has received WHO pre-qualification, and is one of the largest Rabies manufacturing facility in India.

The current programs under development include vaccine candidates designed to address infectious diseases like next-generation Influenza, Measles-Mumps-Rubella-Varicella, Typhoid, DPT-HiB, Hepatitis-B, Hepatitis-A, Hepatitis-E, Japanese Encephalitis, HPV and combination vaccines. Research is also focused on developing a Malaria vaccine.

About Zydus Biologics

Zydus Biologics is the biologics divisions of the Zydus group. The Zydus Biologics division has capabilities to discover and develop therapeutic proteins and monoclonal antibodies.

The division has a cGMP facility for manufacturing therapeutic protein based drugs and has developed and launched several therapeutic protein based drugs. The division also has an 11,000 litre cGMP facility for manufacturing monoclonal antibodies.

Program Schedule

February 4, 2013 Pre-symposium workshop	
08.00 hrs onwards	Registration and Morning Coffee
08.45 hrs	Opening Remarks
09.00 - 10.00 hrs	**Potential of Cellular analysis in Drug Discovery and Early Toxicology studies- The GE Healthcare Integrated Platform** GE Healthcare
10.00 - 11.00 hrs	**Semiconductor sequencing- a boon to translational Research** Life Technologies
11.00 - 11.30 hrs	Tea Break
11.30 - 12.30 hrs	**Paper Trails to Electronic Data Management** PerkinElmer (Informatics)
12.30 - 13.30 hrs	**High Resolution Mass Spectrometry for Pharmaceutical Applications** AB SciEx
13.30 - 15.00 hrs	Lunch, Poster & Networking session
15.00 - 16.00 hrs	**Innovative strategy to locate, screen and identify new chemical entities using STN and SciFinder databases** Sci-Edge Information
16.00 - 17.00 hrs	**Drug transporters in drug discovery: methods and regulatory considerations** Solvo Biotechnology, Hungary
17.00 - 18.30 hrs	**Application of High Resolution Mass Spectrometry and Ion Mobility Separations for Systems Biology Applications** • Introducing the latest Waters MS technology platforms • Protein profiling and biomarker validation • Protein characterization • Metabonomics and structural elucidation • Lipidomics and lipid characterization • Imaging by Mass Spectrometry Waters Ltd.

February 5, 2013

08.30 - 09.00 hrs Morning Coffee
09.00 - 09.30 hrs Inauguration

09.30 - 10.30 hrs

Inaugural Keynote Address

A Chemist's Foray into Translational Research

Dr. Peter Schultz

Director, Calibr Institute, Professor of Chemistry, The **Scripps Research Institute**, USA

Founder of Affymax Research Institute, Syrrx, Kalypsys, Phenomix, Symyx Therapeutics, Ilypsa, Ambrx, Ardelyx, and Wildcat Technologies

10.30 - 11.00 hrs Tea Break

Session 1 : 11.00 – 13.15 hrs

Chairpersons

Dr. Kapil Dhingra

Retired Vice President, Head, Oncology Disease Biology Leadership Team, Roche, Director, KAPital Consulting LLC, USA

Dr. Steven G. Reed

Founder, President, & Chief Scientific Officer, Infectious Disease Research Institute, USA

11.00 - 11.45 hrs

Keynote Address

Peptide Therapeutics and the Quest For Differentiated Medicines

Dr. Pierre Rivière

Sr. Vice President, Global R&D, Pfizer, USA

11.45 - 12.30 hrs

Frontloading Toxicity Detection in order to Lower Costs and Attrition

Dr. Bruce Car

Vice President, Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Company, USA

12.30 - 13.15 hrs

Western biotech & Emerging Market pharma alliances: explosive value creation or hype?

Dr. Stephen Yoder

CEO, Pieris AG, Germany

13.15 - 15.00 hrs Lunch, Poster & Networking session

Session 1 : 15.00 – 15.30 hrs

Chairpersons

Dr. Albert R. Collinson

President and CEO, Theracos Inc, USA

Dr. Bruce Car

Vice President, Bristol-Myers Squibb Company, USA

15.00 - 15.45 hrs

Playing the numbers game in small-molecule lead discovery

Dr. Thomas Franch

CSO, Nuevolution, Denmark

February 5, 2013

15.45 - 16.30 hrs

Studies Towards Development Of PCSK9 Inhibitors For Regulating LDL-R and Cholesterol

Dr. Ajoy Basak

Department of Biochemistry, Microbiology & Immunology, Faculty of Medicine and Interdisciplinary School of Health Sciences, University of Ottawa, Canada

16.30 - 17.00 hrs Tea Break

17.00 - 17.45 hrs

FGF21-Adnectin-Pharmacokinetic Enhancer: A modified FGF21 protein with uniquely extended Pharmacokinetic Profile for the Treatment of Metabolic Diseases.

Dr. Ranjan Mukherjee

Department of Metabolic Diseases, Bristol-Myers Squibb Company, USA

17.45 - 18.30 hrs

Inflammation, Mitochondrial Dysfunction and Insulin Resistance- implications for antidiabetic drug discovery

Dr. Satinath Mukhopadhyay

Professor, Department of Endocrinology and Metabolism, Institute of Postgraduate Medical Education & Research, Kolkata, India.

18.30 - 19.15 hrs **Panel Discussion**

- Dr. Peter Schultz
- Dr. Kapil Dhingra
- Dr. Pierre Rivière
- Dr. Bruce Car
- Dr. Thomas Franch
- Dr. Stephen Yoder
- Dr. Ranjan Mukherjee
- Dr. Satinath Mukhopadhyay
- Dr. Ajoy Basak

08.00 - 08.30 hrs Morning Coffee

February 6, 2013

Session 3 : 08.30 – 12.45 hrs

Chairpersons

Dr. Peter Schultz

Scripps Professor of Chemistry, The Scripps Research Institute, USA

Dr. Shilin Shukla

Director, Gujarat Cancer Research Institute, India

Dr. Johanna Holldack

Chief Executive Officer, Telormedix SA, Switzerland.

08.30 - 09.15 hrs

Keynote Address

Oncology 2013: Recent advances in personalized therapy of cancer

Dr. Kapil Dhingra

Retired Vice President, Head, Oncology Disease Biology Leadership Team, Roche, Director, KAPital Consulting LLC, USA

09.15 - 10.00 hrs

Keynote Address

Combined Targeted Therapy in Sarcomas-Pathways to Success?

Dr. Lee Helman

Head & Senior Investigator, Molecular Oncology Section, National Cancer Institute, USA

10.00 - 10.30 hrs Tea Break

10.30 - 11.15 hrs

Personalized Therapy in Oncology - what they don't tell you

Dr. Purvish M. Parikh

Director of Clinical Research and Education, BSES Municipal Hospital, Mumbai

11.15 - 12.00 hrs

Novelties on BCR/ABL

Dr. Martin Ruthardt

Head, Laboratory for Tumor Stem Cell Biology University Clinic II/Hematology Goethe University Frankfurt; Frankfurt, Germany

12.00 - 12.45 hrs

Disease, Process and Partners: Challenges of Biomarker and Diagnostic Development in Oncology

Dr Anjan Thakurta

Sr. Director, Translational Development, Celgene, USA

12.45 - 13.30 hrs Lunch, Poster & Networking session

13.30 - 14.00 hrs

Popular Talk : Locks, Clocks and Peacocks

Dr. Hiren Shah

Ahmedabad, India

February 6, 2013

Session 4 : 14.00 – 16.45 hrs

Chairpersons

Dr. V. S. Chauhan
Director, ICGEB, New Delhi

Dr. Reinhard Glueck
CSO, Vaccine Technology Centre, Zydus Cadila

14.00 - 14.45 hrs

Keynote Address

Adjuvants to Enable Next Generation Vaccines

Dr. Steven G. Reed
Founder, President, & Chief Scientific Officer, **Infectious Disease Research Institute**, USA

14.45 - 15.30 hrs

TLR7 in oncology and as an adjuvant

Dr. Johanna Holldack
Chief Executive Officer, **Telormedix SA**, Switzerland.

15.30 - 16.00 hrs Tea Break

16.00 - 16.45 hrs

Predicting responses to vaccination with systems biology: What have we learned so far ?

Dr. Jan ter Meulen
Executive Director, Vaccine Research, **Merck Research Laboratories**, USA

16.45 - 17.30 hrs **Panel Discussion**

- Dr. Kapil Dhingra
- Dr. Lee Helman
- Dr. Purvish M. Parikh
- Dr. Martin Ruthardt
- Dr Anjan Thakurta
- Dr. Steven G. Reed
- Dr. Johanna Holldack
- Dr. Jan ter Meulen

17.30 - 18.00 hrs Best Poster Award & Oral Presentation 1 & 2

18.00 hrs **Vote of Thanks**

Speaker Profiles & Abstracts

Dr. Peter Schultz.

Director, Calibr Institute, Professor of Chemistry, The Scripps Research Institute, USA
Founder of Affymax Research Institute, Syrrx, Kalypsys, Phenomix, Symyx Therapeutics, Ilypsa, Ambrx, Ardelyx, and Wildcat Technologies

Dr. Schultz is a pioneer in the fields of synthetic biology and the development of technologies for synthesizing and screening large collections of proteins, druglike synthetic compounds and solid state materials with novel chemical and biological properties. His work spans chemistry, materials science and biomedical research.

Dr. Schultz was director of the Genomics Institute of the Novartis Research Foundation (GNF) in San Diego, Calif. from 1999 to 2010. In addition, he has founded multiple start-up companies including: Affymax Research Institute, Syrrx, Kalypsys, Symyx Technologies, Ilypsa, Ambrx, Ardelyx and Wildcat Technologies.

Dr. Schultz did his undergraduate and graduate work at the California Institute of Technology. After postdoctoral studies at the Massachusetts Institute of Technology, he was on the faculty of the University of California, Berkeley as professor of chemistry, principal investigator at Lawrence Berkeley National Laboratory, and an investigator for the Howard Hughes Medical Institute.

Dr. Schultz has published over 500 scientific publications, and received numerous awards for his work including: the Waterman Award of the National Science Foundation, the 1994 Wolf Prize in Chemistry, the 2003 Paul Ehrlich Prize, and the 2005 Arthur C. Cope Award of the American Chemical Society. He is a member of the National Academy of Sciences and the Institute o f Medicine.

Topic

A Chemist's Foray into Translational Research

Dr. Pierre Rivière.

Sr. Vice President, Global R&D, Pfizer, USA.

Pierre Riviere is also a member of Pfizer WRD Leadership Team. With over 20 years pharmaceutical Industry experience, mainly focused on peptide therapeutics, Pierre Riviere is now leading the transformation of CovX into the peptide therapeutics R & D division of Pfizer. Building upon and beyond its unique CovX bodies technology for extending half-life of macromolecules, CovX is now assembling a versatile and comprehensive peptide drug discovery capability in terms of molecular target drugability, duration of action, targeting and delivering. Before joining Pfizer, Pierre Riviere was President of the Ferring Research Institute and Sr. Vice President, Research for Ferring Pharmaceuticals where he contributed to the discovery of several peptide new chemical entitites, now at various stage of development, either in house or through licenses. He previously led the Gastroenterology drug discovery department of the Institut de Recherche Jouveinal. Pierre Riviere holds a Ph.D. in Biology and Physiology from the Institut National Polytechnique of Toulouse in France and has completed a post-doctoral training in the Department of Pharmacology at the University of Arizona. He is a co-founder of Cara Therapeutics, BioHeatMap, The Peptide Therapeutics Foundation and the Annual Peptide Therapeutics Symposium.

Topic

Peptide Therapeutics and the Quest For Differentiated Medicines

Pharmaceutical and biotech pipelines are heavily weighted towards antibody and small molecule based therapeutics. Peptides in general are often viewed as starting points for drug discovery rather than a viable solution on their own. Lack of oral bioavailability, poor permeability, metabolic instability along with formulation and delivery challenges are often cited as liabilities when considering peptides as drugs. On the other hand, peptides possess unique potential to function as receptor agonists, targeting agents or bifunctional molecules that need to be effectively harnessed in order to develop differentiated medicines. CovX has developed a novel class of peptide-antibody fusion molecules, referred to as CovX-Bodies that provide an elegant solution for half life extension of therapeutic peptides. Evolution of the CovX technology platform and chemical strategies aimed at peptide therapeutics with differentiated target product profiles will be presented.

Dr. Bruce D. Car

Vice President, Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Inc., USA.

Bruce D. Car received Veterinary Medicine with Honours (1983), Masters (1985) degrees, and postgraduate training in pathology at The University of Melbourne, Victoria, Australia. He received his Ph.D. degree in 1989 from Cornell University, New York, studying a pulmonary disease model of extravascular coagulation and fibrinolysis. He attained American College of Veterinary Pathology specialty certification in Anatomic Pathology (1987) and Clinical Pathology (1990), and later certification with the American Board of Toxicology (1995) and was elected a Fellow of the International College of Toxicologic Pathology in 2002. Bruce undertook postdoctoral studies in immunology (1989-1994) at the Theodor Kocher Institute, University of Berne, and ETH/University of Zurich, Switzerland where he studied the role of alpha and beta chemokines in inflammatory disease, and cytokines in immunotoxicology and hematopoiesis. In 1994 he joined DuPont Pharmaceuticals in Pathology and later led the Discovery/Investigative Toxicology group, which he continued after the acquisition of DPC in 2001 by Bristol-Myers Squibb Co. In 2010, Bruce extended his responsibilities to include Metabolism and Pharmacokinetics, Biotransformation, Discovery Analytical and Bioanalytical Sciences, Discovery Toxicology, and Discovery Pharmaceuticals as Vice President, Pharmaceutical Candidate Optimization (PCO); an organization of approximately 300 located in Princeton and Pennington, NJ, Wallingford, Connecticut, and Bangalore, India. PCO's mission is to facilitate the creation of synthetic and biologic drug candidates with optimal developability and risk assessment profiles, and shepherd those compounds through development. Bruce is a frequently invited speaker in the ADME, Toxicology, and Pharmaceutical Discovery fields and has published over 80 manuscripts.

Topic

Frontloading Toxicity Detection in order to Lower Costs and Attrition

Dr. Stephen S. Yoder, JD

CEO, Pieris AG, Germany.

Stephen Yoder is an accomplished deal-maker and IP strategist in the life sciences sector, drawing on significant legal, intellectual property and corporate development experience. Since joining Pieris at the beginning of 2010, the company has matured to a clinical-stage biotech and has closed several pharma collaborations, including prominent alliances with Sanofi Group and Daiichi-Sankyo. Previously, he led the IP and legal departments at MorphoSys AG as General Counsel, and was responsible for several areas of business development, having negotiated several collaborations with the largest pharmaceutical companies worldwide, including the company's 2007 transforming deal with Novartis. Mr. Yoder holds degrees in Molecular Biology (B.S.) and Spanish (B.A.) from Grove City College and a Juris Doctorate, with honors, from George Washington University Law School. As an attorney, he is licensed to practice before the United States Patent and Trademark Office, and in the jurisdictions of Maryland and Washington, DC.

Topic

Western biotech and Emerging Market pharma alliances: explosive value creation or hype?

Converging forces are driving the rationale for R&D alliances between small biotech and established emerging market pharmaceutical companies. For the biotech, emerging market players can be a fresh source of funding in a capitally compressed environment, while potentially allowing a more prominent role in drug development and broader retained rights through collaboration than Big Pharma historically has tolerated. For the emerging market company, novel biologics may be the only way to maintain double-digit CAGR as generics markets mature, and hitching on to an innovative biotech can bring substantial de-risking and time savings relative to organic growth.

But are these two separate universes ready to share a common orbit? While on the surface the ingredients for a symbiotic relationship seem abundant, several factors need to be considered in detail, from costs, timelines, and cultural and regulatory factors that impact the ability to use emerging market-centric activities as a gateway to the more lucrative drug pricing environment of the West. This presentation will provide deeper insights to these and other factors to help stimulate a dialogue on how best to unlock this seemingly pent-up value.

Dr. Thomas Franch

CSO, Nuevolution, Denmark.

Thomas obtained his ph.d in molecular biology from the University of Southern Denmark. In 1999, he co-founded a company based on the application of artificial antisense molecules for the interference of biological processes. He joined Nuevolution 2001 as Sr. Scientist. Over the years, his main responsibility has been the development and integration of all company technologies. Thomas was appointed the CSO of Nuevolution in 2012.

Topic

Playing the numbers game in small molecule Lead Discovery

Initial hit discovery remain a formidable task for a large number of pharmaceutically relevant targets including those involved in protein-protein interactions. The lack of a general design principle for addressing such difficult surfaces with small-molecules often leave programs with few or no hits for optimization. Consequently, finding hits for PPIs is generally considered a numbers game correlating hit-identification with the size and diversity of the libraries screened.

Application DNA-encoded compound libraries allows for the assembly of thousands of fragments into millions of compounds and the subsequent screening of complex mixtures in a pico-scale format. Our data from the screening of such libraries on PPIs documents the value large libraries in the size of multi-millions to billions of compounds in order to obtain significantly improved screening outcome. Interestingly, our results indicate that the number of library fragments and not merely the number of compounds is essential for identification of hits with the best potency and optimization properties.

Data from programs will be presented demonstrating the principle of the DNA-encoding technology and its application for hit identification on both tractable and intractable targets.

Dr. Ajoy Basak

Chronic Disease Program, Ottawa Hospital Research Institute, Department of Biochemistry, Microbiology & Immunology, Faculty of Medicine and Interdisciplinary School of Health Sciences, Faculty of Health Science, University of Ottawa, 725 Parkdale Ave, ON K1Y4E9, Canada

Topic

Studies Towards Development Of PCSK9 Inhibitors For Regulating LDL-R And Cholesterol

Ajoy Basak, Heather Palmer-Smith and Priyambada Mishra

Since its discovery in 2003, human (h) PCSK9 (Proprotein Convertase Subtilisin/Kexin9) has drawn significant attention for its role in the degradation of Low Density Lipoprotein-Receptor (LDL-R). hPCSK9 induced LDL-R degradation reduces LDL-cholesterol uptake by hepatocytes causing an accumulation of circulatory cholesterol in the blood - a condition known as Hypercholesterolemia. Owing to this key biological property, confirmed by various studies including knock out mice, hPCSK9 became a major target for intervention of hypercholesterolemia. Crystal structure revealed that hPCSK9's catalytic domain binds with LDL-R's EGF-A domain leading to re-routing of LDL-R towards lysosome for its degradation. The precise location of catalytic domain(s) of PCSK9 involved in binding with LDL-R's EGF-A domain has not yet been identified. This remains as the major focus of our study since peptides derived from such domains may likely interfere in the binding of PCSK9 with LDL-R, leading to possible reduction of LDL-R degradation. Our studies using human hepatic HepG2 cells and in vitro experiments using synthetic EGF-A peptide suggested LDL-R promoting activity of at least one hPCSK9 catalytic loop peptide. It is proposed that this effect may be mediated via inhibition of hPCSK9 function. The results will be further confirmed using LDL-R uptake and other biochemical studies which may likely lead to the development of new class of small molecule PCSK9 inhibitors as potential therapeutic agents for lowering cholesterol – alternative to non-statin drugs.

This work is funded by Heart & Stroke Foundation Grant no NA 7187 to AB.

Dr. Ranjan Mukherjee

Department of Metabolic Diseases, Bristol-Myers Squibb Company, 311 Pennington-Rocky Hill Road, Bristol Myers Squibb Company, Pennington, N.J. 08534-2130.

Dr. Ranjan Mukherjee did his B.Sc. (Physics Honors) and M.Sc. in Physics at the University of Calcutta, India and completed his Ph.D. in Biology at the University of Delaware, USA. He then did his Post Doctoral training in the laboratory of Prof. Pierre Chambon in Strasbourg, France. Thereafter, he joined Ligand Pharmaceuticals where he demonstrated the possibility of RXR agonists in the treatment of diabetes which led to a collaboration with Eli Lilly in the identification of PPAR and RXR agonists for treating metabolic diseases. He also identified the first selective PPAR modulator as a potential treatment for diabetes. He then moved to DuPont Pharmaceuticals, later acquired by Bristol Myers Squibb where he is currently a Senior Principal Investigator. Among his accomplishments there are initiating and leading discovery programs in Atherosclerosis and Diabetes.

Recently he led the team that delivered FGF21-adnectin pharmacokinetic enhancer (FGF21-Ad-PKE), the first biologic in the BMS diabetes portfolio. This novel approach was presented at the ADA annual conference, Philadelphia in 2012.

Dr. Mukherjee has authored several manuscripts and book chapters, and is an enthusiastic speaker at national and international conferences. He continues to pursue his love of innovative targets for the treatment of metabolic diseases.

Topic

FGF21-Adnectin-Pharmacokinetic Enhancer: A modified FGF21 protein with uniquely extended Pharmacokinetic Profile for the Treatment of Metabolic Diseases.

FGF21 treatment has been shown to improve the diabetic, insulin resistant and dyslipidemic state in several animal models of diabetes and obesity without inducing hypoglycemia, mitogenesis or edema. Its short half life would require multiple, daily injections to be used as a therapeutic agent. We hypothesized that extending the half life of FGF21 would lead to improved efficacy and require less frequent dosing. Adnectins are a new family of proteins based on the 10th type III domain of human fibronectin that can be designed to bind to targets of interest with high affinity and specificity. We have created a novel protein by fusing human FGF21 to an adnectin pharmacokinetic enhancer (FGF21-adPKE) which binds to human and monkey but not mouse serum albumin. This novel FGF21-adPKE retains the property of inducing ERK phosphorylation in β-klotho-expressing cells and glucose uptake in adipocytes. In ob/ob mice, FGF21-adPKE lowers plasma glucose, HbA1c, insulin and triglyceride levels. In cynomolgus monkeys the half-life of FGF21-adPKE was 97 hours, significantly greater than native human FGF21 (4 hours). FGF21-adPKE is produced in E.coli and displays preclinical pharmacokinetic properties that would support once weekly subcutaneous dosing in humans. In conclusion, FGF21-adPKE represents a promising candidate for the treatment of diabetes.

Dr. Satinath Mukhopadhyay

Professor, Department of Endocrinology and Metabolism, Institute of Postgraduate Medical Education & Research, Kolkata, India. Email: satinath.mukhopadhyay@gmail.com

Dr. Satinath Mukhopadhyay is MD (General Medicine) and DM (Endocrinology). He is currently working on insulin signalling, apoptosis of pancreatic beta cells, mitochondrial biogenesis and bioenergetics in non- obese Type2 Diabetes and role of vitamin D in diabetes. He is member of various scientific societies and reviewer for 6 journals. Dr. Satinath Mukhopadhyay has published over 20 journals in last 5 years.

Topic

Inflammation, Mitochondrial Dysfunction and Insulin Resistance- implications for antidiabetic drug discovery

Chronic, low grade inflammation and mitochondrial dysfunction are being increasingly implicated in the pathogenesis of Type2 diabetes (T2D). Our recent studies show that, while mitochondrial dysfunction in the form of deranged bioenergetics and/ or biogenesis may play significant roles in the pathogenesis of obese subjects with T2D, it does not appear to play similar roles in non –obese persons with T2D. Inflammatory cytokines, however, were found to be elevated in all our study subjects with T2D, irrespective of the presence of obesity. Toll-like receptor 4 (TLR4) has a key role in innate immunity by activating an inflammatory signaling pathway. Free fatty acids (FFAs) stimulate adipose tissue inflammation through the TLR4 pathway, resulting in insulin resistance. However, current evidence suggests that FFAs do not directly bind to TLR4 but an endogenous ligand for TLR4 remains to be identified. Here we show that fetuin-A (FetA) could be this endogenous ligand and that it has a crucial role in regulating insulin sensitivity via Tlr4 signaling in mice. FetA (officially known as Ahsg) knockdown in mice with insulin resistance caused by a high-fat diet (HFD) resulted in downregulation of Tlr4-mediated inflammatory signaling in adipose tissue, whereas selective administration of FetA induced inflammatory signaling and insulin resistance. FFA-induced proinflammatory cytokine expression in adipocytes occurred only in the presence of both FetA and Tlr4; removing either of them prevented FFA-induced insulin resistance. We further found that FetA, through its terminal galactoside moiety, directly binds the residues of Leu100–Gly123 and Thr493–Thr516 in Tlr4. FFAs did not produce insulin resistance in adipocytes with mutated Tlr4 or galactoside-cleaved FetA. Taken together, our results suggest that FetA fulfills the requirement of an endogenous ligand for TLR4 through which lipids induce insulin resistance. This may position FetA as a new therapeutic target for managing insulin resistance and type2 diabetes.

Dr. Kapil Dhingra

Retired Vice President, Head, Oncology Disease Biology Leadership Team, Roche, Director, KAPital Consulting LLC, USA

Kapil Dhingra has served as a member of Micromet's Board of Directors since February 2009. In June 2008, Dr. Dhingra founded KAPital Consulting, LLC, a healthcare consulting firm. From 1999 to 2008, Dr. Dhingra served in positions of increasing responsibility at Hoffmann-La Roche, including Vice President, Head, Oncology Disease Biology Leadership Team, and Head, Oncology Clinical Development. Prior to joining Hoffmann-La Roche, Dr. Dhingra worked as a Senior Clinical Research Physician with Eli Lilly and Company. From 1989 to 1996, he served as a Clinical Instructor, Assistant Professor of Medicine at the University of Texas MD Anderson Cancer Center. Throughout his industry career, Dr. Dhingra maintained an active faculty appointment, initially at Indiana University School of Medicine from 1997 to 1999 as Clinical Associate Professor, and, more recently, at Memorial Sloan Kettering Cancer Center in New York from 2000 to 2008. Dr. Dhingra holds an MBBS degree from the All India Institute of Medical Services, and has performed postgraduate work at the All India Institute of Medical Services, the Lincoln Medical and Mental Health Center (New York Medical College), Bronx, NY and Emory University School of Medicine. Dr. Dhingra is currently an advisor to several biotechnology and pharmaceutical companies and serves on the board of directors of Micromet, Algeta ASA, Biovex, Inc., and Coferon.

Topic

Oncology 2013: Recent advances in personalized therapy of cancer

Rapid advances in understanding of the molecular basis of cancer are starting to finally translate to important new therapeutics for cancer. During 2012, a large number of new drugs were approved to treat cancers. These drugs target a variety of signaling pathways. Several of these drugs have produced impressive survival benefit in both hematologic and solid tumors. In this talk, I will discuss some of these important new drugs, the indications for their use and key clinical efficacy and safety data. Among the drugs discussed will be pertuzumab for HER2-positive breast cancer, enzalutamide for prostate cancer, ponatinib for CML, vismodegib for basal cell carcinoma, cabozantinib for medullary thyroid carcinoma and carfilzomib for multiple myeloma. The need to rapidly develop and approve breakthrough drugs for life-threatening diseases is being increasingly recognized by regulators. We can expect to see a continuing acceleration of the diversity of our therapeutic armamentarium against cancer in the years to come.

Dr. Lee Helman

Head & Senior Investigator, Molecular Oncology Section, National Cancer Institute, USA

Dr. Helman received his M.D. from the University of Maryland School of Medicine in 1980 magna cum laude, and was elected to Alpha Omega Alpha. He completed his internship and residency in Internal Medicine at Barnes Hospital Washington University, serving as the Chief Resident, Washington University VA Medical Service in 1983. He began his fellowship training at the National Cancer Institute (NCI) in 1983, where he has remained. He became the head of the Molecular Oncology Section of the Pediatric Oncology Branch, NCI, in 1993, and Chief of the Pediatric Oncology Branch, NCI, in 1997. He was also named a Deputy Director of the Center for Cancer, NCI, in 2001. He served as Acting Scientific Director for Clinical Research, Center for Cancer Research, NCI, in 2005, and named as the permanent Scientific Director in 2007.

Dr. Helman's laboratory currently focuses on three major themes related to the biology and treatment of pediatric sarcomas, specifically rhabdomyosarcoma, Ewing's sarcoma and osteosarcoma: (1) determine the pathophysiologic consequences of IGF signaling; (2) identify the molecular/biochemical determinants of the biology of these sarcomas; and (3) apply preclinical laboratory findings to develop novel clinical studies for these sarcomas.

Topic

Combined Targeted Therapy in Sarcomas- Pathways to Success?

Lee J. Helman, M.D., Center for Cancer Research, National Cancer Institute, Bethesda, MD

It is clear that while newer anti-cancer therapies targeting specific altered signaling pathways (typically kinase signaling) in tumors has led to some success, with rare exceptions, responses are transient. In general, this is due to acquired resistance, either by selection of mutations specifically resistant to the kinase inhibitor, or due to activation of "by pass" signaling pathways that render the initial targeted kinase less critical. We recently completed a Phase II study targeting the IGFI receptor signaling pathway in refractory Ewing's and other sarcomas. We demonstrated an objective response rate of 16 percent, but again most responses were transient lasting less than 18 weeks. To improve on these responses, we have been probing these tumors to identify other critical pathways that might allow combined targeting approaches. Using multiple lines of investigation, we have determined that Src family kinases (SFK) appear to contribute to early resistance to IGFI receptor blockade and may indeed act as a by-pass pathway. Combining IGFI receptor blockade with SFK kinase inhibitors has demonstrated highly synergistic activity both in vitro and in vivo, suggesting that dual IGFI and SFK kinase inhibition may lead to improved therapeutic outcomes.

Dr. Purvish M. Parikh

Director of Clinical Research and Education, BSES Municipal Hospital, Mumbai.

Dr. Purvish M. Parikh is Medical Oncologist & Hematologist. He has MBBS, MD, Ph. D. and MBA degrees from Bombay University. He is director of Clinical Research and Education, BSES Municipal Hospital, Mumbai. He is Editor in Chief of Indian Journal of Cancer and South Asian Journal of Cancer journals. He is member of various societies and boards in India. He is awarded with several awards like National Citizen's Award and Wockhardt Medical Excellence Award. He is author of 16 books and written chapters for 36 books and published 117 research articles. Dr. Parikh has conducted more than 30 ICH GCP/ ICMR compliant clinical trials in cancer as principal investigator.

Topic

Personalized Therapy in Oncology - what they don't tell you

Personalized medicine and targeted therapy have revolutionized the way we approach cancer management. The promise of monoclonal antibodies, initiated 20 years ago, is now becoming a reality. The dramatic success story of imatinib in CML is like a beacon of hope for all cancers.

Today it is not enough to simply have a biopsy confirm the diagnosis of cancer. Histological subtyping is complemented by tumoral, somatic and germline evaluation for immunophenotyping, FISH, mutation analysis, next generation sequencing and imaging of the most sophisticated type. Each specific test provides one more piece of the jigsaw that allows us to select the right treatment strategy and the most appropriate molecule for targeted therapy.

This concept of personalized medicine is not new. But does it live up to the promise? And if not what needs to be done to make it happen. These issues will be discussed using specific examples.

Dr. Martin Ruthardt, M.D.

Head, Laboratory for Tumor Stem Cell Biology University Clinic II/Hematology Goethe University, Frankfurt; 60596, Germany

Dr. Martin Ruthard is a leading clinician and researcher at division of Hematology / Oncology, Clinic of the JW Goethe-University Frankfurt. His research focus is: Chronic and acute myeloid leukemia, Leukemic stem cell, Molecular "targeting" leukemia-inducing oncoproteins. The aim of his laboratory is to develop an interdisciplinary research into the pathogenesis of leukemia and on the basis of the results of basic research to develop novel hypothesis-driven therapeutic approaches or even improving existing treatment strategies.

Topic

Novelties on BCR/ABL

Dr Anjan Thakurta

Sr. Director, Translational Development, Celgene, USA

Anjan Thakurta got his Master's degree from Jawaharlal Nehru University, India and M. Tech. from IIT Delhi, India. He was a Nehru scholar at Cambridge University, U. K., and did his doctoral research in the Genetics dept. and completed his post-doctoral training at Molecular and Cellular Biology Dept. at Harvard University, USA. He worked at the National Cancer Institute, AstraZeneca and Biogen Idec in various roles in Oncology Research and Drug development. Currently he is Sr. Director, Translational Development at Celgene and co-leads the development and implementation of strategies for Biomarker and Companion Diagnostics and oversees R and D/CRO partnerships for Translational Development. In addition, he leads translational development of immunomodulatory agents in Multiple Myeloma and CLL.

Topic

Disease, process and partners: Challenges of biomarker and diagnostic development in oncology

Translational research is a powerful approach that allows the discovery and development of biomarkers and diagnostics at all stages of drug development. While the upside is tremendous, biomarker/diagnostic development adds huge challenges to the clinical development process. In this presentation I will focus on three key areas dealing with disease complexity, process challenges and external partnerships that have significant impact in the development of biomarkers and companion diagnostics. As translational scientists, our ability to respond to these scientific and business challenges is critical to integrate our development efforts and to increase chance of success.

Dr. Hiren Shah, MD

Ahmedabad, India

The house of Ahmedabad-based doctors, Dr. Namita and Dr. Hiren Shah, reflects their love for arts and antiques. From traditional utensils, antique hookahs, old tea pots to myriad locks and objects de arts, every mural has a story to tell. A hobby followed passionately resulted into a creation of museum at their residence. Their collection have got them invited for many international events. Dr. Hiren Shah was the only Indian speaker among 200 delegates at International Puzzlers Party, Australia 2007. He has attended many international events and says that the international acclamation inspired to improve their collection as they realised its global value.

Topic

Popular Talk : Locks, Clocks and Peacocks

Dr. Steven G. Reed

Founder, President, & Chief Scientific Officer, Infectious Disease Research Institute, USA

Steve Reed is the Founder, President, and CSO of the Infectious Disease Research Institute (IDRI). His academic appointments include Professor of Medicine at Cornell University Medical College in New York and Research Professor of Pathobiology at the University of Washington. He serves on several editorial review committees, has served as a member of the Tropical Medicine Review Board of the National Institutes of Health, and is a member of the Vaccine Development Steering Committee of the World Health Organization.

Dr. Reed received a PhD in Microbiology and Immunology from the University of Montana in 1979. That year he was appointed as Scientist of the National Institute of Amazon Research in Manaus, Brazil, where he directed research on tropical diseases. Dr. Reed joined Cornell University Medical College in 1980 as Assistant Professor of Medicine, continuing to work in Brazil as manager of the Cornell-Bahia program in International Medicine. He joined the Seattle Biomedical Research Institute in 1984 where he worked until founding IDRI in 1993. In 1994 he co-founded Corixa Corporation (which was later sold to GlaxoSmithKline, GSK) where he served as Chief Scientific Officer until leaving in 2004. He also founded Dharma Therapeutics, where he served as President from 2005-2008. In 2008 Dr. Reed co-founded Immune Design Corp. Dr. Reed's research interests have focused on the immunology of intracellular infections, and on the development of vaccines and diagnostics for both cancer and infectious diseases. He led the team that, together with GSK, developed the first defined tuberculosis vaccine to advance to clinical trials, and has developed the first defined vaccine for leishmaniasis, as well as the K39-based diagnostic tests currently licensed for leishmaniasis. He has more than 230 original publications, 35 book chapters and reviews, and 105 issued patents on diagnostics, vaccines, and therapeutics of infectious diseases and cancer.

Topic

Adjuvants to Enable Next Generation Vaccines

Safe and effective adjuvants for prophylactic and therapeutic vaccine use are resulting from the identification of small molecules and optimization of their formulations. Effectively engaging macrophages and dendritic cells (DC), leading to T cell responses is essential for developing a new generation of T cell vaccines (e.g. tuberculosis, malaria, HIV), as well as for improving the quality and duration of antibody responses (influenza, HPV, HIV, etc.). The most advanced approaches to new adjuvant development consist of using TLR ligands (TLRL), which when properly formulated can be used in minimal amounts to achieve desired effects. We have developed formulations of synthetic of several TLR4L and have evaluated a variety of these, including oil/water emulsions, micellar, and liposomal, in non-clinical and clinical studies. Thus, selective molecular synthesis and formulation may lead to a new generation of TLR4L- based adjuvants with improved qualities over natural products.

Dr. Johanna Holldack

Chief Executive Officer, Telormedix SA, Switzerland

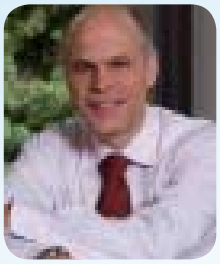
Dr. Holldack has worked in the pharmaceutical industry for more than 20 years, where she has held key positions in both start-up and large pharma companies. Her experience includes management and executive positions at Behringwerke, Chiron, MediGene and Borean Pharma.

Dr. Holldack has a medical degree from Georg-August-University in Gottingen, Germany and is a Board Certified pediatrician. In addition, she has held positions as an assistant professor at the University of Essen, research fellow for the Deutsche Krebshilfe and a research associate at Harvard Medical School.

Topic

TLR7 in oncology and as an adjuvant

A new player in the immune system has been identified less than 20 years ago: the innate immune system. First identified in Drosopholia, later in other animals and humans. A first line defense system, that is hundreds of millions of years old in the evolution. Toll like receptors have been identified as the key players of the innate immune system. Compounds activating the innate immune system have been on the market before the innate immune system had been discovered. They were labeled as immunmodifers. Imiquimod is one of those compounds, that induces interferon through activation of TLR-7 and TLR-8. It is highly efficacious in the treatment of genital warts and skin cancer. In other words the TLR-7 activation is a validated treatment approach in humans. Recent studies make it apparent that the mechanism of action is based on activation of dendritic cells, probably the same mechanism responsible for the vaccine adjuvanticity and the anti tumour effect. It has been demonstrated during the last years, that TLR-7 is highly expressed in autoimmune diseases. The development of weak TLR-7 agonists could therefore be a new means for the treatment of autoimmune diseases. In summary: The development of compounds that bind to TLR7 provides us with stimulatory drugs that can be utilized as vaccine adjuvants or cancer treatments. The group of weak agonists can be novel means for the treatment of autoimmune diseases by bringing the immune system back into balance.



Dr. Jan ter Meulen

Executive Director, Vaccine Research, Merck Research Laboratories, USA

Dr. ter Meulen received his M.D. in 1988 from the Albert-Ludwig University Medical School in Freiburg, Germany. He earned his Dr.Med. in epidemiology in 1990 from Julius Maximilian University in Würzburg, Germany. He conducted research from 1988 until 1992 at the German Cancer Research Center. He held positions at the Bernhard Nocht Institute for Tropical Medicine, and from 1988 until 1999 worked as Research Scientist at EPICENTRE (Groupe Européen d'Expertise en Épidémiologie Pratique) in Paris, France. He is currently Research Scientist of the Projet de Recherche sur les Fièvres Hémorragiques en Guinée at the University of Conakry Institute for Microbiology in Guinea. He is also currently Research Scientist in the Department of Virology of the Bernhard Nocht Institute for Tropical Medicine.

Topic

Predicting responses to vaccination with systems biology: What have we learned so far ?

Poster Abstracts

P001: Ashwagandha powder gives protection in pulmonary hypertension

Gurpreet Kaur, Kashif Hanif

Division of Pharmacology, CSIR-Central Drug Research Institute, Lucknow-226001

Pulmonary hypertension (PH), a chronic debilitating lung disorder, is associated with pulmonary vascular remodeling and progressive increase in pulmonary artery (PA) pressure leading to right ventricular (RV) hypertrophy and heart failure. Current therapies are able to extend life span only up to five years emphasizing the need of new drugs. Powder of Ashwagandha (ASH; Withania Somnifera), a clinically used drug in Ayurvedic medicinal system, shows anti-oxidant, anti-inflammatory and cardioprotection but its effect in PH is still unexplored. Therefore, present study evaluated the effect of ASH on PH which was induced by the single administration of Monocrotaline (MCT, 60 mg/kg, s.c.) in SD rats. Commercially available powder of ASH (50 and 100 mg/kg/day, p.o.) was administered from day 1 post-monocrotaline up to 35 days. MCT caused an increase in right ventricular pressure and hypertrophy which was reversed by ASH dose dependently. ASH treatment reversed the pulmonary artery cardiac remodeling and decreased inflammation. MCT caused endothelial dysfunction in pulmonary arteries and decreased expression of eNOS and both the effects were reversed by ASH. TUNEL studies showed that ASH treatment induced apoptosis in lungs and right ventricle of MCT treated rats. Above results show that ASH attenuates pathophysiology of PH and may serve as a promising drug for future clinical studies in PH.

P002: Cardioprotective effect of T3 hormone in isoproterenol-induced cardiotoxicity

Shilpesh Devada, Priya Ghumatkar, Vinay Mishra, Maulik Patel, Vaibhavi Pawar, Shekhar Kadam, R. K. Ranvir, S. R.Sundar and Mukul Jain

Department of Pharmacology & Toxicology, Zydus Research Centre, Cadila Healthcare Limited.Ahmedabad – 382213, India,

Background: The iodothyronine hormones are essential for normal growth and development and play an important role in energy metabolism. However T3 (3, 5, 3′-triiodothyronine) has attracted relatively little attention in relation to CVS diseases. Objective: To evaluate the cardio protective action of T3 in isoproterenol (ISO) induced cardiac toxicity. Material and Methods: Female Wistar rats were exposed with ISO (100 mg/kg) for 2 days at the interval of 24 h followed by T3 (3 µg/kg) treatment for 3 days. Negative control rats received only ISO (100 mg/kg) for 2 days at the interval of 24 hrs. Vehicle control animals received normal saline. Results: The ISO induced significant changes in heart weight, low-density lipoprotein, CK-MB to TCK ratio, and prolongation of QT interval in electrocardiogram, which were normalized after T3 treatment. Gene expression of cardiac myosin heavy chain alpha (MHC-□) was upregulated in T3 treated group. Microscopic examination of cardiac tissue revealed reduced inflammatory cell infiltration, myonecrosis, vacuolar changes and a trend towards normal cardiac muscle fiber architecture. Conclusion: T3 was found to be cardioprotective in isoproterenol induced cardiotoxicity model.

P003: A modified subtotal nephrectomy model in wistar rats to study renal and anemia related disorders

Shilpesh Devada, Maulik Patel, Vinay Mishra, Vaibhavi Pawar, Sweta Patel, Jitendra H. Patel, G. J. Natraju, Ramchandra Ranvir, S.R.Sundar , Mukul Jain

Department of Pharmacology & Toxicology, Zydus Research Centre, Ahmedabad – 382213, India.

This experimental model was designed to explore the advantages over the existing and established nephrectomy models in the area of kidney disease and anemia for the purpose of determining the efficacy of drugs or new chemical or biological entities. In this model, we have followed subtotal surgical renal ablation technique in rat through a single surgery instead of recommended two surgeries at different time intervals. Study comprised of three groups of male rats viz., Group-I (Sham Control), Group-II (Ablation : whole right kidney on day 1 and 2/3 of left kidney on day 7) and Group-III (Ablation: whole right kidney and 2/3 of left kidney on day 1). Hematological and serum biochemical estimations were studied pre surgery and on day 14, 21 and 28 post surgery. As anticipated under nephrectomised conditions, significant rise in reticulocytes, decline in hemoglobin content and higher levels of serum creatinine and urea was noticed when compared control group in our animal model. Based on above experimental conditions and results, we confirm that the present novel model involves less time to develop with major advantage over the established surgical renal ablation technique in terms more significant and stable effects on the desired endpoints with minimum surgical stress and associated mortality in animal models.

P004: *In vitro* study comparing inhibitory potency of renin inhibitors in presence/absence of plasma

Bhavesh Sharma, Vishal Unadkat, Tulsi Dhakan, †Pravin Thombare, †Jigar Desai, Mukul Jain and Ganes Chakrabarti

Department of Cell & Molecular Biology, † Department of Medicinal Chemistry,
Zydus Research Centre, Ahmedabad

Renin is an enzyme that plays a major role in the Renin-Angiotensin System, a regulatory system in the body, which is responsible to maintain homeostasis of blood pressure. The catalytic role played by renin is thus crucial in mediating blood pressure by the Renin-Angiotensin System.Other antihypertensive drug classes that act at later steps of the ACE pathway in the RAAS system. Angiotensin-converting enzyme inhibitors prevent the production of Ang II and angiotensin II receptor blockers (ARBs) interrupt the binding of Ang II at the receptor without inhibiting the cascade. These classes suppress a negative feedback loop that inhibits renin release and decreases Ang II levels. This leads to a rise in plasma renin concentration (PRC) that results in increased plasma renin activity (PRA). This increased PRA causes an increased formation of Ang I and Ang II, thereby, limiting the antihypertensive benefit of these drugs. Unlike ACE inhibitors and ARBs, aliskiren is able to suppress the renin system at its initiation point, without inducing the reactive rise in PRA. So Screening of Renin inhibitors in presence & absence of plasma is crucial for better understanding the properties of compounds & its relevance *in vivo*.

P005: Development of erythropoietin [EPO] mimetic peptides for the treatment of anemia

Vijay Prajapati*, Rajendra Chopade*, Dipam Patel, Jignesh Pethani*, Ganesh Rahane*, Hardik Shah*, Bhushan Dave*, Rajesh Bahekar, Amit Joharapurkar#, Vishal Patel#, Samadhan Kshirsagar#, Ganes Chakrabarti & Mukul Jain

*Department of Medicinal Chemistry and # Department of Pharmacology, Zydus Research Centre, Sarkej-Bavla N. H. No. 8A, Moraiya, Ahmedabad 382210, Gujarat, India.

Erythropoietin (EPO) is a cytokine synthesized by the kidney that regulates the synthesis of erythrocytes. It is primary regulator of growth and survival of erythroid progenitors which matures into RBCs. The different variants of EPO are present in the market and widely used for the treatment of anemia associated with chronic diseases such as CKD and Cancer. Also the novel erythropoiesis-stimulating agents [ESA] were introduced which are dimeric Erythropoitin Mimetic Peptides [EMP] conjugated with either PEG [eg. Hematide] or with hydroxyethyl starch (HES) [eg. AGEM400]. The uses of these agents are limited due to its cost, frequency of administration and anti-Epo antibodies formation. Moreover, these molecules are high molecular weights and their renal clearance is the major problem in the patient with impaired kidney functions. With these concerns, there is need to develop better agents to stimulate erythropoiesis and which ultimately may be used in the treatment of anemia.

In the present communication, we have developed short chain novel Erythropoitin mimetic peptides. The synthesis of titled compounds (**2a-e**) was carried out by using Solid Phase Peptide Synthesis (SPPS) approach. The *in vitro* EPO mimetics activity of test compounds were evaluated by MTT based assay in TF-1 cell lines and compounds (**2d)** & (**2e)** showed good *in vitro* EPO mimetic activity. Further these test compounds were subjected for *in vivo* activity in Normocythemic mice [BALB/c] & compound (**2e)** exhibited remarkable EPO mimetic activity.

P006: Plaque characterization in the accelerated model of iliac artery atherosclerosis in New Zealand White rabbits following cholesterol diet withdrawal

Khanna V[a], Jain M[a], Singh V[a], Kanshana JS[a]*, Prakash P[a], Barthwal MK[a], Murthy PSR[b] and Dikshit M[a]

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Aim: Effect of long term cholesterol diet withdrawal on atherosclerotic plaque composition in iliac artery of New Zealand White (NZW) rabbits.

Results: Atherosclerosis was induced in NZW rabbits by combination of balloon injury and atherogenic diet (AD) (1% cholesterol and 6% peanut oil) feeding for 8 weeks (baseline) followed by chow diet (CD) for 4, 8, 16, 32, 50 and 64 weeks. Significant elevation in plasma lipids with AD was normalized at 16 weeks of CD. Baseline comparison showed advanced plaque features even after CD period of 8 weeks with significant elevation in intima/media thickness ratio and plaque area showing reversion at late CD periods. Histology (HE and Movat pentachrome) and immunohistochemistry revealed significant increase in CD68, ground substance, MMP-9, pro-inflammatory cytokines and significant decrease in α-actin positive area and collagen staining at 8 weeks CD period indicating maximum plaque instability. These aspects significantly regressed up to 64 weeks of CD. Lipid accumulation (oil red O staining) was maintained till 16 weeks regression period which significantly reduced at later time points. Partial restoration of endothelial functionality was seen after 64 weeks of CD feeding. mRNA expression of MCP-1, VCAM-1,collagen type I and III, MMP-9, TIMP-1, IFN-γ, TNF-α, IL-10 and eNOS was done to correlate the above findings.

Conclusion: The present findings reveal insights of dual phase of plaque instability and subsequent regression on AD withdrawal in this model. The results guide the approach for evaluating investigative molecules on plaque stability/regression, restenosis and relooking at the phenomenon of plaque regression in humans.

P007: Protein model to water networks: a computational study on TGR5 receptor

Mubeen Shaikh[a], Sameer Agarwal[b], Kalapatapu V. V. M. Sairam[a]

a Department of Bioinformatics, b Department of Medicinal Chemistry, Zydus Research Center, Ahmedabad

TGR5 (M-BAR, GPBAR or GPR131) is a plasma membrane-bound, GPCR that is involved in mediating energy and

glucose homeostasis. The aim of this study was to generate a TGR5 receptor protein model that could provide useful insight regarding the binding site, binding site residues and possible role of water. Although it is well appreciated that water molecules play an important role in stabilizing ligand-protein interactions, role of structural and bulk water in TGR5 remains unknown. In this context we have tried to validate role of water molecules by using molecular dynamics simulations. These results are expected to give useful information to undertake rational design of new compounds. In this study network of interactions between ligand-water molecules have been identified that may play important role in biological activity.

P008: Stabilization and identification of transient in-vitro active metabolites of clopidogrel

Poonam Giri, Sanjay Singh, Janmay Shah, Harilal Patel, Mukul Jain

Department of Drug Metabolism & Pharmacokinetics, Zydus Research Centre, Ahmedabad – 382210, India, E-mail: poonamgiri@zyduscadila.com

Clopidogrel is a potent antiplatelet drug, indicated for the prevention of vascular thrombotic events in patients at risk. Hepatic biotransformation of Clopidogrel is required to express the full antiaggregating activity. Majority of antiplatelet activity of Clopidogrel is exerted by its active thiol metabolite.

The objective of this study was the stabilization and identification of in-vitro active metabolite of Clopidogrel in rat and human liver microsomes preparations to correlated formation of active metabolite in rat and human.

In-vitro metabolite identification was carried out in rat and human liver microsomes at 20 µM concentration of Clopidogrel and 2 mg/ml microsomal protein in the presence of 1 mM concentration of co-factor NADPH. The active metabolite was transient in in-vitro preparation and difficult to detect in liver microsomal incubated samples, ~2.5% of 0.4 mM of DL-Dithiothreiotol (DTT) solution was added in incubation mixture to stabilize the active metabolite.

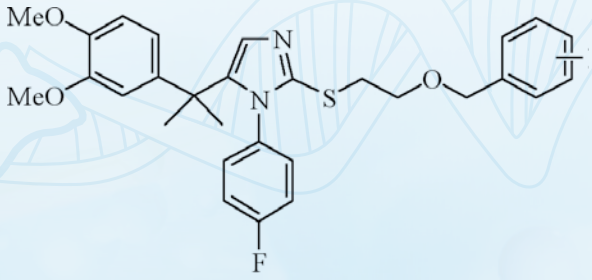
The in-vitro active metabolite of Clopidogrel, with m/z 356 was identified in rat and human liver microsomal incubated samples and its structure was studied by LC-MS/MS (Waters Quattro micro). MS results suggested that in-vitro active metabolite belongs to a very reactive thiol functional group, structural elucidation of the DTT stabilized in-vitro active metabolite was performed and MS/MS fragmentation data obtained with m/z 356 and 358 containing 35Cl and 37Cl isotope, respectively, confirms the structural features of thiol metabolite bearing a chlorine atom.

The use of DTT to stabilize transitory thiol functionality can be applied for qualitative and quantitative characterization in drug discovery and development application.

P009: Discovery of novel TGR5 receptor agonists

Amit Patil, Prashant Deshmukh, Umesh Aware, Ketan Chauhan, Mubeen Shaikh, Hitesh Bhayani, Harsh Bhatt, Kalapatapu V. V. M. Sairam, Priyanka Priyadarsiny, Sameer Agarwal
Zydus Research Centre, Ahmedabad – 382210, India

Takeda G-protein-coupled receptor 5 (TGR5), also known as GPR 131, GPBAR1, GPCR 19, M-BAR or BG37 represents an exciting biological target for the potential treatment of diabetes with simultaneous management of glucose levels and body weight. A novel class of imidazole containing TGR5 agonists is disclosed. Several of these compounds were found to activate human TGR5 (hTGR5) with EC50 values in the low nanomolar range.



Compound 1

P010: Effect of aliskiren on experimental stroke in mice

Rakesh B. Patel[a], Kajri B. Pandit[b], Kanaiyalal D. Prajapati[a], Bhavin M. Sonara [a], Shrikalp S. Deshpande[b], Gaurang B. Shah[b], Vishwanath D. Pawar[a] and Mukul R. Jain[a]

a Department of Pharmacology, Zydus Research Centre, Sarkhej-Bavla National Highway-8A, Moraiya, Ahmedabad – 382 213, Gujarat, India. b Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research, Sector-23, Gandhinagar-382 023, Gujarat, India

In present study, we investigated the effect of aliskiren on experimental stroke (Middle Cerebral Artery Occlusion and Reperfusion) in mice. ICR mice were randomaly assigned to sham group, MCAOR group received vehicle (10 ml/kg, ip for 8 days) and MCAOR receieved aliskiren (10 mg/kg, ip, bid for 8 day).

On day 6 of treatment, MCAOR (2 hr occlusion and 48 hr reperfusion) were performed. Post 48 hr reperfusion, neurological score, convulsions and mortality were observed. Infarct volume, cytokines (IL-6 and TNF-α) and oxidative stress (SOD and MDA levels) were measured in brain. MDA levels were significantly lowered with aliskiren treatment. Mortality and convulsion were less in group of animals receiving aliskiren. Infarct volume and neurological score remained unaffected with the aliskiren. Aliskiren nonsignificantly decreased IL-6 levels in brain homogenates.TNF-α and SOD levels were found to be unaffected with treatment. Aliskiren was not effective for treating stroke developed in mice model of Focal Cerebral Ischemia. However, it did not worsen the condition. Anti-inflammatory and anti-oxidant activiy of aliskiren might be responsible for decreased incidences of mortality and convulsions.

P011: Estrogen and α-lipoic acid do not confer additional benefits in cerebral reperfusion injury

Soni Manessh, Dhote Vipin, Balaraman R.

Faculty of Pharmacy, V.N.S. Group of Institutions, Bhopal, Madhya Pradesh.
M.S. University of Baroda, Vadodara, Gujarat.

Clinical evidences establish the susceptibility of men to stroke induced cerebral damage as compared to women, and that the protection against stroke in women diminishes by the advent of menopause. Although physiological estrogen concentrations are neuroprotective, its supraphysiological concentration exacerbates the cerebral damage induced by ischemia or the following reperfusion injury. A synergistic action of α-lipoic acid with estrogen could be a rationale approach to provide neuroprotection with low concentrations of estrogen in ischemia reperfusion injury. To evaluate the neuroprotective potential of estrogen at low doses in cerebral reperfusion injury, estrogen was administered with α-lipoic acid in rats.

Material and Methods: Male Sprague Dawley rats (300-350 g) exposed to reperfusion injury by occlusion of middle cerebral artery for 2 hour and reperfusion for 22 hours with reopening of artery. Estrogen (8 µg/kg s.c.) was administered alone and in combination with α-lipoic acid (100 mg/kg i.p.) for seven days and 1 hour after ischemia. Neurological deficit, infarct size and edema were observed after 22 hours of reperfusion. Oxidative stress biomarkers, MPO activity, BBB permeability and DNA fragmentation in ischemic hemispheres was assessed.

Results: Estrogen showed significant reduction in infarct volume along with marked improvement in neurological deficit. The activity of antioxidant enzyme, SOD, was significantly enhanced while MDA levels were reduced. Similarly, α-lipoic acid showed marked improvement in GSH levels and attenuated apoptosis. However, the combination of estrogen and α-lipoic acid did not provide any synergistic effect on amelioration of neuronal damage induced by reperfusion injury.

Conclusion: The lack of synergistic neuroprotective actions of combined estorgen and α-lipoic acid treatment indicates that their major therapeutic activities shared vital overlapping neuroprotective mechanisms.

P012: Renoprotective activity of aliskiren in cyclosporine A induced hypertensive nephropathy in dTG mice.

Bhavin M. Sonara, Megha S. Saraswat, Vishwanath D. Pawar, Kanaiyalal D. Prajapati, Rakesh B. Patel and Mukul R. Jain.

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Hypertensive Nephropathy is one of the important causes for the progression of End Stage Renal Disease (ESRD). It is characterized by vasculature damage and compromised function of kidney in response to growing blood pressure. Methods: Hypertensive Nephropathy was produced in double transgenic (C57BL/6-(hREN)/(hAGT)) mice with 20 mg/kg, sc of Cyclosporine A (CsA) daily for 28 days. CsA treated mice were divided in two groups - Vehicle and Aliskiren (10 mg/kg, po, bid). Kidney function test (Blood Urea Nitrogen, serum Creatinine, Urea & Uric acid) and kidney injury biomarker (TNF-α & IL-6) level was assessed in serum, TNF-α, IL-6, TGF-β1 & KIM-1 were assayed in kidney homogenate. Urinary KIM-1 level was assessed as an early biomarker of nephropathy. Results: CsA not only raised the blood pressure but also produced renal damage characterisitcs of hypertensive nephropathy. Significant hypertensive nephropathy and increase in levels of serum biomarkers like BUN, creatinine, urea and uric acid were observed in CsA treated animals when compared with control group. Aliskiren treatment elicited significant renoprotection by preventing the increase in blood pressure and levels of serum biomarkers and also reduced the nephropathic alterations in the kidney histoarchitecture. Aliskiren treatment significantly reduced TNF-α & IL-6 levels both in serum and kidney homogenates with reduction in tissue TGF-β1 levels. Aliskiren treatment significantly reduced KIM-1 levels both in urine and kidney tissue homogenates. Conclusion: A correlation between pharmacological, biochemical and histological findings has been established in mouse model. The present findings have indicated the renoprotective activity of aliskiren in CsA induced hypertensive nephropathy, which may be due to its antihypertensive as well as renin inhibitory action.

P013: Gene expression profiling reveals activation of cell cycle progression in ischemic rat brain: a genomic study

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Cerebral ischemia or ischemic stroke is a third leading cause of death worldwide. Limited treatment options mainly include reperfusion using thrombolytics within 3-5 hours of artery blockade but it is associated with reperfusion injury. There is a need for neuroprotective agent which can block ischemic-reperfusion injury induced neuronal cell death. We have undertaken genomic approch to find out therapeutic target for ischemic stroke. Using DNA microarray, the gene expression changes in ischemic rat brain (two hours of middle cerebral artery occlusion followed by 22 hours of reperfusion) was studied. Gene Ontology (GO) based pathway analysis was done using GenMAPP 2.1 software and most affected pathways were found out. The results showed upregulation of pathways related to DNA damage response, synapse organization and biogenesis, response to oxidative stress and hedgehog pathway in ischemic animals compared control animals. Pathways related to cell cycle arrest, sensory perception of mechanical stimulus, carbohydrate transport and ion transport were found to be downregulated. Amongst these up and down regulated pathways, DNA damage response and cell cycle arrest were found to be interlinked and indicated the activation of cell cycle progression in further analysis. The cell cycle progression related genes were re-quantified using quantitative real time PCR and found to be correlated with microarray data. The upregulation of the cell cycle progression genes in the ischemic brain probably indicate re-entry of post-mitotic neurons into cell cycle, which may cause initiation of neuronal regeneration. Further research could reveal unseen role of this pathway in ischemic brain.

P014: 4-Benzamido-3-carboxamide piperidine based renin inhibitors with improved bioavailability.

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Medicinal Chemistry[1], Pharmacology[2], Cell biology[3], Pharmacokinetics[4]
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The Renin Angiotensin System (RAS) plays an important role in the regulation of blood pressure and fluid electrolyte. Renin is a specific aspartic protease which catalysis key step in RAS, the cleavage of angiotensinogen to angiotensin-I. Hence it has been prominent target for antihypertensive therapy. The first generation peptidomimetic inhibitors like Remikiren have problem of poor pharmacokinetics and high cost of goods. The next generation nonpeptidic inhibitor, Aliskiren sucessfully enter in the market [2007] but it suffers from the drawback of poor bioavailability [~4%] and chemical complexity. Hence there is a need to develop nonpeptidic renin inhibitor with improved bioavailability and comparable invivo efficacy to that of Aliskiren.

Our efforts towards design and synthesis led to 4-benzamido-3-carboxamide piperidine class of compounds. SAR studies of this class led to the identification of candidate with improved bioavailability (>20%), comparable invivo efficacy and better synthetic feasibility than Aliskiren. Synthetic methodology, invitro, invivo potency and pharmacokinetic profile of some of the selected compounds will be presented.

P015: Assessment of antithrombotic activity of Factor Xa inhibitor in various animal models

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Factor Xa is an important protease enzyme in coagulation cascade and placed at the juncture of intrinsic and extrinsic coagulation pathways. Inhibition of this protein will impair the coagulation cascade. The objective of the present work is to evaluate compound 1, a novel orally active Factor Xa inhibitor (IC_{50} 27 nM) in various animal models of thrombosis. In Partial stasis combined with vessel injury induced venous thrombosis model in rats, it showed dose dependent decrease in thrombus weight and increase in prothrombin time, both the effects were significant at 30 mg/kg and higher doses. When we evaluated Compound 1, in $FeCl_3$- induced arterial thrombosis model in rats, it showed a significant increase in time to occlusion at doses 50 mg/kg and higher. In arterio-venous shunt model, we found dose dependent decrease in thrombus weight and the effect was significant at 50 and higher doses. When we evaluated in rat tail bleeding time model, the compound 1 alongwith marketed Factor Xa inhibitor, rivoraxaban, it shows comparable effect on bleeding time and prothrombin time. In conclusion compound 1 showed significant anti thrombotic effect in all in-vivo models of thrombosis at dose 50 mg/kg, p.o. and bleeding time prolongation was comparable to rivoraxaban.

P016: In-silico docking studies of phthalazine-1, 4-dione derivatives as aldose reductase inhibitors.

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Aldose reductase inhibitors are the class of drugs being studied as way to prevent eye and nerve damage in people with diabetes. Their target, aldose reductase, an enzyme that is normally present in many parts of the body and catalyzes one of the steps in the sorbitol(polyol) pathway that is responsible for fructose formation from glucose. Aldose reductase activity increases as glucose concentration rises in diabetes in those tissues that are not insulin-sensitive, which include lenses, peripheral nerves and glomerulus. Sorbitol doesnot diffuse through cell membranes easily and therefore accumulates, causing osmotic damage which leads to retinopathy and neuropathy. The current findings are the molecular-docking studies of series of Phthalazine-1,4-diones. Docking calculations were carried out using DockingServer. The MMFF94 force field was used for energy minimization of ligand molecule. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out on 2FZD protein model i.e. Human aldose reductase complexed with Tolrestat. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 20×20×20 Å grid points and 0.375Å spacing were generated using the Autogrid program. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. The docking calculations showed that free energy of binding are higher & estimated inhibition constants are remarkable for these derivatives when compared with the standard inhibitors.

P017: Design and synthesis of imidazolidine-2,4-dione derivatives as thyroid hormone receptor β selective ligands

Krunal Soni, Preeti Raval, Jaymin Barot, Manoj Hande, Mitul Sorathiya, Anshul Satyanand, Saurin Raval, Debdutta Bandopadhyay, Amit Joharapurkar, Mukul R. Jain

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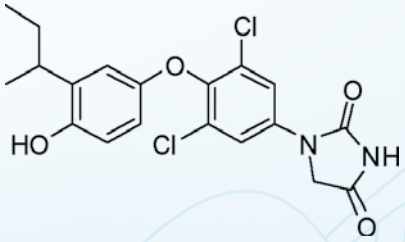
Design and synthesis of novel imidazolidine-2,4-dione substituted derivatives has been reported and their thyroid hormone receptor selectivity has been evaluated among which few compounds has shown significant thyroid hormone receptor β selectivity.



P018: PTP 1B inhibitors: Discovery of DFMP-substituted phosphotyrosine mimetics

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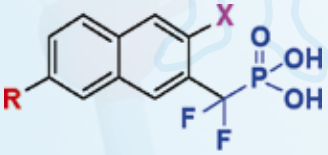
Diabetes is one of the major contributors to ill health. Majority of diabetic people are diagnosed with type 2 diabetes (T2DM) and of these, 90% are obese or overweight. Currently available antidiabetic drugs are not adequate for the safe and effective treatment of T2DM. In this regards, development of new class of selective protein tyrosine phosphatase 1B (PTP 1B) inhibitors were found to be attractive and promising approach. The major disadvantage of developing PTP 1B inhibitor could be achieving PTP 1B selectivity over closely associated PTPs, particularly T-cell protein tyrosine phosphatase (TC-PTP) and lack of oral bioavailability. In the present communication, we described the design and synthesis of DFMP-substituted naphthalene moiety (I & II) as a dual binding site phosphotyrosine (pTyr) mimetic as a potent and selective PTP1B inhibitors, for the treatment of T2DM.



I : X=Br; R= -CH_2Br

II : X=F; R= -CH_2Br

P019: Discovery of novel and nonsteroidal GR antagonist

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Glucocorticoid receptors (GRs), members of the steroid-thyroid-retinoid superfamily, which act as ligand-mediated transcription factors controlling specific gene expression in most mammalian cells. Activation of GR leads to either positive (trans-activation) or negative (trans-repression) regulation of gene expressions. GR agonists have been explored primarily as anti-inflammatory agents. In contrast, the therapeutic potential of GR antagonist remains largely unexploited despite having a strong rational for its role in metabolic disorders. GR antagonism has been validated as a promising therapeutic target for regulating hepatic glucose production (HGP), in animal models and humans, using a non-selective steroidal GR antagonist, Mifepristone. Recently reports on non-steroidal GR antagonist have begun to emerge. In the present communication, attempts were made to design novel nonsteroidal GR antagonist. Some of the test compounds were explored invitro to assess its GR antagonistic activity.

P020: Effect of metformin & sitagliptin co-administration on urinary KIM-1 levels in wistar rats

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Many anti-diabetic drugs in the market are known to cause adverse renal effects. Renal effects are due to excretion of these drugs is via kidney. Kidney injury caused by therapeutic agents or drug combinations requires appropriate monitoring and intervention. Current standards using blood urea and creatinine are considered late indicators of renal injury and are often non-specific and insensitive. KIM-1 is glycoprotein located in the renal proximal tubule epithelial cells. These cells undergo regeneration after various forms of injury and shed KIM-1 antigen in urine. Thus, urinary KIM-1 is an early and specific biomarker for tubular kidney injury. In an effort to identify adverse renal damage at early stage, we estimated KIM-1 from Wistar rats. Animals were orally co-administered with multiple doses of antidiabetic drugs – Metformin & Sitagliptin for 90 days. Treatment associated elevation in urinary KIM-1 were observed at all doses in females which were supported by significantly higher relative kidney weights and histopathological findings at high dose whereas females of intermediate dose revealed increased levels of KIM-1 but no changes were noticed in routine kidney markers as well as histopathological observation, supporting that treatment of Metformin and Sitagliptin is prone to cause adverse effects on kidneys.

P021: High protein diet – a two edged sword

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In today's world, over nutrition is major concern compared to under nutrition. Consumption of diet, high in protein is increasing now-a-days because of the reports suggesting higher satiety from high protein diet (HPD). Still there are many aspects of HPD which needs to be evaluated completely. According to the reports available from in vitro studies, higher protein amount produces amino acids that activate m-TOR (mammalian target of rapamycin). Here, we tried to investigate actions of over-activated m-TOR, in vivo by giving animals HPD (50% casein in total diet) for three months. HPD model was based on the fact that higher protein ingestion produces kidney damage. Animals were considered to have effect of protein when HPD & NPD group shows significant difference between urine & plasma parameters for kidney damage. Expression of markers for proliferation & apoptosis like Bcl-2, Bax, PCNA, caspase-3 was checked in liver tissue of rats fed NPD and HPD. Comparison between two groups suggested increased proliferation in liver of the female rats fed on HPD compared to that on normal diet. It was interesting to observe that females were more prone for increase in proliferation compared to male. Gross histopathological evaluation for liver tissue in male & female on HPD & NPD was performed which supports the result of biochemical as well as western blotting data. Thus, this study provides useful information about impact of our day-to-day life's eating habits on susceptibility for diseases like cancer.

P022: Increased basal insulin secretion by pharmacotherapy may exaggerate insulin resistance

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Type 2 diabetes mellitus (T2DM) is a global health problem. In particular, Asia is at the epicenter of the epidemic as these populations develop diabetes at younger ages and a lower BMI levels than western people. There is a strong relationship between basal insulin levels, obesity, and diabetes in humans. Increasing fasting insulin levels compared with those in lean control subjects have been documented as subject's progress from obesity to impaired glucose tolerance and severe diabetes. The aim of the present study is to find out effect of various anti-diabetic agents and insulin sensitizers on fasting hyperinsulinemia and insulin resistance in diet induced obese and in lean C57 mice. These animals were treated with glibenclamide, metformin, insulin, pioglitazone, exendin-4, sitagliptin and rimonabant for 2 weeks. Serum insulin, glucose were estimated after 18 hour fast. Intraperitoneal tolerance test and Insulin tolerance test were performed, and the insulin secretion capacity of the islets isolated from the chronically treated mice was estimated. The result indicated that glibenclamide is the strong inducer of basal hyperinsulinemia and hence insulin resistance, while Rimonabant caused decrease in basal hyperinsulinemia and increased insulin sensitivity. In conclusion, results of this study indicate that basal hyperinsulinemia induced by pharmacotherapeutic treatments is the strong inducer of insulin resistance, which may result in poor glycemic control in chronic diabetics.

P023: Glucagon and GLP-1 coagonism modulates lipid metabolism independent of the anorectic effect

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Diabetic dyslipidemia is a condition associated with hypertriglyceridemia, low HDL and increased LDL in association with diabetes. In addition to gluco-regulatory effects, GLP-1 is shown to modulate plasma lipid levels in preclinical studies as well as in clinical trials. Glucagon is a hormone related to GLP-1 that diminishes food intake along with elevation in energy expenditure. Glucagon is reported to possess antihyperlipidemic potential partially by inhibiting HMG CoA reductase and hence reduces liver lipids. The lipolytic and thermogenic properties attributed to glucagon, in addition to satiation-inducing pharmacology of GLP-1, is a rationale for the development of synergistic co-agonist. We investigated a rationale combination of GLP-1 and glucagon agonism on the lipid modulation in mice model of diabetic dyslipidemia. Co-agonism improved adiposity and glucose tolerance in diet-induced obese mice. Body weight reduction was achieved by a loss of body fat resulting from decreased food intake and increased energy expenditure. Co-agonist also normalized glucose and lipid metabolism and reduced liver steatosis. We found that co-agonist was effective in reducing body weight along with reduction in serum and hepatic lipids after chronic treatment in male DIO mice. We further explored lipid modulating effect in a pair-fed set up and the results indicate that lipid regulating effect of co-agonist is not dependent on reduction in food intake as against glucose homeostasis.

P024: Treatment with exendin-4 improves the diabetic symptoms and reverses hepatic steatosis in glucokinase activator treated db/db mice

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The glucokinase activators improve the fasting as well as postprandial glucose control and are important investigational mechanism for the treatment of diabetes. However, recent studies have implicated that continuous activation of glucokinase with a small molecule activator can increase hepatic triglycerides and the long term glucose control is not achieved. In this study, we investigated the effect of combination of glucokinase activator with GLP-1 receptor agonist exendin-4 in male db/db mice. The 12 weeks combination treatment in the db/db mice resulted in a significant decrease in body weight gain, food consumption, random glucose and %HbA1c. The decrease in serum glucose and %HbA1c in combination group was more profound and significantly differed than that of alone treatment (GKA or Ex-4) group. This improvement was found to be associated with improvements in hepatic triglycerides, which were increased by glucokinase activator treatment alone. The combination of GKA with exendin-4 reduced the hepatic lipid accumulation, and improved the insulin sensitivity, reduced hepatic glucose production in db/db mice. Overall, our data indicate that combination of glucokinase activator and GLP-1 receptor agonist exendin-4 is a good option for improving the glucose homeostasis with anti-obesity action and more importantly without causing harmful side effects like fatty liver.

P025: Additive effects of CB1 antagonist and GLP-1 agonist combination in diet induced obese mice model

Kartikkumar Patel, Amit Joharapurkar , Nirav Dhanesha, Vishal Patel, Samadhan Kshirsagar, Jaysukh Detroja, #Brijeshkumar Srivastava, #Rajesh Bahekar, and Mukul R. Jain

Department of Pharmacology &Toxicology, # Department of Medicinal Chemistry, Zydus Research Centre, Ahmedabad

Rimonabant a well known appetite suppressant developed for the treatment of obesity blocks the CB-1 receptors in central as well as peripheral nervous system. The clinical development of Rimonabant was haulted due to dose-related side effect profile related to the central nervous system. Recent data indicates that Rimonabant causes dose-dependent increase in blood glucose, which can be partially mediated by the CNS modulation of GI absorption. Several combinational therapies for the treatment of obesity are presently in late-stage clinical trials or being further evaluated by the FDA or other regulatory agencies. The use of combinational therapy may offer the potential for synergistic interactions between compounds to produce a greater degree of weight loss than the sum of the individual effects of each compound. Such combination may also have the potential advantage of minimizing associated dose-dependent adverse effects. Hence, we hypothesized that the insulin secretagogue effect of GLP-1 agonist Exendin-4, along with its antiobesity potential can synergize with the insulin sensitizing action of rimonabant. We have investigated these effects in diet induced obese C57 mice, a model of obesity. Intraperitoneal as well as intracerebroventricular administration of Rimonabant increased serum glucose upon glucose challenge in overnight fasted male DIO C57 mice over four hours with concomitant rise in serum glucagon levels. WIN55,212-2, agonist of CB1 receptors, could not reverse this effect, whereas ICV and IP administration of Exendin-4 reversed the acute hyperglycemia induced by Rimonabant. The combination of suboptimal doses of Exendin-4 and Rimonabant showed an additive effect in the food intake, and the effect was sustained on body weight upon repeated dosing. However, the acute efficacy of both the compounds was additive for inducing nausea like symptoms in conditioned aversion test. Since the suboptimal effects of rimonabant and exendin-4 have complementary and additive effects on glucose and body weight after acute as well as chronic dosing, this combination could be useful in the treatment of diabetes associated with obesity, though associated diabetes.

P026: PP2A /FOXO 1a/ mTOR signalling induces apoptosis of podocytes under insulin resistance

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Podocyte apoptosis and detachment have been strongly associated with the phenomenon of diabetic nephropathy. For the development of insulin resistance (IR), conditionally immortalised human podocytes were cultured in 750 μM palmitate for 24 hrs prior to insulin stimulation for 30 minutes. IR was characterised by the decreased expression of insulin stimulated p-AKT, p-GSK 3β, p-ERK and increased expression of AKT inactivating Protein phosphatase 2A (PP2A) at the protein level. In the present study we investigated the role of PP2A in modulating the mTORc1 activity as it has been shown to be tightly regulated in podocytes. Apoptosis was confirmed by the increased expression of p53 and decreased expression of Bcl2 under palmitate treatment. Palmitate treatment prior to insulin stimulation increased the expression of mTOR, raptor while decreased the expression of rictor indicating the formation of mTORc1 complex. Further, we sought to understand the molecular mechanisms involved in it. Selective PP2A inhibition improved p-AKT, p-FOXO 1a, and decreased the accumulation of mTOR, raptor and p53. To confirm that PP2A is exerting its effect through mTOR we partially inhibited mTORc1 by low dose of Rapamycin. After partial inhibition of mTORc1, we observed increased expression of p-AKT and decreased accumulation of p53 which points toward the beneficial role of PP2A in manipulating the activity of mTORc1 possibly through FOXO 1a at transcriptional level. These results indicate that PP2A, FOXO 1a and mTORc1 axis may be a therapeutic strategy for the prevention of podocyte apoptosis under IR conditions. To the best of our knowledge this is the first report showing the regulation of mTOR through PP2A/FOXO 1a pathway under IR conditions in podocytes.

P027: Teratogenecity of phenteramine hydrochloride in wistar rats

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Phentermine, a sympathomimetic amine chemically related to amphetamine with significant anorectic activity is used widely as an anti-obesity drug. No safety data on teratogenic potential of this drug available till date. To identify it's risk in women of child bearing potential, this study was carried out in pregnant female Wistar rats. Phentermine HCl was administered orally from gestation day 6 to 15 at dose levels of 15 and 30 mg/kg body weight. At the dose level of 30 mg/kg, significant reduction in feed consumption and body weight during the gestation period as a result of pharmacodynamics effects was noticed. Additionally signs of toxicity and treatment related mortality were noticed at 30 mg/kg. No such exaggerated drug related effects noticed at 15 mg/kg. However, Phentemine HCl did not show any adverse changes in uterine parameters and related indices and no developmental anomalies during external, visceral, head razor and skeletal examination in this study at 30 mg/kg. In conclusion, phentermine hydrochloride showed no potential for embryotoxicity and found to be non-teratogenic in Wistar rats up to the dose level of 30 mg/kg bodyweight.

P028: Treatment with a novel pancreatic neogenic peptide in combination with a novel GLP-1 agonist improves the diabetic symptoms in mouse model of type 1 diabetes

Samadhan Kshirsagar, Amit Joharapurkar, Nirav Dhanesha, Vishal Patel, Jaysukh Detroja, Kartik Patel,[#] Rajesh Bahekar, [#]Rajendra Chopade, and Mukul R. Jain

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It is well known that there is a progressive deterioration of pancreatic islets in type 2 diabetes mellitus, and pancreatic islet failure is the very cause of type 1 diabetes. Recently it has been demonstrated that *In vivo* administration of islet neogenesis-associated protein leads to the dose-dependent expansion of beta-cell mass in normoglycemic rodents and dogs, as well as a reversal of hyperglycemia in a mouse model of type 1 diabetes, and hence the Reg3 peptides have been hypothesized to stimulate β-cell neogenesis in the pancreas. It has been indicated to be useful in combination with Insulin. We have discovered a novel neogenic peptide that also helps to regenerate islets. In addition to its incretin related effects, GLP-1 analogues not only regulates mature beta-cell function but also critically regulates beta-cell differentiation, beta-cell proliferation and beta-cell survival. In this study, we have examined the ability of the combination of a novel islet regenerating peptide (ZYIRP) with a novel small peptide GLP-1 agonist (ZYGLP1A) in an animal model. Diabetes in high fat fed C57 mice was induced by giving multiple low-doses of streptozotocin (MLD-STZ). ZYIRP in combination with ZYGLP1A were given twice daily via subcutaneous injections (0.25 mg/kg, each) for 8 weeks. Fasting glucose levels were significantly lower in the combination treated group and displayed a significant decrease in HbA1c, when compared to vehicle treated group. Glucose levels during IPGTT demonstrated improved glucose homeostasis with peak insulin levels were being significantly higher in combination-treated mice. Mice treated with the combination showed healthier islet morphology and significantly higher β-cell mass than the vehicle group. The treatment also improved the pancreatic insulin content. This is the first study to combine and indicate that a novel and small neogenic peptide has additive activity *In vivo* with novel GLP-1 analogue after chronic treatment.

P029: Discovery of isatin derivatives as glucokinase inhibitors

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A series of 5-substituted isatin derivatives which can inhibit glucokinase (GK) were prepared and evaluated. The structure–activity relationship (SAR) of these compounds is also presented. Among compounds tested, Compounds 2 and 9 showed moderate GK enzyme inhibitory activity, with an IC_{50} of 10 and 9 mM respectively, compared to the literature standard Ninhydrine (6 mM). The possible binding modes of compound 2 with GK enzyme were also explored by molecular docking simulation.

P030: Discovery of Indazol-3-one derivatives as dual-acting glycogen phosphorylase inhibitors and glucokinase activators.

Pinkal N. Prajapati[a], Rohit Panchal[b], Amitgiri Goswami[a], S. S. Pancholi[c], Mukul Jain[a], Debdutta Bandyopadhyay[a], Sunil Metiya[a], Sairam Kalapatapu[a], Haresh Ajani[a], Rajesh Bahekar[a].

a Zydus Research Centre, Sarkej-Bavla N. H. No. 8A, Moraiya, Ahmedabad, b S. K. Patel college of Pharmacy, Ganpat Vidyanagar, Mehsana; c Babariya college of pharmacy, Babariya, Vadodara.

A series of 5-substituted Indazol-3-one derivatives, which can simultaneously inhibit glycogen phosphorylase (GP) enzyme and activate glucokinase (GK) enzyme were prepared and evaluated. The structure–activity relationship (SAR) of these compounds is also presented. Among test compounds prepared, compounds 4 and 6 showed moderate activities towards both the enzymes (GK activation and GP inhibition). Compound 6 inhibited hLGP with an IC_{50} of 35.6 mM and activated GK enzyme, with an EC_{50} of 0.77 mM The possible binding modes of compounds 4 and 6 with both the enzymes were also explored by molecular docking simulation.

P031: Antidiabetic effect of roots of *Eranthemum roseum* vahl. R. Br.

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To study the effect of saponin fraction of *Eranthemum roseum* (SFER) on elevated blood glucose along with other biochemical parameters and on oxidative stress generated due to diabetes. Diabetes was induced with single intraperitoneal dose of streptozotocin (STZ) (55 mg/kg). Antidiabetic effect of SFER was tested using different doses like 30, 60, 120 mg/kg. Blood glucose, lipid profile, food intake, urine output and body weight were evaluated at different time intervals during the course of study. Renal lipid peroxidation was estimated for *In vivo* antioxidant activity.Treatment with SFER showed significant decrease ($p<0.01$ and 0.05) in the elevated blood glucose level along with significant reduction of increased urine output and food intake at all three doses. SFER improves the

body weight loss and abnormal changes associated with lipid profile in diabetic condition. Elevated renal lipid peroxidation (LPO) level was significantly attenuated by SFER treatment. This finding suggests that, treatment with SFER attenuate diabetic parameters and symptoms which may be attributed the presence of steroidal saponins as major phytoconstituents.

P032: In-vitro in-vivo correlation and prediction of species and gender difference of a novel antidiabetic agent by microsomal stability

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A novel agent offers a property for treatment of type 2 diabetes mellitus and is a key regulator of glucose homeostasis. In-vitro-In-vivo correlation of species and gender differences of novel antidiabetic agent (NAA) was studied based on in-vitro microsomal stability and in-vivo plasma exposure. In-vitro microsomal stability of NAA was assessed in mouse, rat, dog, monkey and human pooled liver microsomes to investigate species difference. NAA was separately studied in male and female rat liver microsomes to evaluate gender difference based on in-vitro clearance. Pharmacokinetic studies of NAA was established in male and female ICR mice, Wistar rats and Beagle dogs at 5 mg/kg dose by oral route of administration in addition to that a tissue distribution study of NAA was also performed in liver, pancreas, adipose tissue and skeletal muscle. NAA plasma and tissue concentration were estimated using LC-MS/MS method.

In-vitro microsomal stability of NAA was highest in dog > mouse > human > rat > monkey liver microsomes which correlated with highest plasma exposure in Beagle dogs > ICR mouse > Wistar rats.

In-vitro microsomal stability of NAA was higher in female rat liver microsomes than male and fairly translated to higher plasma exposure in female than male rats and further supported by higher tissue concentration of NAA in female compared to male rats.

In-vitro based assumption of gender difference extended to other species, ICR mice and Beagle dogs and well supported by higher plasma exposure in female rats than the male.

So in-vitro microsomal stability studies in male and female liver microsomes of different species can predict gender and species difference for plasma clearance and exposure in-vivo very well.

P033: In-vivo drug-drug interaction study of sitagliptin with clarithromycin in Wistar rats

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A world-wide effort to discover novel therapy for alarming diabetes is progressing. Weight gain is the intrinsic problem with most anti-diabetic therapies. Sitagliptin, an anti-diabetic is expected to be free from weight gain but cause upper respiratory tract infection. Clarithromycin, a macrolide antibiotic is used along with Sitagliptin to avoid respiratory tract infection. Sitagliptin is substrate of CYP3A4 enzyme and Clarithromycin is substrate as well as inhibitor of CYP3A4 enzyme. So objective of this study was to evaluate drug- drug interaction (DDI) of Sitagliptin and Clarithromycin.

In-Vivo DDI of Sitagliptin and Clarithromycin was assessed in male Wistar rats at dose of 10 mg/kg and 26 mg/kg, respectively. Serial blood samples were collected at various time points and centrifuged to separate plasma. Sitagliptin and Clarithromycin plasma concentration were determination by LC-MS/MS method.

The Cmax and AUC of Sitagliptin were increased 45.40% and 72.53%, respectively and clearance was reduced by 34.37% in presence of Clarithromycin after co-administration as compared to Sitagliptin alone. Clarithromycin Cmax and AUC were increased by ~ 27% and 19%, respectively. The increase in Cmax and AUC of Clarithromycin may aid to treat upper respiratory tract infection caused by Sitagliptin.

In-vivo DDI study of Sitagliptin with Clarithromycin reveals that extended plasma exposure and reduced clearance of Sitagliptin after co-administration with Clarithromycin could be due to inhibition CYP3A4 enzyme by Clarithromycin, thus reduction of the metabolic clearance of Sitagliptin. Hence there was a potential drug -drug interaction between Sitagliptin and Clarithromycin and dose adjustment must be taken care for co-administration.

P034: Type-2 Diabetes mellitus mouse model for long term nonclinical safety evaluation

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Nonclinical safety evaluation using healthy animals might have limited value because they are likely to be a poor surrogate for patients with a particular disease. In this context we endeavor to develop

diabetic model by multiple low doses of STZ (40 mpk x 5 days) by intraperitoneal route in female mouse with one month of treatment withdrawal period. Animals were reared under standard controlled environmental conditions and provided ad libitum water and feed. At the end of experiment, STZ treated animals showed impaired glucose tolerance, polycythemia, hyperglycemia, hypoalbuminemia, hyperglobulinemia, higher urea levels, hypokalemia, increased grip strength & pancreatic beta cell necrosis as compared to untreated animals. In conclusion, this in-vivo model may serve as an ideal Type-2 Diabetes Mellitus model for long term nonclinical safety assessment or to address post marketing surveillance concerns without any major diabetic complications.

P035: Pioglitazone treatment protects experimental endotoxemia induced adipose inflammation and insulin resistance in db/db mice

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Activation of various inflammatory molecules have been implicated in insulin resistance in adipocytes and rodent experimental models. Here we hypothesize that pioglitazone might be able to inhibit LPS induced adipose inflammation and insulin resistance in db/db mice. Female db/db mice were treated orally with pioglitazone (3 and 30 mg/kg) for 14 days followed by an intraperitoneal administration of LPS (50µg/kg) and the expression of several genes involved in adipocytes differentiation such as lipo protein lipase (LPL), fatty acid binding protein (aP2), adiponectin, insulin signalling PPAR gamma, suppressor of cytokine signaling-3 (SOCS-3) and representative inflammation genes TNF alpha, IL-6 and monocyte chemoattractant protein-1 (MCP-1) in WAT in db/db mice. LPS induced systemic insulin resistance as was demonstrated by an increase in plasma insulin levels. Our results show that LPS treatment worsens the metabolic characteristics of db/db mice. In adipose, endotoxemia suppressed mRNA expression of PPAR gamma and up regulated SOCS-3 expression which coincided with local activation of innate (IL-6, TNF alpha) and MCP-1 adaptive inflammation. Furthermore, LPS decreased LPL, adiponectin and aP2 transcripts in adipose tissues. All these changes are known to attenuate insulin receptor signaling in model systems. Fourteen day pioglitazone treatment was able to improved glucose tolerance and increased insulin signaling by suppressing SOCS-3 expression in adipose from db/db mice post LPS challenge. Pioglitazone treatment resulted in decreased TNF alpha, IL-6 and MCP-1 expression and up regulated LPL, aP2 and adiponectin expression in adipose tissue indicated improvement in insulin resistance caused by LPS. Therefore, our results indicate that pioglitazone treatment protects adipose tissue from LPS induced insulin resistance and provide strong evidence of its effectiveness against inflammation induced insulin resistance.

P036: Targeting HO-1/CO system: Novel therapeutic potential for diabetes and its complications

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Considerable evidences suggest the importance of HO-1/CO system in various diseases, including coronary artery disease, cardiac hypertrophy, diabetes mellitus, ischemic/reperfusion injury, atherosclerosis and cancer. Modulation of HO-1/CO system improves vascular endothelial function and inhibits smooth muscle cell proliferation by suppressing ET-1 expression and inhibits the atherosclerotic plaque development. Cardioselective overexpression of HO-1 exerts a cardioprotective effect after myocardial I/R in mice and this effect may be mediated via an anti-apoptotic action of HO-1. Moreover, upregulation of HO-1 by hemin improves the myocardial function through inhibition of reactive oxygen species and reduction in creatine kinase. In the isolated hearts subjected to ischemia-reperfusion injury, a significant improvement in the contractile function is seen after pretreatment with CORM-2 through its anti-oxidant properties by reduction in LDH activity, infarct size and ventricular superoxide production. It has been observed that there is an association between the risk of coronary artery disease and HO-1 gene expression in diabetic patients. Moreover, HO-1 induction improves insulin sensitivity and causes adipose tissue remodeling in a model of obesity-induced insulin resistance. Also, CO protects the pancreatic beta-cells from apoptosis and improves islet function/survival after transplantation. The administration of the HO-1 inducer, hemin lowered blood pressure and exerted nephroprotective effects. These findings suggest HO-1/CO as a potential therapeutic target for diabetes and associated complications.

P037: Amorphous vs. crystalline ZYO1: a novel CB-1 antagonist for treatment of obesity

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The endocannabinoid system is known to be hyperactive in obese conditions. Therefore, CB1 receptor antagonists

represent a promising new approach for reducing body weight, and decreasing the co-morbidities associated with excessive adiposity. We have developed a novel orally active CB1 antagonist ZYO1 and further improvised it for better pharmacokinetics.

The present study describes the profile of crystalline and amorphous forms of ZYO1 in rodents. *In vitro* CB-1 antagonism was measured using a binding assay in CHO cells expressing hCB-1 receptors. The CB-1 antagonism was also confirmed by reversal of CB-1 agonist-induced hypothermia in Swiss albino mice. Antiobesity effects of crystalline and amorphous forms of ZYO1 were evaluated using sucrose (5% w/v) consumption models in Zucker fatty rats. The amorphous form exhibited significantly better effect in this model at the same dose level than the crystalline form of the ZYO1. Despite this significant appetite suppressant effect and CB1 antagonism, both the forms of ZYO1 were devoid of nausea like effect up to the 30 mg/kg dose. ZYO1 is a novel CB-1 antagonist that shows good antiobesity effect and improvisation in its pharmacokinetic characteristics result in better efficacy in rodent models.

P038: Design of peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities

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Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, which selectively cleaves the N-terminal dipeptide from the penultimate position of Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Peptide (GLP-1) thus makes them inactive. Inhibition of DPP-IV activity extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying. Because of these multiple benefits of GLP-1 mediated glucose homeostasis, orally bioavailable DPP-IV inhibitors has been developed as promising therapeutic agents for the treatment of Type 2 diabetes mellitus (T2DM).

DPP-IV inhibitors offer a number of potential advantages over existing diabetes therapies, including a lowered risk of hypoglycemia and weight gain. Consequently, various DPP-IV inhibitors such as Sitagliptin (MK-0431), Vildagliptin (NVP-LAF237), Saxagliptin (BMS-477118) and Denagliptin (GW-823093) are in clinic or in clinical development for the effective treatment of T2DM.

Here we report, a peptidomimetic based cyanopyrrolidine derivatives as potent and selective DPP-IV inhibitors. Some of the test compounds (10l and 10m) showed excellent potency and selectivity towards DPP-IV over various serine proteases, without CYP inhibition.

P039: To evaluate the role of Vagal - Brain stem - Hypothalamic pathway in mediating anti-diabetic as well as anti-obesity activities of GPR119 agonist (AR231453) in C57Bl/6 mice

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Current available data on the effects of GPR119 agonists in animals indicate that they could prove valuable agents for treatment of type II diabetes and obesity by improving glucose homoeostasis while concurrently limiting food intake and body weight gain. Although the details of limiting food intake and body weight gain are not yet understood fully. To investigate the mechanistic pathway for above mentioned activities ,we used C57Bl/6 mice, in which ablation of Vagal - Brain stem - Hypothalamic pathway was done by Sub-diaphragmic Vagotomy procedure and AR231453 (a compound of Arena Pharmaceuticals) was used as GPR119 agonist. Results indicate that gastric emptying inhibitory activity of AR231453 was found to be dependent on vagal innervations to G.I tract, liver and pancreas, since AR231453 (20 mg/kg, i.p.) failed to inhibit gastric emptying in vagotomized group; however at same dose it showed significant gastric inhibitory activity in normal group {** ($P<0.01$)}. However glucose lowering activity and acute feed intake lowering activity of AR231453 was found to be independent of vagal innervations since both, normal (55.6 ± 6.3 %) as well as vagotomized (50.3 ± 6.5 %) mice showed significant improvement in BC-AUC glucose Vs. control and significant reduction in acute feed intake at "2 hr" and "4 hr" post dosing of AR231453 (10 mg/kg, i.p.) Vs. control respectively. But further repeated dose detailed study is required to evaluate the role of Vagal - Brain stem - Hypothalamic pathway and anti obesity activity of GPR119 agonist.

P040: Comparative evaluation of rimonabant & sibutramine for their anti-obesity and anti-diabetic activity in High Fat Diet-induced obese (DIO) C57 Mice

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Obesity and Type 2 diabetes are the most prevalent diseases that have reached epidemic proportions in both the developed and developing world. In last decade rimonabant and sibutramine are most talked

drug for the treatment of obesity, but both were banned because of serious safety concerns. The objective of the work described herein was to compare the antiobesity and antidiabetic activity of these drugs in high fat diet induced obese C57 mice model. Rimonabant and sibutramine were evaluated for its ability to reduce body weight, food intake, fat pad weight and antidiabetic activity after 28 days of treatment at 25 mg/kg dose by oral route in C57 mice which were made obese after 24 weeks of high fat diet (Research Diet with 60 % kcal fat). After 28 days treatment of rimonabant and sibutramine caused 31 and 18 % reduction in body weight along with 22 % and 10% reduction in food intake respectively. The terminal fat pad weight has also shown significant reduction, which is higher in rimonabant as compared to sibutramine. The oral glucose tolerance test has shown the worsening of glucose intolerance by rimonabant whereas sibutramine has shown the improvement in glucose tolerance. When we did the calorimetry measurements, data show that rimonabant has significantly increased the oxygen consumption and energy expenditure, whereas sibutramine treatment did not show any significant effect. This data show that rimonabant though has good anti-obesity effect causes worsening of glucose intolerance and sibutramine has did not show any effect on energy expenditure.

P041: Cost-effectiveness of alternate day therapy with atorvastatin and fenofibrate combination in the treatment of mixed dyslipidemia – a randomized controlled trial

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Introduction: Mixed dyslipidemia is strongly associated with the development of atherosclerosis. Combination of statins and fibrates is commonly used for treatment of mixed dyslipidemia. But main problem with these drugs is poor patient compliance, which might be due to adverse effects like myopathy and high cost of drug therapy. Long half-life of atorvastatin and fenofibrate makes them suitable for alternate day therapy. Hence we aimed to study the efficacy, safety & cost-effectiveness of alternate day therapy with atorvastatin and fenofibrate combination in treatment of mixed dyslipidemia.

Methodology: Patients with mixed dyslipidemia, who were eligible for pharmacological treatment, were randomly allotted to two parallel groups – alternate day therapy group (group 1) and daily therapy group (group 2) in 1:1 ratio. Patients in group 1 and 2 received fixed dose combination of atorvastatin 10mg and fenofibrate 160mg on alternate days and daily respectively for 12 weeks. Mean percent change in non-HDL cholesterol, triglycerides and LDL cholesterol from baseline, incidence of adverse effects and cost-effectiveness were compared in both groups.

Results: Among 110 patients randomized, 99 completed the study till 12 weeks treatment duration. There was no statistically significant difference between two groups in the mean percent change of non-HDL cholesterol, triglycerides and LDL cholesterol from baseline to end of 12 weeks. The mean drug treatment cost incurred for 1% reduction in non-HDL level was ` 9.2 (± 1) and ` 17.9 (± 2.5) in alternate day and daily therapy groups respectively ($p<0.001$). Although the study drug was well tolerated in both groups, incidence of adverse events was less in alternate day therapy group.

Conclusion: Our study is the first of its kind to evaluate the combination of atorvastatin and fenofibrate when given on alternate day basis for treatment of dyslipidemia. The study results demonstrate that alternate day therapy with this combination is an effective and safe alternative to daily therapy. Apart from significant cost savings, better patient compliance is expected with alternate day regimen, especially during long term therapy.

P042: Anti-diabetic and anti-obesity activity of TGR5 agonist in various pre-clinical models of type-2 diabetes and diet-induced obesity

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TGR5, a bile acid-responsive GPCR expressed in the GI tract, has been shown to regulate multiple metabolic processes, including hormonal control of energy expenditure. The objective of the work described herein was to assess the antidiabetic and anti-obesity potential of TGR5 activation. Compound 1 (EC50 for TGR5 in mouse is 62 pM and for human is 57 pM) was initially evaluated for its ability to stimulate GLP-1 secretion in C57 mice at 3 and 30 mg/kg dose. Administration of Compound 1 (1 - 30 mg/kg) prior to oral glucose (3 g/kg) meal bolus increased GLP-1 levels and enhanced glucose clearance in C57 mice model. In hyperglycemic, db/db mice model Compound 1 (3 and 30 mg/kg) reduced the non-fasted glucose levels dose dependently and also enhanced glucose clearance in day 1 and day 8 oral glucose tolerance tests. Further, sub-chronic administration (60 days) of Compound 1 (30 mg/kg) to DIO mice reduced the body weight gain without any effect on food intake. When we did the calorimetry it showed the increase in oxygen consumption and energy expenditure. Compound 1 treatment also increased total and active GLP-1 levels and improved glucose clearance. Recent reports showed the activation of TGR5 decreased pro-inflammatory cytokine production by cyclic AMP-mediated inhibition of NF-κB signalling so we tested the

Compound 1 in LPS induced cytokine release in human whole blood and it has shown TNF-alpha inhibition similar to prednisolone. These results support a novel role for TGR5 agonists as a potential new class of agents for the treatment of diabetes and obesity.

P043: GPR119 agonist-A potential tool to conquer diabetes and obesity

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GPR119 is a Gas - coupled receptor expressed predominantly in β-cells of the pancreas and L-cells in the gastrointestinal (GI) tract. This receptor appears to play a key role in the control of postprandial glycemia by stimulating the release of incretin hormones from the gut and enhancing glucose-dependent insulin secretion. In addition, GPR119 may also regulate food intake and GI motility this provides a unique opportunity for a single agent to have anti-diabetic and anti-obesity activity. The present study was aimed to evaluate Compound X, a novel GPR119 agonist for its glucose lowering and antiobesity potential. Compound X increases cyclic AMP levels in CHO cells expressing human GPR119 with EC50 of 57 nM. In C57 mice oral glucose tolerance test, it causes significant reduction in glucose excursion and increases active plasma GLP-1 levels. Further, we tested Compound X, in acute fasting induced food intake model in rat and mice, it shows the reduction in food intake which is correlating to plasma levels. Chronic treatment of GPR119 agonist at 25 and 50 mg/kg, in DIO mice for 4 weeks led to decrease in body weight gain, epididymal fat weight and increase in energy expenditure along with dose dependent reduction of serum triglycerides and LDL-cholesterol levels. In gastric emptying it has shown slight increase at high dose, which might be due to exaggerated pharmacodynamic activity of Compound X. In conclusion, the Compound X, a novel GPR119 agonist demonstrated anti-diabetic and anti-obesity activity in rodent models can be great tool to conquer diabetes and obesity epidemic.

P044: Characterization of a novel & potent TGR5 receptor agonist for the treatment of diabetes.

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Synthetic non-steroidal TGR5 receptor agonists are deliberated as a promising incretin-based strategy for the treatment of diabesity and associated metabolic disorders. In this study, in-vitro efficacy of Compound1 was assessed against human as well as mouse TGR5 receptors using two independent functional assays like Luciferase Reporter Assay and cAMP Assay. In-vitro glucagon like peptide-1 (GLP1) secretion by Compound1 was measured in human intestinal NCI-H716 cell line. Pharmacokinetic study of Compound1 was performed in two different species and further in-vivo efficacy was assessed in pre-clinical model.

Stimulation of cAMP in TGR5 expressing stable cell line identified the NCEs as TGR5 agonists. Compound1 was able to stimulate cAMP against hTGR5 receptors with an EC_{50} of 13.97 nM in hTGR5 cAMP Assay. In addition to it, Compound1 showed potent TGR5 activity with an EC_{50} value of 56.69 pM & 62.67 pM against hTGR5 & mTGR5 receptor respectively in CRE directed Luciferase based RG Assay. Activation of TGR5 receptor due to Compound1 stimulates secretion of the GLP-1 in dose dependent manner in human intestinal NCI-H716 cells. In pharmacokinetic study, Compound1 showed a favorable Pk profile with the Cmax of 9872 ng/mL in C57 mice at 30 mg/kg. Compound1 was able to stimulate GLP-1 secretion more than 3 fold & lowered the serum glucose level dose dependently during glucose tolerance test in mice model.

These results suggest that Compound1 is a potent & orally active TGR5 receptor agonist, which may be beneficial for the treatment of diabetes and associated metabolic disorders.

P045: Modified 9H-fluorene-9-carboxamides as MTP-inhibitors

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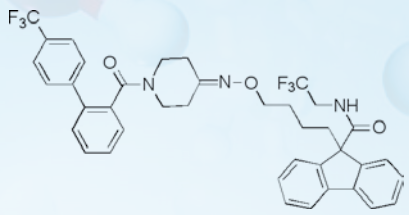
In the present study, successful synthesis of 9H-fluorene-carboxamides as MTP-inhibitors have been described. Selected compounds exhibited promising MTP inhibition *In vitro* as well as TG lowering in Sprague Dawley rats. Among the derivatives synthesized, N-(2,2,2-trifluoroethyl)-9-(4-(((1-(4'-trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl) piperidin-4-ylidene)amino)oxy)butyl)-9H-fluorene-9-carboxamide showed promising activity.



P046: Chemopreventive potential of oryzanol isolated from crude rice bran oil in n-nitrosodiethylamine induced experimental hepatocellular carcinogenesis

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Hepatocellular carcinogenesis is a pathological consequence of persistent oxidative stress leading to DNA damage. Anti-oxidants scavenge free radicals and inhibit neoplastic process. Several bio-active principles, isolated from crude rice bran oil (cRBO), have been reported to possess potent anti-oxidant activity and free radical scavenging property. An *In vivo* study was designed to elucidate the chemopreventive potential of one such bio-active principle; oryzanol (OZ) on the formation of N-nitrosodiethylamine (NDEA) induced pre-neoplastic hepatic nodular incidence, multiplicity, size, and volume, liver tumor markers, tissue lipid peroxidation (LPO) and enzymic anti-oxidant status during experimental hepatocellular carcinoma in mice and to compare its efficacy with 5-fluorouracil. Male BALB/c mice were administered with NDEA (75 mg/kg) injection once a week intra-peritoneally for 3 weeks, followed by NDEA (100 mg/kg) injection once a week intra-peritoneally for the following 3 weeks. OZ (100mg/kg) and 5-fluorouracil (25 mg/kg) were supplemented in the NDEA treated mice every day throughout the experimental period of 32 weeks. Following sacrifice after 16 and 32 weeks, NDEA alone treated mice showed significantly increased nodule incidence and multiplicity, accompanied by enhanced LPO and reduced enzymic anti-oxidant activities. Administration of OZ to NDEA treated mice reduced the nodular incidence and multiplicity, and the levels of liver tumor markers and also improved the levels of enzymic anti-oxidants in a time dependent manner. Histologically, no obvious sign of neoplasia was observed in the OZ supplemented NDEA treated mice. The findings indicate that OZ possesses potent chemopreventive activity against hepatocellular carcinogenesis and its effect is comparable to that of 5-fluorouracil.

P047: Evaluation of anti-angiogenic potential of angiotensin converting enzyme inhibitor in animal model of mammary gland cancer and in various in vivo angiogenesis models.

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Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide. The objective of our study is to investigate anti-angiogenic activity of ACE-I, perindopril in 12-dimethylbenz[a] anthracene (DMBA) induced breast cancer and to evaluate anti-angiogenic potential of perindopril by in corneal micropocket, mouse xenograft model and chick yolk sac membrane Administration of DMBA caused significant increase in cancer biochemical markers, like lactate dehydrogenase and Gamma glutamyl transferase. Perindopril treatment showed significant decrease in LDH and GGT levels. The treatment also showed significant improvement in antioxidant parameters in breast homogenate, inflammatory markers, like C-reactive protein and ESR and decreased hemoglobin levels. Histopathological evaluation showed hyperplastic and well demarcated tubular adenoma in tumor tissue, treatment with perindopril caused inhibition in the development of hyperplasia. In yolk sac membrane model, vascular growth was found to be inhibited by perindopril. In corneal micropocket model of angiogenesis, treatment with perindopril caused inhibition of neovascularisation as compared to control group. In mouse xenograft model, treatment with perindopril also caused reduction in tumor volume as compared to control group. From the present investigations, we can conclude that the perindopril inhibits the neovascularisation in tumor and can be used as neo adjuvant or adjuvant therapy in patients with metastatic breast cancer associated with cardiovascular complications.

P048: Synthesis and antioxidant activity of tetrahydrobenzo[b] pyran and its derivatives

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Tetrahydrobenzo[b]pyran and its derivatives represent the major class of heterocycles and receive a considerable attention due to their various pharmacological actions such as anti-allergic, anti-tumour, anti-bacterial, anti-coagulant, spasmolytic, anti-cancer. They are also used for the treatment of neurodegenerative disease.

Synthesis of tetrahydrobenzo[b]pyaran can be done by various methods. Here we used a piperidine as catalyst for the

synthesis. It involves knoevanagel condensation using Michael addition. A series of analogues were synthesized by condensing different aldehydes with active methylene group present in ethyl cyano acetate, formed an intermediate which reacts with dimedone in the presence of piperidine. These compounds were tested for antioxidant activity by DPPH method.

After screening, it is found that, only PMP1, PMP5 & PMP6 are having moderate antioxidant activity but they have no comparable antioxidant activity. It is observed that none of the compounds is having any potential and comparable anti-oxidant activity with reference to Ascorbic acid.

P049: Protective effect of L-carnitine against cyclophosphamide induced DNA damage in mice

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The study was conducted to evaluate the protective effects of L-Carnitine against chromosome damage induced by anticancer drugs. Carnitine has been proposed as a treatment for many conditions because it acts as an antioxidant and neutralise the free radicals, which damage cells and tamper with DNA. The protective effect towards DNA damage by L-Carnitine was studied through pre and post treatment of the anticancer drug, Cyclophosphamide in ICR mice. Study comprised two groups along with respective positive and negative control groups. In first group, animals were treated with L-Carnitine for two weeks followed by single dose of Cyclophosphamide and animals from second group treated initially with single dose of Cyclophosphamide followed by L-Carnitine treatment for two consecutive weeks. Chromosomal preparations were made from bone marrow cells and evaluated microscopically. Cytogenetic evaluation for structural chromosome aberrations revealed significant reduction in the number of aberrant cells in L-Carnitine treated groups compared to Cyclophosphamide alone treated groups. This clearly indicates the protective effect of Carnitine on healthy cells against the DNA damaging effects of chemotherapy medication.

P050: *In vitro* screening tools to accelerate new drug discovery: alternatives to animal testing

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Screening of biological activities of new therapeutic compounds using *In vitro* assays provides a fast and reliable measure of efficacy and safety. These cell-based assays not only potentially accelerate the research of new therapeutic agents, but may also serve as alternatives to equivalent animal tests *In vivo* by reducing the number of animals and severity of procedures. At Dabur Research Foundation, we have developed a repertoire of *In vitro* models to assess activities of test agents in multiple therapeutic areas. Cytotoxic profile of anticancer compounds is evaluated in a panel of human and murine cancer cell lines with safety assessment in normal cell lines. Murine and human bone marrow cells derived CFU-GM assays are employed to predict hematotoxic side-effects of anticancer drugs, which is ECVAM approved alternative method to determine human MTD for neutropenia. Specialized cell-based screening models have been developed in the area of inflammation, such as Dendritic cells for systemic inflammation, keratinocytes/monocytes for psoriasis, lung/nasal epithelium cells for airway inflammation and allergy, fibroblasts for dermal inflammation and intestinal/colon epithelium cells for gastrointestinal-inflammation. Sophisticated *In vitro* models based on keratinocytes and sebocytes are available to screen anti-acne properties of cosmeticeutical/ dermatological products. Skin health parameters such as anti-aging and anti-wrinkling potential are explored using skin-fibroblast cell lines. These *In vitro* assay systems contribute towards understanding of complex biological action of new compounds at cellular level. Shortlisting of hit/active compounds by in-vitro screening obviates the need of large number of testing animals while meeting 3R's principle of "Reduce, Refine and Replace".

P051: Anticancer potential of chloroform extract of Chitramoola kuligai against human breast cancer (MCF-7) cell line

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"Chitramoola kuligai" is a tamil vernacular name of "Plumbago zeylanica". It is clinically used by the Siddha practitioners from age old days in the treatment of cancer. This drug which is given orally is prescribed after one week of intense metal based chemotherapy in cancer patients. Many recent studies have focused on cytotoxic activity of ethanolic portion of Plumbago zeylanica. This activity is mainly attributed to a constituent 'Plumbagin.' However cytotoxic potential of non-polar fraction of this plant is less explored. In the present study, Plumbago zeylanica was continuously extracted for 24 h with chloroform by cold

maceration technique. The extract was concentrated and evaluated for cytotoxic activity against human breast cancer (MCF-7) cell line, by MTT and SRB assay. IC50 value for the extract was found to be 13.58 and 21.48µg/ml by MTT and SRB assay respectively. IC50 value for the standard doxorubicin was found to be 2.8µM. The results obtained were comparable with respect to the standard anticancer drug doxorubicin. Cytotoxic potential was further explored through DNA fragmentation and nuclear staining. This study shows that even non-polar fraction is highly potent and need to be studied further. Currently fractionation guided assay of the extract is being carried out and efforts are made to characterize the compounds.

P052: In silico design synthesis and pharmacological screening of some quinazolinone leads as dihydrofolate reductase inhibitors for anticancer activity

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Cancer remains an incurable area in Indian and global scenario, hence finding leads for anticancer activity is a noble research. Human Dihydrofolate reductase is a validated target for anticancer therapy. We here report the in silico screening, synthesis and pharmacological evaluation of some prioritized molecules as Dihydrofolate Reductase (hDHFR) inhibitors for anticancer activity. Molecules from the series 3-(substituted-benzylamino)-2-phenylquinazolin-4(3H)-one (QSBR$_{1\text{-}8}$) and 2-(2-substituted-benzylidenehydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (CQSB$_{1\text{-}8}$) were *in silico* screened for Docking Score on V-Life MDS 4.2 Drug Design Software. *In silico* ADME predictions were obtained from Pre ADMET online server. Prioritized molecules QSBR5 and QSBR6 had acceptable Docking Score as compared with Methotrexate and complied with ADME predictions. QSB5 and QSB6 were synthesized and characterized by ^{1}H-NMR, TLC and Mass spectra. QSB$_5$ and QSB$_6$ were subjected to acute oral toxicity studies to determine the LD$_{50}$, compounds were found safe at 2000mg/Kg dose. Further Molecules were evaluated for Human DHFR inhibition assay *In vitro* for anticancer activity. Prior to this assay the compounds were screened for *In vitro* cytotoxicity assay on six human cancer cell-lines following NCI, Bethesda guidelines. This was followed by evaluation of compounds *In vivo* using in cancer cell induced tumor mechanistic model in mice for anticancer activity. Molecules showed comparable anticancer activity as compared with Methotrexate in both *In vitro* and *In vivo* evaluation and can be termed as leads for anticancer activity by hDHFR inhibiton by using rational interdisciplinary approach for rational drug design. The project is funded by Indian Council of Medical Research (Ministry of Health and Family Welfare).

P053: A stable and highly pure polymorphic form of Erlotinib Hydrochloride (Antineoplastic)

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A process for the preparation of stable and highly pure polymorphic form of Antineoplastic drug Erlotinib Hydrochloride. It is a reversible tyrosine kinase inhibitor which acts on Epidermal Growth Factor Receptor (EGFR), use to treat non-small cell lung cancer (NSCLC) and pancreatic cancer. Process includes chlorination of 6,7-bis(2-methoxyethoxy) quinazolin-4-one (BMEQ) with thionyl chloride and dimethyl formamide in dichloromethane at ambient temperature provides the white to off white coloured solid compound 4-Chloro-6,7-bis(2-methoxyethoxy) quinazoline in excellent yield (≥97 %) with good quality which meets with well-defined spectral criteria. This intermediate on further reaction with 3-Ethynyl benzenamine and pyridine in stoichiometric amount in isopropyl alcohol at ambient temperature provides the crude Erlotinib free base in good yield (80%) which on further purification gives the pure Erlotinib free base with high yield ((≥97 %) . Erlotinib free base was treated with ethereal HCl to form the highly pure Erlotinib Hydrochloride as white crystalline powder in excellent yield (≥94 %) with ≥99.9% purity and 99.9 % assay by HPLC. It meets with all parameters of ICH guidelines. The beauty of our commerciable viable process for the synthesis of Erlotinib Hydrochloride involves highly stable form, which is stable upto six months even at accelerated conditions in terms of chemically as well as polymorphic purity and is suitable for global requirement.

P054: Heterogenity and therapeutic targeting of MLL gene in AML: cytogenetic and fluorescence in situ hybridisation analysis

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Chromosomal changes are the signature of gene deregulation in cancer and lead to instability of the genome.

The target gene at 11q23, *MLL*, is disrupted by the translocation and becomes fused to various translocation partners. According to the WHO classification 11q23 abnormalities are frequent cytogenetic abnormalities found in Acute Leukemia (AL) and also in the majority of patients with secondary AL after previous treatment with DNA topoisomerase II inhibitors and show poor prognosis., The present study was performed to clarify the prognosis of patients with AML with 11q23 abnormalities observed with different fusion partners in different FAB subtypes to establish distinct entity and targeted therapy. Bone marrow and peripheral blood lymphocytes of 321 AML patients were carried out by cytogenetics and FISH studies.

Out of 321 patients 12 patients showed rearrangements of 11q23. Mainly observed translocations were t(1;11), t(6;11), t(9;11), t(10;11), t(11;19) once only in different patients, del(11q23)(n=4), i(11q), t(11;17)(n=2). Out of 12 patients, 4 patients showed complete hematological response, no response was observed in 4 patients, 3 patients were lost to follow up and 1 patient with t(11;17) expired. Patient with t(11;19)(q23;p13) showed complete hematological response and is alive.

Total 6 different partners of *MLL* gene were observed. Distinct *MLL* fusion partners suggest a possible role in the tropism of the leukemia because certain partner proteins not only convert *MLL* to an oncogenic fusion protein but also direct the lineage susceptibility for transformation. These fusion genes may alter the normal cellular proliferation and differentiation processes, favoring leukemogenesis.

P055: Cloning of novel and rare chromosomal translocations in acute myeloid leukemia and acute lymphoblastic leukemia

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Important new insights about the pathogenesis of leukemias have been acquired over the last few decades through the analysis of genetic alterations. The molecular analysis of recurring chromosome rearrangements, especially of translocations, deletions and inversions has provided us with valuable insight into pathogenesis of leukemia. Many translocations result in the fusion of genes located at the translocation breakpoints. Unfortunately, especially acute myeloid and acute lymphoid leukemias (AML and ALL) have a very heterogeneous genetic background i.e. great variety of genetic alterations have been detected or are yet to be identified at molecular level.

In the present study, total 50 patients were enrolled with structural rearrangements. Out of 50 patients; 18 novel, 12 rare and 19 recurrent rearrangements were observed. Total 14 breakpoints were identified. The most common nonrandom breakpoints were; 11q23 and 19p13X 8; 1q23X 5; 12p13 X 4; 9q34, 9q22, 11p15, 17q21 and 21q22 X 3; 5q35, 3q26, 8q22, 10p15 and 22q11.2 X2. In 20 patients, variants of standard translocations were also observed. A Novel 4-way balanced translocation was also observed. Data mining was carried out using various databases regarding possible genes present at the chromosomal breakpoints.

In the recent era, innovative functional genetic approaches, such as FISH, M-FISH, m-Band and BAC-FISH, have great potential for the identification of novel cancer genes. It is hoped that the data resulting from these studies will also eventually lead to the development of successful molecular targeted therapies. Molecular methods are emerging as important tools for the diagnosis and stratification of patients with leukemia. However, the importance of rare and novel as well as primary and secondary, aberrations in leukemogenesis and in the prognostication for AML and ALL is still to be determined.

P056: Highly complex chromosomal rearrangements in patients with chronic myeloid leukemia: an indian experience

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During progression of chronic myeloid leukemia (CML) from the chronic to the accelerated phase and/or blast crisis, clonal evolution with nonrandom secondary aberrations frequently observed. Complex chromosomal rearrangements (CCR) are rather rare, and the significance and frequency of CCR are poorly understood. The aim of this study was to identify the role of highly CCR in CML patients and also to determine the chromosomes and chromosomal regions which are involved in CCR at diagnosis and the frequency of nonrandom changes in a large series of 393 CML patients.

Conventional cytogenetics was performed in 393 CML patients, out of that 8 patients showed highly complex chromosomal rearrangements. Fluorescence in situ hybridization (FISH) and multicolor FISH (M- FISH) were also performed to study karyotypes. More than three chromosomes were found to be involved in the CCR. Minimum 4 and maximum 7 chromosomes were involved in CCR. Besides chromosomes 9 and 22, most often involved in CCR were chromosomes 5, 10, 12 and 15 (x3); 1, 6, 11 and 17 (x2) and regions 5q, 10p, 12q and 15q (x3); 1q (x2). There were no recurrent complex translocations. Total 4 patients

were treated with Imatinib Mesylate (IM), and only 2 patients showed complete hematologic response, whereas no cytogenetic response was achieved in any of them.

Precise determination of breakpoints involved in CCR is essential to understand genetic mechanism which play role in leukemogenesis. The presence of several genes and/or miRNAs at the identified breakpoints suggests their potential involvement in the CML pathogenesis. Our data show that the presence of highly CCR is related to poor prognosis. Therefore, we suggest that patients with variant translocations constitute a "warning" category in the imatinib era and high genomic instability of the genome of malignant cells.

P057: Effect of erlotinib, meloxicam and metformin following mono and combination therapy on gene expression in NSCLC xenograft (A-549 cell line) mice model

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More effective and less toxic therapy to prevent tumor progression and recurrence is the requirement of advanced therapeutic approach in present context. Preliminary preclinical and clinical data suggest that targeting multiple pathways in cancer cells might be an effective anti-tumor treatment strategy in non-small cell lung cancer (NSCLC). A tyrosine kinase inhibitor (e.g. erlotinib), COX-2 inhibitor (e.g. meloxicam) and mTOR inhibitor (e.g. metformin) are widely used alone or with other drugs in their respective therapeutic area. The present study evaluated effect of erlotinib (30 mg/kg, p.o), meloxicam (20 mg/kg, i.p) and metformin (100 mg/kg, p.o) on gene expression following mono & combination therapy after repeated administration at 24 h interval for 28 days in NSCLC xenograft model. AKT, AMPK, P070S6K, PTEN and RAF genes expression changes were measured in the tumor tissue from the xenograft. The expression of PTEN and AMPK were upregulated in the treatment group, AKT, P070S6K, and RAF expression were downregulated. The effect of combination treatment showed additive effect when cmpared with the single agent.

The present study therefore opens up a potential new combination regimen. However, clinical studies required to be conducted to determine the true therapeutic benefits.

P058: Effect of telmisartan on vascular endothelial growth factor (VEGF) induced angiogenic responsiveness in normal and diabetic coronary endothelial cells.

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VEGF is a potent endothelial cell selective endogenous mitogen. It has been reported in many clinical trials that VEGF improved cardiac functions, however, it did not show development of new blood vessels. Telmisartan, an angiotensin 1 receptor antagonist and PPAR γ agonist, is widely suggested for treatment of cardiovascular complications. Our objective was to study effects of telmisartan treatment on VEGF induced angiogenic responsiveness in damaged coronary endothelial cells (cECs). Male rats were divided into six groups (n=24), normal rats (saline, 1ml/kg, p.o.), telmisartan treated normal rats (2mg/kg, p.o., for 15 days before isolation of hearts), diabetic rats 30ds. (30 days after administration of streptozotocin-STZ, 60mg/kg, i.p.), telmisartan treated diabetic rats 30ds., diabetic rats 60ds. (60 days after administration of streptozotocin-STZ, 60mg/kg, i.p.) and telmisartan treated diabetic rats 60ds. For purpose of isolation of normal and damage cECs, each group were further divided into three subgroups (n=8), control rat hearts, ischemia-preconditioned rat hearts and ischemia-reperfused rat hearts. We studied angiogenic responsiveness and nitric oxide (NO) releasing properties of cECs using CAM assay and griess method, respectively. VEGF induced angiogenic responsiveness of cECs significantly decreased in diabetic rats as compared to normal rats. Telmisartan treatment showed significant increase in VEGF induced angiogenic responsiveness of cECs of all subgroups as compared to their respective non treated subgroups. Telmisartan also significantly increased VEGF induced nitric oxide release from cECs of all treated subgroups as compared to their respective non treated subgroups. These effects of telmisartan significantly inhibited by pretreatment of cECs with eNOS inhibitor, l-NAME, and PI3K inhibitor, wortmannin, but not with PKC inhibitor, chelerythrine. Our data suggest that telmisartan improves VEGF induced coronary angiogenic activity in normal and diabetic rats via PI3K/eNOS/NO pathway.

P059: Ophiobolin: A multiple pathway inhibitor in cancer

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Multiple pathway inhibition is an attractive concept in cancer treatment, as simultaneous inhibition of multiple survival pathways has the potential for increased efficacy and reduced resistance. The present work describes pathway inhibition activities of ophiobolin isolated from fungal culture Bipolaris setariae on proliferation pathways. In cell based target inhibition assays, ophiobolin inhibited pERK kinase (IC_{50} = 0.32μM) an effector of Ras pathway,

pS6 kinase (IC_{50} = 8.2 μM) an effector of mTOR pathway, protein pRB (IC_{50} = 2.5μM) an effector of CDK pathway. Ophiobolin also inhibited proteasome pathway with IC_{50} of 9.3 μM in Jurkat cell line. In a cell growth inhibition assay, ophiobolin shows cell growth inhibitory activity across a panel of cancer cell lines with an average IC_{50} of 1.5μM and 0.35 μM against solid tumor cancer cell lines and multiple myeloma cell lines respectively. An increase in Sub-G1 phase cell population observed in Triple negative breast cancer cell line MDAMB231 treated with 5 μM ophiobolin for 48 hours indicates that it induces apoptosis and cell death in cancer cells.

Data indicates ophiobolin as a potent inhibitor of multiple survival pathways in cancer and can be considered as a therapeutic agent in treatment of cancer.

P060: Effect of cytotoxic drug (Temozolomide) on pharmacokinetic of novel PARP inhibitor (Olaparib) in Balb/c mice.

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Olaparib (PARP Inhibitor) is chemotherapeutic agent used in treatment of various types of solid tumors. Pharmacodynamics of olaparib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Drugs used in chemotherapy, such as temozolomide (Cytotoxic agent), work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. The objective study is to investigate the effect of temozolomide on the pharmacokinetics of the olaparib in mice. A combination treatment of olaparib and temozolomide would show better therapeutic outcome in tumor bearing animals. *In vivo* drug-drug interaction data were not available in literature for this combination.

Female Balb/c mice were administered with olaparib alone (10 mg/kg) and in combination temozolomide (10 mg/kg and 50 mg/kg). Blood samples were collected at different time using staggered sampling procedure from mice and olaparib and temozolomide were extracted using plasma protein precipitation and analyzed on High Pressure Liquid Chromatography coupled with mass spectrometry method, limit of quantitation of method was 10 ng/ml with linearity range from 10 to 20000 ng/ml. Peak plasma concentrations, exposure, PK parameters of olaparib were calculated in absence and presence of temozolomide, the result showed no significant effect of temozolomide on olaparib pharmacokinetic. This finds would truly predict the PD outcome of olaparib without any dose titration when combination used for chemotherapeutic treatment in animals.

P061: Polyphenol(s) enriched anti-inflammatory nutraceutical derived from underutilized plant parts

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Anti-inflammatory Foods such as whole grains, fruits and vegetables, which provide valuable antioxidant polyphenols, carotenoids, phytosterols, curcumin, vitamins, minerals, omega fatty acids and dietary fibers may play an important role in alleviating inflammation, as well as strengthening the immune system. The polyphenols show significant anti-inflammatory, anti-oxidant and anti-DNA damaging effects. It is suggested that routine consumption of these polyphenols may provide efficient protection. Important dietary sources of polyphenols are onions (flavonols); cacao, grape seeds (proanthocyanidins); tea, apples, and red wine (flavonols and catechins); citrus fruits (flavanones); berries and cherries (anthocyanidins); and soy (isoflavones) prove particularly helpful in alleviating inflammation. Recent studies showed that polyphenols in healthy foods are readily metabolized in different ingredients, which are responsible for their anti-inflammatory properties. Omega fatty acids are also associated with reduced inflammation and improved cardiovascular health. These vegetables and fruits are rich in antioxidant polyphenols and can effectively reduce inflammation. Spices and herbs like cinnamon, basil, clove, parsley, rosemary, mint, turmeric and thyme Nuts and seeds like almonds, walnuts, hazelnuts, oats, flaxseed and sunflower seeds are also known to have anti-inflammatory properties.

Polyphenols can exert their anti-inflammatory properties at multiple levels, through the modulation of mitogen activated protein kinases (MAPK), Akt and NF-κB signaling pathways, inhibition the production of inflammatory cytokines and chemokines, suppressing the activity of cyclo-oxygenase (COX) and inducible nitric oxide synthase (iNOS) and decreasing the production of reactive oxygen and nitrogen species (ROS/RNS). Nutraceutical/functional foods have potential to be used as food supplement and preventive medicine.

P062: Synthesis and biological evaluation of novel indole derivatives as potent CB2 agonist

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A few analogues of novel indole-2-carboxamides are reported. Their activities were evaluated *In vivo* for management of pain in different rodent models. Towards the chemical modifications of the indole-2-carboxamide scaffold, the lead compound 5-Methoxy-1H-indole-2-carboxylic acid [(1S)1,3,3-trimethyl-bicyclo [2.2.1] hept-2endo-yl]-amide showed significant antinociceptive activity with impressive CB2 *In vitro* selectivity. The lead compound also exhibited favorable pharmacokinetic profile.

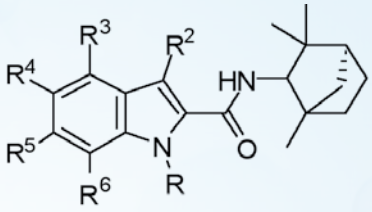

Figure 1: Novel Indole carboxamides

P063: Blockade of tumor necrosis factor-α converting enzyme exacerbates IL-1β and IFN-γ via caspase-1 activation

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TNF-α converting enzyme (TACE) is a member of the ADAM (a disintegrin and metalloproteinase) family and is known as ADAM17, which processes precursor TNF-α in order to release soluble TNF-α (sTNF-α). Inhibition of TACE has been effective as a strategy to inhibit arthritis in animal models however; it has been not translated in the clinic due to lack of efficacy or toxicity. We hypothesized that inhibition of TACE may activate a different pro-inflammatory pathway in human. To investigate this, we studied the effect of TACE inhibitor DPC-333 on cytokine levels in concanavalin A (Con A) activated human PBMC (hPBMC). We have also studied the effects of DPC-333 on cytokine levels *In vivo* in Con A challenged mice or *In vitro* in LPS stimulated mouse whole blood. DPC-333 treatment significantly up-regulated IL-1β and IFN-γ in Con A activated hPBMC. In contrast, pre-treatment with DPC-333 effectively suppressed IL-1β and IFN-γ in mice *In vivo* or *In vitro*. Interestingly, DPC-333 was found to up-regulate mRNA expression of caspase-1 in hPBMC in a dose dependent fashion and selective caspase-1 inhibitor completely restored DPC-333 induced IL-1β and IFN-γ. Furthermore, selective IL-1β receptor antagonist (anakinra) prevented DPC-333 induced IFN-γ. In conclusion, our data demonstrates that blockade of TACE exacerbates IL-1β in a casapse-1 dependent manner *In vitro* in human PBMC and the elevation of IFN-γ is secondarily mediated via IL-1β. This novel finding might explain the possible cause behind the loss of efficacy of TACE inhibitors in human.

P064: Studies on the anti-inflammatory properties of aqueous and alcoholic extract of *Merremia tridentata (L.)* Hall. f.

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Ethnopharmacological relevance: *Merremia tridentata (L.)* Hall. f. Subsp. *hastata* (Convulvulaceae) is one of such plant in the name of "Prasarini "which is used as an anti-inflammatory agent in ancient Ayurveda. Whole plant is used traditionally throughout India in vitiated condition of hemiplegia, hemorrhoids, uropathy, inflammation and general debility.

Aim of the study: To investigate the anti-inflammatory effect of aqueous and alcoholic extract of the whole plant *Merremia tridentata* using carrageenan induced rat paw edema model of inflammation.

Materials and methods: Carrageenan induced rat paw edema model was used for studying the anti-inflammatory activity of aqueous and alcoholic extract of *Merremia tridentata*.

Results: Aqueous and alcoholic extract (500 mg /kg body weight) were used for studying their comparative activity in inhibiting paw edema. Only aqueous extract of the plant significantly reduced the edema in rat.

Conclusion: The study evidently confirmed anti-inflammatory activity of aqueous extract of *Merremia tridentata* and thus supported the traditional claim. The anti-inflammatory activity could be attributed to the phytoconstituents such as flavonoids and saponins present in the aqueous extract of the plant.

P065: The effect of Teripartide (hPTH) and celecoxib cotherapy on an experimental model of osteoarthritis

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OA is generally regarded as a non-inflammatory form of arthritis but considerable data implicate a role for proinflammatory cytokines Several inflammatory factors, including inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and phospholipaseA2, modulate chondrocyte function in OA. The present study investigates the possible protective effect of celecoxib and PTH when used alone and as a cotherapy against OA in rats induced by MIA.

The treatments were initiated on day 7 and continued up to day 34. Meanwhile, weight bearing capacity was measured, blood samples was collected for cell count as well as for hydroxyproline estimation, knee joints were collected for estimation of cytokines, osteocalin and hydroxyproline and tibia-femur with joint was collected for histopathological examinations.

Acute treatment with teriparatide had no effect on pain but it improved weight bearing after chronic treatment. Celecoxib alone as well as in cotherapy with teriparatide showed significant analgesic effect both in acute and chronic treatment. Teriparatide treatment lowered the joint cytokines level and showed significant effect on osteocalcin and hydroxyproline in serum as well as in joint with new bone formation and increasing osteoblastic activity in bone histological analysis. Celecoxib treatment also lowered the joint cytokines level but had minimal effect on osteocalcin, hydroxyproline and bone histology. Cotherapy showed additive effect on cytokines, osteocalcin and hydroxyproline and bone histology.

Therefore cotherapy might be useful in osteoarthritis to solve the purpose of cartilage regeneration and inflammation.

P066: Potential role of kinase inhibitors in an experimental mice model of ovalbumin induced asthma

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Asthma is characterized by chronic eosinophilic inflammation, remodelling and hyperresponsiveness of the airways. Kinases like Phosphoinositide 3 kinase (PI3K) and Janus kinase (JAK3) are involved in mast cell proliferation, activation, recruitment, migration and prolonged survival of inflammatory cells, associated with asthma. Hence, in our study, the efficacy of kinase inhibitors as potential novel anti-asthmatic agents was tested.

We evaluated the effects of PI3K inhibitor (30 mg/kg, p.o) and JAK3 inhibitor (30 mg/kg, p.o) on airway inflammation in an acute and chronic model of ovalbumin induced asthma. Twenty-four hours after the final antigen challenge, bronchoalveolar lavage and histological examinations were carried out. Treatments inhibited the elevated levels of cytokine in BALF in chronic asthma. There was reduction of leucocytes, eosinophils in the bronchoalveolar lavage fluid. Histological analysis of the lung revealed that the infiltration of inflammatory cells, hyperplasia of goblet cells and the collagen deposition were significantly suppressed by the treatments. The treatments decreased airway inflammation in a murine model of allergic asthma, hence, providing an opportunity for the development of novel therapeutics to treat severe asthma.

P067: Free radicals mediate secretory IL-1β production, transcription and processing in monocytes

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Interleukin-1β (IL-1β) driven inflammation plays a pivotal role in many inflammatory diseases and reactive oxygen species (ROS) can modulate IL-1β secretion in monocytes. Therefore, the present study was undertaken to ascertain the role of ROS in IL-1β production, transcription and processing. THP-1 cells were pretreated with DPI (NADPH-Oxidase inhibitor,10μM) and NAC (Free radical scavenger,10mM), then stimulated with PMA (200 nM), LPS and Pam3csk4 (100 ng/ml) and IL-1β secretion was measured by ELISA. PMA (~91 fold), LPS (6.5 fold) and Pam3csk4 (3.2 fold) induced IL-1β production was significantly inhibited ($p<0.001$ with PMA, Pam3csk4 and $p<0.05$, $p<0.01$ with LPS) in the presence of DPI and NAC. Real time PCR showed that PMA, LPS and Pam3csk4 induced IL-1β transcription (~1000 fold,~4000 fold and ~3000 fold respectively)

was significantly reduced ($p<0.001$) in the presence of DPI and NAC. PMA, LPS and Pam3csk4 induced JNK1/2 phosphorylation (~2 fold) and AP-1 activation (~6 fold,~5fold,~4 fold) was significantly inhibited in presence of DPI and NAC. Interestingly not only IL-1β transcription but IL-1β processing was also effected by ROS. PMA, LPS and Pam3csk4 induced caspase activation (~2fold) was significantly abrogated in the presence of DPI and NAC. Role of ROS in IL-1β processing was further assessed by checking the expression of intracellular Pro-IL-1β and IL-1β by immunoblotting. It was found that PMA, LPS and Pam3csk4 induced Pro-IL-1β (2.5 fold) and IL-1β (4 fold) expression was significantly reduced in the presence of DPI and NAC. Thus, the present findings indicate ROS dependent IL-1β production, processing and transcription through JNK-AP-1 axis.

P068: Dissociation between glucocorticoid receptor mediated transactivation and transrepression in vitro: "A new approach for replacement of conventional steroids".

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Glucocorticoids (GCs) are mainly used to suppress disease-related inflammation and are widely used for the treatment of many inflammatory diseases including asthma and arthritis. However, GCs are also associated with debilitating side effects that place limitations on the long-term use of these drugs. The development of a GC with reduced side effects would allow more effective treatments for patients who require long-term suppression of inflammation. GCs influence the expression of genes either by transactivation or transrepression. There is increasing acceptance of the hypothesis that the side effects of steroids are due to transactivation of genes through binding of the GR dimers to DNA, whereas the anti-inflammatory effects are due to transrepression of genes through binding of a single GR to transcription factors or co-activators resulting in gene repression. Compounds that can dissociate the transactivation function of GCs from the transrepression function may have an improved therapeutic index. We have identified selective ligand (compound A) of the glucocorticoid receptor that exert strong *In vitro* TNFα inhibition (transrepression) on LPS induced human monocytic cell line that dissociate from TAT transactivation on steroid induced rat hepatoma cell line. Compound A showed potent anti-inflammatory activity compared with Dexamethasone but lesser side effect with the same.

P069: Ameliorative effect of modified diet in glucocorticoid induced histological changes in spleen and thymus of rats

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This study was designed to investigate the possible ameliorative effect of modified diet on prednisolone induced alterations in the major organ systems after four weeks of treatment in Wistar rats by oral route. Along with a common control group, study comprised of two groups treated with prednisolone at 10 mg/kg with one group supplemented with modified - high calorie diet and another group with normal pellet diet. It's well established that long term use of glucocorticoids accelerates catabolic activity and thereby increase energy expenditure resulting in undesired side effects in humans. Under this hypothesis, effect of modified - high calorie diet was studied in rats under continuous treatment with prednisolone. As anticipated, animals fed with normal pellet diet revealed exaggerated pharmacodynamic effects of prednisolone on lymphoid organs in terms of histological alterations like atrophy and/or lymphoid depletion of thymus and spleen and hematological changes like significantly low total WBC count as well as absolute lymphocyte count ($p<0.001$) and gravimetry changes such as decline in absolute weights of both thymus and spleen ($p<0.001$). However, these adverse effects were substantially reduced in group treated with modified - high calorie diet indicating the requirements of medium to high calorie diet for patient population who have been advised on glucocorticoid treatment for longer period of time. Further this hypothesis can be explored by modifying this high-calorie diet with other essential nutrients to reduce other reported side effects of glucocorticoids.

P070: Curcuma oil mitigates LPF induced TNF production

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Current study was aimed to investigate the anti-TNF effect of Curcuma oil in THP-1 monocytes, mice and human whole blood and mouse model of LPS-induced endotoxemia. Pre-treatment of C.oil (1, 10 and 30µg/ml) for 14h in THP-1 monocytes followed by LPS (100ng/ml) stimulation for 1.5h led to dose dependent inhibition in TNF production (~ 39, 57 and 84% respectively). In addition, C.oil (1, 10 and 30µg/ml) treatment inhibited LPS induced p-38 MAPK activation (1.5, 1.7 and 2 fold respectively) in THP-1 monocytes. In human whole blood, pre-treatment with C.oil (1, 3 and 10µg/ml) for 14h reduced LPS (50 ng/ml) induced TNF production (~12, 38 and 46% respectively).

Likewise, in mice whole blood, C.oil (1, 3 and 10µg/ml) attenuated LPS (50 ng/ml) induced TNF production (~11, 37 and 43% respectively). The anti-inflammatory effect of C.oil was further evaluated in-vivo in mouse model of LPS-induced endotoxemia. Mice were pre-treated with C.oil (100 and 300 mg/kg/day) for 1 week and subsequently LPS challenge (10mg/kg) was given intraperitoneally for 12h. Plasma level of TNF was attenuated (~ 22 and 31% respectively) in C.oil treated group along with reduced plasma nitrite (~ 42 and 47% respectively) and improved endothelial function as compared to LPS alone. In mice, anti-TNF effect of C.oil at the tested dose was comparable to Ibuprofen (2mg/kg). Present study demonstrates anti-TNF effect of C.oil in mice and human blood.

P071: Identification of early biomarkers of arthrtis in adjuvant and streptococcal cell wall induced arthritis models

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The most widely used models of RA are the adjuvant-induced arthritis (AIA and streptococcal cell wall (SCW) induced arthritis. Cytokines such as interleukin-1, tumor necrosis factor alpha, interferon gamma and interleukin-6 play a pivotol role in inflammation and the immune response and are present at high levels in arthritic joints and blood In the current study, we analyzed the kinetics of inflammatory cytokines and cartilage degradation markers in AIA and SCW induced arthritis models. Prednisolone, JAK-3 and P38 inhibitors were administered per oral for 10 days in AIA model and 3 days in SCW model. The injection of complete Freund's adjuvant in AIA model on day 0 (D0) induced a marked, transient increase in IL-6 and TNF alpha (initial phase) followed by a second phase on D10. Initial phase is associated with systemic inflammatory cytokines. Whereas late phase of arthritis was involved in significant increase in local as well as systemic cytokine levels. There was significant improvement in inflammatory cytokine associated paw swelling in JAK-3 inhibitor and prednisolone treated groups. In scw injection, on day 0 (D0) there was significant increase in systemic TNF alpha, IL-6 and IFN gamma after 90 minutes without much change in local tissue cytokine. Treatment groups such as prednisolone, JAK-3 inhibitor and P38 inhibitor significantly reduced IL-6 levels. In conclusion, the results of cytokine evaluation from both the models revealed that IL-6 and TNF alpha may be early predictable biomarkers in arthritic model.

P072: Percutaneous absorption of diclofenac in rats after single and repeated topical application of diclofenac Emulgel

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Historically NSAIDs have been used to assist the resolution of pain in conditions of Osteo arthritis of the knee and sports and soft tissue injuries. A major drawback of using oral forms of NSAIDs such as diclofenac is the high incidence of major adverse effects such as gastrointestinal bleeding, gastric ulceration and renal disease implications. The use of topical formulations of diclofenac is thought to be as efficacious as oral formulations without the risk of systemic side effects. Topical diclofenac is thought to reduce inflammation via inhibition of the COX 2 isoenzyme. This current evidence of the efficacy of topical nanogel formulations of diclofenac for treatment of osteoarthritis of the knee and soft tissue and sports injury. In the present invention, Nanoemulsion and Nanogel has been proposed for the topical application on the site of action by which the drug permeates through the skin because of its small droplets size allowing easy penetration. The nanogel of the present invention adheres well to the skin, spreads easily, dries quickly, and show greater in vivo absorption. Thus, the gel formulations of the present invention provide superior means for delivery of diclofenac sodium through the skin for the treatment of an antiinflammatory and analgesic, as compared to marketed formulations.

P073: Halogenation of resiniferatoxin TRPV1 agonists

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Resiniferatoxin (RTX), isolated from *Euphorbia resinifera*, is an extremely potent irritant tricyclic diterpene which is structurally related to phorbol-related diterpenes except for its homovanillyl ester group at C-20. RTX has proven to function pharmacologically as an ultrapotent agonist for the transient receptor potential vanilloid 1 (TRPV1) channel, displaying 10^3- to 10^4-fold greater potency than the prototypic agonist capsaicin. Structure-activity relations for RTX derivatives have been investigated employing partial modifications starting from RTX or ROPA (resiniferonol orthophenylacetate) based on the three structural regions including the A-region (4-hydroxy-3-methoxyphenyl), B-region (C20 ester), and C-region (diterpene). In the SAR of the A-region of RTX (4-hydroxy-3-methoxyphenyl), any modifications on the phenolic hydroxyl, such as methylation and 2-aminoethylation, led to the reduction in binding affinity and agonism in rat DRG. However, 5-Iodo

RTX, prepared semisynthetically from RTX by iodination, displayed good potency in rat and human TRPV1 and shifted the activity from agonism to antagonism. Previously, we and other groups reported that the halogenation of the aromatic A-ring of TRPV1 agonists also shifted the agonism of the ligands toward antagonism. On the basis of this SAR analysis, we have investigated the halogenated RTX analogues in which 5 position of the 4-hydroxy(or 4-amino)-3-methoxyphenyl group was halogenated with fluoro, chloro and bromo atoms, respectively. In this presention, the synthesis, receptor activities and SAR analysis of halogenated RTX will described.

P074: Protective effect of β- cyclodextrin against acetazolamide induced teratogenicity in pregnant female wistar rats

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The present study was planned to investigate the ameliorative potential of the commonly used drug excipient β-cyclodextrin towards the teratogenic effects of a diuretic, acetazolamide in pregnant female Wistar rats. An effect on embryo-fetal development of acetazolamide at a dose level of 500 mg/kg alone was compared at the same dose level in combination with β-cyclodextrin at dose of 0.75g/kg by oral route. Concurrent control groups treated with vehicle and another group treated with β-cyclodextrin alone was maintained in this study. Embryo and fetal toxicity was primarily assessed through uterine parameters and examination of fetus after C-section. A detailed investigation was made through external, visceral, skeletal and head razor examination in this study. In acetazolamide + β-cyclodextrin treated groups, fetal weight and size were normal and incidences of ectrodactyly and skeletal anomalies were minimal when compared with acetazolamide alone treated groups. This indicates the protective effects of β-cyclodextrin with acetazolamide and its advantages could be further explored with other drugs which could be potentially used during pregnancy when the benefits of the mother outweighs the risks of developing fetus.

P075: Synthesis of N-aryl imidazolidine-2-thione derivatives and their antibacterial evaluation

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Bacterial and fungal infections are the most widespread microbial infections. One of the major medical advances of the last century was the development of effective antibiotics. However, with widespread use of antibiotics came antibiotic resistance. Substituted imidazolidine-2-thiones are of considerable pharmaceutical interest as they display remarkable biological activities. Imidazolidine-2-thione derivatives have been reported to exhibit antimicrobial activity, anti-HIV activity, antifungal activity and so forth. Substituted N-aryl imidazolidine-2-thiones were synthesized by thermal as well as microwave irradiation techniques. Substituted methyl phenyl carbamodithioate was synthesized first by reaction of substituted aniline with carbon disulfide and dimethyl sulphate which was then reacted with bromoethylamine affording different substituted N-aryl imidazolidine-2 thiones. Substituted N-aryl imidazolidine-2 thiones so synthesized were subjected to reaction with different acid chlorides to yield N,N-disubstituted imidazolidine-2-thione derivatives. Structure elucidation of all the synthesized compounds was carried out by IR, ^{1}H NMR, ^{13}C NMR, MASS and C, H, N, elemental analysis. All the synthesized compounds were subjected to antibacterial evaluations against 6 bacterial strains by Agar Well diffusion method. From antimicrobial bioassay it was observed that the presence of bromo atom at para-position influenced the activity strongly against *B. cereus* as compared to its presence at ortho and meta positions as shown by compound 1-(4-bromophenyl) imidazolidine-2-thione. Compounds with halogen atom exhibited significant activity against *P. aeruginosa* as shown by 1- (3- chloro- 4- flurophenyl) imidazolidine-2- thione) and 1- (3- chloro- 4- fluorophenyl)-3- (4- chlorobenzoyl) imidazolidine-2- thione.

P076: Design, synthesis, in-vitro biological and computational evaluation of some 2-Amino-6-nitrobenzothiazole based hydrazones as potential MAO inhibitors

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Monoamine oxidase (MAO) A and B have been documented as potential targets for treatment of neurodegenerative disorders such as Parkinson's and Alzheimer's disease. In recent decades, several nitrogen containing heterocyclic derivatives have been identified as potential MAO-A/B inhibitors but their development as drug candidates is hampered due to lack of selectivity and poor in-vivo profile. Thus, search for selective MAOIs with ideal pharmacological profile still continues. Availability of crystallographic data of MAO-A/B has accelerated discovery of newer MAOIs. Using 3D-geometry of MAO active sites, we designed some 2-amino-6-nitrobenzothiazole based

hydrazones and evaluated them experimentally and virtually for MAO-A/B inhibitory activities. The newly designed compounds (3a-3j) were synthesized via reaction of 2-amino-6-nitrobenzothiazole (1) with ethylchloroacetate and K_2CO_3 followed by reaction with hydrazine hydrate leading to formation of corresponding hydrazide (2). Acid-catalyzed condensation of hydrazide (2) with various aryl aldehydes/ketones yielded final hydrazones (3a-3j). Structures of final compounds were confirmed by FTIR, NMR and elemental analysis. Final compounds were screened for in-vitro MAO-A/B inhibitory properties. All compounds showed significant inhibition against both isozymes at micromolar to submicromolar concentration compared to reference inhibitors clorgyline and selegiline. Computational studies were performed to evaluate binding interactions of these inhibitors against MAO-A/B using AutoDock 4.2. Good correlation between the calculated and experimental results were obtained. Compounds 3g [IC_{50}: 0.42 µM] emerged as most potent inhibitor of MAO-A and 3h [IC_{50}: 0.44 µM] as persuasive MAO-B selective agent. Further studies on lead inhibitors including their in-vivo evaluation and co-crystallization of inhibitor-MAO complex are essential to develop them as possible therapeutic agents for treatment of MAO-associated neurological disorders.

P077: *In silico* screening, synthesis and pharmacological evaluation of some quinazolinones as NMDA receptor antagonists for anticonvulsant activity

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In silico drug designing methods have quenched the thirst of drug discovery as it provides insight for prioritization of the molecules before actual synthesis and pharmacological screening. Literature reveals that NMDA receptor specifically NR2B subunit plays a major role in eliptogenesis. Antagonist at NR2B receptor sight have importance in drug design of anticonvulsant agents. Quinazolinones have inherent drug likeliness for anticonvusant activity. In this research work in silico biological activity spectrum (BAS), ADME prediction, Log P prediction and docking was carried out. 5-[(6,8 dihalogen-substituted/6-halogen substituted/6-unsubstituted-2-methyl-4-oxoquinazolin-3(4H)-yl)methyl]pyrimidine-2,4,6(1H,3H,5H)-trione.(SMMA1-8, SMMB1-8 AND SMMC1-8) were designed and prioritized for actual synthesis and pharmacological screening for NMDA receptor antagonistic activity. The prioritized molecules were synthesized and charecterised by melting point, IR, 1H-NMR, TLC and elemental respectively. AOT was performed to determined LD50 of prioritized molecules, further compounds shall be evaluated for there *In vivo* NMDA (200mg/kg) induced anticonvulsant activity in mice, and shall be compared with anticonvulsant effect produced by the standard Memantine or Ifenprodil.

P078: Modified equilibrium dialysis technique to overcome non-specific binding challenges in in-vitro measurement of plasma protein binding

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Equilibrium dialysis is considered as gold standard method for estimating in-vitro protein binding. Plasma protein binding (PPB) of 15 diversified chemical scaffold exhibited non-specific binding (NSB) was estimated by equilibrium dialysis method using ultra-filtrate as an equilibrium medium instead of traditional phosphate buffers.Experiment was performed in 96-well equilibrium dialysis plate (HTD), donor and receiver side were separated by 12-14 kDa molecular weight cutoff (MWCO) strip membranes. Rats and human plasma was spiked with compounds to get 1 µM final concentrations and dialyzed for 6 hr. at 37oC with 200 rpm shaking against plasma ultra-filtrate, obtained from ultrafiltration of plasma through 10 kDa MWCO filter membrane. Equilibrium time and non-specific binding was assessed in ultra- filtrate before experiments. After dialysis, concentrations from the donor and receiver sides were measured using LC-MS/MS analysis. By using ultra-filtrate in the receiving side, overall mass recoveries were >85%, equilibrium between donor and receiver sides was almost achieved and non-specific binding reduced significantly with good precision among triplicate wells. Protein binding values of known compounds were in good agreement with reported values in the scientific literature. The purpose of this work was to develop a high throughput screening methodology to study protein binding in plasma for chemically divers and lipophilic novel molecules. The modified method was coupled with sensitive and high throughput mass spectrometry to measure precise and accurate protein binding. Thus this method can be applied for screening of molecules for plasma protein binding with minimal non-specific binding.

P079: In-silico investigation of biological potential of phytoconstituents by computational tool pass

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Prediction of Activity Spectra for Substances (PASS) is a computer program used for the evaluation of general biological potential of molecules on the basis of its structural features. The PASS training set consists of about 26000 chemical compounds including drugs as well as toxic substances. Although, the application of PASS as a tool for predicting biological activity spectrum of synthetic substances during discovery of new drugs has been confirmed; its application to predict the biological activities, toxicities & mode of actions of phytoconstituents is limited. The latest version of PASS (10.1) predicts 4130 types of biological activities including, 501 pharmacotherapeutics effects, 3295 mechanisms of actions, 199 metabolic terms, 57 types of toxic effects, 49 transporter proteins and 29 activities related to gene expressions. Results of PASS prediction are obtained as a list of biological activities, having probabilities to be active (Pa) and to be inactive (Pi). The average accuracy of prediction by leave-one-out cross validation method is reported to be 94.7%. The PASS online version is freely available with restricted functionality. While, full version of PASS is available as a commercial programme. PASS can be applied to predict biological and toxic properties of variety of phytoconstituents having well established chemical structures like triterpenoids, flavonoids, glycosides, alkaloids etc. Thus, PASS can be used for exploring the newer mechanisms of actions of known phytoconstituents, high throughput screening of natural product libraries and for finding the novel targets for existing phytochemicals. The present poster highlights application of PASS utility for predicting general activity spectra of phytoconstituents.

P080: Tests for observational batteries and neuropharmacological studies of Roots of *Eranthemum roseum* in a mouse models.

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The *Eranthemum roseum*(Vahl) R.Br.a shrub from Acanthaceaefamily is popularly used in folk medicine for treating. To carry out the Neuropharmacological work, study was rendered on steroidal saponin rich fraction (S) which was obtained by successively partition of methanolic extract with petroleum ether and then with n-butanol. The Steroidal saponin rich fraction of *Eranthemum roseum* (SFER) was administered orally and it was tested on acute toxicity, Modified Irwin battery test, hot plate for central analgesics, rota-rod for muscle co-ordination, open field by video analysis-locomotor and anxiety, Phenobarbital induced hypnosis, milk induced leucocytosis and eosinophil for anti-stress activity and PTZ-Strychnine induced convulsions in mice. The experimental data was disclosed for SFER 30, 60 and 120 mg/kg: (1) Battery of observations showed analgesic, depressant, alteration in motor co-ordination, anxiolytic and hypnotic effect; (2) increased in mean percent of maximum possible effect (% MPE) was observed for hot-plate central analgesics; (3) less significant effect was obtained after testing for muscle co-ordination; (4) SFER failed to reduced anxiety but decreased in locomotion might be predict depressant effect; (5) also potentiation of hypnotic effect by reduction in induction and prolongation in duration of sleep was perceived; (6) SFER was significantly normalized milk induced leucocytosis and eosinophil count in mice; (7)a delay in induction, reduced in duration of convulsion and protection from death was promoted SFER as an anti-convulsant drug when tested on Pentylenetetrazole (PTZ) induced convulsion. SFER had no effect on Strychnine induced convulsion. Our findings were concluded that Saponin fraction of *Eranthemum roseum* presented depressant, analgesics, anti-stress and enhancement of GABA action when assessed particularly for anti-convulsion action and potentiation of sleeping time.

P081: Aminoacid-specific preferential binding site for ligands to Aβ peptides

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Alzheimer's Disease (AD) is a progressive irreversible neurodegenerative disorder and is the basis of loss of reason and ability to care for oneself as seen in the geriatric populations afflicted by AD. It is characterized by deposition of senile plaques composed of neurotoxic β-amyloid (Aβ) peptides either of the Aβ40 or Aβ42 types. The binding sites on these peptides are unclear although many attempts have been made to elucidate these sites. X-ray crystal structures of Aβ peptides are also unavailable. Knowledge of such binding sites could help in the development of agents for AD.

Here we present our research on the utilization of ligands known for binding to these peptides to decipher the binding site on the Aβ peptides using molecular modeling techniques. Initially we find two probable binding sites for reported ligands on Aβ utilizing molecular docking, then induced fit docking and further site mapping for cross-validation of one of these sites as binding site. Quantitative analysis of interactions of individual residues by molecular mechanics and further molecular dynamics simulations signify the relative importance of hydrophobic interactions over hydrogen bonding in binding interactions with this binding site.

Thus we propose an aminoacid-specific binding site on Aβ peptides which could help in understanding the interactions of known ligands with Aβ monomers and oligomers. This preferential binding site might be used in HTVS screening of molecules for binding to Aβ peptides and in the rational design of Aβ ligands with improved binding characteristics for the diagnosis or therapy of AD.

P082: Comparative study of in-vivo release of leuprolide from three month depot microsphere in wistar rats by highly sensitive high-pressure liquid chromatography-tandem mass spectrometry method

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Leuprolide acetate, a highly active agonist of luteinizing hormone-releasing hormone potently inhibits the pituitary-gonadal axis upon chronic administration and is useful in the treatment of hormone-dependent disease. A biodegradable polymer based novel depot formulations were designed to overcome conventional inconvenient daily subcutaneous injection treatment. Novel depot formulations provided sustain release of Leuprolide in serum to achieve greater efficacy over period of three months. A sustain serum levels of Leuprolide would persistently suppressed serum gonadotropin and testosterone levels to get desired effect. Three formulations of Leuprolide acetate, two novel three-month depot and one marketed formulation were administered to male Wistar rats at 4.5 mg/site via subcutaneous route. A serial blood samples were collected from each animals on regular time intervals up to 14 weeks and serum was separated for estimation of Leuprolide levels in serum. Leuprolide was extracted from serum using protein precipitation method and analyzed using high-pressure liquid chromatography coupled with tandem mass spectrometry method. Analytical method showed 100% extraction efficiency of Leuprolide from serum matrix, limit of detection was 0.1 ng/ml and linearity range was from 0.1 to 20 ng/ml. Leuprolide serum concentration were determined from three formulations, two novel three-month depot and one marketed formulation and release profiles were compared. In-vivo release of Leuprolide from different novel formulations can be optimization successfully by application of developed highly sensitive high-pressure liquid chromatography-tandem mass spectrometry method in drug development.

P083: Solid lipid nanoparticles (SLNs) of arteether for improved therapy of malaria

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Introduction: The objective of the present investigation was to evaluate anti-malarial efficacy of SLNs in comparison to Arteether suspension using P. berghei lethal ANKA strain in rodent model.

Experimental: SLNs of Arteether were developed using industrially feasible technique; characterized for physicochemical parameters such as mean particle size, polydispersity index, zeta potential, encapsulation efficiency, drug content, morphology, and in- vitro release studies. *In vivo* anti malarial efficacy testing using 'Peter's four day suppressive test' was performed in infected male swiss mice using lethal strain of P. berghei. On day "0,"the mice were infected by intraperitoneal injection of donor mouse blood diluted with acid citrate dextrose (ACD) buffer to contain approximately 10^6 infected RBCs. The infected animals were randomly divided into four groups (n=8); Group I (SLN s of Arteether; 12 mg /kg), Group II (Arteether suspension ; at recommended oral dose 24 mg/kg , Group III(Blank SLNs),Group IV (control) . The various treatment compounds were suitably diluted with distilled water, shaken to form homogeneous suspensions, and were administered to the animals from day "0" to day "3" of post infection, by oral gavage. On day "4" blood smears were prepared by withdrawing the blood from tail vein, fixed with methanol, and stained with Giemsa's stain to count the parasites. Average percentage parasitemia and percent activity were calculated and subjected to statistical analysis. The survival of animals after 28 days of the experimental work was recorded.

Results and Discussion: The mean particle size of SLNs was found to be 19.91±0.49 nm, with polydispersity index 0.248±0.004. The developed SLNs exhbibited significantly reduced mean parasitemia for a prolonged period as compared to the Arteether suspension (***$p < 0.0001$). The percent survivor after 28 days was superior as that Arteether suspension and control group.

Conclusion: SLNs of Arteether was developed with industrially feasible technology and exhibited a superior antimalarial efficacy.

P084: Formulation and drug release behavior of temperature responsive k-carrageenan submicrogels for poor water soluble drugs.

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Crosslinked K-carrageenan hydrogel submicroparticles (submicrogels) with an average size lesser than 0.5 µm were equipped using reverse microemulsions united with thermally induced gelation. In formulation, water in oil type of emulsion was prepared by using cyclohexane and n-butanol mixture (7.7:2.3) as oil phase and 1 % K- carrageenan as aqueous phase. Emulsion was prepared at temperature more than phase

inversion tempreture of surfactant. The size of the microgels varied with ionic nature of surfactant at an invariable water/surfactant molar concentration ratio. The submicrogels were found to be thermo-sensitive in a temperature series acceptable for living cells (37–45 °C), which leads to reversible volume conversions in response to thermal stimuli. This unwrap the opportunity to investigate the relevance of these submicrogels in smart therapeutics, for example, thermo-sensitive drug carriers. In addition to this, these submicrogels, owing to their hydrophilic nature, were also evaluated for the drug release of poor water soluble agents. Hence to utilize the therapeutic potential of submicrogels , we have estimated the release of cefdinir, (BCS class II) from the submicrogels using In-Vitro techniques. The study showed significantly higher release rate of drug from the submicron particles as compared to conventional formulation, which ultimately verifies the concept of submicrogel mediated release of poorly water soluble drug. Research findings shows that these submicroparticles have the potential to be used as carriers for thermal induced delivery of drugs especially in diseases which shows biphasic and triphasic temperature variations. Further In-Vivo studies of the optimized formulations are envisaged.

P085: Screening of anti-depressant activity of *Indigofera tinctoria* Linn. in swiss albino mice.

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In the present study, Methanolic extract of *Indigofera tinctoria* Linn. was evaluated for preliminary Phytochemical screening & to confirm whether Indigo present in plant constituent is responsible for anti-depressant activity using Nomura water wheel test, Tail suspension test, Open field test at different dose level. The result of present investigation showed that the methanolic extract of *Indigofera tinctoria* & Indigo have increased the wheel turning behavior as compared to that control group & reduced immobility time in tail suspension test & Thigmotaxis effect in open field test. This states that plant may possess anti-anxiety & general stimulant activity instead of Anti-depressant activity.

P086: A novel sensitive and high throughput liquid chromatography tandem mass spectrometry method for determination of lacosamide in human plasma

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Lacosamide, a novel functionalized amino acid used for partial-onset seizures and has a bioavailability of about 100% with minimal first pass metabolism and serum protein binding. As per the literature, there was not much application of lacosamide regarding LC-MS/MS methods in human plasma, a novel liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination Lacosamide in human plasma has been developed and validated. Using Solid-Phase Extraction Procedure, lacosamide and IS were chromatographed on C18 (50*4.6mm) 5µm analytical column. Quantitation was performed on triple quadruple mass spectrometer using electro spray ionization technique and operating in Multiple Reaction Monitoring (MRM) and positive ion mode. The chromatographic run time was 3.80 min and calibration curves were linear over calibration range of 5-5000ng/ml. The method was validated for selectivity, sensitivity, recovery, linearity, accuracy and precision, dilution integrity and stability studies. The recoveries obtained for analyte and IS were reproducible and consistent. Inter-batch and intra-batch coefficient of variation across five validation runs was less than 10%.

The method was applied for comparative bioavailability evaluation of 100 mg of lacosamide tablet after oral administration to 18 human subjects under fasting condition.

P087: Hepatoprotective activity of *Mucuna pruriens* against experimentally induced liver toxicity in rats

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The phytoconstituents of remaining Methanolic fraction of *Mucuna pruriens* fruits were characterized by High Performance Liquid Chromatography (HPLC). The effect of MP against corn oil and paracetamol induced injury to liver was determine and compaired with Silymarine by serum markers measurement,homogenate parameters study and histopathological observations.

Results: The phytochemical screening showed presence of phenols, polyphenols,saponins, galic acid and beta-sitosterol. HPLC analysis showed the presence of gallic acid and beta-sitosterol in MP. The results revealed that Methanolic fraction of *Mucuna pruriens* (MP) significantly prevented the changes in serum markers measurnent, homogenate parameters and histopathological observation s induced by corn oil and paracetamol induced liver toxicity.

Conclusion: By the experimental data obtained it is obvious that phenol and gallic acid present in *Mucuna pruriens* showed significant protection and improvement against liver injury induced by corn oil and paracetamol .

P088: A novel polymer lipid hybrid nanoparticles (Lipomer) for efficient delivery of itraconazole

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The objective of the present study was to formulate, optimize and characterize Polymeric lipid hybrid nanoparticles (Lipomer) containing antifungal drug Itraconazole for the efficient delivery in systemic fungal infection. Polymer lipid hybrid nanoparticles (Lipomer) are composed of liposome and polymeric nanoparticle into a single delivery system. The Polymeric lipid hybrid nanoparticle formulation was prepared by emulsification solvent evaporation method and optimized using Box Behnken statistical design. To prepare Polymeric lipid hybrid nanoparticles, biodegradable Poly-e-caprolactone, soya lecithin and Poly vinyl alcohol (PVA) were used as a polymer, lipid and surfactant, respectively. Polymeric lipid hybrid nanoparticles were characterized for particle size, entrapment efficiency, drug loading, *In vitro* drug release, *In vitro* intestinal cell uptake study using Confocal Laser Scanning Microscopy (CLSM) and Accelerated stability study. Itraconazole Polymeric lipid hybrid nanoparticles revealed nanosize (210 ± 1.8 nm) with entrapment efficiency of 83 ± 0.6 % and negative zeta potential (-11.7 mV). The present study demonstrated that Polymeric lipid hybrid nanoparticles could be promising drug delivery system for Itraconazole with excellent performance features such as nano size, high drug encapsulation efficiency, intestinal permeability and controlled drug release.

P089: HPTLC method for simultaneous estimation of atazanavir and ritonavir & degradation kinetic study of atazanavir

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A simple, validated HPTLC method was developed for the simultaneous estimation of Atazanavir and Ritonavir in their combined dosage forms. Separation was performed on silica gel 60F254 HPTLC plates as stationary phase and mixture of Chloroform: Ethyl acetate: Acetone (5.0:2.0:3.0 v/v/v) as a mobile phase. Densitometric evaluation was performed by absorbance/reflectance mode at 244 nm. The Rf value of Ritonavir and Atazanavir obtained were 0.30±0.02 and 0.58±0.02 respectively. The detector response was found to be linear in the range of 800-2800 ng/spot & 2400-8400 ng/spot for Ritonavir and Atazanavir respectively. The assay of Ritonavir & Atazanavir was found to be 97.08 ± 0.43% and 101.1 ± 1.98% respectively. The developed method can be used for routine quality control analysis of pharmaceutical formulations and drug substances. For Degradation kinetic study of Atazanavir, a simple validated stability indicating HPTLC method was developed. A mixture of Chloroform: Ethyl Acetate: Methanol: Formic Acid (8:2:1:0.5 v/v/v/v) was found to resolve the degraded product(s) from the pure drug (Rf = 0.52 ± 0.02) and detected by absorbance/reflectance mode at 301 nm. The polynomial regression was found to be in the range of 500-5000 ng/spot with regression coefficient of 0.9984. The proposed HPTLC method was utilized to investigate the kinetics of the degradation process in 1.0 N HCl. Arrhenius plot was constructed and energy of activation (Ea) was calculated.

P090: A novel gradient and high throughput liquid chromatography tandem mass spectrometry method for determination of tranexamic acid in human serum

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Tranexamic acid is a competitive inhibitor of plasminogen activation, Tranexamic acid binds more strongly than aminocaproic acid to both the strong and weak receptor sites of the plasminogen molecule in a ratio corresponding to the difference in potency between the compounds.

The objective of this work is to develop high throughput, sensitive, simple protein precipitation with gradient based matrix separation quantitative method to study comparative bioequivalence study of Tranexamic acid formulation with innovator drug. A sensitive and selective liquid chromatography coupled with electrospray ionization mass spectrometry (LC-MS/MS) method has been developed for the estimation of Tranexamic acid from human serum. Mass transition 158.10 m/z-->123.10 m/z and 152.10 m/z-->110.10 m/z were optimized for Tranexamic acid and internal standard (Acetaminophen) respectively. The sample preparation was carried out by using Protein precipitation extraction (PPT) technique. Acetonitrile (100% v/v) was used as precipitating agent. The chromatographic separation was achieved on ACE5 C18, 100*4.6 mm analytical column using mobile phase A : 0.2 % v/v Formic Acid in water (100 % v/v) B : Acetonitrile (100 % v/v)

with gradient elution (Time in min./% B : 0.01/10%,2.30/65%,2.60/85%,3.30/85%,3.50/10%, 5.20/Stop) Flow rate: 1.0 ml/min. with 65 % split to waste). The method was found to be linear over a dynamic range from 0.02 µg/ml to 15.0 µg/ml, with a correlation coefficient (r) 0.9992. The lower limit of quantitation (LLOQ) was 0.02 µg/ml. The absolute recovery for Tranexamic Acid and internal standard were 79.53% to 88.48 % and 86.33 % respectively. Inter day Precision & accuracy was found to be 1.59 % to 5.20 % and 99.21 % to 103.15 % respectively. Method was found to be selective, sensitive, precise and accurate for application of bioequivalence study in healthy human volunteers.

P091: A novel predictive platform for DILI: a combination of *in silico* and *in vitro* methods to predict mechanisms in vivo

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Hepatotoxicity prediction is still a challenge in the drug development process. Methods available at present are inadequate for the prediction of detailed mechanisms leading to the toxicity observed in rodents and humans. We have developed a dynamic systems model of the rat liver to predict DILI. The modeled metabolic network integrates important biological processes involved in fat metabolism (steatosis), energy metabolism (necrosis), oxidative stress (oxidative damage), bile and bilirubin metabolism (cholestasis) quantitatively by a set of differential equations to predict changes in metabolite concentrations. The model was first validated by comparing model simulated outcomes under conditions of normal liver homeostasis and DILI with observations published in literature. We used the model to generate hypotheses for the mechanism behind idiosyncratic toxicity caused by the anti-diabetic drug troglitazone. Sensitivity analysis of the metabolic network identified a set of enzymes and transporters that are predicted to be key players in causing different types of DILI. *In vitro* measurements of these enzyme activities, using a primary hepatocyte system, in absence and presence of well-known hepatotoxic drugs, are used as input to the model. Model simulated outputs of toxicity for each drug matched well with the associated toxic phenotype reported in the literature. The validation of this combinatorial (in silico and *In vitro*) approach using known hepatotoxic drugs e.g. diclofenac, cyclosporin, tamoxifen, etc. yielded additional novel mechanistic insights. The purpose of study is to develop a generalized liver toxicity prediction platform integrating *In vitro* and in silico approaches. Such an approach allows us to predict toxicity, generate mechanistic insights, and mechanism specific biomarkers.

P092: Colchicine intervention abolishes resiliency of aged Fischer 344 rats against chlordecone-amplified carbon tetrachloride hepatotoxicity.

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Aged Fischer 344 (F344) rats are resilient to chlordecone (CD) amplified CCl_4 hepatotoxicity due to prompt, and robust tissue repair response (Murali B. et al., 2004). If this is the underlying mechanism for the resiliency, then antimitotic intervention after infliction of CCl_4-induced liver injury should render those aged F344 rats susceptible to CD + CCl_4 interactive toxicity and mortality. After a non-toxic dietary regimen of CD (10 ppm) or normal powdered diet for 15 days, rats received a single non-toxic dose of CCl_4 (100 ml/kg, ip, 1:4 in corn oil) or corn oil (500 ml/kg, ip) alone on day 16. Thirty h later one group of rats received single dose of colchicine (CLC, 1mg/kg, i.p.) and the other group received distilled water as a vehicle. Liver injury was assessed by plasma ALT, AST, and histopathology during a time course of 0 to 48 h. Liver tissue repair was measured by [^{3}H-CH3]-Thymidine incorporation assay and PCNA immunohistochemistry. Exposure to CLC alone neither caused liver injury nor mortality. CLC administration to CD + CCl_4 group killed 2 out of 3 rats by 48 h, whereas none of the ND + CCl_4 rats receiving CLC died. Liver injury was further increased in CD + CCl_4 + CLC group at 36 h indicating that blocking cell division led to further progression of liver injury, and death. These findings further confirm that it is the stimulation of prompt and robust tissue repair rescued the 14-month old F344 rats from the lethal effect of the CD + CCl_4 combination.

P093: Novel thienopeptide analogs with anthelmintic potential

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S-heterocycles derivatives are well known for their pharmacological potential. Keeping in view the biological potency of thienoic acids as well as utilizing of biodegradability and biocompatibility of amino acids and peptides, present investigation was designed for the synthesis of novel series of thienopeptide analogs. These analogs are widely reported for their pharmacological potential and are associated with antimicrobial, cytotoxic, analgesic and anti-inflammatory activities. The synthesis of novel series of methylated thienopeptide analogs. 5-(2,4-dimethylphenyl)-2-thiophenecarboxylic acid was carried out by diazotization of 2,4-methylaniline followed by subsequent coupling

with 2-thenoic acid in presence of $CuCl_2$. In order to synthesize the novel series of potent peptide analogs, 5-(2,4-dimethylphenyl)-2-thenoic acid was coupled with various amino acids and peptide methyl esters using dicyclohexylcarbodiimide as coupling agent and pyridine as base. Selected ester derivatives were hydrolyzed using lithium hydroxide to get corresponding peptides. Structures of all the newly synthesized peptide analogs were characterized by IR, $^{1}H/^{13}C$ NMR, MS spectral data and elemental analysis, and evaluated for anthelmintic activity. Here in we have explored and utilized the biopotency, biodegradability and biocompatibility of S- heterocycles, amino acids as well as peptides, in the present synthesis of thienopeptide analog. Anthelmintic activity were carried out against earthworms (E. eugeniea, M. konkanensis and P. corethruses),by Garg's method using Mebendazole as a standard and Tween 80 as control. All the hydrolyzed compounds 10-12 were found to be more potent than their corresponding ester derivatives 3, 5 and 8.

P094: Choice of proper enrichment plays a crucial role in maintaining a healthier colony of small laboratory animals.

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Enriched environment in breeding and experiments on rodents is extensively accepted and practiced worldwide as a means of improving animal well-being. Animals can maintain natural behavior when they live in an enriched environment (Mellen and Shepherdson 1997, Loveridge 1998).However there is limited scientific information on choosing the proper enrichment and its effect on animals. The present study was done to evaluate the impact of different kinds of enrichments on reproductive performance and body weight gain of wistar rats. The animals were kept for breeding in cages without and with different types of enrichment items. The reproductive performance parameter like litter size, mortality, pups weaned, survival success, and fertility index were evaluated in different groups of animals. It was observed that the enrichments could be used without altering the reproductive performance and survival success of pups in wistar rats. The growth rate was monitored till 8 weeks of age after weaning. At 8 weeks of age the enriched group showed body weight gain which was significantly ($p < 0.05$) lower than the non-enriched group. The most important observation was that, the animals had positive discrimination for enrichments with large surface area while, there was negative discrimination for round and tubular shape enrichments before they reached adult age. It has been concluded therefore that, the right choice of enrichment may be important to enhance the animal welfare, reduce stress and provide an appropriate gadget for enhancing the physical activity in rodents.

P095: Antiucler potential of *Nelumbo nucifera* stalks

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Background: The contemporary treatments like proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer. However clinical evaluation of this treatment had shown incidence of relapses, side effects, and drug interactions on chronic dosage regimen. In traditional medicinal therapy Nelumbo nucifera gaertn is recomended for the treatment of various diseases such as malaria, haemorrhage anaemia, premature ejaculation. Moreover stalk part of *N. nucifera* has better compatibility with ulcer treatment. The current study was designed to evaluate the antiulcer potential of *N. nucifera* on rats.

Methods: The ulcerations were induced in wistar rats using Pyloric ligation and indomethacin model. The animals were treated orally with methanolic extract of *N. nucifera* and standard drug for 7 days. Parameters evaluated were ulcer index, total volume of gastric juice, free and total acidity of gastric secretion and sectioning of tissues.

Result: The treatment with the methanolic extract of *N. nucifera* significantly reduced the total volume of gastric juice. There was significant reduction in free and total acidity of gastric secretion and also had activity against gastric ulcers in rats. In addition, the histopathological studies showed control animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extract of *Nelumbo nucifera* there was significantly reduction in ulcer ($P < 0.0062$).

Conclusion: Methanolic extract of *Nelumbo nucifera* stalks at a dose of 100 mg/kg and 200 mg/kg p.o. had shown significant antiulcer activity. The isolation, characterization and preclinical evalaution will be further envisaged.

P096: Formulation and evaluation of primaquine-curcumine transdermal patches

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A transdermal patch is a medicated adhesive patch designed to deliver a specific dose of formulation through the skin into the blood stream. Primaquine, an antimalarial agent having short duration of action owing to its high first pass metabolism, it also leads to gastric irritations. Curcumine is an antiprotozoal

herbal drug which has poor gastro intestinal absorption and low oral bioavailability. The study was designed to formulate and evaluate primaquine-curcumine transdermal patches by molding method using various polymers such as polyvinyl pyrrolidone, polyethylene glycol, propylene glycol and dimethyl sulphoxide to produce smooth, flexible and transparent film and to increase the drug release. The combination of these two sparsely bioavailable drugs in a transdermal patch matrix may enhance the penetration, avoid first pass metabolism and improve the bioavailability. Eight formulations were prepared and were investigated for various evaluation parameters. The weight variation was 189.7 ± 3.8 to 212.9 ± 4.7 mg, thickness variation was 0.18 ± 0.1 to 0.22 ± 0.1mm. The formulation pf-2 (primaquine) gave 42.3±2.41% drug release; cf-4 (curcumine) gave 33.7±2.43% drug release where as pcf-6 (primaquine and curcumine) gave 70.2 ± 1.42% drug release. To conclude primaquine-curcumine patches enhanced bioavailability and increase in permeability was observed with the combination of two drugs were given in the same patch. The release rate of the drug through the patches was increased when the concentration of hydrophilic polymer (polyvinyl pyrrolidone) was increased.

P097: Comparison of novel lipid vesicles containing thiocolchicoside for transdermal delivery

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The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carriers i.e. liposomes, ethosomes, transfersomes, having thiocolchicoside. Drug exhibits higher water solubility and higher degradation in GI tract. It is a muscle relaxant drug with lesser transdermal permeation. Drug loaded liposomes and transfersomes were prepared by thin film hydration method by using phosphotidylcholin, surfactant and ethanol as solvent. Ethosome were prepared by ethonolic injection method. Vesicles were characterized for entrapment efficiency, vesicular size, zeta potential, invitro skin permeation studies. The ethosome formulation showed the significantly higher entrapment efficiency (23.16 ± 1%) with significantly small particle size (502 ± 5nm) then liposomes and transfersomes. FT–IR and DSC studies revealed no interaction between the drug and components. Zeta sizer revealed that the ethosomes are sufficiently stable formulation. The invitro skin permeation studies were performed on liposomes, ethosomes, transfersomes formulation, aqueous drug solution and marketed ZYFLEX® thiocolchicoside gel. The results of this study showed, ethosome formulation showed higher cumulative percentage of drug permeation (90 ± 5%) after 24 hours than the other formulations and aqueous drug solution. Ethanol may provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into the deeper layers of skin. Ethanol may enhance drug permeation from ethosomes as compared to liposome and transferosome. However, there was no significant improvement in drug permeation through ethosomes over marketed ZYFLEX®. Results suggest that ethosomes are efficient carrier for transdermal delivery of thiocolchicoside when compared to liposomes and transfersomes. Further in-vivo evaluations of the comparative formulations are envisaged.

P098: Demonstration of various cell types in bone by histological stains and counterstaining techniques.

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The histological evaluation of different cell types in the bone is very important to study effects of drugs in preclinical development. It is useful to develop animal models for diseases like arthritis, osteoporosis etc. and study their pharmacodynamic and toxic effects during risk assessment to humans. The evaluation of subtle histomorphological changes is only possible when tissues are subjected to special stains. In this study we have used different stains to demonstrate osteoblasts, osteoclasts, cartilage & bone formation. The study was carried out on different set of animals and the tibio-femoral joints were collected in 10% neutral buffered formalin. The tissues were decalcified, trimmed, processed, embedded in paraffin and sectioned at 4-5µ thickness. These were further subjected to Haematoxylin & Eosin (H & E), Alcian Blue Haematoxylin (ABH)/Orange G, Safranin O green & TRAP (Tartrate-Resistant Acid Phosphatase) staining respectively. It was found that ABH/orange G-eosin consistently stains cartilage blue, activated osteocytes as bright blue pericellular ring, mature bone orange, & immature bone as orange to red, osteoblasts looks reddish. This method is useful to evaluate fracture healing, osteoarthritis model and bone remodeling. TRAP stain is mostly specific for osteoclasts which appear bright red in color. Safranin O green is perfect to stain cartilage as orange to red which is used to demonstrate necrosis of cartilage.

In conclusion it could be stated that ABH/orange G-eosin & Safranin O green stains can be used for distinct differentiation of different cells of bone & can be advantageous over routine H & E stain.

P099: Formulation and characterization of curcumin loaded transdermal patch for wound healing potential

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Transdermal drug delivery system traditionally uses a patch containing drug substances pressed onto the skin, is non-invasive convenient and painless, and can avoid gastrointestinal toxicity and the hepatic first pass metabolism.

Curcumin enhances capacity of wound healing on the basis of anti-inflammatory property by inhibiting biosynthesis of eicosanoid. The aim of the study is to formulate a transdermal system to prolong the drug release time, reduce the frequency of administration and to improve patient compliance.

Three formulations were prepared by using different polymer ratios and combinations along with plasticizers and penetration enhancers. The patches were evaluated for various parameters like weight variation, variation in patch thickness moisture absorption and invivo wound healing study. The optimized formulation (CPF-1) showed highest percent moisture (4.13 ± 0.6 to 2.80 ± 0.3) absorption than other formulations. *In vivo* study was performed by taking three groups of animal (albino rats) with six rats in each group. Groups were assigned as control, standard and CPF-1formulation. The patches were topically applied on to the wounds once in a day till the wound was completely healed. The result showed wound healing repair is accelerated by applying CPF-1 formulation on the wound area. The enhanced capacity of wound healing with the curcumin could be explained on the basis of anti inflammatory effect of plant.

P100: Synthesis and biological evaluation of thiadiazole derivatives as anticonvulsants

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A series of new 2-(benzoylamino)-N-[5-(phenyl)-1,3,4-thiadiazole-2-yl] benzamide derivatives (PB-1 to PB-4) were synthesized and evaluated for anticonvulsant activity. The spectroscopic analysis was performed by using UV, IR and NMR data. After oral administration of derivatives to rats at dose of 30mg/kg were examined in the maximal electroshock seizure. The order of anticonvulsant activity of the synthesized compounds is PB-4>PB-3>PB-2>PB-1. Compound PB-4 showed maximum anticonvulsant activity due to 4-hydroxy at C-4 position of thiadiazole ring. Synthesized compound PB-4 and standard drug phenytoin showed 60 and 67.17% inhibition of convulsion respectively. Their pharmacophoric groups are similar to phenytoin and possibly the presence of the nitrogen containing heterocyclic ring carbonyl moiety adjacent to the ring nitrogen and electron donating groups are necessary for the anticonvulsant activity.

P101: Formulation and evaluation of orodispersible tablets of primaquine

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One of the aim of our present study is to formulate the fast dissolving tablet of primaquine to avoid GI disturbance, taste masking of drug, achieve quick onset of action & increase patient compliance. Primaquine is an antimalarial drug which is bitter in taste. In the present study taste masking of primaquine was done by entrapment of drug in β-cyclodextrine which forms drug complex. Primaquine orodispersible tablets (ODTs) were prepared by mixing dextrose, PVP K-30, sodium starch glycolate, cross povidone and then drug complex was added in this mixture followed by mannitol and magnesium stearate. A total no. of 6 batches (F1- F6) were prepared and the mixture blends of all the formulation were directly compressed. It can be concluded that the tablet containing cross povidone (F4, F5, F6) exhibit quick disintegration time as compared to tablet containing sodium starch glycolate. ODTs were evaluated for tablet properties like hardness, friability, wetting time, water absorption ratio, content uniformity and disintegration time. All the evaluated parameters are within the prescribed limits and satisfied the criteria of orodispersible tablet. From the result, concluded that tablets containing crospovidone (F4, F5, F6) exhibit quick disintegration time followed by tablets containing sodium starch glycolate. Among six batches, F6 is selected as optimized batch because of its lowest disintegration time and good drug release in comparison to formulation F4 & F5.

P102: Role of neurosteroids in the modulation of obsessive-compulsive disorder in rodents: novel therapeutic target

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Neurosteroids are reported to modulate GABAergic and glutamatergic pathways that influence serotonin and dopamine, neurotransmitters implicated in pathophysiology of obsessive–compulsive disorder (OCD). Fluoxetine used in OCD is reported to increase the levels of neurosteroids like allopregnanolone, whereas OCD patients exhibit higher plasma levels of dehydroepiandrosterone 3-sulphate (DHEAS), a neuroactive steroid having opposite effects to that of allopregnanolone. Hence, it was contemplated that neurosteroids may influence obsessive–compulsive behavior. To test this possibility we studied the influence of various neurosteroids on two behavioral models of OCD, namely marble-burying behavior

in mice and 8-OH-DPAT induced disruption of spontaneous alternation behavior (SAB) in rats. The results revealed that allopregnanolone (1 µg/mouse, i.c.v) and progesterone (20 mg/kg, s.c.) reduced the marble-burying behavior in mice, whereas dehydroisoandrosterone 3-sulphate (DHAS) (5 mg/kg, i.p.) exacerbated same. The effects of allopregnanolone were comparable to that of fluoxetine (10 mg/kg, i.p.). In view of the report that restraint stress increases the levels of allopregnanolone and isolation stress decreases the same, we studied the effect of these stressors on marble-burying behavior; wherein it was found to be less in restraint stress exposed mice, and higher in socially isolated mice. Restrain stress-induced attenuation of marble-burying behavior was blocked by finasteride, a neurosteroid biosynthesis blocker. In rat model of SAB disruption, acute and chronic treatment with allopregnanolone (1 µg/mouse, i.c.v.) reduced 8-OH-DPAT-induced persistent behavior, whereas treatment with DHAS (5 mg/kg, i.p.) had an opposite effect. In conclusion, the studies indicate that neurosteroids can modulate obsessive–compulsive behavior in a bidirectional manner, and could serve as an effective target in the management of OCD.

P103: Formulation and evaluation of fast disintegrating tablets of solid dispersion containing cefdinir

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The present study deals with the effect of different polymer ratio on drug release through solid dispersion (SD) containing cefdinir. It has low aqueous solubility and permeability. In the present study SD of cefdinir was prepared by taking PVP K – 30 as amorphous polymer. Solvent evaporation method was chosen as method of preparation where ethanol was the solvent. Formulations were evaluated for percent drug content and invitro dissolution study. As the drug: polymer ratio increases dissolution rate, increased significantly over pure drug.

In another study effect of incorporation of anionic surfactant into the SD was studied. Incorporation of SLS results in increase dissolution rate by an effective prevention of drug crystallization during dissolution. On the basis of various results formulation containing 1:5 ratio of drug to polymer along with 10 % w/w SLS was optimized. The dissolution rate of SD was found to be increased with incorporation of SLS over dissolution rate of SD without SLS.

Lastly the fast disintegrating tablets were prepared from the optimized SD. Tablets were prepared by direct compression method. Sodium starch glycolate and cross povidone were selected as super disintegrating agents. Tablets were evaluated for weight variation, hardness, friability, wetting time, water absorption ratio, content uniformity and disintegration. All parameters were within the prescribed limit. From the results, it was found that tablets containing cross povidone exhibit quick disintegration time as compared to sodium starch glycolate because of fast water uptake tendency of cross povidone.

P104: Evaluation of anti-anaemic potential of *Annova squamosa* (fruit pulp)

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Objective: Anemia being most rampant in females, it affects majority of pregnant and lactating mothers. Traditionally, medicinal herbs were used to alleviate the symptoms of anemia. The present study was designed to assess antianemic property of *Annova squamosa* (AS) using haloperidol and phenyl hydrazine model for induction of anemia in rats.

Method: Hemolytic anemia was experimentally induced in female Sprague-Dawley rats (100-110 gm) by administration of Haloperidol (0.2 mg/kg b.w.) & phenylhydrazine (single i.p. dose aq. Solution, 40 mg/kg) for 5 days in rats models. *Annova squamosa* methanolic extract was administered orally at various dose levels (250,500 mg/kg) along with the inducing agents for 5 days. The treatment was further continued for next 8 days. The blood samples were collected from retro-orbital on day 5 and 14. Hematological parameters viz. mean RBC count, Hb content, serum iron and protein were assessed.

Result: The treatment of methanolic extract of A.S at the doses of 250 mg/kg and 500 mg/kg significantly ($P < 0.05$) increase RBC count, haemoglobin count, serum iron and serum protein. The alleviation of hematological parameters could be due to the presence of iron in the extract which is estimated by spectrophotometric method.

Conclusion: The observed antianemic and erythropoeitic activity of *Annova squamosa* could be attributed to its observable iron content & reported antioxidant activity. Further characterization & evaluation of the active constituents is envisaged.

P105: Formulation and characterization of mucoadhesive microspheres of atorvastatin calcium

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Atorvastatin a competitive inhibitor of HMG–coA reductase, used in hypercholesterolemia. It has low systemic

availability attributed to poor aqueous solubility and short resident time in GI tract. Aim of formulating the mucoadhesive microspheres of atorvastatin calcium is to increase the gastric residence and increasing the bioavailability. Microspheres were prepared by using carbopol 934P and sodium alginate. Emulsification method was used by taking liquid paraffin as external phase. Seven formulations (A1-A7) were prepared by changing the proportions of both polymers.

Various formulations were evaluated for production yields, invitro mucoadhesion time, % drug entrapment and in–vitro drug release. Production yields of microspheres were found to be between 37±0.32 & 68.2±1.1%. Modified Invitro wash off test was used by taking egg shall membrane for determination of mucoadhesion time. Depending on the evaluation parameters formulation containing 1:1 ratio (A4) of both polymers was found to be best and well optimized. Invitro mucoadhesion time and percentage drug entrapment of optimized formulation was found to be 6±0.11hrs and 64.1±0.1% respectively. The invitro drug release of optimized formulation was best explained by korsemeyer peppas equation. The n = 0.673 which indicate non-fickian transport, the main mechanism for drug release from microspheres. Optimized formulation was also studied for average particle size, and it was found to be 3.82±0.7µm. Mucoadhesive microsphere of atorvastatin calcium was successfully developed. Further in-vivo evaluations of the comparative formulations are envisaged.

P106: Anti-ulcerative colitis activity of Pioglitazone in rats: Role of Nitric oxide

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The protective role of pioglitazone in ulcerative colitis is well establish. However the exact mechanism of this amelioratoryactivity is not very clear. The present study was designed to using nitric oxide synthase(NOS) inhibition investigate the role of nitric oxide (NO) in pioglitazone mediated anti-inflammatory activity in ulcerative colitis. Ulcerative colitis was induced by rectal administration of 2,4,6-tri-nitrobenzene sulphonic acid (TNBS 50 mg/rat). Rats were randomized in five groups: 1) normal control 2) TNBS control 3) Pioglitazone + TNBS treated 4) Pioglitazone + N-nitro-L-arginine methyl ester (L-NAME) + TNBS treated 5) L-NAME + TNBS treated rats. Pioglitazone (PIO, 25 mg/kg/day, p.o) treatment for 18 days reduced mucosal damage induced by TNBS which was reflected by improvement in body weight, morphological grades of colon, reduced myeloperoxidase activity as well as histopathological studies. The administration of L-NAME (40 mg/kg/day, p.o) for 18 days to these animals shown exaggerated mucosal damage as compared to pioglitazone + TNBS treated animals. The absence of beneficial effects of pioglitazone on ulcerative colitis due to L-NAME indicated the implication of nos inhibition in ulcerative colitis.Hence the present study suggests that L-NAME abolised protective effects of pioglitazone in ulcerative colitis by inhibiting nitric oxide production.

P107: Pyranone-thiazolidinedione hybrids for treatment of insulin resistance with hepatoprotective action

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Thiazolidinedione class of compounds do have the risk of serious hepatic adverse effects. We have designed series of compounds, which promote adipogenesis like thiazolidinediones and exhibit hepatoprotective activity. From a series of more than 30 compounds, we shall be presenting detailed studies on these compounds and its thiazolidinedione compounds showing insulin sensitizing and hepatoprotective activity. Some of these compounds showed increased adipogenesis and one compound showed comparable adipogenesis to rosiglitazone. These compounds also showed significantly increased glucose uptake in the fully differentiated adipocytes and also found capable of inhibiting hyperinsulinemia induced insulin resistance development. Histopathological and biochemical studies revealed that these compounds showed hepato-protective activity after acute hepatic injury in swiss mice.

P108: Venturing into novel chemical space based on molecular formula (MF): concept and potential applications

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Historically, structural/constitutional isomers could not gain much popularity as stereoisomers in drug discovery and development. The possible reasons could be distinct physical and chemical properties (e.g., allyl alcohol and acetone, MF: C_3H_6O), altered target binding (e.g., functional group isomers - cyclopropanol and propylene oxide, MF: C_3H_6O) and/or pharmacokinetic (PK) profiles. Recently, there has been much interest in oxetanes (Figure 1) as possible substitute for ketones.1 Surprisingly acetone and oxetane are functional group isomers (MF: C_3H_6O, Figure 1). Oxetanes are much superior to acetone (or ketone) substructure in terms of PK profile. Critical thinking on similar grounds by us led to further investigations

of 'Molecular Formula Search' as a novel way of introducing distinct structural elements into the hit/lead molecules during the early phases of drug discovery. It involves the replacement of a substructure in a lead molecule with another structurally diverse substructure with same molecular formula. Such a search allows exploration of novel chemical space which would not have been possible via conventional substructure and/or similarity searches. Dedicated involvement of an experienced medicinal chemist is required in this approach in selecting appropriate substructures having same MF as the query substructure.The present investigation discusses the concept and the potential applications with the help of suitable examples. An exhaustive comparison of the substructure and similarity search with MF search is also presented. In our opinion, this is the first conscious attempt to address the usefulness of the substructural constitutional isomers in lead discovery and development.

Acetone Oxetane

Figure 1. Functional group isomers: acetone and oxetane

Reference:

Wuitschik, G.; Carreira, E. M.; Wagner, B.; Fischer, H.; Parrila, I.; Schuler, F.; Rogers-Evans, M.; Müller, K.. Oxetanes in Drug Discovery: Structural and Synthetic Insights. J. Med. Chem. 2010, 53, 3227-3246.

P109: Capillary electrophoresis as novel technique for in vitro drug release testing

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The development of novel *In vitro* drug release testing methods is becoming highly important to the pharmaceutical industry. In the early development phase, the amounts of material available for release testing of the drug candidates are often lower and the conventional pharmacopeial methods may not be suitable. This necessitates the development of novel analytical strategies to meet the requirements during the early phase of drug development.

Capillary electrophoresis is presented as an alternative to the current drug release testing methodologies using cubosomes (aqueous dispersions of inverted type of bi-continuous cubic phases) as model drug carrier systems. CE is a separation method facilitating the separation of free and encapsulated drug using microlitre sample volumes while also offering opportunities for simultaneous characterization of the nanoparticulate systems.

Capillary electrophoresis in frontal analysis (CE-FA) mode was used to characterize the nanoparticulate systems (cubosomes). Brompheniramine maleate and chlorpromazine hydrochloride were the model drug substances incorporated in cubosomes with two different compositions 95 % w/w phosphate buffer pH 7.40, 3% w/w of monoolein and 2 % w/w of polymeric stabilizer and 90% w/w phosphate buffer pH 7.40, 6% w/w of monoolein and 4 % w/w of polymeric stabilizer F127 . All the experiments were performed at 25°C.

The interaction of the drug substances with the investigated drug carriers was demonstrated using CE-FA. Chlorpromazine was found to interact more strongly with cubosomes than brompheniramine. This method allowed calculation of encapsulation efficiencies for the drug substance in the cubosomes. Burst release of the drug substances from the cubosomes was confirmed by CE-FA.

CE requires minimum sample volumes, offers sampling and analysis in-line together with high degree of automation establishing its use in *In vitro* drug release testing.

P110: In-vivo screening of nateglinide loaded PLGA nanoparticles.

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The aim of the present study was to design a novel delivery system to maintain peak plasma levels of NTG for the long-term management of diabetes mellitus. Nateglinide (NTG) is an oral hypoglycemic agent with a short half life and lowers blood glucose level by stimulating insulin secretion from pancreas. Nine nanoparticle formulations were prepared by combining nateglinide with poly (lacti-co-glycolic) acid. All formulations were subjected to invitro and invivo characterization. Nanosuspensions of natelinide were evaluated for its improvement in therapeutic potential using streptozotocin (STZ)-induced diabetic model in rats.

Diabetic rats were administered with nanosuspensions for seven days. The blood glucose levels were evaluated in animals after seventh day of treatment for six hours. Whereas Higuchi Leeper model was used for in-vitro assessment of drug release. Glucose levels in conventional formulation were decreased for first one hour of administration and

then rebounded back at around third hour after administration. On the other hand, the decrease in glucose levels in nonosuspension treated animals was sustained for at least four to five hours after treatment. There was significant improvement in glucoregulation of the nonosuspension treated animals from first to seventh day of treatment.

The results indicate that nonosuspension of Nateglinide effectively enhanced the bioavailability by regulating the release of .Nateglinide. This approach of nanoparticles could improve compliance of patients and decreased dosing frequency.

P111: Investigations on *Curcuma amada* roxb. (rhizomes) for wound healing potential

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Background: Wound healing is characterized by intercalating degradation and re-assembly of connective tissue and epidermal layer. In traditional medicinal therapy *Curcuma amada*, is recommended for the treatment of various diseases such as chronic ulcers, cough, constipation, gout, anti-inflammatory and antipyretic. However *Curcuma amada* is reported for wound healing activity. Hence, the present ointment reported was designed.

Method: Wound was inflicted on Wistar rats by using excision wound model. The animals were treated topically with ointment of various extracts (5% and 10% petroleum ether, 5% and 10% ethanolic, 5% and 10% ethyl acetate, 5% and 10% aqueous extract ointmnent) of *Curcuma amada* for 15 days. The healing process was wound contraction, epithelization period and histopathological examinations.

Result: The treatment with the ointment extract of *Curcuma amada* significantly reduced the wound size. There was a significant reduction in epithelization period and also had activity against wound inflicted rats. In addition, the histopathological studies showed control animals had wounds whereas in animals topically applied with the various extracts of *Curcuma amada* ointment there was significant healing activity.

Conclusion: Animals treated with 10% ethanolic extract ointment showed better wound closure and epithelization period as compared to other treated groups in excision wound model. This wound activity of *Curcuma amada* could be attributed to the presence of Ocimene and Myrcene in ethanolic extract. The isolation, characterization and preclinical evaluation will be further envisaged.

P112: Crosslinked nanoparticles of cytarabine: Encapsulation, storage and in-vitro release

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The study demonstrated that nanoparticles of cytarabine can be prepared by modification of ionic cross linking method using sodium tripolyphosphate as cross linking agent. Chitosan molecular weight, polymeric composition and polymer/drug ratio in the nanoparticles did not influence the particle size characteristics.This work presents results of the preparation and characterization of nanoparticles for entrapping cytarabine, a chemotherapeutic agent. The particle size analysis indicated a uniform particle size. The study of the release of drug from nanoparticles exhibited a prolonged release profile as studied over a period of 16 hours. The drug release was constant from the 10th to the 16th hours, which showed that the formulation was successful for long-term treatment. The drug entrapment efficiency of the nanoparticles having the same ratio of polymer and drug was about 90.2%. The physical stability of the nanoparticles was good as studied over a period of 4 weeks. These results are promising for producing nanoparticles by entrapping cytarabine, which can be useful for cancer therapy.

P113: New method for quantitative and qualitative estimation of Amoxycillin Trihydrate

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A simple, fast, sensitive and inexpensive UV-Spectrophotometric method for quantitative and qualitative estimation of amoxicillin trihydrate in pharmaceutical preparations has been developed in HCl media pH 1.2. The amount of amoxicillin was then determined as a change in absorbance at 272 nm. In this work, the assay conditions were studied and optimized and the method was validated. The calibration curve presented an excellent linearity with r2 of 0.997 and regression equations Y = 0.004x + 0.002 (5–50 µg/ml Amoxicillin). The recovery study was carried out by standard addition method. The average percent recovery was found to be 108.83±1.72 for Amoxicillin trihydrate. The proposed methods have been successfully applied to the analysis of the bulk drug and its tablet dosage form. No interference from common excipients in the formulations or degradation products was observed. Finally, since all procedures were performed without the use of any organic solvents or hazardous chemicals which were detrimental to the environment and had a low consumption of reagents, this proposed assay was an ideal green analytical method suitable for the quality control of amoxicillin in pharmaceuticals.

P114: Formulation and evaluation of amoxycillin trihydrate: Three-layer guar gum matrix tablet

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The objective of the study is to design gastro retentive drug delivery systems for Amoxycillin trihydrate. It was prepared with the objective to obtain site-specific drug delivery for the stomach and to extend its duration of action. The sustained release of amoxicillin is desired because of its short biological half-life. The preparation was carried out by using guar gum as a carrier in the form of a three-layer matrix tablet. Amoxycillin trihydrate was chosen as a model drug because of its site-specificity. Matrix tablets containing either 30 % (M) of guar gum were prepared by wet granulation technique using starch paste as a binder. Three-layer matrix tablets of Amoxycillin trihydrate were prepared by compressing on both sides of guar gum matrix tablet granules of Amoxycillin trihydrate with either 50 (TLM) of guar gum granules as release retardant layers. Both the matrix and three-layer matrix tablets were evaluated for weight variation, hardness, friability, buoyancy and in-vitro dissolution studies. Optimized formulation of Amoxicillin trihydrate was found to have increased gastric residence prolonging the release of drug with 74% (matrix tablet) and 81% (three layer matrix tablet) of drug release in 8 hours by diffusion. The mechanism of drug release was found to be diffusion and followed combination of zero order and first order kinetics. Hence gastro retentive drug delivery system of Amoxycillin trihydrate is a promising approach as it can lead to decrease in the frequency of administration and ultimately lead to better patient compliance.

P115: Proniodsome based drug delivery system of Piroxicam

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Piroxicam is a widely used potent non-steroidal anti-inflammatory drug, with due potential for dermal delivery. Permeation of piroxicam from proniosome based reservoir type transdermal gel formulation across excised rat abdominal skin was investigated using Keshery Chein diffusion cell. There was considerable improvement in flux over the control gel formulation. The lipid vesicles were evaluated for entrapment efficiency and vesicle size of niosomes formed. It was observed that Span 60 based formulations produced vesicles of smallest size and higher entrapment efficiency while those of Span 80 produced vesicles of least entrapment efficiency. Incorporation of lecithin further enhanced entrapment efficiency. Proniosomes were prepared by conventional technique and employing maltodextrin and sorbitol as base. The morphology of the proniosomes was studied by scanning electron microscopy. Maximum flux achieved was 35.61 _g/cm2/h, an enhancement of 7.39 times was achieved for transdermal system based on proniosomal gel as compared to control gel. Antiinflammatory studies revealed that proniosome based transdermal drug delivery system of piroxicam were promising carriers for delivery of piroxicam. There was significant reduction in carrageenan induced rat paw inflammation compared to control.

P116: Screening of various antihyperlipidemic herbal drugs and glimepiride for their inhibitory activity on acylco enzymea: cholesterol acyltransferase.

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Elevated level of cholestereol esters, LDL, TG or VLDL levels are observed in hyperlipidemia which can be clinically controlled by reduced absorption of cholestereol from GIT decreased synthesis in liver or increased deposition in to adipose tissue.The cholesterol which is entering in to enterocyte is converted to cholestereryl ester with the help of Acyl-coenzyme A: cholesterol acyltransferase (ACAT) enzyme. The step is necessary for absorb cholesterol to reach lymphatic circulation as well as production of lipoprotein. Various herbal plants have been reported to possess antihyperlipidemic activity. However the mechanism of action of this herbal plant remains obscure. Also several novel sufonyl ureas have been reported to pocess ACAT inhibitory activity. However the ACAT activity to glimperide has not been reported.

Objective: The objective of present study was to screen out various antihyperliidemic herbal drugs and glimpiride for their inhibitory activity on Aceyl-coenzyme A, cholesterol acetyl transferase (ACAT) enzyme.

Method and Result: Effect of various anti hyper lipidemic herbal drug and glimpiride on acyl coenzyme A in liver microsome prepared from high diet rat by measuring cholesteryl ester as product using acyl co A as substrat, were studied to show berberine (IC_{50} < 1µg/ml), capsaicin (IC_{50} < 1µg/ml), citrus limonis (1.79µg/ml), citrus colosynthis, gilimperide, achyranthus aspera and methanolic sub fraction of acyranthus aspera inhibited the ACAT activity. Effet of azadirachta indica, momordica charantia, trigonella foenuma and tinospora cardifollia was much lesser than other studied drugs on ACAT enzyme.

Conclusion: From the present study, it can be concluded that Berberine, Capsaicin, Citrus limonis, Citrullus

colosynthis, Glimpepiride, Achyranthus aspera and Methanolic sub fraction of Achyranthus asprera possess ACAT inhibitory activity. Further study would be required to isolated the active constituents which are responsible for ACAT inhibitory activity.

P117: Evaluation of cell wall contents of probiotics in lipopolysaccharide induced inflammatory bowel disease in rats

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Introduction: Inflammatory Bowel Disease is considered to be component of immune system, mediated by toll-like receptors 4 (TLR4) by recognizing lipopolysaccharide (LPS). TLR 4 are typically expressed on intestinal epithelial cells and other cells of GI tract and expression of which has been shown to be up regulated in IBD. Probiotics can block inflammatory response mediated by LPS/TLR 4 interaction suggests that tested probiotics could provide beneficial effect in IBD. In our study, cell wall contents of various species of Lactobacillus were tested using LPS induced IBD in rats.

Materials & Methods: Colitis was induced by intrarectal administration of Lipopolysaccharide (350µg/rat) for 15 days in rats. Group I was served as normal control & Group II as Model control. After induction of colitis (on day 16), Group III, IV, V VI were treated with 5-ASA (100mg/kg, intrarectally), Cell wall contents of *L.casei* ($1*10^6$ CFU/animal, intrarectally), *L.acidophilus* ($5*10^6$ CFU/animal, intrarectally) and *L.rhamnosus* ($2*10^6$ CFU/animal, intrarectally) respectively for another 21 days. Various parameters like Body weight, Food & water intake, colonic mucosal damage index, disease activity index and anti-oxidant parameters were examined.

Result & Discussion: Post treatment with *L.casei, L.acidophilus & L.rhamnosus* showed significant increased level of Body weight, Food & water intake, Reduced Glutathione, Total protein & decreased level of Nitric oxide, Malondialdehyde, Myeloperoxidase, Colonic mucosal damage index and Disease activity index. Finally in the histopathology there was a protection against the inflammation produced with the administration of LPS.

Conclusion: Cell wall contents of Probiotics have protective effect in IBD.

P118: Role of α-glucosidase inhibitor in male fertility

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Objective: The present study was designed to investigate the role of α-glucosidase in male fertility using acarbose.

Materials and Methods: Rats were divided in four groups. First group served as a control group (saline for 52 days) and second, third and forth groups were treated with acarbose (50, 75 and 100 mg/kg, intravenously once in a day respectively, for 52 days). Food intake, water intake and body weight were measured on day 0 and then weekly. Rats were sacrificed on day 28 and 52 and parameter like α-glucosidase activity, sperm motility, sperm count, epididymal and testes weight were measured. Histopathological examination of testes and epididymis was performed.

Results: α-glucosidase inhibitor, acarbose (100 mg/kg, i.v.) showed significant inhibition of α-glucosidase activity as compared to control group. Sperm motility, sperm count, weight of both testes and epididymis were decreased significantly. Histopathological examination of testes showed significantly lesser density of primary, secondary spermatocytes and spermatozoa count in rats treated with 100 mg/kg acarbose. In epididymis the lining of columnar cell and basal cells were also found to be ruptured in same the sperm cells were found to be clump and disrupt.

Conclusion: The results clearly show that acarbose (100 mg/kg, i.v.) for 52 days produced significant effect on sperm maturation and motility. This activity further explored for male anti-fertility activity.

P119: Evaluation of effect of apocynin in HCl perfusion induced GERD in rats

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Objective: To study effect of apocynin in HCl perfusion induced GERD in rats

Materials and Methods: Female Albino Wistar rats were selected. Animals were treated with 0.5N HCl at the rate of 0.1 ml/5 min for 45 min for different durations to develop the model of GERD. The best suitable duration in which maximum reflux occurs was selected to evaluate the effect of apocynin in GERD. Weekly body weight, LES pressure, intragastric pressure, pH of gastric and esophageal lavage, ulcer index, hydrogen peroxide level in esophageal tissue homogenate were measured. Histopathology of esophagus was also carried out.

Results: Results revealed that the rats treated with 0.5 N HCl at the rate of 0.1ml / 5 min for 3 consecutive days once a day showed significant reduction in LES pressure on day 4 and maximum reduction on day 11 but was found to be increased on day 18. Ulcer index was also higher on day 11 and was found to be reduced on day 18. So treatment with 0.5N HCl at the rate of 0.1ml / 5 min once a day was selected for the main study. Drug treatment was given for 7 days and observations were made on day 11. Results of main study showed that rats treated with apocynin showed significantly higher LES pressure gradient against intragastric pressure, less reduction in body weight, decreased ulcer index, low hydrogen peroxide level in esophageal tissue homogenate. Histopathological studies revealed reduced basal cell hyperplasia and inflammation of esophagus as compared to disease control group. There were signs of esophageal regeneration. However, in rats treated with omeprazole, there was significantly higher LES pressure gradient against intragastric pressure but ulcers were not healed and mild inflammatory changes were seen. Hydrogen peroxide level in esophageal tissue homogenate was also higher as compared to that in normal control and disease control group. Epithelial regeneration was also absent.

Conclusion: From results, it can be concluded that apocynin may increase LES pressure by reducing hydrogen peroxide level in esophagus resulting from inhibition of NADPH oxidase and may give promising results in GERD.

P120: Evaluation of modulatory effect of fluoxetine on anti-inflammatory activity nicotine in experimental model of rheumatoid arthritis

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Nicotine cholinergic and serotonergic receptor subtypes have been characterized in B and T lymphocytes, macrophages, and dendritic cells. Nicotine and serotonin have been reported to regulate cytokine release. Nicotine mediated through α7 receptor and TNF-α inhibition in experimental model of rheumatoid arthritis.

Objective: To evaluate the modulatory effect of Fluoxetine on anti-inflammatory activity of α7 receptor activation by nicotine in experimental model of rheumatoid arthritis.

Materials and methods: In present study, effect of drugs was studied on Complete Fruend's Adjuvant (CFA) induced arthritis in Rats. The effects of Nicotine (400µg/kg, intraperitoneal), Fluoxetine (20mg/kg, intraperitoneal) and combination of Nicotine and Fluoxetine were evaluated for 21 days. The effect of drugs were evaluated by change in paw volume, body weight and arthritic index, change in erythrocyte sedimentation rate (ESR), detection of serum rheumatoid factor (RF), serum C- reactive protein (CRP), blood cell count (total WBC count, total RBC count and haemoglobin), brain 5-HT level and by histopathology of knee joint.

Results: Rats treated with CFA showed significant higher paw volume, ESR, RF, CRP and lower 5-HT level as compare to normal control. Treatment with Nicotine, Fluoxetine and combination of both drug decline serum biochemical parameters and increased 5-HT level. Combination of nicotine and fluoxetine (post) treated animals was significantly higher as compared to fluoxetine and combination of nicotine and fluoxetine (pre) treated animals. This effect exerts due to effect of fluoxetine as SSRI as well as synergic effect of fluoxetine on nicotine anti-inflammatory activity.

Conclusion: Nicotine has beneficial role in CFA treated animals due to its activity on nAchRsand can down regulate the inflammation by decreasing the release of cytokines including TNF-α by activated macrophages. Fluoxetine inhibits TNF-α and decrease pain by increasing 5-HT level in brain and inhibition of serotonin transporter expressed on macrophage. Treatment with combination of nicotine and fluoxetine was significantly more beneficial as compared to nicotine alone.

P121: Antidiabetic and antiobesity activity of embelin and pamoic acid in high fat diet (HFD) fed rats.

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Introduction: Type 2 diabetes mellitus and obesity are characterized by impaired insulin signaling with insulin and leptin resistance. PTP1B plays a critical role in development of insulin and leptin signaling through dephosphorylation of IR/IRS1 and JAK2 respectively.

Objective: To evaluate the anti-diabetic and anti-obesity activity of Embelin and Pamoic acid (PTP1B inhibitors) in high fat diet (HFD) fed rats.

Materials and methods: Animals were randomly divided into five groups based on oral glucose tolerance test (OGTT). Group II, III, IV and V were fed with high fat diet (HFD) for 84 days to induce Type 2 diabetes mellitus and obesity. Group II animals served as a model control, group III, IV and V were treated with pioglitazone (3mg/kg p.o.), embelin (50mg/kg) and pamoic acid (5mg/kg i.p.) respectively for 21 days (3 weeks). Group I animals were fed with normal pellet diet (NPD) and served as normal control. Various parameters such as oral glucose tolerance test

(OGTT), fasting plasma glucose (FPG), plasma insulin, plasma triglyceride(TG), plasma total cholesterol (TC), insulin tolerance test (ITT), body weight(BW), food intake, water intake, body mass index (BMI) and fat pad analysis were assessed at regular time interval.

Results and discussion: Treatment with embelin showed significant ($p<0.05$) decrease in FPG, fasting plasma insulin, TG, body weight, food intake, BMI and fat weights- epididymal fat, intrascapular fat, visceral fat. Similarly, significant ($p<0.05$) decrease in FPG, fasting plasma insulin, TG, body weight, food intake, BMI and fat weights- epididymal fat, intrascapular fat, visceral fat were observed with pamoic acid treatment.

Conclusion: Study results provide clear evidence of anti-diabetic and anti-obesity activity of embelin and pamoic acid in high fed diet rats.

P122: Lymphatic delivery & uptake: Importance of nano approach for preclinical screening

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Objective: 1.The aim was to explore preclinical plasma kinetic performance of developed nanocarriers (NC). 2. To explicate the absorption mechanism of NC through GIT by executing preclinical mesenteric lymph node bioavailability studies. 3. To study the influence of cellular features of cancerous cells on therapeutic efficacy and cellular uptake level of (NC) by *In vitro* anticancer activity, clonogenic assay, confocal and flow cytometric experiments.

Introduction: Breast cancer is one of the oldest afflictions and even today a massive world's population is at risk of this disease [1]. Underscoring factors like compromised efficacy due to unfavourable pharmacokinetics and adverse effect/toxicity calls for development of improvised nanocarrier system.

Experiments: Preclinical kinetic studies: A single dose oral pharmacokinetic studies were performed in female Wistar rats to evaluate the oral bioavailability of developed NC. Preclinical biodistribution in mesenteric lymph node: This preclinical study was performed to study the uptake pathway of NC in GIT. *In vitro* anticancer studies: MCF-7, MDA-MB-231 & ZR-75-1 cell lines were used for cytotoxicity and clonogenic assay.

Confocal & flow cytometer studies: *In vitro* cell uptake studies of NC were carried out in MCF-7 and ZR-75-1 cell line to elucidate the cellular uptake behavior.

Result & discussion: NC showed improved pharmacokinetic profile. Mesenteric lymph node biodistribution studies confirmed the uptake pathway of NC majorly occurs via lymphatic system. Different breast cancer cells responded differently for NC. Confocal studies and flow cytometric analysis experiment gave a better insight on cellular uptake behaviour of NC.

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Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index