

CHAPTER 33

MEDICAL SCIENCES BIOCHEMISTRY

Doctoral Theses

01. KARE (Pawan Kumar)
Assessment of Renoprotective effect of Angiotensin Converting Enzyme Inhibitor Therapy in Patients with Diabetic Nephropathy having different Receptor for Advanced Glycation end products (RAGE) Genotypes and Mechanistic Approach to Advanced Glycation end products-Induced Renal Cell Damage in Vitro.

Supervisors : Prof. A. K. Tripathi, Prof. B. D. Banerjee, Prof. O. P. Kalra and
Dr. V.K. Arora

Th 23674

Contents

1. Introduction 2. Aim and objectives 3. Study Design 4. Strengths and limitations of the study 5. Summary and conclusions. Bibliography, appendices and publications

02. MAHMOOD AHMAD KHAN
Assessment of Anti-Rheumatic Activity of Withania Somnifera Dunal (Ashwagandha) Root Extracts in Animal Model.

Supervisors : Prof. Rafat S.Ahmed and Prof. Vinod K. Arora

Th 23673

Contents

1. Introduction 2. Extraction / preparation and analysis of different extracts of withania somnifera root 3. Anti-rheumatic effect of different extracts of withania somnifera root 4. Effects of withania somnifera root extract on cartilage destruction 5. Anti-inflammatory activity of withania somnifera root 6. Anti-oxidant activity of withania somnifera root. Summary and conclusion, references and list of publications

03. MOOL KUMAR
Biochemical Studies on Antidiabetic Effect of Leaves of Ficus Infectoria.

Supervisor : Dr. Rimi Shukla, Dr. (Mrs) J.K. Gambhir and Dr. Sonal Sharma

Th 23669

Contents

1. Introduction and review of literature 2. Aims and objectives 3. Materials and methods 4. Preliminary studies with aqueous extract of leaves of ficus infectoria 5. Isolation and characterization of antidiabetic compound 6. Studies on mechanism of action of 'FI-PC' 7. Toxicity assessment and safety profile of 'FI-PC' Summary and conclusion, references.

- 04 ROHILLA (Akshay)
Identification and Evaluation of Inhibitors against important Drug targets of Mycobacterium Tuberculosis.
 Supervisors : Dr. Garima Khare and Prof. Anil K. Tyagi
Th 23671

*Abstract
 (Verified)*

Tuberculosis remains one of the deadliest diseases in the world killing around 10.4 million people in 2015 worldwide. Long duration of the treatment by the frontline drugs often leads to non-compliance ultimately leading to the generation of drug resistant *Mycobacterium tuberculosis* (*M. tb*) strains. Thus, there is an urgent need to identify new inhibitory molecules to fill the pipeline. We targeted essential proteins involved in iron homeostasis inside *M. tb*. Virtual Screening, pharmacophore development and structure based similarity search lead to the identification of various inhibitory scaffolds against IdeR, an essential transcriptional regulator of *M. tb*. Compound I-108 exhibited IC of 24 µg/ml in EMSA, MIC of 17.5 µg/ml against the growth of *M. tb* *in vitro*, negligible cytotoxicity to various mammalian cell lines and intraphagosomal inhibition of *M. tb* at 125 µg/ml. We also targeted PptT (4'Phosphopantetheinyl transferase) of *M. tb*. PptT carries out phosphopantetheinylation type post translational modification of various proteins in *M. tb*. Virtual screening and structure based similarity search led to the identification of two inhibitory scaffolds against PptT. N, N-diethyl-N'-(2- methylquinolin-8-yl) propane-1, 3 di amine emerged as one of the potent inhibitory scaffolds with 5 analogues exhibiting IC < 1 µg/ml. PS40 emerged as one of the most potent inhibitory molecule with IC of 0.25 µg/ml, MIC of 10 µg/ml, negligible cytotoxicity to various mammalian cell lines and intraphagosomal inhibition of *M. tb* at 40 µg/ml. We carried out homology based 3-Dimensional structure determination of MbtA, MbtB and MbtE (Mycobactin synthases) followed by docking studies. MIC studies led to the identification of few inhibitory molecules with negligible cytotoxicity against mammalian cell lines tested. Thus, *in silico*, *in vitro* and *ex vivo* studies were carried out against IdeR, PptT, MbtA, MbtB and MbtE to identify various inhibitory molecules against *Mycobacterium tuberculosis*.

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1. Introduction 2. Review of literature 3. Aims and objectives. Summary and conclusions, appendix and publications

- 05 SHARMA (Karuna)
Evaluation of Genetic Polymorphism (SNP-1562 C/T) of Matrix Metalloproteinases -9 (MMP-9) and First Trimester Maternal Serum Level of Biomarkers (PAPP-A, free β-hCG, and MMP-9) and Proinflammatory Cytokines (TNF-α and IFN-γ) for the Prediction of Preeclampsia.
 Supervisors : Dr. Ritu Singh and Dr. Jayashree Bhattacharjee,
 Dr. Usha Gupta, Dr. Manish Kumar,
 Dr. Vishwajeet Rohil

Th 23672

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1. Introduction 2. Review of literature 3. Aims and objectives 4. Materials and methods 5. Results 6. Discussion 7 Conclusion 8. Reference 9. Appendices

06. TYAGI (Vipin)
Gene-Environment Interaction in the Etiology of Preterm Birth.
 Supervisors: Prof. B.D. Banerjee, Prof. Kiran Guleria and Pro. Debabrata Ghosh
Th 23782

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1. Introduction 2. Aim and objectives 3. Review of literature 4. Materials and methods 5. Results 6. Discussion 7. Conclusion 8. Strengths, limitations and future aspects. Bibliography and appendices

07. VERMA (Vaishali)
Strategies for Selection of Cognate Antigen- Antibody Pairs for M. Tuberculosis Proteome.
 Supervisor : Prof. Vijay K. Chaudhary
Th 23670

*Abstract
(Verified)*

Due to their exquisite specificity towards the target, antibodies find tremendous utility in basic research, diagnostics, and therapeutics. It was conceived that the availability of a comprehensive collection of monoclonal antibodies directed against the whole proteome of any organism can serve as an extremely valuable resource for studying localization, function, and quantification of the expression levels of different proteins and will facilitate numerous applications pertaining to both infectious and non-infectious diseases. The conventional approaches for antibody production allow selection of antibodies against one target at a given time. In addition, these approaches require purified preparation of the target protein, which may not be available for the whole proteome. In essence, to obtain specific monoclonal antibodies at genome-scale, an efficient strategy is required that circumvents the need for purification of every protein individually, and allows the selection of antibody library against antigen library, followed by identification of cognate antigen-antibody pairs in high-throughput format with minimal background. The work embodied in this thesis describes a streamlined and robust strategy to produce protein targets at genome-scale, selection of recombinant antibodies from a naïve human antibody library, and finally to decipher the target for each selected recombinant antibody in a high-throughput manner. The first part of the strategy involves the selection of specific binders from a naïve human phage-displayed antibody library available in the laboratory against a mixture of mono-biotinylated protein fragments obtained from an ORF-selected DNA fragment library. The final part of the strategy employs a combination of phage display and NGS technology for the determination of the specificity of individual antibodies selected against a mixture of proteins in a high-throughput format. The strategy has been successfully optimized using *M. tuberculosis* as the model, and is expected to enable rapid selection of specific monoclonal antibodies against a large number of targets in parallel.

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1. Introduction and review of literature objective and scope 2. Construction of an ORF-selected M. tuberculosis 30 gene fragment library using split Beta-lactamase complementation system 3. Characterization of a phage displayed naïve human antibody library 4. Development of vectors soluble expression of protein and antibody 5. Development of system for high-throughput selection of human antibody fragments against M. tuberculosis protein fragments and identification of cognate antigen-antibody pairs 6. Summary and conclusions. Appendices