# वार्षिक प्रतिवेदन

# ANNUAL REPORT

## 2008-09

# वार्षिक प्रतिवेदन

# Annual Report

## 2008-2009


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CENTRAL DRUG RESEARCH INSTITUTE

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Published by Dr. A.K. Goel, Deputy Director, Technical Information, Industrial Liaison & Planning Division on behalf of Director, Central Drug Research Institute, Lucknow

## THE CHARTER

Development of new drugs and diagnostics

Cellular and molecular studies to understand disease processes and reproductive physiology

Development of contraceptive agents and devices

Systematic evaluation of medicinal properties of natural products

Development of technology for drugs, intermediates and biologicals

Dissemination of information in the field of drug research, development and production

Consultancy and development of technical manpower

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# From the Director's Desk

Dr. Tushar K. Chakraborty, Director, CDRI

Since I joined this institute about two months back on 19th December, 2008, writing the preface for the Annual Report for the year 2008-09 is a rare opportunity and indeed a great privilege.

Following a decade of change and modernization under Director, Dr. C.M. Gupta, and a spell under the Director, Dr. Rakesh Tuli there are onerous tasks left by my immediate predecessors to complete. However, during his short stay of one and a half year, Dr Rakesh Tuli not only ably handled the affairs of the institute and spent maximum possible time on institute's research programs with matching zeal of his predecessor, Dr. C.M. Gupta, but also left a remarkable effect on the institute staff. I have noticed in the institute's ambience, freshness and tranquility of nothing short of high academic excellence and committed enthusiasm of scientists, technicians, administration, and everyone else. I express my profound thanks to Dr Tuli for his service to this institute.

The institute mandate covers the full 'drug discovery to development' domain encompassing single molecules, plant products, diagnostics, vaccines, process technologies, human resource development and S&T services. I am truly awed by the institute's diversity reflected through its programs and their objectives, the quality and visible depth of science and overall achievements. This scenario creates a model which is not replicated anywhere else in the world: a full drug R&D setup under one roof! Indeed this model has been a challenge to maintain, and shall be a greater challenge to sustain in the future.

Currently the institute is in a critical phase of development requiring completion of new campus and making it fully functional. In the past the institute has fulfilled its mandate to a great extent but now it requires a great deal of work to be carried out to make it worthy of expectations of a truly world class institution. Global drug R&D today is not the same as it was two decades earlier when the institute's current set up probably came into existence. Today, drug R&D is one of the most challenging endeavors primarily because drugs are for

consumption in humans afflicted by disease. Saving life of a sick man being top priority, all issues of quality of drugs, their efficacy and safety are foremost in drug R&D. Therefore, issues of cost-effective process technologies (green technologies!), chiral purity of molecules, regulatory compliances (GLP, GMP, GCP, etc) are of foremost importance.

The existing institute being housed in a palace, the building had limitations to establish full laboratory systems, but as the institute shifts to new campus and pending issues are resolved the demands of world class drug research institute shall be a major priority. On this account I shall be open to the comments and advice from our stakeholders to make new CDRI achieve its ultimate aims. Therefore, to enable new campus fully compliant we have to ready ourselves with best quality systems and management practices now.

Beginning the Xth Five Year Plan (1997-2002), the CSIR had adopted a system of network projects in order to utilize collective strengths in infrastructure and capability within and outside CSIR system to handle multidisciplinary problem areas. In addition, NMITLI projects have been undertaken to push up country's technological leadership in select areas. These have obviously broadened the horizon of research capability within the system to collectively tackle problems in drug research. Drug research starts with the search of a novel molecule that acts on a known or new (novel) target and ends with completion of multicentric clinical efficacy trials (phase III) involving high stakes in terms of cost and time. Therefore, linkages with national/international agencies/institutions and industry can possibly offset the need for technology input in every possible area and reduce cost and time of drug development. The institute shall invite greater collaboration with sister laboratories like IICT, NCL, IMT, IGIB, IICB, etc.

We shall shortly carry out in-depth SWOT analysis to find out our relative strengths and weaknesses, and develop suitable short term and long-term strategies to overcome weaknesses and build upon strengths. The institute today has a flagship program on parasitic diseases which I believe is the reflection of the institute's traditional strength in tropical infections. Already the institute has developed two antimalarial drugs (Arteether and Aablaquin) and process technology for Artemether (antimalarial) and some novel antimalarial molecules are in pipeline. But out of the large portfolio of parasitic infections the institute needs to develop some commercial products for leishmania, filariasis, tuberculosis, etc. Undoubtedly we have to work harder in these areas.

Progress has been made on the front of most projects. The products in clinical trials have moved forward. Dossier on Arteether (blood schizontocidal antimalarial) for its use in children was submitted to DCGI, Compound 80/574 (hypolipidaemic) data compilation for phase-III clinical trials has been completed, CDR-134D123 (antihyperglycemic) phase-I trial has concluded, CDR-134F194 (antihyperglycemic) has completed regulatory pharmacology and toxicity in monkeys and phase-I initiation is on anvil, and compound 97/78 (antimalarial) phase I trial progressed well. Several products were studied for pharmacokinetic, pharmacodynemic and toxicity studies.

Biological screening is one of the major strengths of the institute in terms of test systems and animal models, including macrophage mouse model for TB. Under CVS-CNS, two potential antithrombotic molecules (S-002-333 and S-000-20) and one anti-hyperglycemic (S-007-1261) are currently under evaluation. Combination therapy of institute's new antimalarials

(compounds 97/78 & 99/411) in combination with piperaquine and lumefantrine in rodents and simian models are in progress. In reproductive health area four compounds have been found to promote peak bone mass (PBM) and a plant has yielded two novel compounds (K058 and K100) out of which K058 holds therapeutic potential as an anti-osteoporotic agent.

Basic research is the principal driver of innovation, and innovation comes from out of box thinking. Thinking beyond set boundaries is the foreground which unleashes productive crops of innovative technologies. Hence we ought to give highest preference to out of box thinking in any sector of innovation chain, be it a molecule, a formulation, a process know-how, a delivery system or a novel mechanism of action of a candidate molecule. Application of new approaches in drug design and discovery is an important activity at this institute, including search for novel targets and their possible use. Results from virtual screening of target specific anti-tubercular compounds using ligand (against enzymes TMPKmt and dihydrofolate reductase of *M. tuberculosis*), followed by structure interaction fingerprints, has allowed prioritization of virtual hits.

We shall need to think off-board to launch new initiatives on all fronts, research, management, even business development. To achieve on this front all concerned laboratories need to be well organized with well set goals and objectives. A holistic plan for overall business development through all forms of activities, covering grant-in-aid projects, sponsored projects from industry, collaborative projects with national/international agencies, contractual research, S&T services (including HRD) etc have to be given a serious thought and handled through a single window for proper monitoring. Every scientist need to be accountable for business generation in the area of his activity by working on projects or providing S&T services. Implementation of a Business Plan shall demand organizational preparedness to meet concerns for quality and time lines.

I find the institute has unique distinction in terms of its overall function, activities and achievements. Likewise the previous years, the institute honored its scientists for academic achievements for filing patents abroad and for publications in high impact factor journals. Several institute scientists received honors and awards from prestigious academic bodies and organizations. To recognize contributions of Indian researchers in drug research the institute also honored scientists from other institutions, viz., Dr. Souvik Maiti (IGIB), Dr. Brijesh Kumar Srivastava (Zydus Research Centre), Prof. Chinmay Sarkar Dey (NIPER) with the '*CDRI Award-2008*'. It is no less surprising the institute in true strength of its innovative strength received the '*CSIR Technology Award-2008*' for 'Innovation and discovery of guggulsterones and development of analogs with novel mechanism of action'.

I thank all the institute staff for their valuable contributions and hope they shall continue to work even harder during the years ahead to build a new CDRI.

(Tushar K. Chakraborty)

# Significant Achievements

# Significant Achievements

The Annual Report of the year 2008-09 highlights the salient features of the contributions made by CDRI in all the areas of research vis-à-vis its mandate. During the period of review, CDRI continued to make strides in scientific achievements as well as managerial reforms. Dr. Rakesh Tuli, having additional charge as the Director, laid down the office and Dr. Tushar Kanti Chakraborty, an eminent and world renowned scientist from IICT, Hyderabad took over as Director on 19th December 2008. Dr. Chakraborty stressed upon the need for new modes of operation in consonance with new demands of government vis-à-vis dynamic changes taking place world over in the field of drug research. The Institute continued in exploring and promoting the available leads on candidate drug molecules, standardized fractions, development of new screens and liaison with new industrial partners. A brief summary of the notable features of the present Annual Report are presented below:

## 1. Business Development and Contract Research

The Institute signed a collaborative cum licensing agreement with TVC Sky Shop Ltd., Mumbai in respect of CDR-134D123 and CDR-134F194 for the management of diabetes and antidyslypedimia. Centchroman, as a female non-steroidal contraceptive and *Bacopa monniera* for the management of Age Associated Memory Impairment in elderly subjects and Attention Deficit Hyperactivity Disorder in children, were also licensed to them.

28 Days toxicity studies of Herbal Medicament in Rhesus Monkey were completed in collaboration with Themis Medicare Ltd., Mumbai and was found to be safe.

The material transfer agreement for validation of RKT-1 variants for examining HIV-1 infected cells for disruption of NEF PACS-1 interactions was signed with Oregon Health & Science University, Portland and another material transfer agreement for procuring a plasmid DNA for vitamin D receptor (CMX VP16-VDR) for mammalian two hybrid based screenings was executed with Nihon University School of Medicine, Japan.

A MOU has been executed with Institute of Molecular Medicine, New Delhi for developing animal models by using drugs that modulate NMDA receptor to induce Schizophrenia like symptoms in mice which are close to human symptoms.

A MOU has been signed between CDRI and Uttar Pradesh Dental College and Research Centre, Lucknow for antibacterial testing under study entitled "Antimicrobial effectiveness of three root canal irritants against *Enterrococcous faecalis,* as *in vitro* study".

M/s Natural Remedies Private Ltd., Bengalooru is evaluating the data on Osteojuvenate for management of bone disorders in order to collaborate with CDRI under confidential agreement.

## 2. Progress in R&D Activities

During the year 2008, a total of 1659 new were synthesized chemical entitles. Botany Division surveyed 10 areas and 29 field stations and collected 22 new terrestrial plants, 14 repeat samples and 18 voucher specimens. Bulk collection of a marine sample Xylocarpus sp. was made for technology transfer studies. An on line synthetic compounds code entry software was developed by the IT Unit. This software provides individual password protected 24 hour access to the chemical database, upload of structure and spectral data. A new database of Mycobacterial transcriptional regulators was developed during the reporting period. This database is an integrated systems biology platform that gives an insight to the transcription regulators of four pathogenic Mycobacterium species.

## 2.1 Clinical Trials & Pharmacokinetic Studies

Clinical trials continued on candidate drug products. CONSAP (contraceptive cream) has been licensed to Hindustan Latex Limited. Dossier on Arteether (blood schizonticidal) has been submitted to DCGI for approval to use it in children suffering from *P. falciparum* malaria and marketing permission is awaited from DCGI. During the year, clinical trials on Picroliv (hepatoprotective) continued at KGMU, Lucknow. Results of interim analysis indicate faster clinical recovery of tuberculosis in patients treated with picroliv in comparison to patients receiving placebo. Adverse events were also monitored during the trials and no major side effects were reported with picroliv therapy when compared to the placebo group. With regard to 80-574 (hypolipidemic), 175 cases completed the phase III multi-centric clinical trials and data compilation has been concluded. Future course of action depends on inputs from Cadila Pharmaceuticals Ltd. Phase-I clinical trials on 97-78 (antimalarial) are in progress and so far 13 healthy male volunteers have completed the study. A new set-up for phase I clinical trials with 4 beds and ICU facility are operational. Phase-I clinical studies in 32 cases on the marine product CDR-134D123 (antihyperglycaemic) have concluded. M/s TVC Skyshop Ltd. has been licensed to market this product and future strategy is being worked out.

Pharmacokinetic and metabolic studies were also undertaken on candidate substances. Gonadal uptake and tissue distribution, plasmaRBC partitioning, serum stability and toxicokinetics data on 99-373 (anti-resorptive) has been generated to further strengthen the pharmacokinetic data on this molecule. Its absorption, distribution, metabolism and excretion data has been submitted for inclusion in the dossier for Phase-I trials. Multiple dose pharmacokinetic and toxicokinetic studies on 99-411 (antimalarial) were completed. Complete regulatory pharmacokinetic data on this molecule was submitted for inclusion in the dossier for phase-I trials.

Plasma pharmacokinetic data on biologically active osteogenic marker component K095 from NP-1 (Plant 1020) and its synthetic analog S-006-1709 were generated in female SD rats for establishing the pharmacodynamic correlation. Principal Component Analysis (PCA) and absolute quantification of biologically active marker component K058 from CDRI plant 914 has been accomplished by LC-MS/MS. Further bio-analytical assay method is being developed to undertake pharmacokinetic evaluations. Comparative preliminary pharmacokinetic data on anti-thrombotic lead molecules S-000-20 (racemate), S-007-867 and S-007-1175 (isomers of S-000-20) and S-002-333 (racemate) was generated for lead selection.

## 2.2 Pre-clinical Safety Evaluation and Regulatory Toxicology

Regulatory toxicity studies were carried out on in-house and outside products. Amongst candidate drugs of CDRI, product CDR-134F194, 99-373, Herbal Medicament, NP1 and 99-411 were screened for their preclinical safety profiles. In addition to it, two projects (viz. NWP0037 and GTP) were also carried out in Toxicology Division. Under project NWP0037, compounds AP20am15 and AP20am16 were screened and under GTP 2$^{nd}$ Batch of Kajjali Yoga, Ras Sindoor and Vasant Kusumakar were studied for their toxicity profiles.

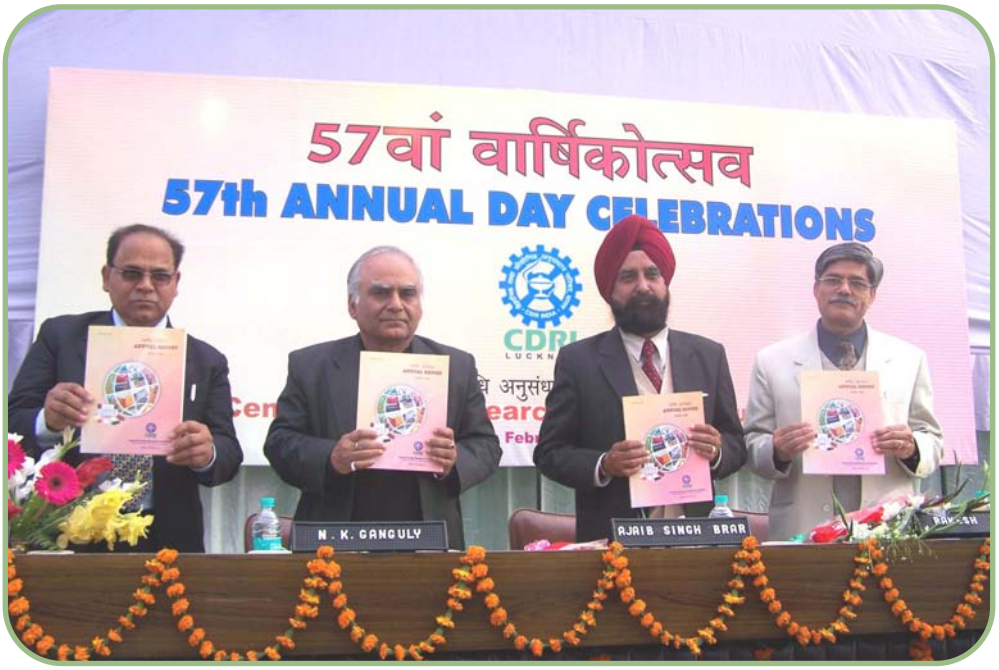
## 2.3 Biological Screening

Over 2500 samples were screened for antitubercular activity *in vitro* and 18 new synthetic molecules showed a minimum inhibitory concentration (MIC) of = 3.12 µg/ml with no *in vitro* toxicity towards vero cells or mouse macrophages. Their evaluation using mouse macrophage model of TB indicated that most molecules were able to kill the bacilli residing within the phagocytic vacuoles. In the *in vivo* screening (using mouse model of TB), 2 molecules have shown significant enhancement in the mean survival time (MST) as well as >10 fold reduction in viable bacilli within the lungs of infected animals. In anticancer screening, 3 synthetic molecules showed an *in vitro* activity against cervical and breast cancer cell lines. CDRI molecule K095 is a potential drug for osteoporosis. It was found to be a specific agonist for estrogen receptor (ER) alpha and beta and activates these receptors at concentrations comparable with Estradiol. However, this compound does not show any estrogenic/anti-estrogenic action in uterus indicating an alternative mechanism. CDRI

Dr. T.K. Chakraborty taking over charge as Director, CDRI on 19th December, 2008. Also seen in the picture (R-L): Dr. Rakesh Tuli, Outgoing Director, CDRI, Dr. A.K. Saxena, Senior Deputy Director, CDRI and Mr. B.D. Vashisth, COA, CDRI.

Dr. T.K. Chakraborty , Director, CDRI felicitating Dr. Rakesh Tuli, Outgoing Director, CDRI during farewell to the latter. Seen on the dais Dr. A.K. Saxena, Senior Deputy Director, CDRI.

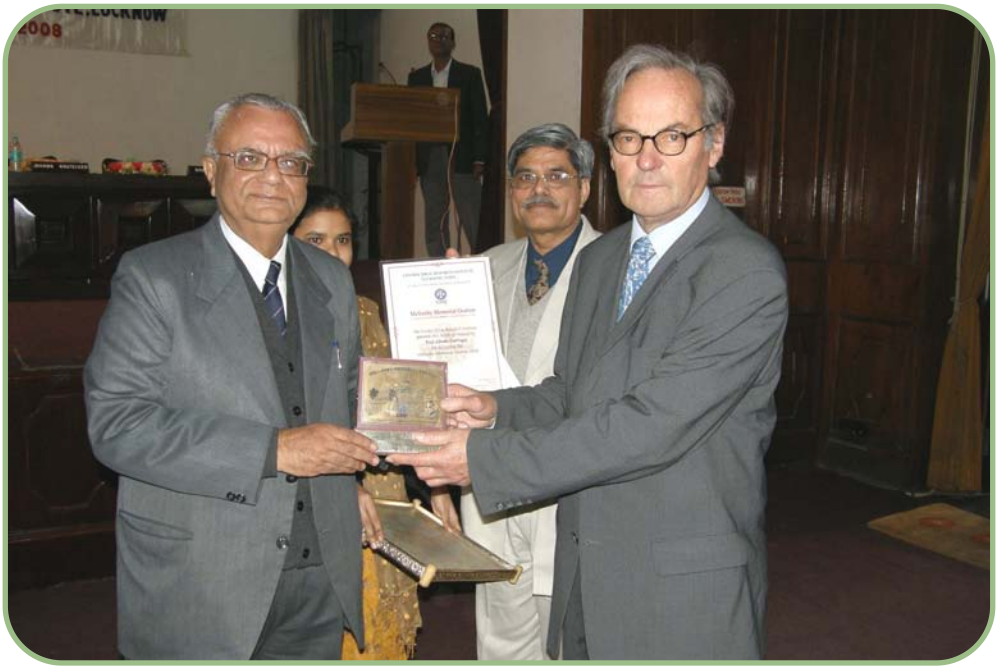

Prof. N.K. Ganguly, Former DG, ICMR addressing the audience during 57th Annual Day Celebrations.




Release of Annual Report 2007-08. Dignitaries on dais (R-L): Dr. Rakesh Tuli, Director, CDRI, Prof. A.S. Brar, Vice Chancellor, Lucknow Univeristy, Prof. N.K. Ganguly, Former DG, ICMR and Dr. Zaka Imam, Senior Deputy Director, CDRI.

Launching of 'Mycoview' PCR based tuberculosis diagnostic kit during the Annual Day

Exchange of Collaborative-cum-Licensing Agreements between Director, CDRI and Mr. Vinod Agarwal, CMD, TVC Skyshop Ltd., Mumbai in respect of Antidiabetic agents and Memory enhancer.



Prof. C.L. Khetrapal, Director, CGMR, SGPGI, Lucknow presenting a scroll of honour and memento to Prof. Johann Gasteiger, University of Erlangen, Nurnberg, Germany for delivering 33rd Mellanby Memorial Oration 2008.

Dr. Rakesh Tuli, Director, CDRI felicitating H.E. David M. Malone, High Commissioner, Canada during his vist to CDRI

molecule K1485 induces apoptosis in ER-positive as well as ER-negative breast cancer cells. This compound was found to be a specific agonist for the orphan nuclear receptors ERR alpha, beta and gamma. It has also shown a better anticancer activity than the published ERR agonist DY-131.

Tamoxifen is widely used as endocrine therapy, though the mechanism of its action is not clearly known. ER alpha positive breast cancer cell line MCF7 cells were induced with Tamoxifen and cell lysates were subjected to identification of differentially appearing proteins. The initial proteomic screening has suggested that Tamoxifen downregulates Ubiquitin Ligase E3A isoform (UBE3A) at mRNA as well as protein levels.

### 2.4 Cardiovascular, Central Nervous System & other Disorders

Two potential anti-thrombotic molecules S-002-333 and S-000-20 and their d and l-enantiomers are currently being evaluated in athero-thrombosis models. A total of 646 test samples were investigated for anti-thrombotic, anti-ischemic, antihypertensive, appetite suppressant, anti-dementia, anti-depressant, anti-stress, anti-anxiety, CNS, anti-hyperglycemic, anti-dyslipidemic, anti-inflammatory, anti-ulcer and anti-histaminic activities and those found active are being perused further. Further, S-007-1261 showed promising anti-hyperglycemic activity in STZ diabetic rat model. A model of macrophase foam cell formation in atherosclerosis has been established for studying atherothrombotic mechanism.

Molecular and functional characteristic of NOS isoforms was studied in human blood cells. NOS were found localized in cytoplasm, granule and open canalicular systems in the enucleated platelets. Studies have been performed to understand regulation of neutrophil maturation and free radical generation.

Focal cerebral ischemia model was used to analyse the role of certain biomolecules like PARP, neurotrophins, calcineurin NMDA, glutamate uptake inhibitors and endoplasmic reticulum to identify targets for analyzing the mechanism of antistroke drugs and for developing target based drug candidates for cerebral stroke.

Melatonin significantly improved memory and increased insulin receptor (IR) expression in hippocampus of STZ (icv) induced memory impaired rats. The IR gene expression was also studied in different brain areas CA1, DG and CA3 of hippocampus in rat by RT-PCR. Gene expression level was found higher in CA1 and CA3 regions in trained rats as compared to control. The results indicate the involvement of brain insulin receptors (IR) in memory functions. Studies suggest that a7-nicotinic receptor is inhibitory to LPS induced neuro-inflammation in rat. Meltonin showed protective effect against reflex esophagitis due to down regulation of pro-inflammatory cytokines TNF-a, IL-1í and IL-6 in rat.

### 2.5 Filariasis

320 Marine samples were tested *in vitro* against *Brugia malayi* and 32 were active and were short listed for follow-up. One hundred and two compounds received under CDRI-TDR collaborative project were tested *in vitro* and the active ones are being followed up. The protein profile of Wolbachia depleted worms was compared with that of untreated parasites harbouring Wolbachia in 2D-gel. About 100 protein spots could be visualized in Coomassie gel, of which 56 showed differential expression thus analyzed by MALDI-TOF and interesting findings have been reported.

Immunization with recombinant *B. malayi* myosin (BmAF-Myo) resulted into a significant reduction in mf density, worm establishment with embryostatic effect in both mastomys and jird. Major cross-reactive molecules of *B. malayi* and *L. donovani* have been identified and being characterized. In the studies related to biochemical and molecular biology, *Brugia malayi* hexokinase, Acetylcholine esterase, DEAD box RNA Helicase, Phosphoglycerate mutase and Trehalose 6 phosphate synthase were picked up as antifilarial drug/protein targets and therefore cloned and characterized. *S. cervi* antigen(s) equivalent to circulating filarial antigen was purified by the monoclonal antibody developed against filarial circulating antigen.

## 2.6 Leishmaniasis

Eight marine extracts have shown significant activity in vitro against intracellular amastigotes and on the basis of high SI selected for *in vivo* trial in hamster model. One pure compound of plant 4555 K009 exhibited >90% antileishmanial efficacy. The putative pathway responsible for the death of *L. donovani* on treatment with this compound has been observed to be mediated by apoptosis-like cell death in Leishmania parasite.

Picroliv *per se* showed no antileishmanial efficacy. However, when given with suboptimal doses of miltefosine and paromomycin, it enhanced their efficacies which can be correlated with remarkable production of toxic oxygen metabolites and increased CMI responses.

Of the 18 soluble *L. donovani* proteins that have been identified as major immunostimulatory proteins through proteomics seven viz. enolase, calreticulin, p45, protein disulphide isomerase, triose phosphate isomerase, nucleoside diphosphate kinase, Elongation Factor-2 have been cloned, sequenced and over-expressed. DNA encoding N-terminal domain of ppg gene yielded significant prophylactic efficacy to the tune of 80% against the *L. donovani* challenge in hamsters. The efficacy was supported by a surge in IFN-γ, TNF-α, and IL-12 mRNA levels along with a rise in the level of Leishmania specific IgG2 which was indicative of enhanced cellular immune response.

Biochemical characterization of purified Leishmania-actin was carried out. Primers for deletion mutants and point mutants of Leishmania-cofilin were designed and cloning and characterization of these mutants is currently under progress. The antibodies against denatured squalene synthase were raised in rabbit and sera checked by ELISA. Truncated primers of this enzyme were designed to purify the protein in soluble form by removing the N and C terminus patches. Its biophysical characterization is underway. Triose Phosphate Isomerase was subcloned in pET-28a (+) and over-expressed in BL21 (DE3) vector; checked for over-expression on 12% SDS-PAGE and confirmed by western blotting using anti His antibodies to get protein in soluble form. Folding stability of recombinant trypanothione reductase was characterized. 3D model construction and validation of leishmanial dipeptidylcarboxypeptidase was carried out. Cloning and sequencing of differentially expressed gene (DEG-II), identified through micro-array, was carried out.

## 2.7 Malaria

Bio-evaluation of over 800 novel synthetic compounds representing several prototypes against *Plasmodium falciparum in vitro* model yielded promising leads for new drug development. Identified leads were pursued further against *in vivo* rodent models and two prototypes have been identified for lead optimization. With a view to develop combination therapy regimens, studies employing candidate antimalarial compounds 97-78 and 99-411 in combination with piperaquine and lumefantrine have been continued in rodent and simian malaria models. Observations against rodent models have been successful in optimizing regimens providing total parasite clearance with two to four fold lower doses of the individual components. Besides, curative response has also been obtained in combination studies with short duration regimens. Studies are also underway to validate the suitability of *P. cynomolgi* monkey malaria model to study immuno-prophylactic potential of *P. vivax* vaccine proteins since these two parasites share high homology between prime vaccine candidates including CSP and MSP proteins. Cloning, expression and characterization of these two proteins from both parasites are in progress. Studies on characterization of transketolase as novel drug target have been successful in demonstrating inhibition of recombinant PfTk protein activity by specific transketolase inhibitors, thus opening an opportunity to synthesize target specific molecules. Molecular biology studies have been directed towards identification and analysis of biochemical pathways operative within the apicoplast, a non-photosynthetic plastid of secondary endosymbiotic origin, to provide novel sites for drug intervention against malaria. Due to its essentially prokaryotic nature, the processes of DNA replication, transcription and translation within the apicoplast are also validated drug targets. The prokaryotic nature and putative red algal origin of the apicoplast


Dr. Rakesh Tuli, Director, CDRI addressing the audience during 11th Dr. B. Mukerji Memorial Lecture. Also seen on the dais (R-L): Prof. A.K. Mahapatra, Director, SGPGI, Lucknow, Prof. Rajendra Prasad, Rector, JNU, New Delhi and Dr. Zaka Imam, Senior Deputy Director, CDRI.

Dr. Rakesh Tuli, Director, CDRI presenting a memento to Prof. Rajendra Prasad, Rector, JNU, New Delhi on the occasion of 11th Dr. B. Mukerji Memorial Lecture.

Prof. P.N. Tandon, President, NBRC, Manesar inaugurating the CSIR Foundation Day Celebrations by lighting of the lamp. Also seen on the dais (L-R): Prof. Samir Bhattacharya, Dr. Rakesh Tuli and Dr. Ashwani Kumar.


Dr. Rakesh Tuli, Director, CDRI addressing the audience during CSIR Foundation Day. Seen on the dais (L-R): Dr. U.C. Lavania, Scientist, CIMAP, Prof. Samir Bhattacharya, INSA Senior Scientist, Shantiniketan, Prof. P.N. Tandon, President, NBRC, Manesar and Dr. Ashwani Kumar, Director, IITR.

A View of the CSIR Techolnogy Award-2008 for Innovation function. On the dais are (L-R): Dr. O.P. Asthana (CDRI), Dr. Ram Pratap (CDRI), Dr. Vikram Kumar(Director, NPL), Prof. Bartha Maria Knoppers (Faculte de Droit, CRDP, Canada), Hon'ble Sri Kapil Sibal (Union Minister of S & T and Earth Sciences), Prof. Samir K. Brahmachari (DG, CSIR) and Dr. Ashim Ghatak (CDRI).


A view of the celebration of 66th CSIR Foundation Day at CDRI. Dignitaries seen on the dais are (L-R): Dr. S.K. Puri, Dr.(Mrs). Madhu Tuli, Prof. Samir Bhattacharya, Prof. P.N. Tandon and Mr. B.D. Vashisth


Prof. P.N. Tandon, President, NBRC, Manesar addressing the audience during Vaigyanik Jagrukta Abhiyaan. Seen on the dais are (R-L): Dr. Rakesh Tuli, Director, CDRI, Prof. Samir Bhattacharya, INSA Senior Scientist, Shantiniketan and Dr. Vinod Bhakuni, Scientist, CDRI.


Prof. Samir Bhattacharya, INSA Senior Scientist, Shantiniketan addressing the audience during Vaigyanik Jagrukta Abhiyaan. Seen on the dais are (R-L): Dr. Rakesh Tuli, Director, CDRI and Dr. Vinod Bhakuni, Scientist, CDRI.

suggested the possible involvement of a histone-like protein ('heat unstable' or HU). HU proteins are small basic proteins of prokaryotic origin that are structurally distinct from eukaryotic histones. Results have shown the involvement of *P. falciparum* Chr.9-encoded bacterial histone-like protein (PfHU) in DNA compaction in the apicoplast. Atomic Force microscopic study of PfHU-DNA complexes shows protein concentration-dependent DNA stiffening, intermolecular bundling and formation of DNA bridges followed by assembly of condensed DNA networks. These results provide the first functional characterization of an apicomplexan HU protein and give additional evidence for red algal ancestry of the apicoplast.

Studies on three additional putative apicoplast-targeted proteins continued. Nuclear genes annotated as prokaryotic translation elongation factors EF-Ts and EF-G and the apicoplast-targeted putative [Fe-S] complexation protein SufC were investigated. Interactions between recombinant EF-Tu and EF-Ts were analysed by fluorimetry and the ability of apicoplast EF-Ts to mediate nucleotide exchange (GDP/GTP) on *E. coli* EF-Tu was investigated using FRET with Mant-GDP. *P. falciparum* EF-Ts could mediate GDP/GTP exchange on both *P. falciparum* and *E. coli* EF-Tu. FRET analysis using a stopped-flow device revealed fast kinetics of nucleotide exchange reaction. Components similar to the *E. coli* Suf system have been identified in P. *falciparum* and are likely to function in the apicoplast. While the SufB homolog (ycf24) is encoded by the apicoplast genome, homologs of the other components of this pathway are nuclear-encoded. We have studied two interacting components of this pathway-SufB and SufC. Case control studies have been carried out for analysis of SNPs related to susceptibility / resistance / severity of *P. falciparum* malaria infection in populations from endemic and non-endemic regions across India. Association of specific promoter SNPs of the TNF gene as well as the Fc?RIIa receptor with susceptibility to severe *P. falciparum* malaria has been revealed from these studies.

## 2.8 Microbial Infections

127 Synthetic compounds were screened against *Mycobacterium tuberculosis* by radiometric BACTEC method. Two synthetic compounds, with MIC 0.79 and 3.125 µg/ml bactericidal mode of action and cytotoxicity to Vero cells and macrophage cell line, have been identified for *in vivo* evaluation. Genes of *Mycobacterium tuberculosis* expressed in hypoxia conditions *in vivo* were investigated by expression of reporter genes by selecting up-regulated promoters in *in vitro* simulated conditions of anaerobic persistence. Transcriptional analysis by Real Time PCR revealed 6-15 fold induction in non replicating conditions compared to 2-5 fold in replicating cells. Few genes have been selected for functional analysis by mutation. A murine infection model of *Mycobacterium fortuitum* for persistence and reactivation has been developed which can be used to screen compounds against acute and latent infection. The model was used to demonstrate the role of Rpf proteins of *M. tuberculosis* on resuscitation of bacilli in NRP state in vivo and used as rapid *in vivo* screening system for isolation of a transposon insertion mutant of *M. fortuitum* attenuated in virulence and persistence. The mutant was complemented by Rv3291c of M. tuberculosis. Resuscitation of dormant BCG by Rpf proteins of *M. tuberculosis* and *M. luteus* was demonstrated. Transcriptome and differential proteome analysis of BCG cells in extended stationary phase and Rpf mediated resuscitated cells was attempted to identify pathways and differentially expressed transcripts and proteins. Acetohydroxyacid synthase and dihydroxyacid dehydratase, both essential genes belonging to BCAA pathway are being explored as drug targets for TB. A method for improved 2-D separation of *M. tuberculosis* proteins for proteome analysis has been developed leading to identification of three new proteins. Role of Rv3080 in growth of mycobacteria was demonstrated by expression of the gene in fast growing *M. smegmatis.* Infection of macrophages with *M. tuberculosis,* BCG and *M. tuberculosis* H37Ra down-regulated the expression of PKC. SiRNA mediated knockdown of PKC drastically reduced phagocytosis of BCG and M. smegmatis by macrophages while their intracellular survival was increased. The presence of SigF and seven sigH paralogs was demonstrated in *M. smegmatis.* sigF was found to be expressed throughout the growth while sigH and its paralogs were found to be differentially expressed during growth stages in *M. smegmatis* and in response to different stress conditions.

A total of 696 compounds/extracts were evaluated for antifungal and antibacterial activity. One marine extract against fungi (MIC 1.9-62.5 µg/ml) and five synthetic compounds having antibacterial activity (MIC 0.19-0.78 µg/ml) were identified. Monoclonal antibodies against cell wall proteins of *C. albicans* and *Aspergillus fumigatus* have been developed. Two monoclonal antibodies having candidacidal activity were identified and are being evaluated for diagnostic potential. One of the proteins recognized by MAB 1A1 has been identified as Glyceraldehyde-3-Phosphate dehydrogenase. Laboratory derived amphotericin B resistant strain of *C. albicans* was found to over express virulence factors, extra cellular aspartyl proteinase and phospholipase.

### 2.9 Natural Products

Regulatory pharmacology and toxicity studies in monkeys for CDR-134F194, which is in the preclinical phase, have been completed and initiation of phase-I clinical trial is in progress. Confirmation of antihyperglycaemic activity has been carried out in 8 plants and several fractions and pure compounds have been isolated. Seven plant extracts, their fractions and pure compounds are under detailed bio-evaluation for antidyslipidemic screening. Ethanolic extract and n-butanol fraction of plant 1020 showed promising osteogenic activity. 8 Compounds from chloroform fraction and 16 compounds from n-butanol fraction have been isolated. These compounds were bio-evaluated and five compounds exhibited promising osteogenic activity. Three active compounds have been synthesized and in vivo osteogenic activity validated. Synthesis of hybrid forms of lupeol (antimalarial and anticancer), solanesol (antitubercular) were designed and synthesized. Analogs of aromatic turmerone were designed where conformational mobility of its side chain was restricted, synthesis of these were initiated for antithrombotic and neuroprotective activities.

### 2.10 Newer Approaches in Drug Design and Discovery

Isocitrate lyase (Icl), an enzyme that plays an important role in the regulation of isocitrate flux and anaplerotic replenishment of pool of substrate required for biosynthetic process in *Mycobacterium tuberculosis* is a potential drug target for the antituberculosis drugs. During this period, role of divalent cations in modulation of functional and structural properties of the enzyme have been investigated by biophysical methods. The results obtained from the studies provide insight into the possible mechanism of divalent cation-induced changes in structure, function and stability of MtbIcl. Further, an integrated approach to prioritize target specific antitubercular compounds using ligand and structure-based virtual screening has been explored followed by structure interaction fingerprints to prioritize the leads for the enzyme TMPKmt and dihydrofolate reductase of *M. tuberculosis.* This has resulted identification of inhibitors against these enzyme. These results suggest that structure based virtual screening coupled with the structure interaction fingerprints should be a valuable tool for prioritization of virtual screening hits.

A significant milestone has been achieved by solving structure of the potential drug target protein peptidyl-tRNA hydrolase from *M. tuberculosis* H37Rv (MtPth) in solution by NMR spectroscopy. The ensemble of 40 structures representing the solution structure of MtPth has been deposited in PDB under ID 2JRC.

There has been significant progress in the X-ray crystal structure determination of proteins, involved in the thiol based redox metabolism pathway of kinetoplastida. Two of the proteins have been successfully cloned, expressed, purified and crystal diffraction data collected.

Inhibition of PTP1B which attenuates insulin signaling by catalyzing de-phosphorylation of insulin receptors (IRs) has gained significant attention as a new target for diabetes therapy. We have been working on the lead optimization of a dipeptide by developing peptidomimetics to improve its potency and selectivity. During this period, six new compounds have been synthesized. Compound S-008-752 has shown better inhibitory activity and selectivity compared to the reference compound.

Prof. Samir K. Brahmachari, DG, CSIR during visit to the new campus; Inspecting the model of New Campus.

Prof. Samir K. Brahmachari, DG, CSIR visiting the construction site of New Campus.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Dr. Rakesh Tuli, Director Felicitating Dr. Shailesh Nayak, Secretary, MoES, Govt. of India during his visit to CDRI


Prof. Chinmoy Sarkar Dey, NIPER, Mohali receiving the CDRI Award-2008 for excellence in drug research from Dr. Rakesh Tuli, Director, CDRI. Also seen in the picture Dr. Nitya Nand, Former Director, CDRI.


Dr. C.M. Gupta, Former Director, CDRI and Dr. Rakesh Tuli, Director, CDRI releasing the CDRI-NLAC News Letter during Training Course in Laboratory Animal Science.

## 2.11 Reproductive Health Research

A novel synthetic compound S-003-296 is being developed as a much safer non-detergent vaginal *spermicidal*. The *in vivo* contraceptive efficacy of S-003-296 was taken up in rabbits. The *in vivo* efficacy of the compound in a vaginal formulation (suppository) is in progress.

Four compounds (S-006-1709, S-007-1500, S-008-398 and S-008-399) were found to be active *in vivo* in promoting peak bone mass (PBM) in female Sprague Dawley rats. All four compounds act as estrogen mimic in osteoblasts but without estrogen-like effect in uterus. Minimum effective dose varies from 1.0-10.0 $mg.kg^{-1}.day^{-1}$ b.w. doses for these four compounds. S-006-1709 appears to signal via estrogen receptor (ER)- and has good oral bioavailability.

As new leads, two novel compounds (K058 and K100) have been isolated from a plant 914. Both these compounds exhibit strong osteogenic effects *in vitro.* K058 promotes acquisition of PBM in developing rats and also stimulates bone formation in ovariectomized (OVx) rats. Minimum effective dose (MED) of K058 is 1.0 $mg.kg^{-1}$ $day^{-1}$ b.w. Unlike osteogenic synthetic series mentioned above, K058 does not signal by ER, instead activates aryl hydrocarbon receptor (AHR). AHR has recently been shown to regulate osteoblast differentiation as AHR-null mice have lower acquisition of PBM compared with wild type controls. K100 possesses anti-adipogenic effect *in vitro*, in addition to be osteogenic. None of these compounds affect osteoclast functions in vitro. Given novel chemistry, small molecule and MED as low as 1.0 mg.kg-1.day-1 b.w., K058 holds tremendous therapeutic promise in osteogenic mode for anti-antiosteoporosis.

## 2.12 Technology Development

### 2.12.1 Chemical Technology

Compounds 99-411 (Antimalarial), S-003-296 (Spermicidal) and natural products viz. CDR-134BS479C (Antidiabetic) and Herbal Medicament (for management and cure of Cerebral Stroke) were prepared in additional quantities and supplied to Pharmaceutics Division. The process of production of picroliv has been demonstrated to M/s DIL Ltd., Mumbai on 10 kg scale and 716 g picroliv prepared during demonstration was handed over. An improved process for Centchroman has been standardized at bench scale and the process is ready for demonstration.

### 2.12.2 Fermentation Technology

A strain of actinomycetes group, showing broad spectrum antibacterial activity, was isolated from the soil samples. 16S rRNA homology studies characterized the strain as *Streptomyces triostinicus.* Two compounds were chemically characterized as Actinomycin V and D. Studies on the chemical characterization of other two compounds is progressing. Artificial neural network (ANN) and genetic algorithm was applied to optimize the medium components for the production of actinomycin V. Maximum antibiotic yield of 452mgl-1 (38.6% higher) was obtained with the GA optimized medium.

A heparinase producing bacterium was isolated from the soil samples collected from the hilly areas of India, was characterized biochemically and identified as *Acinetobacter calcoaceticus* by 16S rRNA homology. The intracellularly produced constitutive heparinase enzyme was isolated from the periplasmic space of the culture by freeze fracturing and purified 51.2 fold by ion exchange and gel filtration chromatography. *In-situ* gel digestion of the purified protein with trypsin did not show any homology with heparinase I from *Pedobacter heparinus,* the commercially available enzyme. This indicates the novelty of the enzyme.

### 2.12.3 Pharmaceutical Technology

A process for preparation of Herbal Medicament starting from 25 kg of plant material was successfully demonstrated to M/s Themis Medicare, Mumbai at their Wapi (Guajrat) plant. Also the quality control parameters of CDRI compound 97-78 were demonstrated at IPCA Laboratories Ltd. Mumbai. Inhalation safety/toxicity studies of microparticles containing anti-tuberculosis drugs were carried out in non-human primates (*Macaca mulatta*). These indicate suitability of the formulation for human trials. Indian patent application is under preparation by industrial partner, M/s Lupin. A layer-by-layer based ultrathin

polyelectrolyte nanoreservoir has been developed. The possibility has been explored for the delivery of kaempferol and of macromolecules taking Bovine Serum Albumin as a model drug. Studies related to nanometer-sized delivery systems for anticancer agents, tetanus toxoid, glucagon-like peptide I and RNAi against human SOCS-3, antimalarials targeting RBC and blank nanoparticles capable of crossing the female cervix if instilled into the vagina are in progress.

### 3. Publications and Patents

During the reporting period, 205 research papers were published from the Institute in different national and international periodicals having average impact factor 2.466. Besides, several papers and poster presentations were presented in seminars, symposia and conferences. During the year, the Institute continued to obtain success in its high quality innovative approaches. This is well reflected in filing of 7 foreign and 12 Indian patents and grant of 14 foreign and 7 Indian patents.

### 4. Technical Services Provided

The Sophisticated Analytical Instrument Facility continued to provide its services all over the globe. During the year, 632 users utilized the analytical services. There were 592 users from Colleges/Universities while 32 and 8 users were from research laboratories and industries respectively while 345 users were from the Institute itself. Total number of internal samples amounted to 22695 while 8070 external samples were analyzed this year. Institute continued to provide *in vitro* and *in vivo* biological screening facilities to R&D institutions, academic organizations, Universities, industrial houses, etc. on payment basis.

The National Laboratory Animal Center continued to supply healthy animals for research for institutional use and to outside agencies. This year, over 35000 animals were supplied. Tissue and Cell Culture Unit provided 155 T-25 Cell Culture Flasks of various cell lines to the scientists at Eastern Medikit Ltd., Gurgaon and Shriram Institute of Industrial Research, Delhi. The Laboratory Engineering Services Division provided engineering services for infrastructural needs and support services of various divisions towards setting up of new facilities, scientific instruments, renovation, up-gradation and repair/maintenance of laboratories, infrastructures, buildings and services.

Information Technology Unit provided systems and network administration; operation and maintenance of internet and mail services; operation and maintenance of software (for Stores & Purchase system, online compounds code register); LAN Infrastructure; development of MoES Database application software; Web Hosting for Intranet Web Site; Secured Web Access within CSIR; digital knowledge repository of DLS Division; R&D database application of MSB Division; network planning for New CDRI; up-gradation of ICT infrastructure; and Planning & Implementation of IT Setup for NIPER, Rae-Bareli.

### 5. Human Resource Development

A R&D proposal Rural School Health Education Program: Integrating with Diverse Inputs: Reaching the Un-reached was jointly submitted by CDRI and NBRI, Lucknow as a consequence to a presentation made by Dr. O.P. Asthana in the brain storming meeting on Sanitation and Health at CSIR Headquarters, New Delhi on 11.9.08. The proposal has been approved for 5 years (2008-2012).

During the year of report, 36 scientists were deputed to different organizations for advancement of their knowledge in their area of specialization. A total of 44 research fellows submitted their thesis and many of them were awarded their Ph.D. degree while 4 students were awarded the M.D. degree. 3 Scientists from Nigeria were provided short term training under International Bilateral Cooperation. Seven students from Birla Institute of Technology & Science, Pilani were provided six months training on monthly stipend while 3 students were provided two months training under cooperation with Indian Academy of Science, Bangalore. Four industry/ academia sponsored personnel were trained in Toxicology and Pharmacology Divisions of the Institute. A total of 225 university / college sponsored students were imparted training in different divisions and the tenure of the training ranged from 2 to 12 months.

## 6. Memorable Events

### 6.1 Mellanby Memorial Lecture

The 33rd Mellanby Memorial Oration was delivered by Prof. Johann Gasteiger, University of Erlangen, Nurnberg, Germany on the topic "Explorations into Biochemical Pathways for Drug Design" on February 17, 2008. In his presentation, he pointed out that the living species have to survive in a hostile environment, both intrinsically and extrinsically. The intrinsic issues of human body are dealt by biologists; the chemists take care of the issues that affect a man's body from outside. Prof. Gasteiger added that it is important to have biologists and chemists on a single platform to understand the complexities of human body and nature and accordingly design new therapeutic agents for the cure of human diseases. Prof. C.L. Khetrapal, Director, Centre of Biochemical Magnetic Resonance, SGPGIMS, Lucknow presided over the function.

### 6.2 CDRI Annual Day

The Institute celebrated its 57th Annual Day on February 17, 2008. Dr. Rakesh Tuli, Director, enumerated the achievements made by the institute during the year 2007-08. The highlights covered attainments in basic research, as reflected through publication of research findings in high impact journals, filing and grant of patents in India and abroad, products in pipeline and licensing of products to the industry. Two antidiabetic and one memory enhancer agents were licensed to M/s TVC Skyshop Ltd. A PCR based tuberculosis diagnostic kit, licenced to Biotron Health Care Ltd., Mumbai was formally launched. Prof. N.K. Ganguly, Former DG, ICMR and Chairman, Research Council was the chief guest. In his address, Prof. Ganguly suggested government to introduce a bill to award a special status to scientific institutes and scientists. He emphasized the need for better salaries and working conditions for scientific workers at par with international pharma players as they have an active role to play in nation building. Prof. Ajaib Singh Brar, Vice Chancellor, Lucknow University, who presided over the function, emphasized the need for strengthening the education system and promoting scientific temper in children as there is a steep decline in the number of youth opting for basic sciences. A large number of employees, completing 25 years of continuous service, were felicitated on the occasion. Academic Incentive Awards were given to several scientists in chemical and biological sciences for publishing their research findings in high impact factor journals and filing/grant of foreign patents during the year. Two research fellows were given the Dr. M.M. Dhar Memorial Prize 2007 for best thesis presentations in biological sciences.

### 6.3 Dr. B. Mukerji Memorial Lecture

Dr. B. Mukerji Memorial Lecture, 11th in the series, was organized on March 4, 2008. Dr. Bishnupada Mukerji was the first Indian Director of CDRI. The lecture was sponsored by Sachin and Sikta Pradhan Foundation, Bethesda, USA and was delivered by Prof. Rajendra Prasad, Rector, Jawaharlal Nehru University, New Delhi. The topic of his presentation was Fungal Infections in India: Molecular Mechanism of Emerging Antifungal Resistance. Prof. Prasad stated incidence of fungal infection is increasing in India due to lack of awareness and specialized expertise and the investigations on the subject are still limited. Besides few sporadic reports, there is a paucity of planned studies on this subject in India. In addition, there is very limited surveillance of fungal infections in Indian hospitals. The early diagnosis of fungal infections is very essential in AIDS/Burn or in other immuno-compromised patients. The function was presided over by Prof. A.K. Mahapatra, Director, SGPGI, Lucknow. In his presidential remarks he said that critical examination of dead patients reveals that fungal infections are more common in various disease conditions. He informed that about six million people die from tuberculosis every year and many divulge fungal infections due to poor resistance.

### 6.4 CDRI Award 2008

A presentation ceremony of the CDRI Award 2008 for excellence in drug research was organized on April 7, 2008. This award was instituted in the year 2004 to recognize excellence in contribution of Indian researchers below 50 years of age to the broad areas of drug research. This year, three eminent scientists viz. Dr. Souvik Maiti, Institute of Genomics and Integrative Biology, New Delhi; Dr. Brijesh Kumar Srivastava, Zydus Research Center, Ahmedabad and Prof. Chinmoy Sankar Dey from National Institute of Pharmaceutical Education & Research, Mohali

(Punjab) were honored and they delivered their award orations. Dr. Nitya Nand, Ex Director, CDRI presided over the function.

### 6.5 World Hypertension Day

The World Hypertension Day was celebrated on May 15, 2008 at the Institute under the auspices of Indian Society of Hypertension. Prof. M.K. Mitra, Prof. Ashok Chandra from CSMU, Lucknow and Dr. A. Ghatak, Deputy Director, CDRI delivered their presentations on the occasion.

### 6.6 CSIR Foundation Day Celebrations

As per the practice in the past, all the four Lucknow based CSIR laboratories viz. CDRI, NBRI, IITR and CIMAP jointly celebrated the CSIR Foundation Day on September 26, 2008. Earlier in the day, an exhibition was organized at NBRI exhibiting major achievements of all the laboratories. It was inaugurated by Prof. K.C. Upadhyay, Former Vice Chancellor, M.S. University, Vadodara and Professor, School of Life Sciences, JNU, New Delhi. The exhibition remained open and a large number of scientists from academic institutes, students and general people visited it and discussed their concern with experts. The main function was organized at Scientific Convention Center, Lucknow. Dr. Rakesh Tuli, Director, CDRI and NBRI, welcomed the dignitaries while Dr. Ashwani Kumar, Acting Director, IITR presented an overview of Lucknow based CSIR laboratories. The Foundation Day Lecture was delivered by Prof. Samir Bhattacharya, INSA Senior Scientist, School of Life Sciences, Visva Bharti, Shantiniketan. The topic of his presentation was Molecular Mechanism of Insulin Resistance and Type 2 Diabetes. Prof. P.N. Tandon, President, National Brain Research Center, Manesar presided over the function. On this eventful day, CDRI received the CSIR Technology Award - 2008 on “Discovery of guggulsterone and development of analogues with novel mechanism of hypolipidemic agents”. The award carries a cash award, a plaque and a citation.

Besides the scientific activities, prizes and cash awards were given to the children of staff members for winning essay competition and securing meritorious positions in different examinations held at national levels by Dr (Mrs.) Madhu Tuli. Members of the staff, who had superannuated in the last one year, were honored with certificates and a shawl whereas staff members who completed 25 years of continuous service were awarded wrist watches. Earlier during the day, Prof. P.N. Tandon inaugurated Vaigyanik Jagrukta Abhiyaan to create scientific awareness among the scientists and research scholars.

### 6.7 Vigilance Awareness Week

A one week program Vigilance Awareness Week was organized at the Institute during November 3-7, 2008. All members of staff took an oath for being vigilant and deliver their best for the welfare of mankind and Nation. An essay competition and a debate contest were organized during the course of program. Suitable prizes were given to the winners.

## 7. Symposia

### 7.1 National Symposium on 'An Update of Male Reproduction and Infertility'

A two day national symposium “An Update of Male Reproduction and Infertility” was organized on March 13 14, 2008 at the Institute. The focus of the symposium centered on basic understanding of spermatogenesis, genetic basis of male infertility, endocrine disruptors, environmental reproductive toxicology, male contraceptives and current trends in male infertility. Luminaries from academic institutions, scientists and medical profession participated in the program. Over 110 participants and 15 speakers participated in the symposium. Several topics viz. 'Clinical aspects of male infertility', 'Genetic basis of male infertility', 'Male contraception', 'Andropause and male infertility', Male reproduction', Cell biology of male reproduction', Environmental toxicology and male infertility' were discussed at length during the program.

### 7.2 Recent Advances in Female Reproductive Health Research

A two day symposium on "Recent Advances in Female Reproductive Health Research" was organised on December 11-12, 2008. The symposium was designed in keeping with the Institute's mandate and an update of recent advance in female reproductive health research and fertility regulation.

The objective of the symposium was to provide a common interactive forum to basic scientists, clinicians for exchange of current research ideas, view and advances in female reproductive health research including fertility regulation. The elaborative scientific programme included invited lectures abd poster sessions covering themes viz. Basic aspects fo female reproduction, Female reproductive disorders, Female infertility: causes and management, Advances in female contraceptive. About 15 eminent speakers delivered lectures on various topics during the symposium.

## 8. Training Programs Organized

8.1 The Division of Laboratory Animals organized a four weeks Training Course in Laboratory Animal Science from April 28 to May 23, 2008 as part of human resource development program of the Institute. The valedictory function was organized on May 23, 2008.

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## 9. Awards and Honors

Team CDRI received the CSIR Technology Award - 2008 for Innovation for "Discovery of guggulsterone and development of analogues with novel mechanism of hypolipidemic agents". The award carries a cash award, a plaque and a citation. The award was presented to CDRI on 26th September 2008 at National Physical Laboratory, New Delhi. Dr. Ram Pratap received CDRI Incentive Award for Great Britain Patent "Process for preparing guggulsterones" (2007). Dr. Atul Kumar was the recipient of OPPI-2008 Award. Dr. (Mrs.) Vinita Chaturvedi received the Immunology Foundation Prize (2007). Dr. Srikanta Kumar Rath, Scientist, Genotoxicity Laboratory was awarded First Genomic Pioneer Award from India in 2008 for his significant contributions in the field of molecular toxicology and understanding of disease genomics by developing assays for genotoxicity using oligonucleotides and real-time approaches. Dr. Ashim Ghatak received the Dr. Coelho Memorial Oration in Experimental Medicine - 2008 from Association of Physicians of India. Dr. (Ms.) Saman Habib was honored with Prof. B.K. Bachhawat Memorial Award by National Academy of Sciences. Dr. J.K. Saxena was given the Zoological Society of India Award - 2008. Dr. Atul Goel was the recipient of Alexander von Humboldt Fellowship by Federal Republic of Germany and CDRI Incentive Award for one of his research papers. Dr. P.R. Mishra received the INSA-DFG Fellowship. Bioorganic & Medicinal Chemistry, Elsevier Ltd., UK honored Dr. S.B. Katti for one of his most cited papers. Best poster awards were given to Drs. S.K. Puri, Rakesh Shukla, Khashif Hanif, G. Palit, Anuradha Dube, Kalpana Murthy and R.P. Tripathi in their respective fields of presentations at different seminars. Four research students were also given different awards; details thereof are given in relevant section of this Report.

## 10. Other Activities

In order to increase security and monitor the movement of employees and outside visitors, both the campuses of the Institute were equipped with an Electronic Access Control System with the aim to control authorized access and deter un-authorized entry, control and record movement of men and material in a designated area. The system was formally inaugurated by the Director, Dr. T.K. Chakraborty on January 9, 2009. CDRI Staff Club actively organized various sports, cultural, literary and welfare activities. The Institute participated in Shanti Swaroop Bhatnagar Indoor Tournaments at CGCRI, Kolkata and a total of 9 players qualified for SSBMT finals in Badminton (Men), Table Tennis (Women) and Carrom. Mr. Mohd. Salim received the best player award in the tournament. Children of the staff members participated in various events organized by CDRI club and the winners were suitably awarded.

Dr. Rakesh Tuli, Director, CDRI inaugurating Cricket match between Director's XI and COA's XI during Annual Sports 2008

Director's XI and COA's XI cricket teams with respective patrons during Annual Sports 2008

# SECTION I

# Progress in Research Projects

# Regulatory Studies

# Area: Clinical Trials & Pharmacokinetic Studies 1

(Coordinator: Dr. O.P. Asthana)

*Clinical trials of CDRI candidate drugs including clinical pharmacokinetic studies in human volunteers and post marketing surveillance.*

1.1 Clinical trials of candidate drugs/products

1.2 Pharmacokinetic and metabolic studies of synthetic compounds and natural products

## 1.1 Clinical trials of candidate drugs/ products

### 1.1.1 Consap (Contraceptive Cream)

Product licensed to Hindustan Latex Limited (HLL).

Decision to manufacture and market consap cream is awaited from HLL.

### 1.1.2 Arteether (Blood schizontocidal)

Dossier submitted to DCGI for approval to use Arteether in children suffering from *P. falciparum* malaria.

Marketing permission is awaited from DCGI.

### 1.1.3 Compound 80-53 [Aablaquin] (Antirelapse Antimalarial)

Status quo, as per data reported last year. Future of this novel drug depends upon initiatives from NPIL/CSIR.

### 1.1.4 Picroliv (Hepatoprotective)

1.1.4.1 Study-1

During the year, clinical trials in patients of tuberculosis receiving MDT continued at KGMU, Lucknow.

1.1.4.2 Study-2

A total of 64 patients included in the trials; 29 patients (18 females, 11 males; mean age 28.33 years) received placebo; 35 patients (8 males, 27 females; mean age 26.86 years) received picroliv.

Placebo group: 29 patients completed 6 months study.

Picroliv group: 35 patients completed 6 months study.

1.1.4.2.1 Results of interim analysis

Clinical recovery of tuberculosis patient was faster in patients treated with picroliv in comparison to patients receiving placebo.

Liver function tests performed before and during treatment revealed no appreciable difference in both the groups i.e. placebo /picroliv.

Adverse events were also monitored during the trials and no major side effects were reported with picroliv therapy when compared to the placebo group.

1.1.4.3 Study-3

A total of 46 patients included in the trials (31 females, 15 males); 7 patients completed 6 months study; 7 patients completed 5 months study.

26 patients completed 3-4 months study; 6 patients dropped out.

Codes are yet to be opened for detailed data analysis.

Total no. of patients 188 included in the study; 112 patients completed 6 months study; 26 patients completed 5 months study; 44 patients completed 3-4 months study; 6 patients dropped out.

## 1 Area: Clinical Trials & Pharmacokinetic Studies

### 1.1.5 CT-1 (Antidiabetic)

Status quo as per the data reported last year. Initiative from NPIL is awaited.

### 1.1.6 Compound 80-574 (Hypolipidemic)

175 Cases completed the phase III multi-centric clinical trials at SGPGI and KGMU, Lucknow, Seth GSMC, Mumbai and PGIMER, Chandigarh. Data compilation concluded. Future course of action depends on inputs from Cadila Pharmaceuticals Ltd.

### 1.1.7 CDR-134D123 (Antihyperglycaemic)

Phase-1 clinical studies completed. So far, 32 cases have completed the trial.

TVC Skyshop Ltd. has been licensed to market this product. A review meeting was held at Seth GSMC, KEM hospital Mumbai on 22nd Dec 2008. CDRI team, TVC group and investigators from Seth GSMC discussed the results of this trial and future strategy is being worked out.

### 1.1.8 Compound 97-78 (Antimalarial)

Visited PGIMER, Chandigarh in February, 2008 to assess the status of new GCP complaint facility for Phase I Clinical Trial.

New set-up for phase I clinical trial (4 beds) with ICU facility is operational.

Formulation and case record forms have been delivered by IPCA, coded supplies for double blind trial has been supplied.

Phase-I clinical trial is in progress. So far 13 healthy male volunteers have completed the study.

### 1.2 Pharmacokinetic and metabolic studies of synthetic compounds and natural products

### 1.2.1 Compound 99-373 (Anti-osteoporotic)

a. Tissue distribution studies in gonads (ovary and uterus).
b. Bone uptake studies in female SD rats.
c. Distribution kinetics between plasma and blood cells of rats.
d. Toxico-kinetic study in male and female rhesus monkeys.
e. Serum stability study.
f. Serum stability study of metabolites (M-1 and M-2) of 99-373.

### 1.2.2 Compound 99-411 (Anti-malarial)

a. Multiple dose PK studies in male and female monkeys by oral routes.
b. Toxicokinetic study of 99-411 in male/female monkeys.

### 1.2.3 S-002-853 and S-002-857 (Anti-diabetic)

a. Assay validation of S-002-853 / 857 in rat urine
b. Assay validation of S-002-853 in rat feces
c. Excretion of parent compounds (S-002-853 / -857) in rat urine
d. Excretion of compound S-002-853 in rat feces
e. Detection and characterization of metabolites of S-002-853 in rat feces
f. Detection and characterization of metabolites of S-002-853 in rat urine.

### 1.2.4 S-006-1709 (Anti-osteoporotic)

a. Bio-analytical LCMS/MS "Method Development".
b. Plasma pharmacokinetic studies in female SD Rats.

### 1.2.5 Plant 1020 (NP-1: Anti-osteoporotic - Osteogenic)

(a) Bio-analytical LCMS/MS Method Development for marker component K095.
(b) Plasma pharmacokinetic studies in female SD rats.

### 1.2.6 Plant 914 (Anti-osteoporotic - Osteogenic)

(a) LCMS/MS analytical Method Development for marker components K012, K058, K068 and K100.
(b) Finger printing and absolute quantification of marker components K012, K058, K068 and K100 in crude extract and acetone fraction.

### 1.2.7 New Leads: PK studies

(a) PK studies of two anti-thrombotic compounds in NZ rabbits:

- S-000-20 (Racemate)
- S-002-333 (Racemate)

(b) Analytical & bio-analytical methods for the isomers S-007-867 and S-007-1175 (Isomers of S-000-20).

(c) PK studies of S-007-867 & S-007-1175 in male NZ rabbits.

(d) Analytical method for the isomers S-004-1032 and S-007-1558 (Isomers of S-002-333).

(e) Plasma pharmacokinetic studies of antimalarial lead molecule S-006-309 (in rats).

# 2 Area: Preclinical Safety Evaluation and Regulatory Toxicity

(Coordinator: Dr. O.P. Asthana)

i. *Toxicology profiling of candidate drugs according to internationally accepted methods for studying local, systemic, reproductive, genetic toxicity and other special toxicity if required.*

ii. *In addition to various basic researches, deployment of alternative test systems which can be less or non cost and time intensive and can also reduce, refine or replace the use of animals in toxicity testing.*

iii. *To provide vital information on safety/mechanism of toxicity/ metabolism of drugs.*

2.1 Regulatory Studies

2.2 Basic Experimental Toxicology Studies

## 2.1 Regulatory Studies

### 2.1.1 CDRI products

2.1.1.1 99-411 (Antimalarial)

- 28 day repeat dose toxicity study in rhesus monkey. Product found safe up to 80 mg/kg body weight dose.

- *In vivo* micro-nucleous test in mice. Found safe.

- Chromosomal aberration assay in mice. Found safe.

2.1.1.2 NP-1 (Anti-osteoporotic)

- Single dose toxicity study in rat by oral route.

2.1.1.3 Herbal Medicament (Anti-stroke)

- 28 day repeat dose toxicity study in rhesus monkey. Found safe at the highest dose of 400 mg/kg body weight.
- *Salmonella* reverse mutation assay. Product found safe.
- Male fertility study in rats is in progress.

2.1.1.4 Compound 99-373 (Anti-osteoporotic)

- Teratogenecity and fetotoxicity studies in rats and rabbits are in progress.

2.1.1.5 CDR-134F194 (Antidiabetic)

- Micronucleus assay in mice. Found safe.
- Male fertility study in rats: Except affecting the libido at high dose level, the product did not reveal any adverse effect on reproduction and fertility of rats.

### 2.1.2 Products received under network project NWP0037

2.1.2.1 AP20am 15

- 28 Day repeat dose toxicity study in rat. Found safe at highest dose of 1000 mg/kg body weight.

2.1.2.2 AP20am 16

- 28 Day repeat dose toxicity study in rat. Found safe at highest dose i.e. 1000 mg/kg body weight.

### 2.1.3 Products received under Golden Triangle Project

2.1.3.1 Kajjali Yoga

- Single dose toxicity study in rat. Found safe at the dose of 5.0 g/kg body weight.

2.1.3.2 Ras Sindoor

- Single dose toxicity study in rat. MTD is 5 g/kg body weight.

2.1.3.3 Vasant Kusumakar

- Single dose toxicity study in rat. Found safe at limit dose level of 5.0 g/kg body weight

## 2.2 Basic Experimental Toxicology Studies

### 2.2.1 Determining Embryotoxicity Potential of a NCE by Embryonic Stem Cell Test (EST)

Over the past 20 years, more than 30 different culture systems have been proposed as test for developmental toxicity. The results from them were not promising while showing a high number of false positive / negative results. Since protecting human beings is the prime aim, it is controversial that these tests could get widespread acceptance for their regulatory use, thus, the use of omnipotent embryonic stem cell lines have shown more promising results in this context. Two experiments were carried out in this context i.e. *ES cell differentiation assay* and *Cytotoxicity assay in ES cells and 3T3 cells.* For present two studies 5-FU was taken as test compound and $ID_{50}$ & $IC_{50}$ in terms of embryotoxicity were found to be 0.03 µg / ml for *ES cell differentiation assay* and *0.20 and 0.110* µg / ml in *Cytotoxicity assay* for 3T3 cells and ES cells respectively.

Figures Showing Different Steps of the Assay for $ID_{50}$ of D3 ES Cells


*Fig.:* 1. Es cell suspensions ($3.75^1$ x 104 cells/ml) from undifferentiated ES cells (D3) in a 60-mm maintenance plates.

2. 20 pl of cell suspension (750 cells) containing the appropriate test chemical concentration or solvent or D3 assay medium was dispensed with the help of a multi-channel pipet on the inner side of a 100-mm tissue culture Petri dish lid. 25 to 35 drops per lid was pipeted.
3. The lid was turned carefully into its regular position and was put on top of a Petri dish filled with 5 ml PBS, thus giving a look of hanging drops.
4. The Petri dishes with hanging drops were then incubated for 3 days in a humidified atmosphere with 5% CO2 at 37°C.
5. 1 Embryo body (EB) (in a small volume, ?40 ?l, with blue tip or cut yellow tip) was added in each well. These 24-well plates were incubated for 5 days in a humidified atmosphere with 5% CO2 at 370C and then assayed for the endpoint i.e. on day 10 of the assay.

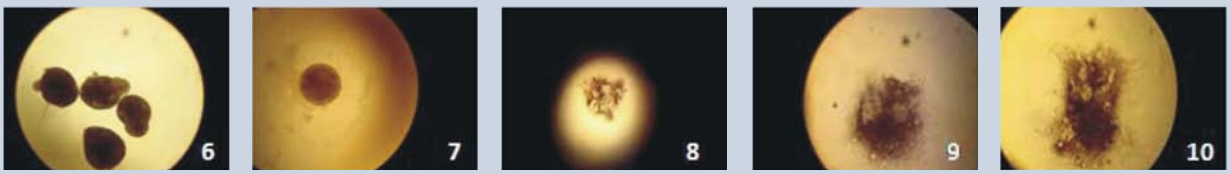



*Fig.:* 6, 7, 8, 9 & 10 - 3, 5, 8, 9 & 10 Days old ES (D3) cells respectively after culture. From day $8^{th}$. The grown cells showed little beating like heart and on $10^{th}$. Days the beating was observed to be quite high and well noticeable.

### 2.2.2 Determining Teratogenicity by Mouse Limb Bud Culture

Limb buds from 12 day old mouse embryos were used for the study. The limb anlagen were grown *in vitro* for 6 day with the medium changed once after 3 days of culturing. 15 to 20 limbs were cultured in Bigger's Media and incubated at 37°C in the presence of 50% $O_2$, 5% $CO_2$ and remaining volume of Nitrogen. This whole procedure was performed in rotating bottle. During this procedure also known or unknown test sample was incorporated, however, the cultured limbs were processed for alcian blue staining subsequently evaluated with under stereo zoom microscope for its morphology.

Figures Showing Different Steps involved in Mice Limb Bud Culture for Detecting Teratogenicity

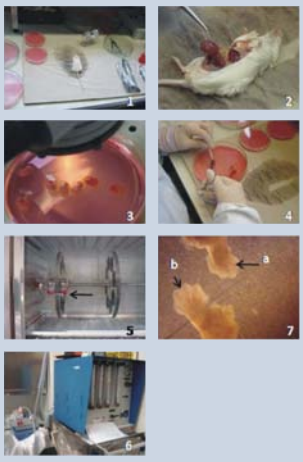


*Fig.:*
1. Mice Ready for dissection
2. Cut open abdomen of mice showing placement of embryas.
3. Dissected out Embryos (12 days old) from Uterus.
4. Separating out the Limbs of embryo after dissecting it out of uterus.
5. Rolling Bottle incubator where the limbs were incubated at 37°C Temp and Gases (5% Co2, 50% O2 and 45% N2) after putting it in bottle % filled with Bigger's Media.
6. Supply Unit of Gases which was attached with incubator.
7. a-Limb Bud after dissection and before culture.

   b- Limb Bud after 6 days culture.

### 2.2.3 Transcriptomic profiling of Antimalarials

Malaria is a major health concern especially in endemic areas of India. CDRI has been contributing significantly by producing new molecules for the treatment of the disease. However, most of the antimalarial molecules are toxic in nature. Therefore, toxicity studies at transcriptomic level using cDNA microarrays were initiated to study antimalarial molecules which may help us making new molecules free of side effects. The liver transcripts were analysed following Primaquine and Bulaquin treatment in mice. Primaquine [8-(4-amino-1-methylbutylamino)-6-methoxyquinoline], is an important anti-relapse antimalarial derivative of 8-aminoquinoline, and extensively used to combat liver stages of *Plasmodium vivax* and *Plasmodium ovale* responsible for malarial relapses. Bulaquin ([N-(3-acetyl-4-5-dihydro-2-furanyl)-N4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine], CDRI 80/53) is a potent antimalarial analogue of Primaquine that causes only one-third of methaemoglobinaemia, as compared to Primaquine and therefore considered three to four times safer for human consumption. Following Primaquine and Bulaquin dosing, affected genes were identified. Based on upregulation and down regulation of expression of genes at global scale, affected pathways were identified. Additionally, the data were compared with traditional biochemical markers and histopathology of liver.

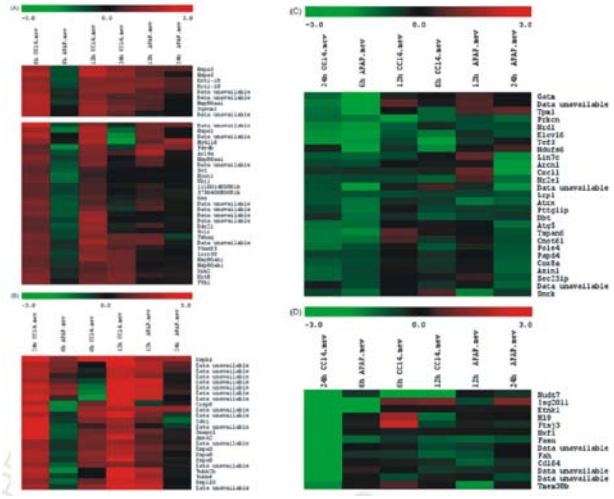
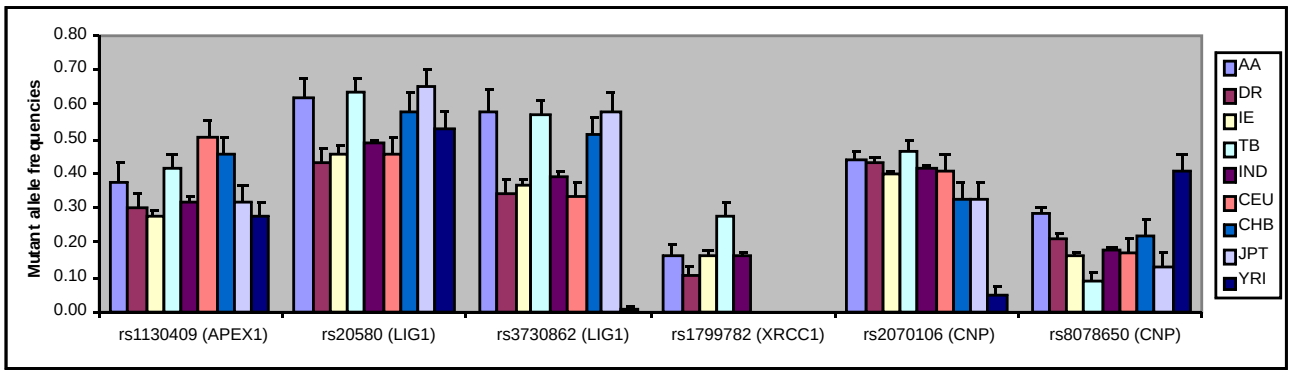


Fig. : Part of *k*-means clusters showing statistically significant ($p < 0.01$ and twofold) up regulation (red) and down regulation (green) of genes

The results obtained in the present work indicate that Primaquine and Bulaquin lead to transcriptional alterations in the hepatic genes after acute dosing and can be used as a signature of hepatic tissue response in the absence of traditional markers of hepatic stress *[Toxicology 239 (2007) 96107; Basic & Clinical Pharmacology & Toxicology 103 (2008) 522-9; Environmental Toxicology and Pharmacology 26 (2008) 150-16].*

### 2.2.4 Single nucleotide Polymorphisms associated with cancers

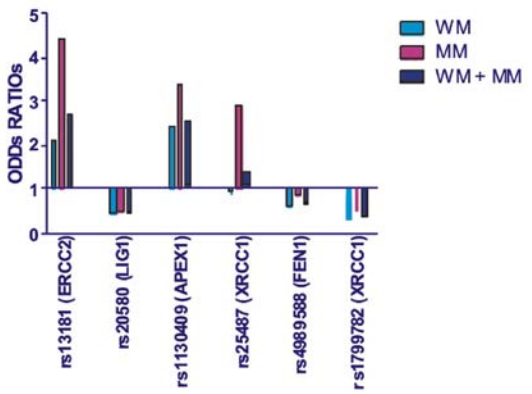
Genetically isolated populations are considered




Comparison of mutant allele frequencies within the Indian population and between the Indian linguistic subpopulation clusters and the world population data obtained from Hapmap project AA-Austro-Asiatic, D- Dravidian, IE- Indo-Europeans, TB- Tibeto-Burman, IND_ Overall mutant allele frequency of the Indian population, CEU- CEPH (Utah residents with ancestry from northern and western Europe), YRI- Yoruba in Ibadan, Nigeria, JPT- Japanese in Tokyo, Japan , CHB- Han Chinese in Beijing, ChinaNo Hapmap data was available for the SNP rs1799782 (XRCC1)

to be important in dissecting complex diseases and mapping underlying genes. The Indian Genome Variation Consortium (IGVC) was set up by CSIR to build a resource that would enable us to address such questions. The investigation was conducted in 55 Indian populations representative of the ethnic, linguistic and geographic diversity of India based on 405 SNPs, selected from a set of 75 genes spread across all chromosomes and a 5.2 Mb segment of chromosome 22 spanning 49 genes. This is the largest single study conducted on Indian populations in terms of numbers of populations, candidate disease genes and biparental SNPs assayed *Journal of Genetics 87 (2008) 320*. The Genotoxicity laboratory of CDRI participated extensively in this programme. In continuation to this programme, we have studied DNA repair genes, which are gaining worldwide attention as low penetrance candidate genes for genetic association studies on human cancers owing to their critical role in the maintenance of genome integrity in both somatic and germinal cells through the minimisation of replication errors, removal of DNA damage and reduction of deleterious rearrangements arising through aberrant recombination.

Mutations in DNA repair genes in the form of single nucleotide polymorphisms are suggested to be involved in the modulation of DNA repair capacity may cause reduction of activity leaving the genome unrepaired resulting in genomic instability and cancer and therefore, their relatedness to cancer risk is being increasingly explored. Hence, selected nonsynonymous SNPs located in the exonic regions of some vital DNA repair genes were further analyzed using Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing analysis for association with the risk of Squamous Cell Carcinomas of the Head and Neck (SCCHN) and Breast cancer in a subpopulation cluster matched (Indo-Europeans + Caucasoids) case-control based genetic association study among north Indian subpopulations. Gene expression analysis was performed to assess the overall change in expression of the selected genes among SCCHN patients with respect to normal healthy controls.

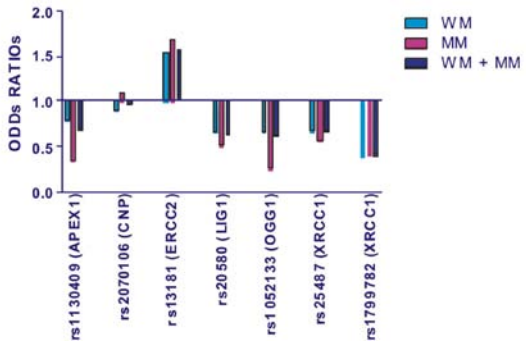



Representation of genetic association of selected SNPs with the risk of Breast cancer determined in terms of odds ratios of mutant genytypes

OR>1 denotes positive asociation

OR<1 signifies protective/negative association with Breast cancer risk

## 2 Area: Preclinical Safety Evaluation and Regulatory Toxicity




Representation of genetic association of selected SNPs with the risk of SCCHN determined in terms of odds ratios of mutant genotypes

OR>1 denotes positive association

OR<1 signifies protective/negative association with SCCHN risk

Eight genes, mostly involved in the human DNA repair mechanism, viz. Apurinic/apyrimidinic Exonuclease/endonuclease (APEX1), Flap Endonuclease 1 (FEN1), Proliferating Cell Nuclear Antigen (PCNA), DNA ligase I (LIG1), X-ray repair Cross Complementing in Chinese Hamster 1 (XRCC1), human 8-oxoguanine DNA glycosylase (hOGG1), Excision Repair Cross-Complementing Rodent Repair Deficiency, Complementation Group 2 (ERCC2) and the gene 2',3'-Cyclic Nucleotide 3' Phosphodiesterase (CNP) (refer to above figures), were selected for the study mainly on the basis of their relevance as functional and positional candidates in many cancers and complex diseases [*Oncology Research 17 (2008) 127-135].*

# Project Area Studies

# Biological Screening 1

(Coordinator: Dr. Sudhir K. Sinha)

*The main objectives of this project area are: (a) anti-TB screening (b) anti-cancer screening (c) high-throughput screening (HTS) and (d) development of new screening models.*


1.1 Screening for anti-tuberculosis activity

1.2 Screening for anticancer activity

1.3 Target based screening of compounds active against osteoporosis, cancer and diabetes

1.4 Identification of drug targets for breast cancer


## 1.1 Screening for anti-tuberculosis activity

Over 2500 new samples (1997 extracts from plants/microbes under CSIR Networked Project, 232 marine extracts under MoES project, and 284 molecules synthesized by CDRI) were screened for anti-TB activity. In the *in vitro* assays (Microplate Alamar Blue and Agar Microdilution), 2 plant extracts were considered active against *M. tuberculosis* H37Rv, with a minimum inhibitory concentration (MIC) of 25 and 50 µg/ml. On the other hand, 18 synthetic molecules showed an MIC of = 3.12 µg/ml. The active molecules did not show *in vitro* cytotoxicity towards Vero cells or mouse bone marrow derived macrophages.

Twelve of the *in vitro* active synthetic molecules were evaluated using the *ex vivo* mouse macrophage model of TB. When used at their 4 x MIC (in vitro), they showed 5 to 40 fold reduction in the intracellular colony forming units (CFU) of *M. tuberculosis.* These results indicated that the molecules were able to reach and kill the bacilli residing within the phagocytic vesicles. Also, the intracellular *(ex vivo)* efficacy of molecules was variable even though all were similarly effective *in vitro.*

Ten active molecules (including 5 repeat samples) were subjected to *in vivo* screening in the mouse model of TB, of which 3 have shown a statistically significant ($P<0.01$) enhancement in the mean survival time (MST) of animals infected with *M. tuberculosis* H37Rv. Further, the active molecules showed >10 fold reduction in CFU of the lungs of infected mice.

Two purified fractions - RTR101 and RTR128 of *Withania somnifera* (Ashwagandha, being studied under a CSIR Networked Project) had earlier shown an immunostimulatory activity. Based on this finding, the prophylactic potential of these fractions was determined against *M. tuberculosis* infection in mice. Both fractions were given at 30 mg/kg oral dose for 14 days, following which the animals were infected (i.v.) with *M. tuberculosis* H37Rv. MST was 17.16 days for the group of untreated mice, 20.14 days for RTR101 treated mice and 20.85 days in RTR128 treated mice. However, the difference in MST between control and experimental groups was not statistically significant.

## 1.2 Screening for anticancer activity

Approximately 600 new samples were screened for their anticancer activity *in vitro* using a set of cancer cell lines procured from ATCC. Initially, the solubility of extracts in aqueous medium was determined by laser nephalometry and only the soluble ones were bio-evaluated. Samples showing 80% growth inhibition (at 50 µg/ml culture concentration) by MTT or SRB assay were considered as 'hits' and categorized according to their selective

cytotoxicity, using Vero cells as a 'non-cancer' control. With these criteria, 3 of the synthetic molecules showed activity against cervical and breast cancer cell lines and were selected for further evaluation. 23 Marine samples showed selective activity in the initial screening which is being confirmed by repeat collection.

### 1.3 Target based screening of compounds active against osteoporosis, cancer and diabetes

Mammalian two-hybrid/one-hybrid and cognate reporter based assay systems have been successfully optimized for screening a number of nuclear receptors, viz., GR, ERs, PRA and B, TR, ERRs, FXR, LXRs, PPARs, VDR, HNF4, LRH-1, SF-1, SHP and Dax-1.

CDRI molecule 1020-K095 has shown both osteogenic and anti-osteoclastogenic activity in rats. Hence it is a potential candidate drug for osteoporosis. Following screenings, it was found that this compound is a specific agonist for estrogen receptor (ER) alpha and beta and activates these receptors at concentrations comparable with estradiol. However, this compound does not show any estrogenic/anti-estrogenic action in uterus indicating an alternative mechanism. Detailed study into the potential role of 1020-K095 and its synthetic analog 1709 in bone-morphogenic protein receptor type 1 (BMPR1) is also being investigated as 1709, despite being a selective ER beta agonist, also shows osteogenic properties. Moreover, further screening of in-house synthesized osteogenic analogs of 1020-K095 and 1709 has been done and they were found to show distinct levels of ER agonism. Their detailed mechanistic aspects are also being studied.

Another osteogenic CDRI molecule 914K058 was found to be an aryl hydrocarbon receptor agonist and its mechanism of action is being elucidated.

CDRI compound S-006-1485 inhibits growth and induces apoptosis in both ER-positive and ER-negative breast cancer cells. This compound was found to be a specific agonist for the orphan nuclear receptors ERR alpha, beta and gamma. This compound has also shown a better anticancer activity than the published ERR agonist DY-131. Its detailed mechanism of action is being studied by RNAi and real-time PCR methods.

A number of compounds, showing anti-hyperlipidemic activities, were screened in cell-based assays for their PPAR and LXR modulating activities. Five compounds were found to modestly activate PPAR alpha or PPAR-gamma or LXRs and potential of these compounds as anti-atherogenic molecules is being investigated.

*In vitro* GST-pulldown-based assay system has been optimized for screening of compounds which disrupt p53-MDM2 interactions. This assay is extremely important in screening anti-cancer drugs which aid to apoptosis by stabilizing p53.

### 1.4 Identification of drug targets for breast cancer

Tamoxifen is widely used as endocrine therapy. However, the molecular mechanism of its action is not clearly known. ER alpha positive breast cancer cell line MCF7 cells were induced with tamoxifen (for 24 hrs) and whole cell extracts were subjected to 2D gel electrophoresis. The differentially appearing proteins were excised, trypsin digested, and identified by LC-ESI based mass spectrometry. A total of 17 proteins were identified, few of them being already known to be regulated by modulation in their expression and a role in breast cancer. These include Ring Finger Protein 17 and Ubiquitin Ligase E3A isoform (UBE3A) which are reported to be up-regulated in ER alpha positive breast cancer.

The initial proteomic screening has suggested that tamoxifen down-regulates UBE3A isoform. It was also shown, with the help of RT-PCR and western blotting, that tamoxifen down-regulates UBE3A in MCF7 cells at mRNA as well protein levels. Using immuno-precipitation, we further showed that tamoxifen indeed down-regulates UBE3A in these cells, as the amount of precipitated UBE3A protein

was significantly lower in the treated cells. These data suggest that UBE3A might be a target of tamoxifen and may be used for development of targeted therapeutics against breast cancer.

Interacting proteins of ER alpha, which may be modulating its function to contribute in breast tumor pathophysiology, are being screened. For this, both in vitro (GST-pull down) and *in vivo* (co-immunoprecipitation) approaches are being carried out. Both GST empty vector and GST tagged ER alpha from bacteria have been purified and GST pull down is underway.

# 2 Cardiovascular, Central Nervous System and Other Disorders

(Coordinator: Dr. Ram Raghubir)

*The research activity pursued under the above project area includes design, synthesis and development of new drugs for various diseases of cardiovascular system (stroke, thrombosis and hypertension), central nervous system (dementia and stress) and other disorders (diabetes, lipid disorders, inflammation and gastric ulcers). The project area also covers regulatory pharmacological studies of the candidate drugs, development of suitable, better and predictable screening models for the evaluation of plant and marine extracts, fractions and synthetic compounds. Besides, this neuro-chemical and molecular investigations are also vigorously persuaded for developing newer molecular targets for drug discovery and analyzing the possible mechanism(s) of action of newer drugs. Developing new target based assays is of prime concern which may eventually help in the development of new target based drugs.*


2.1 Cardiovascular System

2.2 Central Nervous System

2.3 Other Disorders


## 2.1 Cardiovascular System

### 2.1.1 Development of Anti-stroke Agents

Seventeen synthetic, two NMITLI extracts, three single molecules of genomic extract NMITLI-118 R and Gugulipid were subjected to bio-evaluation for anti-stroke activity using MCAO model.

### 2.1.2 Anti-stroke potential of CDRI molecules

The neuro-protective efficacy of synthetic compounds S-006-1510, 1506, 1512, -1514 was assessed by administering them at 50 mg/kg p.o. and CDR-134K211 at 50 or 100 mg/kg p.o. 1 hour prior to MCAO and 3 hours post reperfusion. None of the compounds demonstrated significant anti-stroke potential.

### 2.1.3 Anti-stroke activity of Gugulipid

Anti-stroke potential of Gugulipid was evaluated in the focal cerebral ischemia model in rats. A dose of 25-50 mg/kg p.o. was administered 6 hours post reperfusion. It produced dose dependent neuroprotective effect with significant reduction in cerebral infract volume, blood MDA level, which was reflected by decrease in neurological deficit. The elevation in GSH level is also indicative of reduction in oxidative stress by Gugulipid. Thus Gugulipid seems to be a potent anti-stroke agent; significantly effective even 6 hours post ischemia/reperfusion injury.

### 2.1.4 Anti-stroke potential of Withania

The brain uses large quantity of oxygen making it particularly susceptible to oxidative stress and that this has been implicated as one of the major cause for neuronal cell death in a number of neurodegenerative disorders including stroke. We have investigated the neuroprotective effect of *Withania somnifera* extract, NMITLI-118 R. This was administered at the doses of 50 mg/kg and 100 mg/kg 1 hour before MCAO and 3 hour post reperfusion. The neurological deficit, blood MDA and GSH levels were estimated after ischemic insult. Further,

## Antistroke Potential of Bioactives from Nature

'Cerebral Stroke' is third leading cause of death and long term disability worldwide. Disappointingly, over a hundred neuroprotective drugs have shown significant efficacy in preclinical studies but were found ineffective in clinical trials in humans

Since dawn of civilization, nature has served as a best source of medicines to treat different ailments. As a part of ongoing drug development programme, CDRI has focused research on neuroprotective potential of bioactives from plants.

I. NMITLI 118R: It offered neuroprotection on pre as well as post treatment in rat model of focal cerebral ischemia. Administration of 50 and 100 mg/kg NMITLI 118R 1 hr before ischemia showed almost 80 % decrease in cerebral infarct. Further, a dose of 100 mg/kg also showed significant neuroprotection post ischemia. Besides decreasing infarction size, NMITLI 118R also enhanced cerebral blood flow significantly.

II. Withanolide: It is a single molecule obtained from this very extract and produced more marked neuroprotective effect.

III. Guggulipid: A CDRI lipid lowering drug recently showed promising memory improvement in experimental model of memory deficit and also offered significant neuroprotection in experimental stroke in view of its antioxidant action and presence of phytosphingosine.

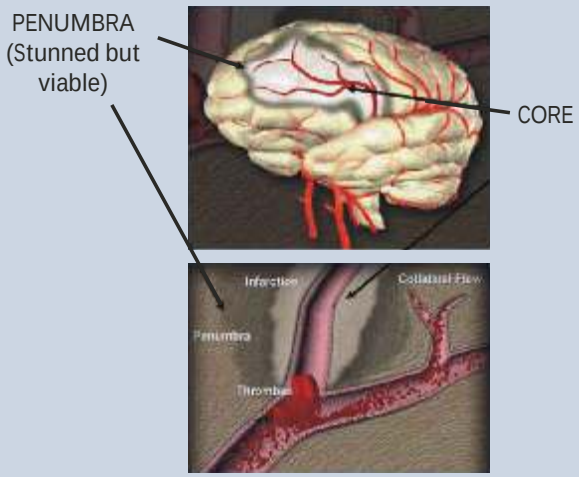



Fig 1. A view of Ischemic Stroke

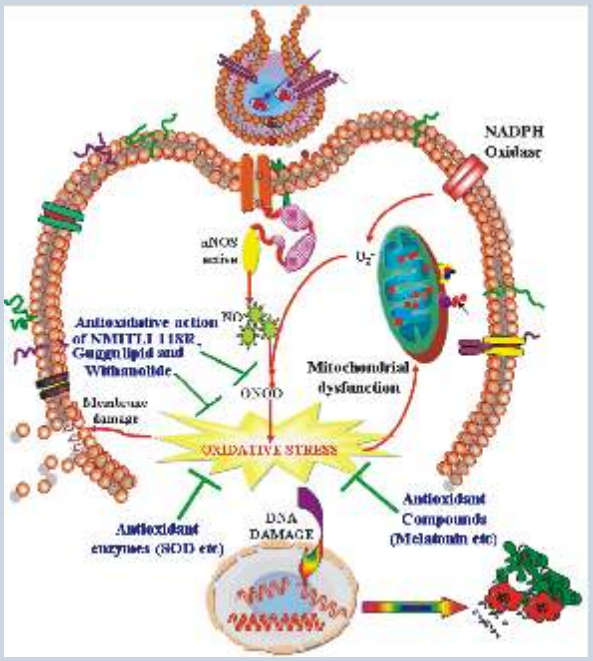



Fig 2. Possible mechanism of neuroprotective effect of NMITLI 118R, Withanolide and Guggulipid

Publication

1. Nakka VP, Gusain A, Mehta SL, Raghubir R. Molecular mechanisms of apoptosis in cerebral ischemia: multimultiple neuroprotective opportunities. Mol Neurobiol. 2008; 37:7-38.

2. Mehta SL, Manhas N, Raghubir R. Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev. 2007; 54:34-66.

### Patents for memory enhancing effect of Guggulipid

1. US Patent: 6896901
2. European Patent 1224938

brain sections were stained with triphenyl tetrazoluim chloride (TTC) and the damage manifested as infarct was quantified using Biovis Image Analysis. Brain of NMITLI-118R treated animals was also used for staining with haematoxylin and eosin to access the brain cellular damage. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) was also used to assess apoptotic cell death.

Withanone, a single molecule of NMITLI-118R, was also used at 50 mg/kg and 100 mg/kg p.o. post treatment at 6 hours of reperfusion. Pretreatment with withanone prevented motor impairment and significantly decreased the elevated level of MDA. It was also observed that TUNEL positive cells indicating DNA fragmentation was also significantly decreased in striatum followed by cortex in ischemic rats at 2/24hr of I/R injury.

### 2.1.5 Effect of NMTLI-118R on blood flow and brain damage in rat model of focal cerebral ischemia

In this study, effect of NMTLI-118-R on blood flow and brain damage was studied in rat model of focal cerebral ischemia. Male SD rats were subjected to 1 hr ischemia followed by 24 h of reperfusion. Rats were assessed for neurological deficit (ND) before sacrifice. Brain sections were stained with TTC and the cerebral infarct was quantified using Biovis Image Analysis. Blood flow of rats was monitored before and during ischemia and after reperfusion injury at different time points.

NMTLI-118R was administered at a dose of 50 mg/kg p.o. one hr before ischemia. Results show that there was significant damage to brain by 1hr of ischemia followed by 24 hr of reperfusion. Oral administration of NMTLI-118R significantly decreased infarct size and ND. Before MCAO, blood flow in cortical region was 424 ± 80.77 blood perfusion units (BPU). During ischemia, there was 83.16 ± 5.7 % decrease in blood flow in cortical region in MCAO control rats. Pretreatment of rats with NMTLI-118R, increased blood flow to 848 ± 69.05 BPU (a 200 % increase). During ischemia, blood flow decreased by 70.59 ± 5.44 % only. The rats pretreated with NMTLI-118R showed an enhanced blood flow as compared to control MCAO rats at varying time points. These results indicate that an increase in cerebral blood flow may impart neuroprotection in NMTLI-118R treated rats.

### 2.1.6 Development of anti-thrombotic agents

Total 63 compounds were tested at 30 µM/kg (po) against collagen in adrenaline induced thrombosis in mice. Nine compounds exhibited appreciable protection against thrombosis.

### 2.1.7 Anti-thrombotic molecules under product development

Active molecules S-000-20 and S-002-333, picked up by anti-thrombotic screening in various *in vitro* and animal models, were evaluated in athero-thrombosis model. D- and L- enantiomers of these compounds were synthesized and evaluated *in vitro* and *in vivo* for the anti-platelet activity. During this period, work was undertaken on S-000-20 and its enantiomers. Among the two, S-007-867 seems to be better and is being evaluated as a potential anti-platelet agent for its efficacy in various *in vitro* and animal models of thrombosis and athero-thrombosis.

### 2.1.8 Studies on GTP formulations

Two GTP formulations (GTP-0008 and GTP-0125) were tested for their BP lowering effect in rats. BP lowering effect of GTP-0125 was evident in conscious spontaneously hypertensive rats following 30 days treatment as assessed by non invasive blood pressure (NIBP) monitoring/recording system.

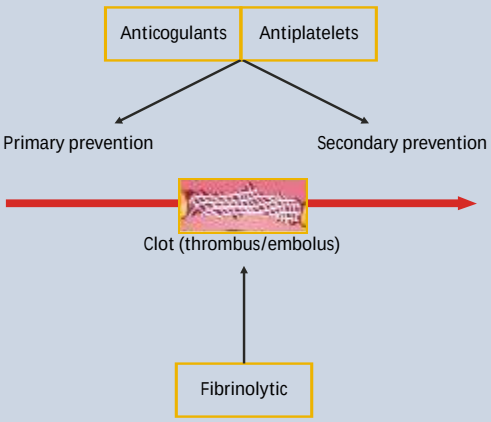
### 2.1.9 Myocardial ischemia reperfusion model in rat

An animal model for myocardial ischemia by the ligation of left anterior descending (LAD) coronary artery and the removal of ligature to induced reperfusion injury was standardized in anesthetized Sprague-Dawley male rats. A prominent infarct zone in the myocardium of rats was observed after 30 min to 3 hrs. Serum levels of CK-MB and LDH were significantly increased after reperfusion. The TTC staining showed approximately 20% infarct in all experimental animals while control animals

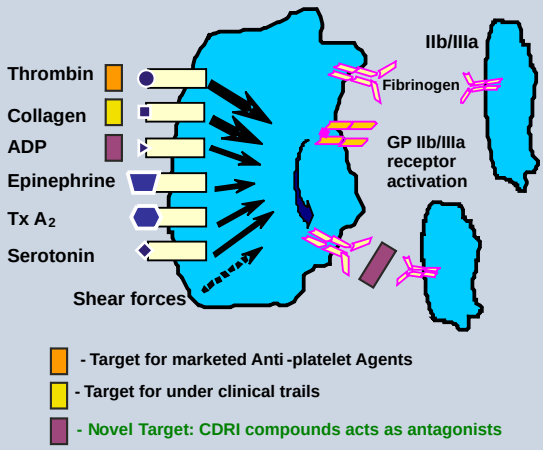
## Anti-thrombotic drug development at CDRI

Anti-thrombotic drugs, include anti-platelet drugs, anticoagulants and thrombolytic drugs, which are used for prevention and treatment of ischemic events following intravascular thrombosis. These include acute coronary syndromes (ACS), myocardial infarction (MI), stroke, venous thromboembolism (VTE) and peripheral arterial occlusion (PAO). Under the anti-thrombotic drug development programme at CDRI, few compounds have been identified which interfere with collagen induced platelet activation, a novel target for new anti-platelet drugs.

The efficacy of CDRI synthetic compounds (S-000-20 (enantiomer S-007-867) and S-002-333) has been compared with standard anti-platelet drugs (Aspirin, Ticlopidine and Clopidogrel) in various experimental models of thrombosis in mice, rats, hamster and rabbits as well as against collagen induced human platelet adhesion and aggregation. Efficacy of these compounds was also compared with standard anti-platelet agents in athero-thrombosis model in hamster. These compounds exhibit anti-adhesive properties which is not seen in other anti-platelet agents, while anti-thrombotic efficacy was either comparable or better than marketed anti-platelet agents in various test models used for the evaluations at CDRI




Intra-vascular thrombus formation and its prevention by anti-coagulant (heparin, warfarin), anti-platelet (aspirin, ticlopidine, clopidogrel) and fibronlytic (streptokinase, urokinase) drugs




Mechanism of action of platelet aggregation and target of new and existing anti-platelet agents

exhibited uniform deep red colored TTC staining with no sign of infarction.

### 2.1.10 Macrophage foam cell formation in Atherosclerosis

Macrophage foam cell formation is a critical and hallmark feature of atherosclerosis. Efforts were made to elucidate the role of macrophage and its scavenger receptors in foam cell formation, lesion development and endothelial dysfunction.

Monocytic THP-1 cells were treated with PMA and Ox-LDL to generate foam cells. Differentiation of the monocytic cells to macrophages was evident by a significant increase (2 fold) in the Cd11b expression. Differentiated cells showed enhanced foam cell formation (> 2 fold) on Ox-LDL treatment. Semi-quantitative RT-PCR analysis suggested a significant increase in CD-36 (>10 fold) and CD-68 (>3 fold) mRNA expression on PMA treatment. Although a trend of increase in cla-1 expression was observed, it was not significant. Ox-

LDL treatment further potentiated the CD-36 expression (>2 fold) however no change in cla-1 and CD-68 was observed. FACS analysis of CD-36 also showed up-regulation of this receptor (2 folds) both after PMA and Ox-LDL treatment. Pretreatment with lovastatin (10µM) reverted the PMA and Ox-LDL induced CD-36 up-regulation both at mRNA and protein levels. Macrophages isolated from high cholesterol high fat (HCHF) treated hamsters showed enhanced macrophage foam cell formation as evident by the significant increase (3 fold) in the cellular lipid contents. A significant increase (around 3 fold, $p<0.001$) in the atherosclerotic lesion formation was observed as assessed by staining the aorta with lipid specific Oil Red O dye indicated enhanced macrophage foam cell formation in HCHF fed hamsters. At the same time, a significant reduction in relaxation induced by acetylcholine (3nM to 30mM) ($p<0.001$), assessed by aortic ring and organ bath studies, showed endothelial dysfunction in the HCHF treated hamsters. Results from the present study indicate up-regulation of specific macrophage scavenger receptors and foam cell formation under hyperlipidemic conditions that may lead to endothelial dysfunction.

### 2.1.11 Antihypertensive activity

Under CSIR-Network project, 743 samples and 10 plant extracts were tested for their antihypertensive activity in anesthetized and conscious SHR rats by Data Acquisition System and NIBP system respectively. These materials did not show any significant antihypertensive activity.

### 2.1.12 Discovery groups

Antihypertensive activity of RJM/0035/P10/A001/F003/K002A was studied by NIBP in SHRs. Oral administration of 50 mg/kg RJM/0035/P10/A001/F003/K002 decreased blood pressure by 15.00 ± 5.51 % for nearly 3 hours, which returned to baseline in four hours. Antihypertensive activity of RJM/0035/P10/A001/F003/K002B was studied on Grass polygraph in SHRs. Oral administration of 5-10 mg/kg RJM/0035/P10/A001/ F003/K002B on i.v. administration produced fall in blood pressure, which was short lived and produced tachyphylaxis.

An active marker K002 produced dose dependent fall in blood pressure on iv administration but at 50 mg/kg orally was not effective.

### 2.1.13 Basic Studies in CVS

#### 2.1.13.1 NADPH Oxidase and its role in cerebral ischemia/reperfusion injury

Cerebral stroke is a clinical syndrome caused by impairment of cerebral blood flow due to temporary or permanent disruption of blood supply to the brain. It leads to a number of hemodynamic, biochemical and neurophysiologic alterations, which brings behavioral and pathologic disturbances. Reperfusion i.e. restoration of blood supply to the ischemic tissue which results in oxidative stress due to staggering increase in Reactive Oxygen Species (ROS). Over the last decades, a vast number of studies showed that the main source for ROS induced damage is NADPH Oxidase (NOX). It is a transmembrane multimeric enzyme and have important role in pathophysiology of cerebral ischemia.

The study was conducted on male SD rats subjected to 1hr MCAO followed by different time points of reperfusion injury viz. 0, 3, 6, 12, 24, 72 and 168 hr in SD rats. Cerebral blood flow was measured before and during ischemia and after reperfusion using laser doppler flow meter. The results showed 80% decrease in cerebral blood flow after ischemia. Blood pressure was also measured before, during ischemia and after reperfusion using NIBP (non invasive blood pressure) unit and also by invasive method, a 14% increase in BP was observed and which returned to basal level after approximately 1 hr. ROS and viability assay were analyzed all time points with the help of FACS. Apocynin, inhibitor of NADPH Oxidase was used at a dose of 2.5mg/kg, ip (1, 2.5, 5 mg, iv) at the time of reperfusion to assess role of NADPH oxidase neuroprotective mechanism.

#### 2.1.13.2 Role of acid sensing ion channels in the pathology of cerebral ischemia

Since its inception, excitotoxic theory has been the centre of attraction for developing drug

targets for treatment of cerebral ischemia. But recently, acidotoxicity has also emerged an important mechanism contributing to the pathology of cerebral ischemia. Excitotoxicity is mediated by NMDA receptors, whereas acidotoxicity is mediated by acid sensing ion channels (ASICs) and both cause intracellular calcium accumulation by promoting calcium influx during cerebral ischemia. This burden of calcium inside neurons leads to further aggravation of various signaling pathways causing neuronal death.

Studies were undertaken to analyze the effect of ASIC inhibition alone and in combination with NMDA receptor antagonist in MCAO model of rats. It was found that ASIC inhibition with Flurbiprofen resulted in a significant improvement of neurological deficit, significant reduction in infarct area decreased MDA and nitrite levels. Further, its neuroprotective effect was more pronounced when administered in combination with NMDA antagonist, Ifenprodil. There was further decrease in neurological deficit, infarct area, MDA and nitrite levels. ASIC expression studies were also done and it was found that its expression was increased at late time points of I/R injury (i.e. 6, 12 and 24 hrs post ischemia). These results indicate significant role of acid sensing ion channels in patho-physiology of cerebral ischemia and requires further studies.

2.1.13.3 Modulation of Endoplasmic reticulum stress is neuro-protective after cerebral ischemia

It has been suggested that Endoplasmic Reticulum (ER) stress induced neuronal cell death plays an important role in stroke pathophysiology. Attempt was made to analyze the status of I/R induced ER stress and its modulation on the outcome of ischemic insult in a rat model of focal cerebral ischemia. The brain regions and time dependent changes were seen in the expression pattern of biomarkers related to cytoplasmic dysfunction e.g. HSP70 and ER stress GRP78, Caspase-12, CHOP/GADD153, ATF-4, Processed xbp1 mRNA using reverse transcription polymerase chain reaction (RT-PCR)/western blotting in affected brain regions. Ischemic brain damage was assessed by TTC staining. Apoptotic DNA fragmentation was monitored by TUNEL. It was found that I/R injury induced marked ER stress as evidenced by the upregulation of ER stress related markers. Further, intraperitoneal (1 mg/kg) administration of Salubrinal selectively inhibited dephosphorylation of eIF2α subunit significantly increased the phosphorylation of eIF2α leading to reduced brain damage after I/R injury. Therefore, the treatment with Salubrinal tends to inhibit the ER stress and seems to be neuroprotective after I/R injury, therefore inhibition of ER stress may serve as a novel therapeutic target for neuroprotection.

2.1.13.4 Glutamate Transporters: potential target for neuro-protection

Glutamate induced excitotoxicity and ionic imbalance are the major early events of ischemic brain stress. In order to protect brain cells from glutamate excitotoxicity, glutamate transporters may be of use to sequester it from the extracellular space, hence the present study was undertaken to investigate the role of glutamate transporters and their inducers following cerebral ischemia/ reperfusion (I/R) injury.

The role of glutamate transporters especially GLT-1 and their inducers was analyzed in glutamate homeostasis following cerebral I/R injury. Pretreatment with ceftriaxone and rosiglitazone also showed alteration in GLT-1 protein both at transcription and translational levels following I/R injury. Further, pretreatment with these inducers also lead to significant reduction in cerebral infarct area. The ceftriaxone has also increased the activity of glutamine synthetase enzyme. Pretreatment with GLT-1 specific blocker dihydrokainate abolished the effect of ceftriaxone on glutamine synthetase activity which incidated that the ceftriaxone showed its protective effect by increasing the expression and activity of glutamate transporter protein on plasma membrane of astrocytes.

2.1.13.5 Studies on the role of NF-kB in cerebral stroke

NF-kB is a dimeric transcription factor that has a fundamental role in the processes of normal

development, nerve transmission, immunity, apoptosis, cell survival and inflammation. As cell death and inflammation are the leading detrimental outcomes of ischemia-reperfusion induced damage, it is crucial to have a proper understanding of the role of NF-kB in this setting. However, its role in the pathophysiology of cerebral ischemia is not clear. The activation of NF-kB in terms of its nuclear translocation and DNA binding was explored after 1 hour ischemia and different time points of reperfusion in rat middle cerebral artery occlusion (MCAO) model. The effect of pyrrolidine dithiocarbamate, an NF-kB inhibitor, on the expression of MMP-9 and MMP-2 is being studied in normoglycemic MCAO rats. Also, as hyperglycemia is one of the major predisposing factors for the occurrence of stroke, NF-kB expression was also investigated in hyperglycemic rats subjected to 1 hour MCAO followed by 6 hours of reperfusion. Its expression and nuclear translocation was found to be altered in the brain of hyperglycemic rat MCAO model.

2.1.13.6 Calcineurin activates the mitochondrial dependent apoptotic cascade in focal cerebral ischemia/reperfusion injury

Cerebral ischemia is a consequence of a transient or permanent reduction in cerebral blood flow (CBF) leading to a reduction in adenosine triphosphate (ATP) levels. This causes ionic imbalance and this result into excessive Ca2+ influx, leading to mitochondrial Ca2+ overload, leakage of mitochondrial membrane, cessation of already compromised ATP production, and burst of oxygen free radicals. Elevated intracellular Ca2+ activation of calcineurin (PP2B), a Ser/Thr protein phosphatase.

Efforts were made to delineate the involvement of calcineurin in the modulation of the death cascade following cerebral I/R injury by using MCAO model in SD rats. The study suggested that expression of calcineurin at transcriptional as well as translational level was unaltered following I/R injury of different severity in terms of varying reperfusion time. However, pretreatment with a specific inhibitor of calcineurin, FK506 (1 mg/kg i.v. just after MCAO), has shown protection in terms of reduced neurological deficit scores, lowered MDA levels and reduced cerebral infarct size after 2/24h I/R. Moreover FK506 pretreatment blocked BAD dephosphorylation and abolished activation of mitochondria dependent pathway by decreasing cytochrome c release from mitochondria. There is further evidence that FK506 caused inhibition of cyt.c release from mitochondria, possibly via up-regulation of phosphorylation of BAD, after focal cerebral ischemia and reperfusion. These findings extend previous *in vitro* observations and implicate the involvement of calcineurin activation and BAD dephosphorylation as upstream, pre-mitochondrial signaling events leading to apoptosis in cerebral ischemia/reperfusion injury. Furthermore, activation of the mitochondria dependent apoptotic cascade in cerebral ischemia/reperfusion injury seems to be partly regulated by calcineurin mediated dephosphorylation of BAD.

2.1.14 Studies on neutrophils

(a) Effect of NO on the cell cycle of HL60 cells

Nitric oxide (NO) regulates a wide array of cell functions, proliferation, cytostasis and apoptosis. Role of NO in cell proliferation is however less explored, which has been suggested to be dependent on the intracellular redox status, NO concentration and the cell type. Effect of NO donors (DETA-NO, SNAP and SNP) on the promyelocytic HL-60 cell line was therefore undertaken. NO was found to proliferate HL-60 cells at lower concentration (1-100 µM) as confirmed by thymidine incorporation, BrdU labeling and cell cycle analysis regardless of any effect on the apoptosis, while higher concentrations (250-1000 µM) promoted apoptosis as evident by change in mitochondrial potential, caspase activation, tunnel assay and PI labeling. To investigate further the mechanism involved in proliferation of HL-60 cells by NO, expression profile of various CDK/cyclins including CDK2, CDK6, Cyclin A, Cyclin B, Cyclin D1, p15, P27 was monitored. Expression of Cyclin B was increased at 10-50 µM by DETA-NO, while most of these cell cycle regulators decreased at 1mM DETA-NO. The effect of NO donor was redox sensitive as effect was abolished by pre-treatment of cells with DTT, while NO donor treated cells exhibited

increase in the intracellular GSH content. NO also augmented nitrosylation of various proteins suggesting nitrosylation of important targets. The results obtained indicate NO mediated biphasic modulation of cell cycle in HL-60 cells.

(b) Neutrophil extracellular trap formation by NO

High availability of NO at the inflammatory/ infection site is noticed often with oxidative stress and neutrophil extracellular traps (NETs) contents, but role of NO remains unexplored in NETs formation. Incubation of adhered human neutrophils with DETA-NONOate led to NETs release in a time and concentration dependent manner, as assessed by confocal microscopy and by measuring extra-cellular DNA and NET-bound elastase, which was blocked by N-acetyl cysteine, suggesting role of free radicals. A time and concentration dependent augmentation in free radical formation by NO donors was measured by using DCF-DA. NO mediated formation of NETs and free radicals was significantly attenuated by pretreatment of neutrophils with diphenyleneiodonium, a dual inhibitor of NADPH-oxidase/NO synthase (NOS), 4-aminobenzoic acid hydrazide, a myeloperoxidase inhibitor and 7-nitroindazole, a NOS inhibitor, suggesting enzymatic free radical generation.

(c) Nitric oxide synthase distribution in human blood cells

Molecular expression and catalytic status of nitric oxide synthase (NOS) have been reported in human blood cells with contradictions but the sub-cellular distribution of NOS remains poorly defined. NOS isoforms expression was investigated by RT-PCR and immunoprecipitation (IP)/ Western blot (WB), nitric oxide (NO) formation by L-[H3] arginine and DAF-2DA and NOS sub-cellular distribution by immunogold electron microscopy in human blood cells (neutrophils, PBMCs, platelets and RBCs). Neutrophils and PBMCs expressed all the NOS isoforms, while platelets expressed iNOS and eNOS but RBCs had only eNOS mRNA transcript. IP/WB revealed the presence of nNOS, eNOS and iNOS proteins to a variable extent in all the human blood cells. Highest and lowest NO generation was from neutrophils and RBCs respectively, which was mainly due to the calcium-dependent NOS. However, NOS catalysis in PBMCs and platelets was calcium independent. The relative abundance of NOS isoforms in the human blood cells correlated with NO generation potential. Neutrophils exhibited predominant presence of NOS in the cytoplasm, granules, mitochondria, membrane and nucleus. Treatment of isolated neutrophils with LPS for 4 hrs led to NFκB mediated induction of iNOS expression. RBCs contained NOS in the cytoplasm while, monocytes and lymphocytes displayed NOS distribution in cytoplasm and nucleus. NOS were localized in the cytoplasm, granules and open canalicular systems in the enucleated platelets. We have thus explored and demonstrated the molecular and functional characteristics of NOS isoforms in the human blood cells and their sub-cellular distribution.

2.1.15 Studies on macrophages Inflammation, hyperlipidemia and thrombogenicity in atherosclerosis: role of macrophages

Monocyte derived macrophages play an important role in the initiation and progression of atherosclerosis by participating in inflammatory response and accumulating modified lipids to form foam cells. The objective of the present study was to understand the role of macrophages in atherosclerosis.

THP-1 floating monocytic cells differentiated into adherent macrophage like cells on PMA treatment (200 nm, 72hours). Status of NO, macrophage scavenger receptors, protein kinases, were monitored by conventional methods. Differential expression of macrophage scavenger receptors was observed in these cells and NO levels were significantly increased (~ 3 folds). Differentiated cells showed enhanced foam cell formation at 48 hrs of Ox-LDL treatment (~2 fold). LPS (1 µg/ml, 1, 2, 4, 8 and 12 hours,), Ox-LDL (40 µg/ml, 48 hrs) treated THP cells showed a significant increase in NO levels (~2 fold). Altered expressions of several protein kinases were observed in differentiated cells. Macrophages from high fat (3% cholesterol + 15% saturated fat) fed hamsters also showed significant foam cell formation. Increased plasma total cholesterol

(p<0.001), HDL (p<0.01), LDL (p<0.01), TGs (p<0.05) and lipid positive oil red O staining indicated well developed hyperlipidemia and atherosclerosis. Hypercoagulability of the blood was evident by the significant reduction in prothrombin time (p<0.001), activated partial thromboplastin time (p<0.01), and a significant increase in thrombin induced whole blood aggregation (p<0.05). Results from the present study indicate a role of inflammatory and protein kinase pathway in macrophage foam cell formation and atherosclerosis. Role of thrombogenicity in the above process is still to be deciphered.

## 2.2 Central Nervous System

### 2.2.1 Drug Development

2.2.1.1 CSIR-Coordinated program

(i) Anti-dementia: 33 new samples were tested by scopolamine induced memory deficit in passive avoidance test in mice. Among identified active samples, NBR herbal was evaluated in combination with anti-oxidant in STZ model of dementia in rats. Combination of herbal sample NBR with known antioxidant, Melatonin and with herbal antioxidant, supplied by NBRI, was significantly effective in pr etreatment and post-treatment schedules.

(ii) Anti-depression: 18 samples were tested by swimming despair test in mice. However, none was found to be significantly active.

(iii) Anti-anxiety activity: 9 compounds were screened for anti-anxiety activity using Elevated Plus Maze test in mice and none of them were found to be active.

2.2.1.2 Anti-stress activity:

Seven pure derivatives of Eugenol were screened for their anti-stress activity in AS model, 5 of them were found active. Also 3 extracts (4735, 3833 and 4740A001) were screened, only 4740A001 was found effective in both AS and CUS model. Four formulations (I, II, III and IV) were received under SMM007 project, out of which formulation II, III and IV were found effective against AS model.

2.2.1.3 NMITLI [Ashvagandha]

Samples tested for CNS, memory and anti-depression activity, 2 samples showed significant anti-dementic activity.

2.2.1.4 Appetite suppressants: 15 compounds were tested on scheduled fed rat. One was found active.

2.2.1.5 GTP: Memory enhancing effect of sample GTP-0111 was evaluated by Scopolamine induced memory deficit in passive avoidance test in mice.

2.2.1.6 MOES Project: A total of 161 samples were studied for their effect on gross behavior in mice.

### 2.2.2 Basic Studies in CNS

2.2.2.1 Studies on learning and memory

2.2.2.1.1 Role of brain insulin receptor (IR) in learning and memory functions in rodents

(a) [i] Effect of donepezil and melatonin on brain IR in Scopolamine induced amnesia model in mice

Effect of donepezil (anticholinesterase antidementia) and melatonin (antioxidant) on memory deficit, acetylcholinesterase (AChE) activity and insulin receptors (IRs) level in brain areas of scopolamine induced amnesic mice was studied under this program. Memory was tested by passive avoidance (PA) test in Swiss adult male mice. A significant increase in transfer latency time (TLT) in 2$^{nd}$ trial as compared to 1$^{st}$ trial was considered as successful learning. The results suggest that anti-amnesic effect of donepezil and melatonin may be mediated through enhancement of cholinergic activity and increase in IR level by donepezil and melatonin indicates a possible involvement of brain IR in behavioral functions particularly memory.

[ii] Intra-cerebroventricular streptozotocin induced dementia model

Effect of donepezil and melatonin on brain IRs, memory functions and its correlation with acetylcholinesterase (AChE) activity and oxidative stress in different brain regions were investigated in intra-cerebroventricular (ICV) streptozotocin (STZ) induced dementia model. Memory deficit was found

in STZ group as indicated by no significant decrease in latency time antagonized by donepezil and melatonin. IR protein level (WB) was found significantly increased in trained group as compared to control; whereas STZ decreased IR level significantly as compared to trained rats in hippocampus, which indicates that IR is associated with memory functions. STZ induced decrease in IR was reversed by melatonin but not by donepezil. Melatonin *per se* did not show any significant change in IR level as compared to control. AChE activity (DS and SS fraction) was found to be increased in hippocampus in STZ group as compared to trained which was inhibited by donepezil and melatonin. Increase in MDA level and decrease in GSH level was obtained in STZ group indicating oxidative stress, which was attenuated by donepezil and melatonin. Effectiveness of antioxidant, melatonin but not of anti-cholinesterase, donepezil against STZ induced changes in IR indicates that IR is more affected with oxidative stress than cholinergic changes.

(b) IR gene expression by RT-PCR in different regions of hippocampus in ICV STZ induced dementia model

Hippocampus region of the brain plays a key role in regulating the memory processes. Therefore, the IR gene expression was studied by RT-PCR in different regions - CA1, DG and CA3 of hippocampus. In CA1 and CA3 region, the IR gene expression was found significantly higher in trained (control and CSF treated) groups as compared to control not subjected to training whereas STZ showed significant decrease in IR expression only in CA3 region as compared to trained groups.

2.2.2.1.2 Effect of melatonin on histological changes in brain regions of icv streptozotocin treated rats

The present study was designed to examine the effect of melatonin on intracerebroventricularly (ICV) administered streptozotocin induced neurodegeneration in rats. The results indicate that melatonin is effective in providing protection against neuronal damage induced by STZ (ICV).

2.2.2.1.3 Okadaic Acid ICV induced memory impairment in rat: A suitable experimental model to screen anti-dementia activity

Memory impairment has been reported in the population using sea foods containing dinoflagellates (*Helicondria Okadai*) which are natural source of polyether toxin Okadaic acid (OA). Therefore, it was planned to evaluate OA ICV as an agent to develop an experimental model of dementia. Effect of ICV administered OA was investigated on memory function by water maze test in SD rats. The results of present study indicate that anti-dementia drugs effectively protects against OA ICV induced memory deficit and oxidative stress which are the important factor in dementia. Therefore, OA ICV induced memory deficit can be used as an experimental model for screening of anti-dementia activity.

2.2.2.1.4 Role of Renin-angiotensin system in memory function

Renin-angiotensin system, besides blood pressure regulation, affects learning and memory as evidenced by improvement of cognition in hypertensive patients being treated with AT1 receptor blockers like Candesartan. The present study examined the influence of Candesartan on memory impairment induced by intracerebral streptozotocin (IC STZ) in mice. The results suggest that AT1 receptors facilitates STZ induced memory deficit.

2.2.2.1.5 Effect of curcumin on ICV STZ induced dementia model

Effect of curcumin, a potent free radical scavenger, was investigated against the intracerebral (i.c.) streptozotocin (STZ) model of memory impairment in mice. Treatment with curcumin for 14 days, following STZ (0.5 mg/kg, i.c.) administration, improved memory which was assessed by Morris water maze and step-through passive avoidance tests. Apart from memory deficit, STZ (i.c.) injected mice showed increased oxidative stress as evidenced by increased malondialdehyde (MDA) and decreased glutathione (GSH) levels. Curcumin at dose of 20 and

50 mg/kg decreased oxidative stress by significantly decreasing MDA and increasing GSH levels. Further, curcumin attenuated reactive nitrogen and oxygen species generation in the STZ (i.c.) treated mice. STZ (i.c.) treatment affected cholinergic system by increasing acetylcholinesterase activity which was reversed by curcumin treatment dose dependently. In mice with significant memory deficit induced by STZ (i.c.) administration, post treatment of curcumin (50 mg/kg, p.o.) for seven days improved spatial memory as shown by decreased latency period in water maze test. The improved performance of mice after curcumin treatment was not accompanied by any change in locomotor activity. In view of its efficacy and apparent low toxicity, this Indian spice component shows promise for the prevention of neurodegenerative diseases.

2.2.2.2 Rotenone induced oxidative stress

Rotenone is a pesticide derived from the roots of plants from the Leguminosae family. It is known to induce neurotoxicity and studies related to its effect on different rat brain regions were undertaken. In the study, pattern of rotenone effect suggest that behavioral impairment is not directly related to biochemical changes in all the four regions. Moreover, impairment in mitochondrial enzyme system occurring at 24 hrs can be linked to impaired motor coordination. Oxidative stress can be considered as a late onset process in rotenone induced neurotoxicity. Lack of uniform biochemical response by different doses of rotenone in the brain areas indicate that sensitivity to oxidative stress may not be the prime factor in initiating neurotoxicity while the mitochondrial enzyme changes may play a major role in rotenone induced neurodegeneration.

2.2.2.3 Development of NMDA receptor modulated psychosis model in mice

The primary aim was to develop a psychosis model in mice showing positive symptoms, negative symptoms and cognitive defects as observed in schizophrenic patients. We have used NMDA antagonist, Ketamine at a dose of 100 mg/kg i.p. to develop psychotic symptoms in mice. Positive symptoms have been observed in terms of hyperactivity of animal through digiscan animal activity monitor. Negative symptoms were observed through forced swim test and passive avoidance test was used for investigation of cognitive deficits. To validate this model, we have used typical (Haloperidol) and atypical antipsychotic drugs, which showed improvement in these symptoms.

2.2.2.4 Role of dopaminergic system in stress

In order to elucidate the role of dopaminergic system in stress, we evaluated the mRNA expression of D-1 and D-2 like receptor during acute (AS) and chronic unpredictable stress (CUS) in dopamine (DA) rich brain regions i.e. striatum and frontal cortex. We observed a significant down regulation of D-1 like receptor mRNA expression in frontal cortex in CUS with no change in AS. However, in striatum it was up regulated in both AS and CUS. On the other hand, down regulation of D-2 like receptor mRNA expression was observed in AS and CUS in both the brain region consider in our study. Studies are in progress to further correlate this differential pattern of Dopamine receptor mRNA expression during AS and CUS.

2.2.2.5 Effect of different stressful condition on CRF and POMC gene expression in various brain regions

Evaluation of CRF and POMC mRNA expression profile during acute stress (AS) and chronic unpredictable stress (CUS) in Pituitary (PT), Hypothalamus (HT), Cortex (FC) and Hippocampus (HP) of rat brain, we observed an up regulation of CRF mRNA expression in all four brain regions during CUS, while only in PT during AS. Thus the increased CRF mRNA expression during CUS might reflect the more stress sensitized state. Further, AS significantly up regulated the POMC mRNA expression in PT and HT, and also an up regulation in PT, FC and HP during CUS might suggest its role in stress response as these brain regions are known to be involved in various neurological disorders including chronic stress like conditions such as CUS.

## 2.3 Other Disorders

### 2.3.1 Development of anti-inflammatory agents

One GTP sample and 3 plant extracts were evaluated for anti-inflammatory activity in Carraginin induced paw oedema model in rats. None showed any appreciable activity. Total of 48 samples of NWP-37 have been screened for COX and TNF inhibition activity in whole blood assay and one compound showed mild activity.

### 2.3.2 Effect of Ibuprofen (NSAID) on LPS induced neuroinflammation

Ibuprofen (25-100 mg/kg, po) showed dose dependent protection in normalizing enhanced release of TNF-α, IL-1β, GSH and MDA in Striatum, Cerebral Cortex, Hippocampus and Hypothalamus regions of brain whereas there was no effect AChE activity in LPS induced Neuroinflammation.

### 2.3.3 Effect of cholinergic agonists and antagonists on LPS induced neuroinflammation

The effect of nicotine, a nicotinic receptor agonist; oxotremorine, a muscarinic receptor agonist and methllylcaconotine (MLA), a α7 nicotinic acetylcholine receptor antagonist was studied on LPS induced proinflammatory cytokines in different areas of rat brain. Oxotremorine showed no effect whereas nicotine was able to block the pro-inflammatory cytokines induced by lipopolysaccharide (LPS). MLA was able to antagonize the protective effect of nicotine demonstrating that α7 nicotinic acetylcholine receptors is responsible for attenuation of LPS induced pro-inflammatory cytokines.

### 2.3.4 Effect of antidementia drug donepezil in LPS induced changes in expression of COX-2 and iNOS using western blotting

Donepezil was found to regulate only the iNOS protein expression level induced by LPS, whereas there was no protection on the COX-2 protein expression level induced by intracerebroventricular injection of LPS.

### 2.3.5 *In vitro* studies on neuroinflammation

Astroglial cells (C6) in culture on stimulation by LPS upregulate cytokines, ROS, RNS, expression of inflammatory genes iNOS, COX-2, TNF-α, and GFAP by activating NF-kB and CHOP transcription factors. LPS significantly decreased cell viability and reduced GSH level with increase in ROS generation and NO release. LPS also up regulated the expression of iNOS, COX-2, GFAP, and TNF-α, down regulated mPGES-1 expression via activating NF-kB and CHOP transcription factors. Treatment of Gugulipid increased cell viability and GSH level, reduced ROS and NO production as well as attenuated GFAP, iNOS, COX-2 and TNF-α expression, up regulated mPGES-1 expression and also inhibited NF-kB and CHOP activation in these LPS stimulated astrocytoma cells, indicating anti-inflammatory and antioxidant properties of Gugulipid which may be a useful potential therapeutic drug for neuroinflammation.

### 2.3.6 Role of PPAR-γ in gastric ulcer healing

In the present study we have examined the effect of pioglitazone on MAPK signaling pathways, particularly p38 MAPK and JNK in relation to its ability to attenuate chronic gastric ulcer induced inflammatory responses. Ulceration resulted in a significant increase in the phosphorylation of p38 and JNK signifying its activation. Furthermore, treatment with pioglitazone (40 mg/kg, p.o) significantly inhibited their levels of phosphorylation. Thus the involvement of PPAR-γ in the control of inflammation and inflammatory-gene expression, associated with chronic ulceration, might be mediated largely through their transrepression capabilities which are exerted through phosphorylation of members of the MAPK family like p38 and JNK.

### 2.3.7 Effect of melatonin against experimental reflux oesophagitis

In order to evaluate the inflammatory nature of reflux esophagitis we examined the alteration in mRNA level of pro-inflammatory cytokines TNF-α, IL-1β and IL-6. These cytokines have important role in inflammation mediated tissue damage and are used as sensitive markers for evaluating severity of inflammation. Expression levels of the above mentioned cytokines were up-regulated with induction of reflux esophagitis. It was observed that

RE was associated with over expression of IL-10 and melatonin pretreatment did not affect its expression when compared to RE control.

### 2.3.8 Anti-ulcer activity

2.3.8.1 Elucidation of molecular mechanism of AP76P (WGI 76P) in gastric ulcer healing

In order to evaluate the antisecretory potential of anti-ulcer compounds, we standardized an assay system to estimate proton pump (H+ K+ ATPase) activity using gastric microsomes isolated from rats. Further to validate, the effect of standard anti-ulcer drug, omeprazole on proton pump activity was estimated. It was observed that omeprazole significantly inhibited activity of proton pump within a concentration range of 10-50 µg/ml.

2.3.8.2 Anti-ulcer activity of *Tectona grandis* crude extract

The crude extract of the plant 4483C003 (125 500 mg/kg p.o.) was screened for anti-ulcer activity against cold restraint, aspirin, alcohol, pyloric ligation and histamine induced gastric and duodenal ulcers in all the models. This plant showed significant anti-ulcer activity comparable with standard anti-ulcer drugs. Its 4 fractions (4483/F008, F009, F010, F011) were tested at the dose of 40 mg/kg p.o. against cold restraint induced gastric ulcer. Fractions F010 and F011 were found to be active against CRU and thus was further tested against alcohol induced gastric ulcer model where it showed significant protection.

### 2.3.9 Development of antidiabetic agents

A total of forty eight compounds were evaluated for their effect on oral glucose tolerance in normal rats (SLM) at 100 mg/kg oral dose. Out of these twelve compounds have shown marked improvement (>30%) on oral glucose tolerance in the normal rats. Five of the compounds viz. S-001-469, S-006-1405, S-007-1261, S-005-473 and S-005-1443 earlier found active in SLM showed significant improvement on OGTT in diabetic rats and db/db mice. A number of compounds were evaluated against the five selected enzyme screens (glucose-6-phosphatase, glycogen phosphorylase, alpha-glucosidase, protein tyrosine phosphatase and dipeptidylpeptidase-IV) and *in vitro* effect on glucose uptake by L-6 muscle cells at 10 µM concentration. None of the tested compounds showed significant activity against glucose-6-phosphatase. Out of 55 compounds evaluated against glycogen phosphorylase, 13 compounds showed over 50% inhibition. Further, six out of 89 compounds showed over 50% inhibition on protein tyrosine phosphatase, whereas none among 42 showed any effect on dipeptidylpeptidase-IV. The $IC_{50}$ values of each of the inhibitory compounds have been determined.

Efforts to combat global T2DM epidemics at CDRI


Out of 53 evaluated for their *in vitro* effect on glucose uptake by L-6 muscle cells, seven among these viz. S-006-1506, S-006-1508, S-006-1512, S-007-324, S-007-325, S-007-326 and S007-327 enahanced glucose uptake to the tune of 67.2, 51.9, 51.3, 53.5, 69.5, 33.0, 35.4 and 45.5 at 10 µM concentration compared to 63.9% by metformin (100 µM) and 61.6% by Rosiglitazone at 50 µM. These active compunds will be evaluated for their effect on blood glucose profile in streptozotocin-induced diabetic rats, db/db mice and other validated models.

# 3 Filariasis

(Coordinator: Dr. Shailja Bhattacharya)

*Lymphatic filariasis has been and still is a major public health problem in India. The disease though is not fatal, but in chronic state, it is disabling and a cause of social stigma. Development of a macrofilaricide and/or female worm-sterilizing agent is today's urgent need.*

*The project is being pursued with the objective to develop orally active macrofilaricides and female worm sterilizing agents, to define biochemical and immunological functions of parasites and host and to utilize genomic information in the identification of molecular targets for in vitro screening and rational design of potential antifilarials and also to understand pathogenesis of the disease.*


3.1 Development of macrofilaricidal agents

3.2 Immunological studies on filariasis

3.3 Molecular cloning and characterization of *Brugia malayi* enzymes / proteins

3.4 Evaluation of immunomodulatory activity in synthetic agents and natural products.


## 3.1 Development of macrofilaricidal agents

### 3.1.1 In house project

320 New marine samples were tested *in vitro* and 32 were found active at 31.25 µg/ml against *Brugia malayi in vitro*. Further short listing of samples led to selection of six samples viz. IIC-857A001; AU1-312A001; AU2-440A001; CSM-4147A001; CSM-4151A001 and CSM-4156A001 retaining activity at a lower concentration of 15.6 µg/ml.

### 3.1.2 Follow-up activity of marine samples

CDR-332F012 showed activity on adult *B. malayi in vitro* up to 7.8 µg/ml concentration when tested at serial two fold dilutions starting from 31.25 to 3.9 µg/ml.

### 3.1.3 *In vivo* activity against *Brugia malayi* in *Mastomys coucha*

(a) AU2-370A001 when administered at 500 mg/kg x 5 days by oral route, 50% of the treated mastomys died before completion of observation period. Two mastomys which survived had low worm recovery (6.5% over 18.0% from control) showing 63.9% adulticidal activity with no significant microfilaricidal or sterilization efficacy. At a lower dose of 250 mg/kg, p.o. x 5 days (in *Mastomys* infected with *B. malayi*) the adulticidal efficacy was found to be 61.1%.

(b) AU2-321A001 at 250 mg/kg, p.o. x 5 days in *Mastomys/B. malayi* s.c. model demonstrated ~38.8% adulticidal efficacy.

*(c) CDR-332F012* on oral administration at 250 mg/kg for 5 consecutive days in *Mastomys coucha* infected subcutaneously with *B. malayi,* appeared toxic causing mortality of 3 out of 4 infected treated animals within the observation period. Further testing at a lower dose is underway.

### 3.1.4 *In vitro* activity of new plant extracts against adult B. malayi

Twenty three new CDRI plant extracts were tested at 31.25 µg/ml *in vitro* on adult *B. malayi*, however, only two 4745 A001 and 4726 A001 were found active in both motility and MTT assay.

### 3.1.5 *In vivo* activity of earlier picked up plants against *B. malayi in mastomys*

4726 A001 earlier picked up *in vitro* when administered orally at 1 g/kg x 5 days, showed toxicity in mastomys. Lower dose of 500 mg/kg is now being tried.

Plant 4722 A001 showed promising adulticidal activity in *B. malayi*/jird model. Activity is being confirmed.

### 3.1.6 Network project NWP0037

2775 Products were received during the period of report. 2482 Products screened against *B. malayi* adult worms and 3 ($LC_{100}$: 62.5 g/ml) of them were picked up for *in vivo* activity evaluation.

Fraction F001 of a plant product NBR 0010 P04 (earlier reported to be effective against adult worms) evaluated in *B. malayi*/ *M. coucha* model showed dose related adulticidal efficacy.

### 3.1.7 WHO project: Development of new macrofilaricidal and/or embryostatic agents

#### 3.1.7.1 *In vitro*

One hundred and two compounds, received from WHO, were tested at 10 µg/ml concentration on adult worm and microfilariae of *B. malayi* simultaneously. Of these, 23 were found to be highly active, 23 moderately active while remaining 56 were inactive on adult/mf parasites using both parasite motility and MTT reduction as two activity indicating parameters. In case of microfilariae, 31 were highly active, 15 moderately active and 56 inactive. Majority of these compounds were active against both the life-stages with only a few showing differential activity. $IC_{50}$ and cytotoxicity data ($CC_{50}$) of the active agents was further evaluated.

#### 3.1.7.2 *In vivo*

Clorsulon was tested at 200 mg/kg, sc x 5 days in *B. malayi*/ *M. coucha* model. 4 out of 6 animals showed 19-59% fall in mf count on day 7 post treatment (p.t.), remaining 2 showed rise in mf level over 0 day count. Adult worms remained unaffected. DEC at 50 mg /kg, sc x 5 days produced 83% reduction in mf count on day 7 p.t. but did not affect the adult worms.

### 3.1.8 *Wolbachia* as an antifilarial drug target

#### 3.1.8.1 A comparative antifilarial profile of doxycycline, tetracycline and Rifampicin on human lymphatic filariid *Brugia malayi* in rodent models

Tetracycline and doxycycline were administered orally at various dose schedules in, i) mastomys (*Mastomys coucha*) carrying a subcutaneous infective larval (L3) induced infection and, ii) intraperitoneally L3 infected jirds (*Meriones unguiculatus*) in the close vicinity of adult *B. malayi* and microfilariae in the peritoneal cavity. The micro- and macro- filaricidal efficacies, wolbachial status and fecundity of recovered female worms were also determined at each time frame. Tetracycline at 200 and 20 mg/kg for 90 consecutive days in mastomys and jirds respectively exerted marked antifilarial efficacy with 100% female worm sterility following complete wolbachial depletion. Doxycycline, however, showed 100% microfilaricidal activity with significant adult worm mortality without w*olbachial* clearance or any effect on female worm fecundity in just 60 and 15 days regimen in mastomys and jirds, respectively. The rapid and remarkable antifilarial efficacy of doxycycline achieved in the present study cannot solely be attributed to its anti-rickettsial property and therefore other pharmacological activities, like direct action of this antibiotic may also be involved. Rifampicin had activity similar to tetracycline.

#### 3.1.8.2 Efficacy of liposomized antibiotics alone and in combination

In order to increase efficacy of these antibiotics with minimal toxicity and to reduce the dose amount and its frequency, liposomized formulation of doxycycline and rifampicin were prepared. The efficacy was found to be comparatively

very high over tetracycline and the serum concentration of both remained above their MICs up to 48 hours post injection of a single dose of the formulation. At 10 mg/kg these were administered at every 72 hour in a total of five doses. The formulations alone or in combination with DEC in five doses were very effective against microfilaraemia as well as adult parasites as compared to free drugs. A combination of two liposomized antibiotics with standard drug DEC acted synergistically in providing significantly higher antifilarial efficacy.

## 3.2 Immunological studies

### 3.2.1 Tetracycline treatment targeting *Wolbachia* affects expression of an array of proteins in *Brugia malayi parasite*

We have tried to identify parasite proteins which are affected when *Wolbachia* is targeted by tetracycline. For this *Wolbachia* depleted parasites (*Brugia malayi*) were obtained by tetracycline treatment of infected Mongolian jirds (*Meriones unguiculatus*) and their protein profile after 2D electrophoretic separation was compared with that of untreated parasites harbouring *Wolbachia*. Approximately 100 protein spots could be visualized followed by Coomassie staining of 2D gel, out of which 56 showed differential expressions. These proteins were subjected to further analysis by MALDI-TOF for their identification using NCBI, MSDB and Swiss-Prot databases. Our study unravels two crucial findings; i. the parasite or *Wolbachia* proteins which disappeared may be essential for parasite survival and can be used as drug targets, ii. The down regulated parasite proteins which were stress related can be studied further to understand their role in parasite *Wolbachia* relationship. Work is in progress to identify remaining proteins whose expression is altered.

### 3.2.2 Vaccination with recombinant heavy chain myosin (BmAF-Myo) of *B. malayi*

Immunization with BmAF-Myo resulted in to a significant reduction in microfilarial burden and adult worm establishment accompanied with embryostatic effect in both mastomys and jird models as reported earlier. The findings suggest that immune-protection by recombinant myosin was conferred through both humoral and cellular arms of immunity as indicated by an increased antibody titer with predominance of IgG2a and IgG2b isotypes along with elevated level of IgG1 apart from significant proliferation of lymphocytes, increased nitric oxide production and profound adherence of splenocytes causing cytotoxicity to microfilariae and infective larvae. BmAF-Myo vaccination was accompanied by increased production of Th1 cytokines, down regulation of Th2 cytokines with increased expansion of CD19, $CD4^+$, CD8a and NK cells with increased expression of CD80/86 without affecting CD30 cells. The recombinant *B. malayi* myosin appears to be a promising vaccine candidate against human lymphatic filarial infection.

### 3.2.3 Vaccination with adult *B. malayi* sub cellular fractions

Vaccination with sub-cellular fractions of sub periodic human filariid, *Brugia malayi,* was carried out. Highest level of protection was conferred in animals vaccinated with the mitochondria rich (MT) and nucleus rich (NR) fractions in which microfilaraemia and worm burden were significantly reduced. Mastomys vaccinated with MT and NR fractions displayed a significant increase in antigen-specific serum immunoglobulin G (IgG, IgG2a, IgG2b and IgM), antigen-specific lymphoproliferation along with enhanced release of nitric oxide. There was an increased population of CD4+ and CD8a+ T cells and an elevated level of pro-inflammatory cytokines; interferon gamma (IFN-?), tumor necrosis factor alpha (TNF-a) and interleukin-1 beta (IL-1í ) thus indicating that both NR and MT contain proinflammatory molecules which evoke a protective Th1 type of immune response.

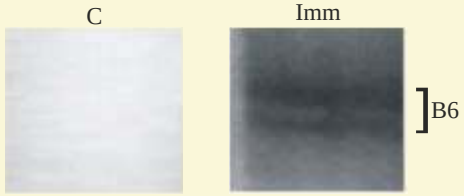
### 3.2.4 Immuno-regulation in experimental *B. malayi* infection in rodents

A single sensitization of mice with either of *Wolbachia* intact or depleted *B. malayi* antigen reduced the T cell responsiveness, which was more pronounced in presence of Wolbachial components in filarial antigen. Multiple sensitizations with *Wolbachia* intact *B. malayi* antigen reversed the immunological scenario characterized by a remarkable down regulation of Treg and CD4

populations expressing CTLA-4 associated with dramatic increase in the total CD4 and CD8. Down regulation in regulatory T and CD4 cells expressing CTLA-4 population indicated selective clonal anergy apparently due to wolbachial presence. It was interesting to observe that macrophages were also affected in the same fashion as T cells showing similar tolerance. Sensitization of peritoneal macrophages with *Wolbachia* intact *B. malayi* antigen also induced nitric oxide release which was down regulated after multiple sensitizations generating the tolerance in macrophages which did not even respond to bacterial LPS.

### 3.2.5 Characterization of inflammation-modulating molecules of *B. malayi* adults

(a) Earlier we have shown that B6 fraction (54.3-67.8 kDa) *B. malayi* adult extract was predominantly reactive with IgG2 and IgG3 of clinical cases and IgG1 and IgG2a of chronic experimental filarial infection. Presently we found that in *M. coucha* immunized with B6, there was mixed Th1/Th2 type responses with greater predisposal towards Th1 response and it imparted protection against establishment of *B. malayi* infection and survival of intraperitoneally implanted adult worms. 2DE and MALDI TOF analysis of the fraction revealed that most of the proteins were immunostimulatory.




Immunoblot of *Brugia malayi* B6 fraction

(b) Three NO stimulating fractions (B8: 45.2-48.6kDa; B11: 33.4-38.4kDa and B12: 28.4-33.4kDa) of *B. malayi* adult extract have also been identified and their immunological profile is being investigated.

(c) A Sephadex G-200 eluted fraction, BmAFI, of *B. malayi* adult worm extract predominantly stimulated release of IL-10 from host cells. Preimmunization of *M. coucha* with BmAFI facilitated the survival and development of 3rd stage infective larva ($L_3$) in the hostile peritoneal cavity (p.c.) of the animals. In non-immunized animals no parasite stage was detectable in p.c.

### 3.2.6 Cross reactive antigens of filarial and leishmanial parasites

Some filarial and leishmanial antigen molecules were identified to be dominantly cross reactive with the sera of filaria and leishmania infected hosts. The molecules are being isolated for further study.

## 3.3 Biochemical and molecular biological studies

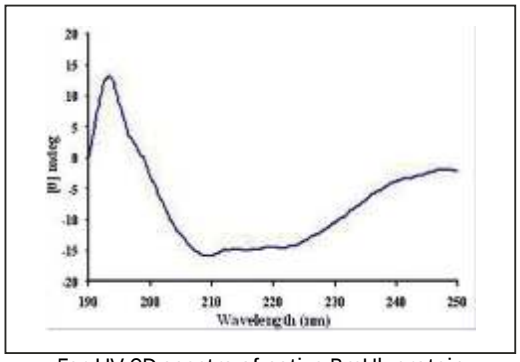
### 3.3.1 Cloning and characterization of antifilarial drug/protein targets

(a) Filaria Hexokinase

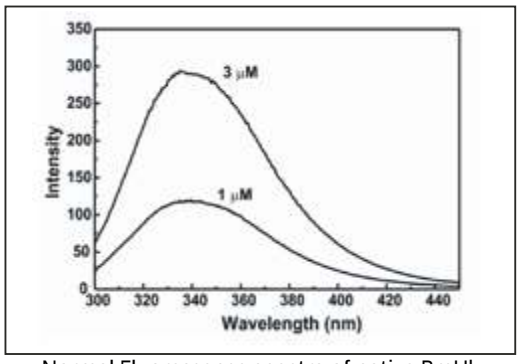
Hexokinase (ATP: D-hexose-6-phosphot-ransferase, EC 2.7.1.1.) is the key regulatory enzyme of glycolytic pathway catalyzing the transfer of a phosphoryl group from ATP to glucose to form glucose-6-phosphate. Full length cDNA sequence derived from 3'RACE and 5'EST of *Brugia malayi* hexokinase was cloned and characterized. The recombinant BmHk is a tetramer with a subunit molecular mass of 72 kDa. Expression of hexokinase in filarial parasite, western blotting was performed using lysate of parasite and microfilariae. Fluorescence microscopy was employed to further support the expression and localization of hexokinase in parasite.

With an aim to get an insight into the structural organization of BmHk, we have carried out a detailed characterization of the structural and functional changes associated with the GdmCl-and urea-induced unfolding of BmHk. The effect of increasing concentrations of GdmCl and urea on the enzymatic activity of BmHk was studied. In case of GdmCl, biphasic curve corresponding to two independent transitions centered at about 0.3 and 0.8M GdmCl respectively were observed, while in case of urea no significant change in enzymatic activity of BmHk was observed up to 1M. However, on

increasing the concentration of urea from 1M to 4M, a sharp decrease in enzymatic activity was observed.




Far-UV CD spectra of native BmHk protein

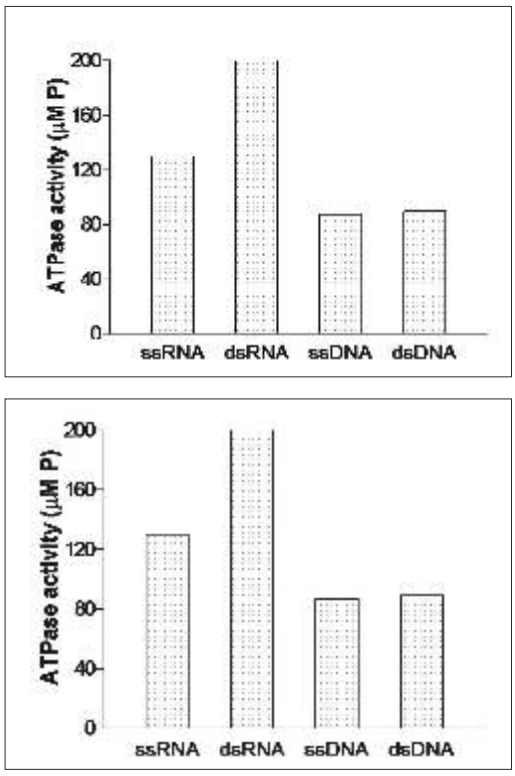



Normal Fluorescence spectra of native BmHk

Far-UV CD studies on denaturant-induced unfolding of BmHk were also carried out to study the effect of denaturant on the secondary structure of the protein. In order to study the effect of denaturant on the structural properties of BmHk, tryptophan fluorescence studies were carried out in the presence of increasing concentrations of denaturant. We also studied the effects of increasing concentrations of GdmCl or urea on the molecular dimension of BmGk by size-exclusion chromatography. The refolding experiments were performed to study the reversibility of enzyme activity of the GdmCl-and urea-denatured BmHk.

(b) DEAD box RNA Helicase of *B. malayi*

DEAD box proteins are putative RNA unwinding proteins found in organisms ranging from mammals to bacteria. We identified a novel immunodominant cDNA clone, BmL3-Helicase, encoding DEAD box RNA Helicase by immunoscreening of a larval stage cDNA library of *Brugia malayi*. The cDNA sequence exhibited strong sequence homology to *Caenorhabditis elegans* and *C. briggasae* RNA Helicase, a prototypic member of the DEAD (Asp-Glu-Ala-Asp) box protein family. The clone also showed similarity with RNA Helicase of *Wolbachia*, an endosymbiotic bacterium of filarial parasite. It was over expressed as ~50 kDa His-tag fusion protein, and ATP hydrolysis assay of recombinant enzyme showed that either ATP or dATP was required for the unwinding activity, indicating BmL3-Helicase as an ATP/dATP-dependent RNA helicase.




ATPase activity of the recombinant His-tag BmL3-Helicase

The recombinant protein also demonstrated cross-reactivity with human bancroftian sera of various disease categories and did not show reactivity with normal sera from non endemic area.

The presence of BmL3-Helicase in various life stages of *B. malayi* was confirmed by immunoblotting of parasite life-cycle extracts with polyclonal sera against the BmL3-Helicase, which showed high levels of expression in microfilaria, $L_3$, and adult (both male

and female) stages.

BmL3-Helicase protein in *B. malayi*


Immunoblotting of L3-Helicase


Immunoblotting with *W. bancrofti* human sera

The three-dimensional model of the protein was constructed based on the crystal structure of human RNA Helicase to assess the structural homology and to facilitate designing of inhibitors. In the absence of an effective macrofilaricidal agent and validated antifilarial drug targets, RNA Helicases could be utilized as a rational drug target for developing agents against the human filarial parasite.

(c) Cofactor independent Phosphoglycerate mutases of *Brugia malayi*

Phosphoglycerate mutases catalyze the interconversion of 2- and 3-phosphoglycerate in the glycolytic and gluconeogenic pathways. They exist in two unrelated forms that are either cofactor (2,3-diphosphoglycerate)-dependent or cofactor-independent. Since the independent form is absent in mammals, it becomes a potential antifilarial drug target. We have cloned and expressed independent phosphoglycerate mutase from human-parasitic nematode *Brugia malayi*. Forward and reverse primers of iPGM flanking the extremes of gene were designed and the gene was amplified using cDNA of *Brugia malayi* adult worms. The amplified product was cloned in PTZ 57 R/T (TA vector). The gene was further sub-cloned in pET28a and transformed in different *E. coli* strains viz. BL21(DE3), Rosetta, and C41 host cells for optimizing expression. Maximum ex-pression of the recombinant protein (~60 kDa) was observed at 37$^0$C at 6h after induction with 1mM IPTG in BL21(DE3). Maximum protein was found in soluble fraction and was purified using Ni-NTA column. Characterization studies are underway.

(d) Trehalose - 6 - phosphate synthase (TPS) of *Brugia malayi*

The synthesis, accumulation and utilization of trehalose by nematodes are important in interaction with their external environment, in egg-hatching, cryopreservation, resistance to desiccation and in osmoregulation and thus may provide new targets for attacking nematode parasites in mammal. One of the enzyme of trehalose synthesis is TPS which was cloned and expressed for characterization. Forward and reverse primers of TPS flanking the extremes of gene were designed and the gene was amplified using cDNA of *Brugia malayi* adult worms. The amplified product was cloned in PTZ 57 R/T (TA vector). The gene was further sub-cloned in pET28a, transformed in different *E. coli* strains and maximum expression of the recombinant protein (~60 kDa) was observed at 37$^0$C at 5h after induction with 1mM IPTG in BL21(DE3). The maximum protein was found in soluble fraction and was purified using Ni-NTA column. Further characterization studies are underway.

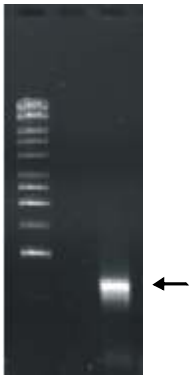
(e) Cloning of Acetylcholinestrase gene from filarial parasites

Characterization of parasite acetyl cholinesterase (AchE) at molecular level requires large quantities of purified AchE and it is very difficult to obtain by conventional methods. Therefore, cloning and expression of filarial AchE was attempted. To identify the gene coding for filarial AchE, PCR was done using AchE specific primers designed from the conserved sequences of AchE from related organisms and immunoscreening of *B. malayi* cDNA expression library with anti-pAchE antibodies.

### 3.3.2 PCR amplification of filarial AchE gene

Earlier we have identified inserts of 0.8 kb and 0.6 kb of AchE gene with *S. cervi* genomic DNA. The phage DNA from *B. malayi* and *W. bancrofti* cDNA library was used with different primer pairs to amplify the AchE gene. Both *B. malayi* and *W. bancrofti* cDNA showed PCR amplification of one insert of 0.8 kb of AchE gene, which was cloned into pGEMT-easy vector. Sequencing of these clones showed homology

to esterase but not to specific AchE sequence. Efforts are in progress to PCR amplify AchE gene using degenerate primers from the conserved AchE sequences (catalytic sites).



PCR amplification of *S.cervi* Ache gene fragment, 1kb ladder in the left lane

### 3.3.3 Immunoscreening of *Brugia malayi* gt11 cDNA expression library

Since we did not get much success with the PCR amplification of AchE gene, the immunoscreening of *B. malayi* cDNA library was done using anti-pAchE polyclonal antibodies showing high reactivity with AchE from filarial parasites but not with host AchE. The plaques (pfu) recognized by the anti-pAchE polyclonal antibody were tested in PCR. Out of 33 plaques picked, 5 pfu (cDNA clones) showed consistent results. Two PCR products of 0.7 and 0.55 kb were identified consistently. The 0.55 kb PCR fragment was cloned in pGEMT vector and sequenced which showed around 50% homology to *C. elegans* AchE and around 30% to *N. brasiliensis* AchE. Cloning and sequencing of the other PCR fragments is in progress and efforts are also under way to amplify the full length AchE gene by RT-PCR using degenerate primers.

### 3.3.4 Characterization of filarial parasite AchE

Efforts were made to test the effect of AchE inhibitors on the purified parasite enzyme in order to optimize the conditions and later on these can be used to study the effect of inhibitors on recombinant AchE. The effect of different concentrations of two inhibitors Bw284c51 (true AchE inhibitor) and Iso-OMPA (pseudo AchE inhibitor) on parasite AchE isozymes was studied spectrophotometrically and the Bw284c51 strongly inhibited (around 80%) the activities of both pAchE1 and pAchE2 isozymes while the other inhibitor Iso-OMPA did not have any significant inhibitory effect on parasite enzyme. The purified parasite enzyme showed Ki of 9.3 and 250 µm for BW284c51 and ISO-OMPA respectively.

The immunoprecipitation of filarial parasite AchE has been done using monoclonal antibody that recognized the parasite AchE bands under non-reducing conditions. The immunoprecipited parasite AchE showed a protein band of ~70 kD on SDS-PAGE. The immunoprecipitated enzyme will be used for N-terminal sequencing studies.

### 3.3.5 Identification and characterization of *S. cervi* antigen/s equivalent to human filarial circulating antigen

In our previous studies on identification of S. cervi antigen(s) equivalent to circulating filarial antigen, we have purified *S. cervi* antigen recognized by the monoclonal antibody detecting filarial circulating antigen. The immunoscreening of *B. malayi* gt11 cDNA expression library with polyclonal antibodies against the purified antigen led to the identification of four cDNA clones. One cDNA clone, showing homology to B. pahangi and Loa loa antigens was sub-cloned in pRSET plasmid vector for the expression of protein. Optimum expression was observed at 0.1 mM IPTG at 37°C for 3 h. SDS-PAGE and immunoblotting analysis revealed a fusion protein of 55 kD. Efforts are under way to purify and characterize the recombinant protein.

## 3.4 Evaluation of immunomodulatory activity in synthetic agents and natural products

Three CDRI plant extracts and 16 synthetic compounds received from outside sources were evaluated *in vitro / in vivo* in preliminary study. Plant extracts, 4740 A001 showed immunostimulant activity while 4742 A001 and 4742 A002, exerted immunosuppressive activity. Three synthetic compounds, MO-01 (at 10 µg/ml), MO-02 and MO-03 (at 100 µg/ml) increased ROS content in peritoneal macrophages in FACS study in concentration dependent manner.

# 4 Leishmaniasis

(Coordinator: Dr. Anuradha Dubey)

*Visceral Leishmaniasis (VL) is a chronic and infectious disease which often becomes epidemic and leads to a heavy loss of human lives in many parts of the world, including India. In the face of new challenges of drug resistance, treatment failures, occurrence of relapses and convergence of HIV related VL cases; there is an urgent need to search for new and better alternatives of chemotherapy. Our program, therefore, envisages screening of synthetic compounds as well as extracts from plants and marine sources for antileishmanial activity, development of diagnostic kit of high specificity and sensitivity, studies in molecular mechanism of virulence and drug resistance and search for newer specific biochemical and molecular targets.*


4.1 Development of potential antileishmanial agents

4.2 Development of reporter gene based screening models/assay system

4.3 Immunobiological studies

4.4 Cloning, over expression and characterization of *Leishmania donovani* drug targets

4.5 Drug resistance mechanism.


## 4.1 Development of potential antileishmanial agents

### 4.1.1 Antileishmanial screening

Novel synthetic moieties comprising 212 compounds representing several prototypes viz. quinoline analogues (aminoquinolines, quinolinooxoacetamides), imidazolopyrimidines, triazines, í-carbolinopyrimidines, chromanochalcones, aryl chalcones, terpenyl chalcones, pyrimidines, styryl pyrimidines, trizinopyrimidine, aplysinopsin derivatives, indole derivatives, pyrazolines, quinazolines, benzylidene derivatives, aryloxylazoles, arylketomethylazoles and oximinoetherazoles were synthesized during the year for their evaluation against *Leishmania donovani in vitro* and *in vivo*.

A total of 544 agents were screened against *L. donovani* infection. Of the 212 synthetic compounds screened *in vitro* against promastigotes, 136 synthetic compounds were found active at 10 µg/ml concentration. Among these, 5 were found cytotoxic and of the rest nontoxic compounds, 89 exhibited significant activity at 10 µg/ml concentration against intracellular amastigotes (80-100 % inhibition in parasite). In addition, concentration response profiles of active molecules were determined for $IC_{50}$ calculation. On the basis of significant sensitivity index (SI), 33 compounds were identified for *in vitro* efficacy evaluation. Several New compounds reprensenting arylketomethylazoles and oximinoetherazoles, quinolinooxoacetamide, imidazolopyrimidines, trizino-pyrimidines, í-carbolinop yrimidines, pyrazolines, quinazolines and

aplysinopsin derivatives have also been identified. SAR studies with these prototypes resulted in selection of a few new analogues having >30 SI. These will be tried for *in vivo* evaluation in hamster model.

Of the 321 marine extracts, 21 extracts were active against promastigotes and of these, only 11 extracts have shown promise against intracellular amastigotes. On the basis of SI, 8 highly active extracts were identified for *in vivo* trial in hamster model. Activity of two extracts, reported active last year, could not be confirmed against amastigotes with repeat samples.

Based on leads from *in vitro* screening results and also compounds/plant extracts received directly for *in vivo* evaluation, a total of 33 synthetic compounds representing five different prototypes were evaluated *in vivo* against *L. donovani* / hamster model. Of these, 14 have shown moderate activity (54-79% inhibition). Out of 11 plant extracts, one crude extract viz. 4554 and its pure compound K037 have shown promising *in vivo efficacy*. The fractions of two marine extracts - CDR-332 and CDR-347 (CDR-332F012, CDR-347F011 and CDR-347F012) which have shown activity *in vitro* have also shown promising activity in hamsters at a dose level of 250 mg/kg x 5.

#### 4.1.2 Follow-up studies with active antileishmanial agents

A pure compound of plant 4555 K009 exhibited >90% antileishmanial activity *in vitro* as well as *in vivo*. Several morphological and biochemical parameters were assessed to confirm the putative pathway responsible for death of *L. donovani* on treatment with this pure compound. It was found that 4555 K009 is mediating apoptosis-like cell death in *Leishmania* parasite. Various assays like exposure of phosphotidylserine, tunnel assay, DNA fragmentation confirmed our observation. Further studies are ongoing on target site of this compound.

#### 4.1.3 Combination therapy

There are currently no vaccines for either VL and CL. Chemotherapy is inadequate and suffers from problem of drug resistance and severe toxicity. So, there is an urgent need for development of new drug combinations. In the present study, a rational approach was adopted which can modulate the immune response to overcome the negative control systems and boost the positive killing responses. Efficacy of antileishmanial agents viz. paramomycin and miltefosine alone and in combination with a potent immunomodulator, picroliv (a standard preparation from the roots of *Picrorriza kurrooa*) in different dose regimens was evaluated. Picroliv *per se* has shown no antileishmanial efficacy (9.36%) but it enhanced leishmanicidal efficacy of both miltefosine and paromomycin separately. Animals treated with combination of all the three drugs have shown significant enhancement in efficacy (98.8%). Increased lymphocyte proliferation, toxic oxygen metabolite generation and phagocytosis responses were witnessed in the animals treated with this combination. Present study thus establishes the possible use of Picroliv as adjunct to antileishmanial chemotherapy.

### 4.2 Development of reporter gene based screening models/assay system

#### 4.2.1 Stable green fluorescent protein (GFP) transfectants

The application of enhanced and stably expressed GFP in *L. donovani* clinical isolates in the development of antileishmanial drug screening assays using Fluorescence Activated Cell Sorting (FACS) and 96-well microplate fluorometer has been explored. The $IC_{50}$ of several standard antileishmanial drugs were found to be same as observed with other reporter and non-reporter based assays.

#### 4.2.2 Axenic amastigotes of *L. donovani* cells expressing luciferase reporter gene

Luciferase tagged *L. donovani* transformed amastigotes were not able to maintain the luciferase expression both in absence of G418 and after several subcultures.

## 4.3 Immunobiological studies

### 4.3.1 Identification of Th1 stimulatory proteins for immunoprophylactic potential

The characterization of immunomodulatory F2 sub-fraction (89.9-97.1 kDa) by 1-DE and MALDI-TOF-MS have revealed that out of total 18 proteins that have been identified; 5 were hypothetical/ unknown proteins. Other major immunostimulatory proteins were Elongation factor-2, p45, Heat shock protein (HSP)-70, HSP-83, Aldolase, Enolase, Triosephosphate isomerase, Disulfideisomerase and Calreticulin. The cloning and over-expression of these 18 proteins was initiated in order to narrow down the specific immunostimulatory proteins for their evaluation as cocktail vaccine. Out of these, seven have been cloned, sequenced and over expressed.

### 4.3.2 Proteophosphoglycan of *L. donovani*

A partial gene (1.5 kb) of Proteophosphoglycan (PPG3) of *L.donovani* was amplified sequenced and further sub-cloned in bacterial (pET28a) and mammalian expression (pcDNA3) vectors. The PPG expression in *E. coli* Rosetta strain was confirmed by western blotting using anti-His antibody. In mammalian cells (BHK) the expression was checked at mRNA and at protein levels by RT-PCR and fluorescence microscopy respectively and at DNA level from immunized hamster muscle tissues using PCR and southern blotting. This DNA encoding N-terminal domain of *ppg* gene was further evaluated in hamsters as a vaccine against the *L. donovani* challenge. The prophylactic efficacy to the tune of 80% was observed in vaccinated hamsters and all of them could survive beyond 6 months post challenge. The efficacy was supported by a surge in IFN-?, TNF-a, and IL-12 mRNA levels along with extreme down-regulation of IL-4 and IL-10. A rise in the level of *Leishmania* specific IgG2 was also observed which was indicative of enhanced cellular immune response. The results suggest N-terminal domain of *L. donovani* ppg as a potential DNA vaccine against visceral leishmaniasis.

## 4.4 Cloning, over expression and characterization of *L. donovani* drug targets

### 4.4.1 Squalene synthase (SSN)

Squalene Synthase (SSN) enzyme of sterol biosynthetic pathway is an attractive drug target. Truncated primers for LdSSN were designed to purify the protein in soluble form by removing the N and C hydrophobic patches. The cloning and over expression was carried out but none of the recombinant gave overexpressed protein. Hence, the clone having complete ORF of squalene synthase gene was taken and protein was partially purified using phenyl sepharose column chromatography. The catalytic activity of SQS was assayed by measuring the conversion of [$^3$H] FPP to [$^3$H] Squalene. $K_m$ and $V_{max}$ of LdSSN substrates were found to be comparable to the SSN of other trypanosomatids. In order to understand the mechanism of ligand binding and the interaction between the substrates and LdSSN, a three dimensional (3D) homology of LdSSN was made based on the x-ray crystallographic structure.

### 4.4.2 Triose phosphate isomerase (TIM)

TIM activity was assessed in recombinant enzyme LdTIM (fused with Nus tag) using glyceraldehyde 3 - phosphate as a substrate, but enzyme did not show any activity. Further subcloning in pET-28.a (+) vector showed that all the protein was present in inclusion bodies, so further studies were carried out to get protein in soluble form by using various parameters and additives viz. Tween 80, glycerol, sorbitol, sucrose, ethanol and glucose induced with IPTG (0.1 mM-1 mM) at lower temperature (18°C -20°C) but still were unable to get protein in soluble form which was confirmed by the western blot. A very little amount of soluble protein was found when culture was induced with 1 mM IPTG at 0.6 O. D, grown at 20$^0$C in LB media as confirmed by western blot. Optimization of conditions to get more active soluble LdTIM is underway.

### 4.4.3 Arginase (Arg)

Polyamines are ubiquitous organic cations found in virtually every eukaryotic cell and play critical roles in key cellular processes such as growth,

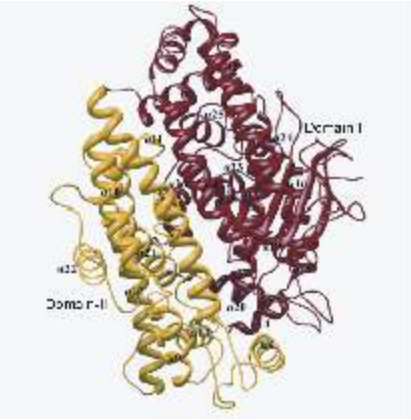
differentiation and macromolecular biosynthesis. Ornithine, the first amino acid from which polyamines are generated is produced from arginine by agrinase enzyme. The lethal nature of agrinase knockouts establishes that *L. mexicana* promastigotes have only a single avenue for ornithine biosynthesis so arginase from *L. donovani* genomic DNA was amplified. The amplicon of 990 bp was cloned in the pGEMT easy cloning vector. The complete sequence of *Ld*Arg ORF (990 bp) was confirmed by nucleotide sequencing of recombinant pGEMT-Arg clone. The nucleotide sequence of *Ld*Arg has been deposited in Gene Bank under Accession No. DQ-649412.

### 4.4.4 Trypanothione reductase (LdTR)

Folding stability of recombinant trypanothione reductase was studied in presence of urea and guanidine hydrochloride. Urea induced unfolding was non-reductive in nature and led to the formation of partially folded intermediate. This intermediate species lacks catalytic activity and characteristic conformation of native LdTR but has significant secondary structure and could be partially reactivated. Guanidine hydrochloride induced denaturation was irreversible in nature. Reactivation and cross-linking experiments clearly demonstrated that the loss of activity at higher denaturant concentrations was coincided by dimer dissociation or structural unfolding.

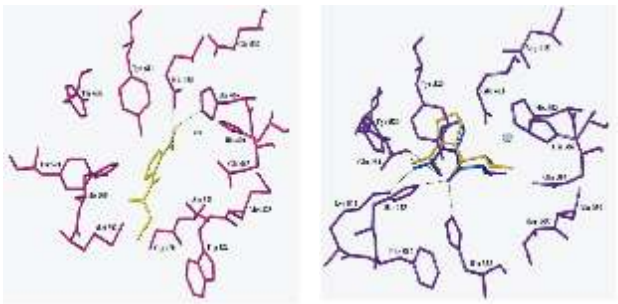
### 4.4.5 Dipeptidyl carboxypeptidase (DCP)

The docking studies with captopril revealed that binding mode of inhibitor with LdDCP was different from EcDCP and ACE which correlated reasonably well with experimental Ki values.



1-D homology model of LdDCP with subdomain I and II displayed in red and deep yellow color respectively, α-helices and β-sheet are shown as serpentine and arrow while the rest of the molecule represented as a rope.

The molecular electrostatic potentials (MEP) further suggested potentially important structural differences between the active sites of the three functionally similar enzymes. Results of current study will be useful in development of new antileishmanial agents by either *de novo* drug design or virtual screening of large small molecule databases.



A-LdDCP A-LdDCP

Binding mode of captopril docked into the active site of Ld-DCP and ACE

### 4.4.6 Actin network in *Leishmania* parasites

The studies on actin network of *Leishmania donovani*, through characterization of actin and various actin binding proteins have revealed its role in cell division and motility. The studies were continued and *Leishmania*-actin has been expressed and purified in Baculovirus-insect cell expression system and explored for its biochemical characteristics and comparative analysis with conventional actin. The results reveal a remarkable divergence in its biochemical behavior *in vitro* and suggest that *Leishmania* contains an unconventional form of actin. This protein besides being present in the cytoplasm is also present in nucleus and kinetoplast of this parasite. Its role has been examined in these organelles which revealed that *Leishmania*-actin directly binds with DNA (both the supercoiled and relaxed forms). It was further demonstrated that *Leishmania*-actin possesses topoisomerase-I like activity and relaxes supercoiling of the plasmid DNA. For *in vitro* studies of *Leishmania*-coronin, recombinant baculovirus were generated and expression of coronin was established in SF9 cells. Constructs were further designed that expressed two domains (head and tail) of coronin to establish its structural and functional properties. The

tail domain could be successfully cloned and expressed in *E. coli* whereas; expression of head domain is developed in the baculovirus-insect cell expression system. Studies of tail domain revealed that coronin exists mainly in the pentameric form and a small fraction of it exists as decamer. After characterization of *Leishmania*-ADF/cofilin homolog using rabbit muscle-actin, some of its properties were analyzed with *Leishmania*-actin as well. Analogous to other ADF/cofilins, *Leishmania*-ADF/cofilin homolog binds and depolymerizes *Leishmania* F-actin. Using ADF/cofilin null mutants, it was shown that this homolog also binds with monomeric *Leishmania*-actin. Various deletion constructs were also generated for expression in *E. coli* for its biochemical analyses and for expression in ADF/cofilin null mutants to pin-point involvement of different domains in the flagellar function.

## 4.5 Drug resistance mechanism

### 4.5.1 Cloning and characterization of antimony resistance related genes

Using micro array technique, few genes were identified which exhibited up-regulation in SAGresistant field isolates. These genes were named arbitrarily as SRRG1, SRRG2 and SRRG3. In the present study the gene named SRRG-I (SAG resistance related gene-1) was amplified using genomic DNA as template. A 870 bp amplified fragment was cloned and sequenced.

### 4.5.2 Elucidation of mechanism of drug resistance

Membrane ATPase activity in SAG resistant and sensitive field isolates was compared. SAG resistant field isolates showed increased activity as compared to sensitive ones. Efforts are in progress to confirm the data with more isolates.

# 5 Malaria

(Coordinator: Dr. S.K. Puri)

*Malaria is a major health problem in many tropical countries, including India. Inspite of tremendous gains witnessed in biomedical research during 20th century, malaria continues to provide barriers to the global health community. The global problem of malaria is largely due to the emergence of parasite resistance to limited armamentarium of antimalarial drugs. The progress in our understanding of mechanism of action and resistance to traditional drugs, the emergence of artemisinins as one of the most important antimalarial class of compounds and determination of the genome sequence of malaria parasite promise a more optimistic future for antimalarial drug development. The focus of our research program is aimed towards development of novel, orally effective chemotherapeutic agents for treatment of drug resistant malaria; exploration of suitable drug combinations with available agents; validation of novel parasite-specific drug targets as a result of an improved understanding of the parasite biology and characterization of drug resistant parasites and SNP's linked to disease endemicity.*


5.1 Chemotherapy of Malaria

5.2 Immunology of Malaria

5.3 Biochemistry of Malaria

5.4 Molecular biology of Malaria


## 5.1 Chemotherapy of malaria

### 5.1.1 Synthesis

Novel synthetic moieties comprising more than 800 compounds representing several prototypes viz. endoperoxides, í -carbolines, substituted quinoline, isoquinoloine, triazine and pyrimidine derivatives, peptide deformylase and fatty acid synthesis inhibitors, chalcones, pyrrolidines, pyranones, lactones and certain hybrid molecules with above prototypes were synthesized during the year for evaluation against *in vitro* or *in vivo* experimental malaria models. In addition, 17 extracts/fractions from natural sources were prepared and evaluated for antimalarial activity.

### 5.1.2 Screening

5.1.2.1 (a) Screening against *Plasmodium falciparum in vitro*

A total of 410 new synthetic compounds were screened against *Plasmodium falciparum* (strain 3D7) *in vitro* at various concentrations ranging between 10 ng/ml - 10 µg/ml. The screening strategy adopted growth inhibition assay procedure for lead generation and chemical moieties exhibiting inhibition in maturation of ring stage parasites into the schizont stage (MIC) during 36-40 hr incubation period were identified. Compounds exhibiting inhibitory activity at 1 µg/ml or lower concentrations were selected for microfluorimetric assay employing

*SYBR Green nucleic acid dye to determine* concentration response profile for the identified molecules. This assay has been standardized for use in lieu of previously employed radioactive hypoxanthine-uptake based assay. Promising molecules were selected for *in vivo* assay. A number of novel compounds representing quinoline-urea, indoquinolines, quinoline-pyrrolidine hybrids, í -carbolines and acridino-triazine derivatives have been identified with $IC_{50}$ values ranging < 25 ng/ml.

Screening of 625 samples representing marine fauna against *in vitro* model yielded 6 crude extracts with MIC values 10 µg/ml. In addition, nearly 2800 samples of natural origin comprising plant, fungal or bacterial extracts were evaluated under a CSIR coordinated network program and 8 plant extracts showing schizont maturation inhibition at 10 µg/ml concentration were identified for follow up.

(b) Control of Mycoplasma contamination in *P. falciparum* cultures

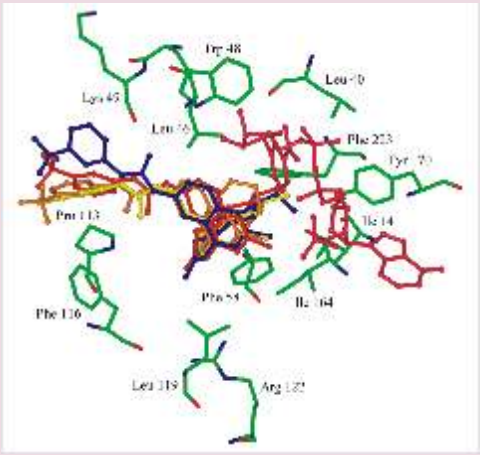
Mycoplasma is a common contaminant interfering with the growth of cell cultures. Mycoplasma is known to attach to human erythrocytes by adhering to sialic acid containing proteins and glycolipids and once established, it hinders the long term *in vitro* cultivation of *P. falciparum and* may be a cause of artefactual recordings specifically in drug response assays. To overcome this difficulty, a comparative efficacy of four commonly used antibiotics namely Plasmocin (macrolide), Biomyc-1-2 (Tetracycline) and Biomyc-3, and Mycoplasma Removing Agent (quinolone derivatives) was determined for elimination of mycoplasma from *P. falciparum* cultures. Our studies showed that three of these agents exhibit anti-plasmodial activity of their own and are not recommended for use duing antimalarial assay protocols. Mycoplasma Removing Agent (MRA) did not interfere with the growth of malaria parasites and hence can be safely used for elimination of mycoplasma from *P. falciparum* culture.

5.1.2.2 Screening against *Plasmodium yoelii* (N-67) - Swiss mice model

A total of 44 synthetic compounds representing 4 different prototypes identified after activity response against *P. falciparum in vitro* were evaluated against chloroquine resistant *P. yoelii* (N-

## Search for new pharmacophores for antimalarial activity

Several rationally designed 2-methyl-6-ureido-4-quinolinamides and 6-ureido-4-anilinoquinazolines elicited promising antimalarial activity against *P. falciparum in vitro* and CQ resistant *P. yoeilli nigeriensis in vivo.* (*Bioorg. Med. Chem.* **2009, *17*, 203-221;** *Bioorg. Med. Chem.*

**Conformations of the most active compounds docked in pf DHFR enzyme**

67) Swiss mice model. Several anilino quinoline triazine derivatives have shown very promising response at 50 mg/kg dose including curative activity with two compounds at 50 mg/kg after oral administration. Other promising categories where none of the molecules provided total clearance of parasites, but exhibited above 90% parasite clearance after 4 day treatment regimen include acridinotriazine, í-carboline and 4-aminoquinoline derivatives. None of the 8 plant extracts evaluated against the same model showed any promising activity up to 500 mg/kg dose.

5.1.2.3 Screening against *Plasmodium yoelii* (MDR) - Swiss mice model

Screening of new peroxide generating derivatives continued and 144 new compounds were screened at 96 mg/kg x 4 day, by both p.o. and i.m. routes, against multi-drug resistant *P. yoelii* in Swiss mice model. Compounds exhibiting curative response during the 28 day observation period were revalidated and assayed at lower doses. Six novel endoperoxide derivatives showing curative efficacy at 24 mg/kg x 4 day regimen were identified as the promising leads for follow up studies. Besides, 28 semi-synthetic artemisinine derivatives were also evaluated for improved oral efficacy response and one compound showed curative activity at 12 mg/kg dose by both oral and intramuscular routes. In addition to the endoperoxide derivatives, 39 *in vitro* selected novel moieties, representing quinoline-pyrrolidine hybrid derivatives, were screened for optimization of new leads. Two compounds showing curative activity at 50 and 100 mg/kg dose levels against multi-drug resistant parasites have been identified for further optimization.

### 5.1.3 Combination studies with compounds 97-78 and 99-411

Synthetic endoperoxide compounds 97-78 and 99-411 had been shown earlier to exhibit curative activity against *P. yoelii* - Swiss mice model and *P. cynomolgi* rhesus monkey models. Phase-I clinical trials with compound 97-78 have been initiated at PGI, Chandigarh. Drug combinations studies continued against *Plasmodium yoelii* Swiss mice model employing combinations of these two endoperoxide compounds with two antimalarial drugs piperaquine and lumefantrine. An overview of the recent clinical trials has revealed that these two antimalarials have been extensively employed as partner drugs with available artemisinine derivatives. Observations on monitoring the curative response against rodent models with these combinations have been successful in optimizing regimens providing total parasite clearance with two to four fold lower doses of the individual components. The curative response has also been obtained in combination studies with short duration regimens. Studies are underway to monitor response with combination regimens against simian malaria model.

## 5.2 Immunology of malaria

### 5.2.1 Cloning and expression of Merozoite surface protein-1 and Circumsporozoite protein of *Plasmodium vivax* and *P. cynomolgi B*

*Plasmodium vivax* has been recognized as the second most important human malaria parasite that accounts for over half of all malaria cases outside Africa (15-30 million). Some of the major constrains for development of *P. vivax* vaccines are; lack of *in vitro* culture system and requirement of a highly specialized monkey model for *in vivo* testing. *P. cynomolgi bastianelli*, a parasite of rhesus monkeys, is a closely related species to *P. vivax*. The two parasites share similar clinical course of infection, reticulocyte specific invasion, a dormant liver hyponozoite stage and similar genomic GC content. There is high homology of prime vaccine candidates (CSP, MSP1, AMA1 and EBA) between these two parasites. Therefore, studies have been initiated with merozoite surface protein-1 (MSP-1) and circumsporozoite protein (CSP) of *P. vivax* and *P. cynomolgi B* for cloning, expression and evaluation of protective potential in *P. cynomolgi* rhesus monkey model system.

The *P. cynomolgi B* $MSP1_{19}$ insert was subcloned in pGEX-6P1 expression vector and

optimum expression of *P. cynomolgi B* $MSP1_{19}$ recombinant protein was obtained using 0.1 mM IPTG at 37°C for 4 h. SDS-PAGE analysis showed a fusion protein of 40 kD. The *P. cynomolgi* $MSP1_{19}$ recombinant protein showed reactivity with immune monkey sera in ELISA. The $MSP1_{42}$ gene fragment from *P. vivax* malaria infected blood *was* PCR amplified using the specific primers based on sequence of *P. cynomolgi* $MSP1_{42}$. PCR product of 1.2 kb was observed for $MSP1_{42}$ of *P. vivax.* Purified PCR product was cloned into pGEM-T Easy vector and sequenced. The *P. vivax* $MSP1_{42}$ sequence showed 97% homology with $MSP1_{42}$ from Sal1 and Belem strains of *P. vivax* and 78% homology to *P. cynomolgi* $MSP1_{42}$. The *P. vivax* $MSP1_{42}$ subcloned in pGEX-6P1 expression vector and SDS-PAGE analysis showed a fusion protein of 70 kD.

CSP gene fragment from *P. cynomolgi B* malaria parasites were PCR amplified using the specific primers based on sequences from other strains of *P. cynomolgi and* PCR product of 1.3 kb was observed. *P. cynomolgi* B CSP was cloned into pGEM-T Easy vector and sequenced. The *P. cynomolgi* B CSP sequence showed 98% homology with CSP from Berrok strain of *P. cynomolgi* and 67% homology to *P. vivax* Sal1 strain. Subcloning and expression of *P. cynomolgi B* CSP insert was done in pGEX-6P1 expression vector and SDS-PAGE analysis showed a fusion protein of 72 kD. CSP gene fragment from *P. vivax* malaria infected blood was also PCR amplified using the specific primers based on sequence of *P. vivax* and PCR product of 1 kb was observed. Purified PCR products were cloned into pGEM-T Easy vector and sequenced. The *P. vivax* CSP sequence showed 65-67% homology with CSP from Sal1 and Belem strains of *P. vivax* and *P. cynomolgi* B parasites. However, region-I (RI) and region-II (RII) of the CSP gene, which are involved in recognition, binding and invasion of hepatocytes by sporozoites, showed 92-95% homology between these two parasites. Studies are in progress to purify and characterize these proteins and evaluate their protective potential.

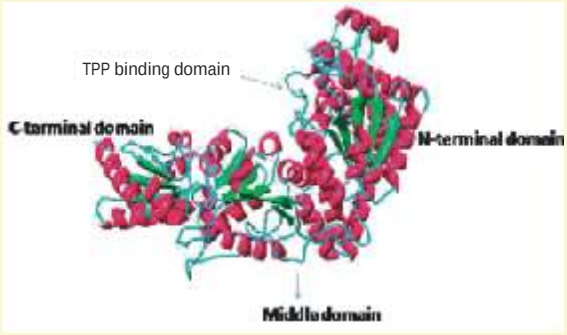
## 5.3 Biochemistry of malaria

### 5.3.1 Studies on *Plasmodium falciparum* transketolase as a drug target

Transketolase is the key enzyme of the non-oxidative part of the Pentose Phosphate Pathway (PPP), which catalyzes the reversible transfer of two carbon units from ketose phosphates to aldose phosphates. Together with transaldolase, it provides a link between the glycolysis and PPP, providing precursors for nucleotide, aromatic amino acid and vitamin biosynthesis.

**Transketolase : novel drug target for malaria chemotherapy**

A homology model of *P. falciparum* transketolase revealed close structural resemblance with the crystal structure of homologue transketolase of *S. cereviseae*




Model structure of PfTk

In our earlier studies we have cloned, over-expressed and characterized *P. falciparum* transketolase (PfTk). The PfTk protein was localized both in the cytoplasm and nucleus of the parasite by confocal microscopy. The availability of catalytic active recombinant PfTk opened an opportunity to study the effect of various inhibitors on PfTk activity. The influence of known inhibitors of transketolase i.e., p-hydroxyphenylpyruvate and oxythiamine pyrophosphate on the activity of PfTk were carried out. p-Hydroxyphenylpyruvate (HPP) acts as reversible and competitive inhibitor with respect to hydroxypyruvate and exerts a significant influence on the dissociation of holo-PfTk into the apoenzyme and

coenzyme. Oxythiamine pyrophosphate (OP) also competitively inhibited PfTk with thiamine pyrophosphate. Moreover, both HPP and OP exhibited anti-malarial activity *in vivo* against the rodent malarial parasite *Plasmodium yoelii*. Both the inhibitors significantly suppressed the day four mean parasitemia value at different dose levels. In order to explore the structural similarity of PfTk with existing transketolase structures, a homology model of PfTk was constructed. Using the homology model of PfTk, binding property of both the identified inhibitors in PfTk was optimized on the basis of prior information available for template. The binding properties of these inhibitors were used to perform a pharmacophore search of CDRI's 3D compound database to identify new effective PfTk inhibitors. Studies on synthesis of prototype inhibitors are proposed to be undertaken.

### 5.3.2 Role of drug metabolizing enzymes in antimalarial drug resistance

The cytochrome $P_{450}$ super family of enzymes is the major drug metabolizing group present in malaria parasite. These are bound to the microsomal portion of the cell and play an important role in determining the final outcome of antimalarial drug regimen. During malaria infection the level of the enzyme changes depending on the level of parasitaemia present in the host. The multi-drug resistant parasite *P. yoelii nigeriensis* (MDR) shows resistance to a number of quinoline antimalarials including chloroquine, amodiaquine, mefloquine, halofantrine, quinine and quinidine. The level of cytochrome $P_{450}$ in *P. yoelii nigeriensis* infected mice livers initially showed an increase with an increase in the level of parasitaemia. However, the increase occured only upto 30% of infection level, after which the enzyme showed a sharp decrease with increasing parasitaemia. At the terminal stage of infection, the percentage decrease in the level of enzyme compared to normal was 33%. A comparison of the enzyme levels in the drug resistant and drug sensitive *P. yoelii* strains showed that cytochrome $P_{450}$ was present in higher amounts in drug resistant strain than in drug sensitive strain. The high level of enzyme in drug resistant parasites would result in a faster rate of metabolisation of antimalarials and thereby provide a mean to the parasites to survive. Besides, studies on the activity of a related enzyme Aminopyrine-N-Demethylase showed a steady decrease with the increase in the infection in the host. When compared to the activity found in normal mice livers, the decrease was found to be 73.2%. This decline in the enzymatic activity can be attributed to the disruption of hepatic microsomal system as a consequence of the steady progression of *Plasmodium yoelii nigeriensis* MDR infection in mice.

Our studies have also shown a marked increase in the activity of glutathione reductase. During the initial stages of infection, the increase in the activity was only 14% but with the progression of parasitemia, the enzyme activity increased and was recorded to be 3 times higher as compared to controls at 65% parasitemia.

## 5.4 Molecular biology of malaria

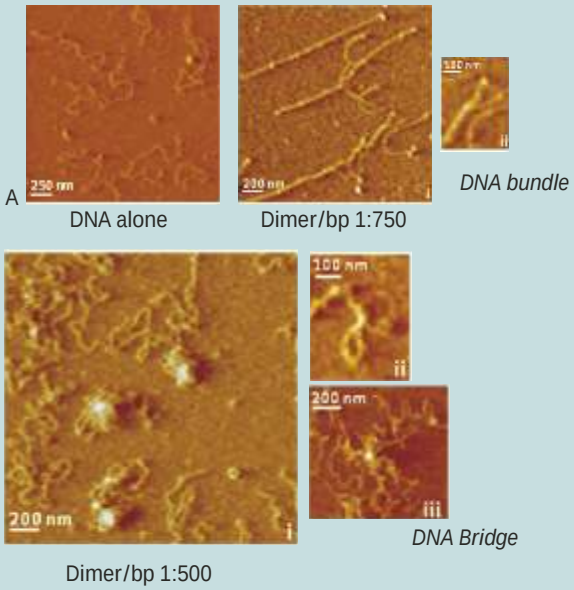
### 5.4.1 Identification and analysis of proteins involved in *Plasmodium falciparum* apicoplast DNA replication and organization

Apicomplexans, including the pathogens *Plasmodium* and *Toxoplasma*, carry a non-photosynthetic plastid of secondary endosymbiotic origin called the apicoplast. Biochemical pathways operative within this organelle provide novel sites for drug intervention against malaria. Due to its essentially prokaryotic nature the processes of DNA replication, transcription and translation within the apicoplast are also validated drug targets. The *P. falciparum* apicoplast contains a 35 kb, circular DNA genome (plDNA) with limited coding capacity. Previous work from our laboratory has shown that the ~35 kb, A+T-rich, circular double-stranded plDNA molecules of *P. falciparum* replicate via the D-loop/bi-directional *ori* mechanism at the late trophozoite-early schizont stage of the intraerythrocytic cycle. PlDNA replication origins (*ori*) localize within the inverted repeat (IR) region of the plDNA molecule. The fact that a single plDNA circle is ~12 µm in circumference and several molecules have to be

packed into an organelle with a diameter of only ~0.3 µm as well as replicate and divide into daughter molecules without getting tangled is indicative of the involvement of a DNA-compacting protein in plDNA organization.

## DNA organization by PfHU, an apicoplast-targeted histone-like protein

*P. falciparum* nuclear-encoded histone-like protein (PfHU) promotes concatenation of linear DNA and inhibits DNA circularization in contrast to bacterial HUs that bend DNA. Atomic Force Microscopy of PfHU-DNA complexes showed protein concentration-dependent DNA stiffening, intermolecular bundling and formation of DNA bridges followed by assembly of condensed DNA networks (Figure). Results provide first functional characterization of an apicomplexan HU protein and give additional evidence for red algal ancestry of the apicoplast. (*Nucleic Acids Research*, 2008, 36 (15): 5061-5073)




The dearth of information on proteins involved in organization of the *P. falciparum* apicoplast genome prompted us to investigate putative candidates from the parasite genome database. The prokaryotic nature and putative red algal origin of the apicoplast suggested the possible involvement of a histone-like protein ('heat unstable' or HU) that is the primary organizational component of bacterial nucleoids, dinoflagellate chromosomes as well as red algal chloroplast genomes. A gene encoding a HU-ortholog that carries a conserved BHL domain (bacterial histone-like domain) together with a predicted N-terminal apicoplast targeting sequence was identified on Chr.9 of the *P. falciparum* nuclear genome. HU proteins are small basic proteins of prokaryotic origin that are structurally distinct from eukaryotic histones, belong to the DNABII family of DNA-binding proteins, and exhibit hetero- or homo-dimeric DNA binding. HU proteins also have regulatory effects on DNA replication, recombination and transcription. In bacteria, HU proteins together with the structurally related IHF (integration host factor) and other DNA-binding proteins, organize chromosomal DNA into periodic nucleosome-like structural units.

Our results reveal that the *P. falciparum* Chr.9-encoded bacterial histone-like protein (PfHU) is involved in DNA compaction in the apicoplast. PfHU is associated with apicoplast DNA and is expressed throughout the parasite's intra-erythrocytic cycle. The protein binds DNA in a sequence non-specific manner with a minimum binding site length of ~27 bp and a $K_d$ of ~63 nM and displays a preference for supercoiled DNA. PfHU is capable of condensing *E. coli* nucleoids *in vivo* indicating its role in DNA compaction. The unique 42 aa C-terminal extension of PfHU influences its DNA condensation properties. In contrast to bacterial HUs that bend DNA, PfHU promotes concatenation of linear DNA and inhibits DNA circularization. Atomic Force Microscopic study of PfHU-DNA complexes shows protein concentration-dependent DNA stiffening, intermolecular bundling and formation of DNA bridges followed by assembly of condensed DNA

networks. These results provide the first functional characterization of an apicomplexan HU protein and give additional evidence for red algal ancestry of the apicoplast.

### 5.4.2 Functional analysis of proteins putatively involved in apicoplast specific pathways

Studies on three additional putative apicoplast-targeted proteins continued. Nuclear genes annotated as prokaryotic translation elongation factors EF-Ts and EF-G and the apicoplast-targeted putative [Fe-S] complexation protein SufC were investigated. The current annotations for these molecules are based on sequence identity and lack functional confirmation. Additionally, apicoplast translation is a validated drug target while the Sufs may play a critical role in maintaining oxidative potential of the apicoplast. Following progress has been made in these programmes:

(i) Translation in the apicoplast: Translation elongation factor Tu (EF-Tu) is encoded by the apicoplast while its predicted interacting partner, EF-Ts, is nuclear-encoded. We have previously established translation activity in the apicoplast by localizing EF-Tu in the organelle. The effect of the prokaryotic translation inhibitor, thiostrepton, on parasite growth and apicoplast EF-Tu levels in infected RBCs has also been studied. Interactions between recombinant EF-Tu and EF-Ts were analysed by fluorimetry and the ability of apicoplast EF-Ts to mediate nucleotide exchange (GDP/GTP) on *E. coli* EF-Tu was investigated using FRET with Mant-GDP. *P. falciparum* EF-Ts could mediate GDP/GTP exchange on both *P. f*. and *E. coli* EF-Tu. FRET analysis using a stopped-flow device revealed fast kinetics of nucleotide exchange reaction. The *P. falciparum* putative apicoplast translation factor EF-G (domains I-IV) was also cloned and expressed as a recombinant protein in *E. coli*. Its GTP-binding and hydrolysis properties as well as ribosomal interaction were studied. The effect of specific inhibitors acting at the interface of EF-G/ribosomal interaction is currently being analysed.

(ii) [Fe-S] complexation pathway of the apicoplast: Components similar to the *E. coli suf* system have been identified in *P. falciparum* and are likely to function in the apicoplast. While the *sufB* homolog (*ycf24*) is encoded by the apicoplast genome, homologs of the other components of this pathway are nuclear-encoded. We have studied two interacting components of this pathway-SufB and SufC. The region of the *P. falciparum sufC* gene encoding the predicted processed protein was expressed and purified and SufC ATPase activity was characterized. An internal portion of SufB, the predicted interacting partner of SufC, encoded by the apicoplast genome was also expressed and interaction between the two Sufs was investigated. Additionally, a putative SufS encoded by nuclear genome was cloned and expressed. An assay for measuring its cysteine desulfurase activity in the presence of a SufE homolog is currently being standardized.

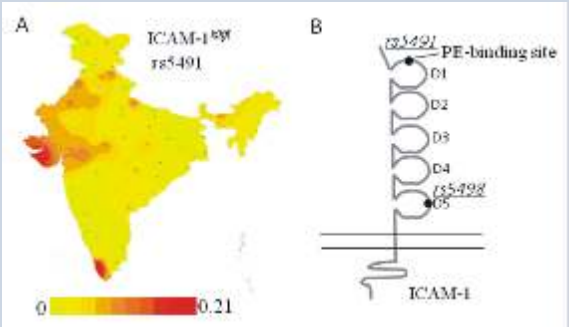
### 5.4.3 Analysis of SNPs related to susceptibility/resistance to *P. falciparum* malaria in populations across India and case-control studies for genotype-disease association in *P. falciparum* endemic and non-endemic regions in India

The association of severity of malaria with several human genetic factors is well documented and the disease has been the selective pressure behind several erythrocytic defects. Malaria susceptibility/resistance has been correlated with polymorphisms in more than 30 genes, some of which have exhibited differential association in distinct populations of the world. *P. falciparum* blood infection levels and fever episodes have been linked to chr 5q31-33 and chr10. While most human gene polymorphism-disease association studies for malaria susceptibility have been carried out on populations from Africa and south-east Asia, there is limited information on malaria-associated gene polymorphisms in Indian populations. We have carried out case-control studies for determining disease association of specific SNPs with severity of *P. falciparum* malaria in India. Samples of patients and ethnically-matched controls have been collected from field surveys and hospitals in Uttar Pradesh (non-endemic region), Chhattisgarh, and Orissa

(endemic regions). Genotyping of candidate SNPs and cytokine and CR1 level analysis in patients and controls followed by association statistics has indicated interesting variations from reports from other countries. Association of specific promoter SNPs of the TNF gene as well as the Fc?RIIa receptor with susceptibility to severe falciparum malaria has been revealed from these studies.

### Genetic variation in host adhesion molecules and pathogenesis of *P. falciparum* malaria

Frequency distribution of seven selected SNPs of *ICAM1*, *PECAM1* and *CD36* determined in 552 individuals drawn from 24 populations across India revealed association of the ICAM1 rs5498 (exon 6) G allele (Figure) and the CD36 exon 1a A allele with increased risk of severe malaria while CD36 rs1334512 (-53) T allele as well as the TT genotype are associated with protection from severe disease. (*Malaria Journal*, 2008, 7:250)




Host adhesion molecules play a significant role in the pathogenesis of *P. falciparum* malaria and changes in their structure or levels in individuals can influence the outcome of infection. The association of SNPs of three adhesion molecule genes, *ICAM1*, *PECAM1* and *CD36*, with severity of *P. falciparum* malaria in a malaria-endemic and a non-endemic region of India was investigated. The frequency distribution of seven selected SNPs of *ICAM1*, *PECAM1* and *CD36* was determined in 552 individuals drawn from 24 populations across India. Association of the ICAM1 rs5498 (exon 6) G allele and the CD36 exon 1a A allele with increased risk of severe malaria was observed (severe versus control, OR=1.91 and 2.66, p=0.02 and 0.0012, respectively). The CD36 rs1334512 (-53) T allele as well as the TT genotype associated with protection from severe disease (severe versus control, TT versus GG, OR=0.37, p=0.004). Interestingly, a SNP of the *PECAM1* gene (rs668, exon 3, C/G) with low minor allele frequency in populations of the endemic region compared to the non-endemic region exhibited differential association with disease in these regions; the G allele was a risk factor for malaria in the endemic region, but exhibited significant association with protection from disease in the non-endemic region. The data highlights the significance of variations in the *ICAM1*, *PECAM1* and *CD36* genes in the manifestation of *P. falciparum* malaria in India. The *PECAM1* exon 3 SNP exhibits altered association with disease in the endemic and non-endemic region.

# Microbial Infections 6

(Coordinator: Dr. Ranjana Srivastava)

*The objective of the project area covers the screening of synthetic compounds and natural products for antitubercular, antifungal, antibacterial and antiviral activities, development of diagnostics for tuberculosis and fungal infections, development of vaccines for cholera and tuberculosis, rapid in vitro and in vivo molecular screens for drug screening, construction of mycobacterial vectors, novel antigens and drug targets, basic studies on mycobacterial, bacterial and fungal proteins and virulence genes.*


6.1 Cholera

6.2 Tuberculosis

6.3 Fungal Infections

6.4 Viral Infections


## 6.1 Cholera

Two regulatory elements, VC0973 and VC0974 have been further analyzed. Interaction between two proteins was demonstrated by bacterial two hybrid system. The PCR amplified sequence of both genes from *V. cholerae* genome was cloned in plasmid pKT25 and pUT18c and reconstitution of í -galactosidase activity was determined. With appropriate negative and positive controls, protein-protein interaction between two proteins was demonstrated.

## 6.2 Tuberculosis

### 6.2.1 Synthesis and screening

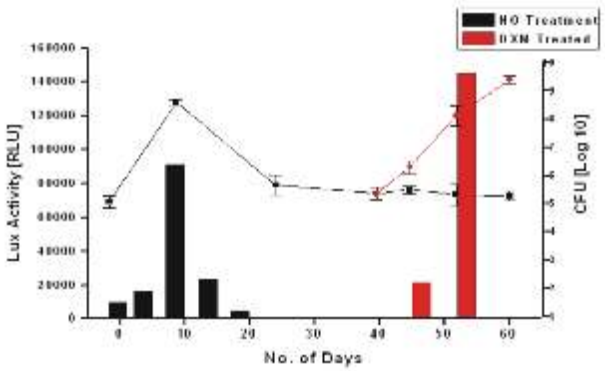
Synthetic compounds comprising several prototypes viz. monosaccharide derived acyclic and cyclic deoxy sugars, 1,2-diarylindane derivatives, aryloxycyclopropyl methane, alkenoyl glycosides, hydroxamate derivatives, synthesized during the year, were evaluated for antitubercular activity *in vitro*. A total of 127 compounds were screened against *M. bovis* BCG and *M. tuberculosis* using lux based reporter assay. Recombinant *M. tuberculosis* expressing firefly luciferase was constructed by expressing firefly luciferase gene in mycobacterial integrative vector under control of $hsp_{60}$ promoter. The recombinant plasmid was stably integrated into the genome thus obliviating the need for providing selective pressure for maintenance of plasmid. The assay is based on luminescence determination using luciferin as substrate and provides a good correlation between bioluminescence and viability of recombinant *M. tuberculosis*. The compounds were tested at various concentrations ranging between 1.56 µg to 12.5 µg/ml and inhibition of bioluminescence was measured.

Out of 127 compounds tested, 25 were identified having activity in the range of 0.79-6.25 µg/ml against *M. tuberculosis* $H_{37}Rv$. The activity was further confirmed by radiometric BACTEC system using *M. tuberculosis* $H_{37}Rv$ as test strain. Cytotoxicity of the active molecules (MIC 0.79 6.25 µg/ml) was tested in Vero cells, mouse (J774A.1) and human macrophage (THP1) cell line by resazurin based fluorometric cell viability assay. Resazurin detects cell

viability by converting from a nonfluorescent dye to the highly red fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. Two compounds have been selected for *in vivo* evaluation in experimental murine model on the basis of MIC (0.79 -3.125 µg/ml), bactericidal mode of action and cytotoxicity to Vero cells, mouse macrophage and human macrophage cell line.

### 6.2.2 Development of murine infection model for persistence and reactivation

A murine infection model for *Mycobacterium fortuitum* has been developed in which course of acute infection; non-replicating persistent state and reactivation can be delineated. *M. fortuitum*, lodge and proliferate in kidney and produce characteristic disease symptoms like lethargy, spinning of head and restlessness. An infecting dose was optimized which produced least mortality (25%) and all disease symptoms. Upon infection, the bacilli proliferated in kidney with high metabolic activity for upto 10 days producing characteristic disease symptoms. Acute phase of infection was followed by non-replicating phase (NRP) which showed decline in tissue bacillary load, minimal metabolic activity, loss of disease symptoms and resistance to ciprofloxacin suggesting latent state of infection. The mice at this stage were treated with dexamethasone to cause reactivation which resulted in proliferation of bacilli, reappearance of disease symptoms and susceptibility to ciprofloxacin. Peak levels of TNF-a, IFN-?, and IL-2 were observed 10 days post inoculation, concomitant with the peak bacillary load in kidney, suggesting recruitment of Th1 immune response to control the increased bacillary burden along with simultaneous increase in serum concentrations of cytokines IL-4 and IL-5 upon reactivation. IL-4 and IL-5 constitute predominant cytokines of Th2 response of the host. The model can be used as rapid *in vivo* model for screening of drugs active against replicating and latent bacilli.




*In vivo* activity in murine infection model

### 6.2.3 Generation of rationale based screen system

Recombinant *M. aurum* strain showing an increased reporter gene expression after treatment with FAS-II pathway inhibitors has been constructed to serve as a secondline screen for characterization of compounds showing antimycobacterial activity in a first-line screen.

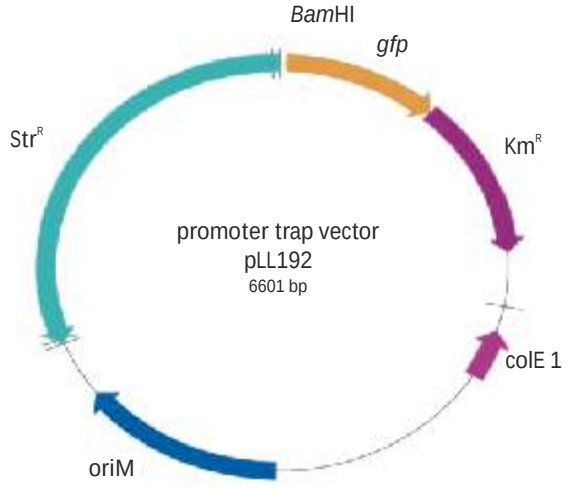
### 6.2.4 Persistence and resuscitation

#### 6.2.4.1 A transposon insertion mutant of *Mycobacterium fortuitum* attenuated in virulence and persistence in murine infection model

From a Tn*pho*A insertion library of *M. fortuitum*, a mutant MT13 was isolated by *in vivo* screening in *M. fortuitum*-murine latency model based on mortality and loss of symptoms. The mutant was attenuated in virulence with lesser bacterial burden, milder and delayed spinning of head and no mortality of mice. The significant feature of the mutant was its failure to persist in kidney and thus the persistent bacillary load characteristic exhibited by the wild type strain was not observed. The insertion of transposon in MT13 was mapped in a region of the genome, which showed homology to rv3291c of *M. tuberculosis,* annotated as transcriptional regulatory factor and reported to be upregulated in nutrient starvation and anaerobic persistent states. Complementation of MT13 with *rv*3291c resulted in restoration of wild type characteristics including persistence in kidney suggesting the role of rv3291c homolog in virulence and persistence of *M. fortuitum*.

6.2.4.2 Use of reporter vector to identify genes of *M. tuberculosis* expressed during anaerobic persistence

A promoter trap shuttle vector pLL192 was constructed in lab containing an artificial bicistronic operon composed of promoterless green fluorescent protein gene followed by kanamycin resistance. A promoter library of *M. tuberculosis* was constructed in plasmid pLL192 and subjected to hypoxic condition (dissolved oxygen <1%) in a controlled fermenter to monitor differential expression of mycobacterial promoters in aerobic and hypoxia conditions. On the basis of green fluorescence and kanamycin resistance, seven promoters were identified which were upregulated in hypoxia condition. The expression levels of each corresponding gene were individually confirmed by RT-PCR analysis. The upregulated genes include Rv0050 (penicillin binding protein), Rv1511 (GDP-D-mannose dehydratase), Rv1489, Rv2257, Rv2258, Rv0467 (isocitrate lyase) and Rv2031 (alpha crystalline homolog). Few genes have been selected for generation of knockout mutants in *M. tuberculosis* for validation as persistence targets *(Tuberculosis (Edinb) 88(6): 518-25; Tuberculosis (Edinb).88(3):171-7)*.




6.2.4.3 Resuscitation of dormant mycobacteria by resuscitation promoting factors (Rpf): whole genome expression profiling during extended stationary and resuscitation phase

The resuscitation of aged, dormant BCG cells generated during extended stationary phase of growth was demonstrated by recombinant Rpf proteins cloned from *M. tuberculosis* and *Micrococcus luteus*. For establishing extended stationary phase and its resuscitation by Rpf, *M. bovis* BCG expressing firefly luciferase was employed. The cells were grown in Sautons medium at 37°C. The culturability of cells decreased with time and after five months of cultivation, cells became nonculturable and were treated as extended stationary phase. The nonculturable cells could be resuscitated by purified Rpf protein from *M. tuberculosis*/ *M. luteus* which was observed as rise in optical density at 600 nm and increase in RLU as opposed to control cells which showed negligible increase in optical density and RLU.

Gene expression changes were followed during transition of *M. bovis* BCG from stationary phase to resuscitation phase by whole genome expression profiling by microarray and differential gel electrophoresis. A total of 765 genes were up regulated and 814 genes were down regulated in Resuscitation versus Extended stationary phase conditions of growth. Mapping of differentially regulated genes onto KEGG pathways revealed that ABC transporters pathway, tryptophan metabolism, tyrosine metabolism and steteleno amino acid metabolism were Up regulated and Ribosome pathway, Purine metabolism, Pyrimidine metabolism, Protein export, RNA polymerase were down regulated. The validation of selected up and down regulated genes during resuscitation and extended stationary phase is in progress.

The proteome analysis of BCG cells during extended stationary phase and Rpf promoted resuscitation by differential gel electrophoresis (DIGE) was attempted which has resulted in identification of BCG proteins, differentially, commonly and uniquely expressed during resuscitation and extended stationary phase of growth. The analysis is in progress.

6.2.4.4 Role of Rpf in reactivation of latent infection

The role of Rpf in reactivation of nonreplicating persistent (NRP) bacilli in *M. fortuitum* murine latency model was demonstrated. Recombinant *M. fortuitum* expressing RpfC and RpfE of *M. tuberculosis* were constructed by cloning *rpf* genes in integrative mycobacterial vector under control of mycobacterial P*hsp*60 promoter resulting in constitutive expression of the cloned genes. The mice were infected with Rpf expressing recombinant strains and course of infection was followed. In mice, recombinant *M. fortuitum* strains behaved different from *M. fortuitum* in tissue bacillary load, persistence of disease symptoms and ciprofloxacin sensitivity during 25 to 60 days of infection period. While non-replicating persistent wild type bacilli were resistant to ciprofloxacin *in vivo* in NRP state, bacilli expresssing Rpf were sensitive to the drug during the same period of infection in mice.

### 6.2.5 Drug targets

Acetohydroxyacid synthase (AHAS) and Dihydroxyacid dehydratase (DHAD) belonging to BCAA pathway (essential genes) are being explored as drug targets. In *M. tuberculosis*, five open reading frames (ORFs) have been identified on the basis of their homology to the catalytic and regulatory subunits of AHAS from other organisms. These ORFs have been annotated as ilvX, ilvB1, ilvB2, ilvG and ilvN. Four genes (ilvB1, ilvB2, ilvG and ilvX) have sequence homology to the catalytic subunit and ilvN to regulatory subunit. DHAD is coded by single gene. All six genes are expressed *in vitro* and *in vivo* in mice. The expression of these genes varied during various stress conditions viz. hypoxia, acidic pH and stationary phase. In order to define the function of genes, two approaches have been made. In first approach, knockout mutants are being generated for individual genes in *M. tuberculosis* and in second approach, *ilv*B1, *ilv*B2, *ilv*G, *ilv*X, *ilv*N and *ilv*D genes have been cloned and overexpressed in *E. coli* for purification of recombinant proteins and development of assays. In addition, genes expressed during anaerobic persistence and infection viz. *rv*0050, *rv*3291 and *rv*3711 are being validated by mutant generation.

### 6.2.6 A method for improved 2-D separation of *M. tuberculosis* proteins for proteomic analyses

To improve the 2-D gel electrophoretic separation of *M. tuberculosis* proteins, three important reasons for their poor resolution under these conditions were addressed: (a) electro-endosmotic flow (EEF) of water during isoelectric focusing (IEF), (b) migration of dithiothreitol (DTT) especially in alkaline pH gradients, and (c) protein precipitation during IEF. Addition of optimal concentrations of graded amounts of isopropanol (to counteract EEF), DTT (to take care of its loss during electrophoresis ) and glycerol (to take care of protein precipitation) in the sample buffer prior to IEF on the IPG strips provided a remarkably improved resolution of the *M. tuberculosis* proteins from cytosol or the cell membrane. With the new sample buffer, more proteins are visible on the respective 2-D gels, as the intensities and number of the spots increase.

Since drug targets (proteins), especially those involved in cell wall biogenesis, are expected to be found in the cell membrane of *M. tubercuolsis*, proteome of *M. tuberculosis* membrane proteins was analyzed. Proteomic analysis of *M. tuberculosis* membrane proteins after partitioning them into detergent and aqueous phases of Triton X114 has been earlier reported which led to identification of only a small subset of membrane proteins predicted by the *M. tuberculosis* genome. The triton X114 insoluble portion of the *M. tuberculosis* membrane was analyzed by 2-D gel electrophoresis, followed by proteomic analysis of 28 protein spots (using MALDI TOF-TOF). Peptide mass data from 14 spots showed significant matches with the *M. tuberculosis* ORF database. Three of these proteins were new to the M. *tuberculosis* proteome, not described earlier.

### 6.2.7 Interaction of macrophage PKCs with Serine Threonine Kinases of mycobacteria

Serine threonine kinases are involved in growth, development, division, differentiation and in regulation of immune responses. *M. tuberculosis* H37Rv contains 11 eukaryotic like serine threonine kinase genes. Among them two are cytosolic (PknK and PknG) and nine transmembrane proteins. The polymorphic studies show that PknK (Rv3080) is present in slow growers and or in pathogenic mycobacteria. The cloning of *rv*3080 (PknK) in mycobacterial *E. coli* shuttle vector downstream to hsp60 promoter and its overexpression in *Mycobacterium smegmatis* $mc^2$ 155 (MS) strain , resulted in dramatic loss in the growth (4-10 folds) and a significant delay in the colony formation were observed, demonstrationg the role of Rv3080 in growth of mycobacteria. The temporal transcripts were quantitated at different phases of cultures. The gradual increase in the copy number of *pkn*K transcripts were observed when the cultured were at stationary phase suggesting that increased amount of PknK slows down the growth in BCG. Disruption of *rv*3080 in tubercular mycobacteria is being attempted in order to look for the definitive role of *rv*3080 in slowing the growth and further develop as a target or study and control pathogenesis.

Infection of macrophages by *M. tuberculosis* $H_{37}$Rv, BCG and *M. tuberculosis* $H_{37}$Ra was found to selectively down regulate the expression of PKCα while infection by *M. smegmatis* did not. SiRNA mediated knockdown of PKCα drastically reduced phagocytosis of *Mycobacterium bovis* BCG (BCG) and *M. smegmatis* (MS) by macrophages while their intracellular survival was increased. To test whether enhanced survival by pKnG involves inhibition of PKCα, THP-1 cells were infected with recombinant *M. smegmatis* expressing *M.tb* specific pKnG (MS-pKnG) and the level of macrophage PKCα was determined. THP-1 cells infected with MS pKnG showed decreased expression of PKCα when compared to control cells. Real time PCR analysis of THP-1 cells infected with MS-pKnG and Rv showed decreased level of PKCα mRNA while expression of pKnG mRNA in Rv was increased. In normal THP-1 cells survival of MS-PknG was enhanced when compared to MS while in PKCά deficient THP-1 cells, MS and MS-pKnG survived equally which were higher than the survival of MS in normal macrophages. The phagocytosis of MS and MS-pKnG by THP-1 cells and observed reduced phagocytosis of MS-pKnG.

### 6.2.8 Expression of SigF and SigH in *Mycobacterium smegmatis*

The presence of *sigF* gene in *M. smegmatis* and its expression was studied. It was observed that unlike reported late-stage expression in *M. tuberculosis* and *M. bovis*, *sigF* was expressed throughout the growth in *M. smegmatis*, by and large, at the same level, but its expression varies upon exposure to different stress conditions. The presence of *sigF* orthologs in non tuberculous mycobacteria and its continued expression throughout the growth suggests that apart from regulating the expression of virulence factor genes in pathogenic mycobacteria, *sig*F is likely to have more roles in the mycobacterial physiology. A deletion mutant of *sigF* in *M. smegmatis* has been generated and studies are in progress to map its regulon.

*M. smegmatis* genome contains seven *sigH* paralogs. To analyze the role of these paralogs in *M. smegmatis*, the expression of *sigH* paralogs at different stages of growth and under various stress conditions was analyzed using quantitative real time RT-PCR. *sigH* and its paralogs were found to be differentially expressed during growth stages and in response to different stress conditions. Variable expressions *sigH* paralogs during growth stages suggest a role for these sigma factors in regulating stage-specific gene expression. Several of them are induced in response to heat and oxidative stress, which is a central feature of *sigH* sigma factor.

## 6.3 Fungal Infections

### 6.3.1 Antifungal and antibacterial screening

A total of 696 compounds / extracts were evaluated for *in vitro* antifungal and antibacterial activity by microboth dilution method. One marine extract (MIC 1.9-62.5 µg/ml against fungi) and five synthetic compounds (0.19-0.78 µg/ml against bacteria) and S-008-0913 (MIC 0.78-12.5 µg/ml against fungi) were found to be active.

### 6.3.2 Generation of monoclonal antibodies against *Candida albicans* and *Aspergillus fumigatus*

6.3.2.1 Monoclonal antibodies against *C. albicans*

The 24 hybridomas produced earlier using spleens of mouse immunized with HF pyridine extracted cell wall proteins of *C. albicans* were followed for their characterization. After single cell cloning, the monoclonal antibodies were evaluated for *in vitro* anti *C. albicans* activity. 10 Hybridoma clones which exhibited >80% reduction in CFU were selected for further studies. Of these, 2 monoclonal antibodies exhibited specificity to *Candida* sp. as determined by Western blotting. Further candidacidal activity of these MAbs was estimated by MTT assay and nearly 95% reduction in viability was observed for both the MAbs. The therapeutic role of these MAbs (MAb 2D2 and MAb 2A11) is being evaluated *in vivo* against mouse model of candidiasis.

Two hybridomas obtained from another fusion experiment using spleens of mouse immunized with secretory proteins of *C. albicans* were subjected to single cell cloning by limiting dilution method. These two MAbs were used for development of ascites in Balb/c mice. Of these one MAb (1A1) was studied for its diagnostic potential. The monoclonal antibody (MAb 1A1) recognized two proteins (~ 46KDa and ~37kDa) of *C. albicans*. This MAb cross reacted with other strains of *C. albicans* as well as other pathogenic yeast forms but not with mycelial pathogens. The 37 kDa protein was identified as Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by N-terminal sequencing. This protein is constitutively expressed by the pathogen and is released into blood stream during pathogenesis. Further work is being carried out to identify and characterize the other protein (46kDa).

6.3.2.2 Therapeutic potential of monoclonal antibody NE5 generated against *Candida albicans*

Twenty two paratope derived dodeca-peptide sequences were identified *in silico* with the help of 'Predicted antigenic peptides' software and synthesized by solid phase method of synthesis on Resin-Rink Amide (RAM) resin. One of the peptide showed *in vitro* activity against two strains of *C. albicans* by CLSI (NCCLS now CLSI). Its $IC_{50}$ is being evaluated.

6.3.2.3 Monoclonal antibodies against *Aspergillus fumigatus*

For generation of monoclonal antibodies against secretory proteins of *A. fumigatus*, spleenocyte of mouse immunized with total secretory proteins of A. *fumigatus* were fused with SP2/0 myeloma cells to produce hybridomas. After primary screening by ELISA, 20 positive hybridomas were selected. 4 hybridomas producing monoclonal antibodies with high titer were selected by western blotting. The MAbs produced by these hybridomas were identified as IgM isotypes. The 4 hybridomas (AK-9, AK-11, AK-14 and AK-56) were propagated in ascites as well as in culture medium and cryopreserved. Further characterization of these monoclonal antibodies for therapeutic as well as diagnostic potential is under progress.

### 6.3.3 Genetic analysis of amphotericin B resistant strain of *C. albicans*

Amphotericin B resistant strain of *C. albicans* developed in laboratory condition was confirmed for stability of acquired resistance *in vitro* as well as in

*vivo.* The strain exhibited reduced germ tube formation, enhanced expression of virulence factors like extracellular secreted aspartyl proteinase and extracellular phospholipase and reduction in ergosterol content and germ tube formation capacity.

### 6.4 Viral infections

Marine extracts were tested for *in vitro* activity against Japanese encephalitis (JE) virus in Vero cells. The *in vitro* antiviral activity of extracts was determined as inhibition of viral cytopathic effect in Vero cells infected with JE virus and plaque reduction assay.

# 7 Natural Products

(Coordinator: Dr. Rakesh Maurya)

*Chemical and pharmacological investigations of Indian medicinal plants and marine flora / fauna for isolation of active constituents to obtain new therapeutic agents.*


7.1 Bioactivities of medicinal plants

7.2 Modification of natural products

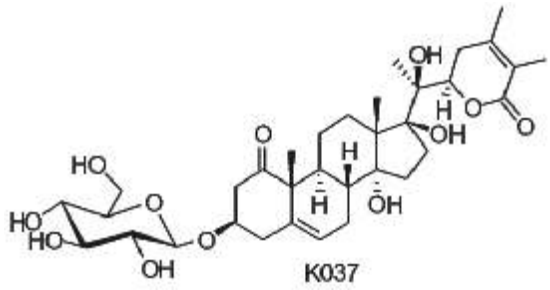

## 7.1 Bioactivities of medicinal plants

### 7.1.1 Antihyperglycaemic/ Antidyslipidemic activity

#### 7.1.1.1 Plant 4554 (Antihyperglycaemic)

Ethanolic extract (C002) and aqueous extract (C003) were evaluated for antihyperglycaemic activity in sucrose loaded rat model at 250 mg/kg p.o. dose level; the percent antihyperglycaemic activity was calculated to be around 13.5 and 34.4 respectively.

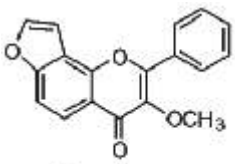
The aqueous extract (C003) was evaluated for antihyperglycaemic activity in STZ-S model at 250 mg/kg p.o. dose level, the percent antihyperglycaemic activity was calculated to be around 25.1 (5h) and 24.3 (24h). 4 Compounds K028, K029, K037 and K041 have been isolated, evaluated for antihyperglycamic activity. Only compound K037 showed significant antihyperglycamic activity. *(Bioorganic & Medicinal Chemistry Letters 2008, 18, 6534-6537)*.



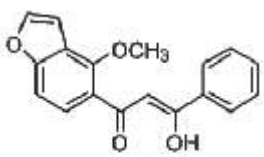

K037

#### 7.1.1.2 Plant 3200 (Antihyperglycaemic)

In streptozotocin-induced diabetic rats, single dose treatment of pongamol and karanjin lowered the blood glucose level by 12.8% ($p < 0.05$) and 11.7% ($p < 0.05$) at 50mg /kg dose and 22.0% ($p < 0.01$) and 20.7% ($p < 0.01$) at 100 mg/kg dose, respectively after 6 h post-oral administration. The compounds also significantly lowered blood glucose level in db/db mice with percent activity of 35.7 ($p < 0.01$) and 30.6 ($p < 0.01$), respectively at 100 mg/kg dose after consecutive treatment for 10 days. The compounds were observed to exert a significant inhibitory effect on enzyme protein tyrosine phosphatase-1B (EC 3.1.3.48) (*Journal of Ethnopharmacology 2008, 118, 435-439*).




karanjin

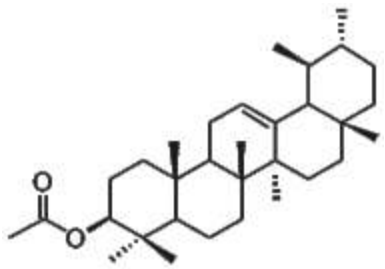



pongamol

#### 7.1.1.3 Plant 3247 (Antihyperglycaemic)

Bioactivity guided isolation on the fruits of 3247 resulted in the identification of antidiabetic active principle, α-amyrin acetat. It lowered the blood glucose levels by 18.4 and 17.0% at 5 and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rat (STZ-S) model at the dose of 100 mg/kg body weight. Fifteen novel derivatives of α-amyrin

were prepared and their antihyperglycemic activity profile was assessed. The *p*-chlorobenzoic acid derivative (22.1 at 5h and 25.6 at 24h) and nicotinic acid derivative (18.2 at 5h and 27.6 at 24h) showed potent antihyperglycemic activity at 100 mg/kg body weight (*European Journal of Medicinal Chemistry* 2008, doi: 10.1016/ j.ejmech. 2008.09.011).



a -amyrin acetate

7.1.1.4 Plant 4699 (Antihyperglycaemic)

Ethanolic extract (A001) showed significant antihyperglycaemic activity in Sucrose loaded rats (28.8%) and Streptozotocin-induced diabetic rats (16.5% at 5h; 22.5% at 24h) models, at 250 mg/kg p.o. dose levels.

Fraction (F021) was evaluated for antihyperglycaemic activity in STZ-S model at 250 mg/kg p.o. dose level; the percent antihyperglycaemic activity was calculated to be around 30.2% (5h) and 33.8% (24h). Two compounds K022 (new) and K023 (known) were isolated from active fraction F021. New compound (K022) showed significant activity.

7.1.1.5 Plant 4725 (Antihyperglycaemic)

Crude extract (A001) showed promising antihyperglycaemic activity in sucrose loaded rats (28.5%) and streptozotocin induced diabetic rats (22.1%) models, at 250 mg/kg p.o. dose levels.

7.1.1.6 Plant 4726 (Antihyperglycaemic)

Crude extract (A002) showed promising antihyperglycaemic activity in sucrose loaded rats (35.9%) model, at 250 mg/kg p.o. dose level.

7.1.1.7 Plant 1554 (Antihyperglycaemic)

Crude extract (C002) showed promising antihyperglycaemic activity in sucrose loaded rats (29.3%) model, at 250 mg/kg p.o. dose level.

7.1.1.8 Plant 4665 (Antihyperglycaemic)

Earlier the crude extract (A001) had shown antihyperglycemic activity. The extract was fractionated and evaluated for antihyperglycemic activity. The fraction (F003) has exhibited 25.2% and 24.7% sugar lowering at 5 hours and 24 hours respectively in STZ model at a dose of 250 mg/kg.

7.1.1.9 Plant 4659 (Antihyperglycaemic and Antidyslipidemic)

Earlier we have identified antihyperglycemic and antidyslipidemic active principle (K007) from plant 1703. We have also identified the same active principle (K007) in plant 4659. Further work is in progress to isolate the compound in larger quantities for further studies.

7.1.1.10 Plant 4698 (Antidyslipidemic)

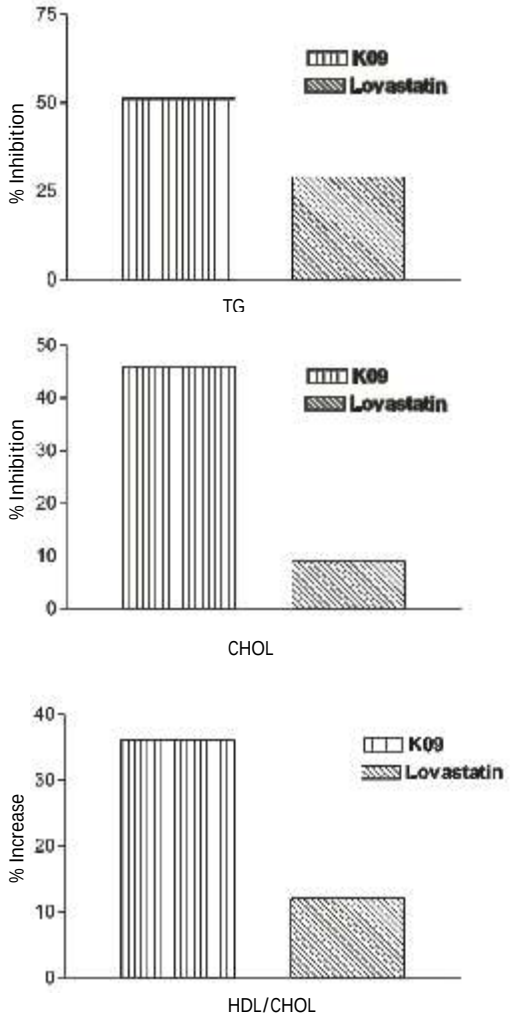
Ethanolic extract (A001) at 500 mg/kg dose, was found to reduced serum cholesterol by 27%, serum LDL and TG were decreased by 33% and 49 %, respectively. Therefore, ethanolic extract was fractionated into four fractions; F002, F003, F004 and F005 Fractions. Fractions F002 and F005 were found to be active. 2 Compounds (K035, K036) from hexane fraction (F002) and 2 compounds (K046, K047) from chloroform fraction (F003) have been isolated. K046 and K047 showed significant antidyslipidemic activity.

7.1.1.11 Plant 4499 (Antidyslipidemic)

Twelve derivatives of K027 were prepared and five compounds evaluated for their antidyslipidemic activity. The compound K027D4 significantly lowered 40% ($P<0001$) in triglycerides, 30% ($P<0.05$) in glycerol, 24% ($P<0.05$) in cholesterol quantity and also improved the HDL-Cholesterol by 5% in dyslipidemic hamster model at the dose of 50 mg/kg body weight.

7.1.1.12 Plant 4655 (Antidyslipidemic)

Ethanolic extract (A001) and its four fractions (F002, F003, F004 and F005) have been evaluated for antidyslipdemic activity. The activity is localized in hexane fraction (F002). Four diterpenes K006, K007, K009 and K010 isolated from Fraction F002. Pure compound K009 showed significant lipid lowering profile at 25mg/kg body weight dose and possess much better cholesterol lowering property than known drug lovastatin at the same dose (Patent No. 031NF2008).




7.1.1.13 Plant 4400 (Antidyslipidemic)

Ethanolic extract (C002) showed significant antidyslipidemic activity with lowering of TG, TC, Gly and increase in HDL and HDL/TC. Isolation of compounds from the active fractions (F004, F005) is under progress.

7.1.1.14 Plant 4666 (Antidyslipidemic)

From the aqueous fraction of plant 4666, compound K007 was isolated and its antidyslipidemic activity was studied in hamster model. Compound K007 showed significant activity.

7.1.1.15 Plant 4714 (Antidyslipidemic)

Ethanolic extract (C002) showed significant antidyslipidemic activity. Four fractions (F002, F003, F004 and F005) are under evaluation.

7.1.1.16 CDR-134F194 (Antihyperglycaemic)

CDR-134F194, which is in the preclinical phase, the regulatory pharmacology and toxicity studies in monkeys have been completed. IND has been filed for initiation of phase-I clinical trial .

7.1.1.17 CDR-150 (Antidyslipidemic and Antihyperglycaemic)

Antidiabetic activity was reconfirmed in the extracts C003 and C004 prepared from the repeat collections. Isolation of compounds from active fraction is in progress.

### 7.1.2 Anti-stress activity

7.1.2.1 Plant 38 (Anti-stress)

Three new compounds named ocimumosides A, ocimumosides B and ocimarin from n-butanol fraction (F005) were found to be effective in normalizing all parameters of acute stress (hyperglycemia, increased corticosterone, CK and adrenal hypertrophy).

7.1.2.2 Plant 4740 (Anti-stress)

Ethanolic extract (A001) was found to be effective in normalizing parameters of acute stress. Four fractions (F002, F003, F004 and F005) were evaluated and the activity was localized in butanol fraction (F004).

### 7.1.3 Anti-ulcer activity

7.1.3.1 Plant 4483 (Anti-ulcer)

Ethanolic extract (4483 C003), at 250 mg/kg, po the showed significant protection against cold restraint, aspirin and pylorocligation induced ulcer

model. Activity confirmed in repeat experiment. Fractions F008, F009, F010 and F011 were evaluated in CRU model at 40 mg/kg, p.o. Fraction F010 and F011 showed significant protection. Isolation of compounds is in progress.

7.1.3.2 Plant 4738 (Anti-ulcer)

Ethanolic extract (A001), at 250 mg/kg, po the showed significant protection against cold restraint, aspirin and pyloricligation induced ulcer models. Activity guided fractionation and isolation is in progress.

### 7.1.4 Antiparasitic activity

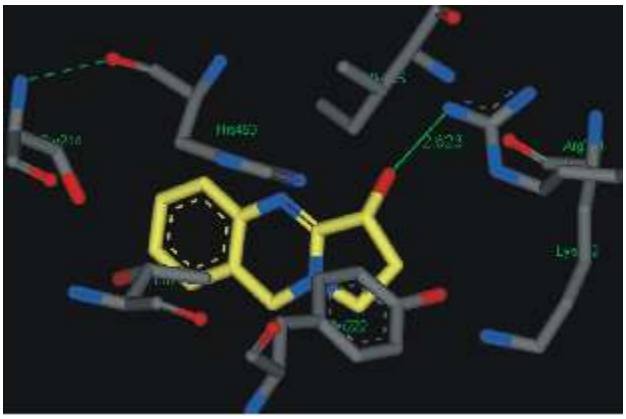
7.1.4.1 Plant 4601 (Antileishmanial)

Ethanolic extract (C003) and fractions (F004-F008) were evaluated both *in vitro* and *in vivo* against *Leishmania donovani*. The oral administration of ethanolic extract to hamsters infected with *L. donovani* resulted in 75.5 ± 3.7 % inhibition at 500 mg/kg x 5 whereas at 250 mg/kg x 5 dose, showed slightly lower (55 ± 7.2 %) antileishmanial effect.

Fraction F007 exerted 61.8 ± 3.6 % inhibition of parasite at the dose of 250 mg/kg x 5, p.o. 6 Compounds (K012, K013, K015-K018 isolated from F007) were evaluated *in vitro*. 3 Compounds (K013, K015 and K017) showed $IC_{50}$ (µg/ml) 47 ± 6.8, 56 ± 7.5 and 27.4 ± 3.7 respectively against intracellular amastigotes.

7.1.4.2 Plant 4666 (Antileishmanial)

Peganine hydrochloride dehydrate isolated from the seeds of 4666 exhibited *in vitro* activity against both extracellular promastigotes as well as intracellular amastigotes residing within murine macrophages in *Leishmania donovani*. Furthermore, it also exhibited *in vivo activity*, 79.6 (± 8.07) % against established VL in hamsters at a dose of 100 mg/kg b.wt. Besides being safe, it was found to induce apoptosis in both the stages of *L. donovani* via loss of mitochondrial transmembrane potential. Molecular docking studies suggested that a binding interaction with DNA topoisomerase I of *L. donovani* (binding energy of 279 kcal/mol) forms a stable complex, indicating a possible role in apoptosis. The compound also inhibits *L. donovani* topoisomerase I in experimental studies (*Journal of Antimicrobial Chemotherapy* 2008, 62, 9981002).



7.1.4.3 Plant 4382 (Antileishmanial)

The plant extract has exhibited antileishmanial activity. Fractionation and isolation of active compounds is in progress.

7.1.4.4 Plant 4613 (Anti-filarial)

Different class of compounds (seven) were isolated and characterized. Out of these olenonic acid and lantadine derived from mixture of lantadines, showed significant antifilarial activity *in vitro*.

### 7.1.5 Anti-osteoporotic activity

7.1.5.1 Plant 1020 (Anti-osteoporotic)

Ethanolic extract and *n*-butanol fraction of plant 1020 showed promising osteogenic activity. 8 Compounds (K084, K090, K095, K103, K105, 113, 115, sitosterol) from chloroform fraction 16 compounds (K010, K035, K039, K040, K051, K052, K053, K054, K064, K080, K082, K098, K111, K130, K135, sitosterol glucoside) have been isolated from n-butanol fraction. These compounds were evaluated only five compounds (K051, K052, K054, K080 and K095) were found to show promising osteogenic activity. The three active compounds (K052, K054 and K080) have been synthesized and *in vivo* osteogenic activity validated. K052, K080 and K095 have been combined based on their differential mode of action for *in vivo* osteogenic activity.

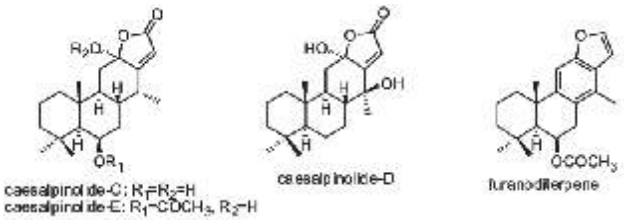
7.1.5.2 Plant 914 (Anti-osteoporotic)

Ethanolic extract (C002) administered to overiectomized (OVX) rats showed improvement in BMD. 4 Compounds K012, K058, K068 and K100 have been isolated. Administration of K058 and K012 to ovariectomized (OVX) rats, showed improvement in BMD. Administration of K058 and K012 to immature rats showed better peak bone mass achievement.

### 7.1.6 Anticancer activity

7.1.6.1 Plant 4690 (Anticancer)

Ethanolic extract A001 has shown antibreast cancer activity. Four fractions (F002, F003, F004 and F005) were prepared and evaluated for anticancer activity. Three new cassane diterpene hemiketals, caesalpinolide-C, caesalpinolide-D, caesalpinolide-E and one new cassane furanoditerpene were isolated from active fraction F005. The isolated compounds were tested for their antiproliferative activity against MCF-7 (breast adenocarcinoma), DU145 (Prostate carcinoma), C33A (Cervical carcinoma) and Vero (African green monkey kidney fibroblast) cells (*Phytochemistry* 2009, doi: 10.1016/*j.phytochem.2008.12.008).*




7.1.6.2 Plant No. 4655 (Anticancer)

Two ditepenes (K019, K006) have been isolated and characterized from hexane fraction (F002), both compounds showed anticancer activity.

## 7.2 Modification of natural products

Synthesis of hybrid forms of lupeol, isolated from *Crataeva nurvala* are continued for antimalarial and anticancer activities.

Novel hybrid forms of solanesol were designed and synthesized for antitubercular activity.

Analogs of aromatic turmerone were designed where conformational mobility of its side chain was restricted, synthesis of these were initiated for antithrombotic and neuroprotective activities.

New curcuminoids derived from thymol were prepared for anticancer activity.

9 New compounds have been synthesized for antimalarial activity; out of these two compounds have shown significant antimalarial activity.

# Newer Approaches in Drug Design and Discovery

8

(Coordinator: Dr. S.B. Katti)

*The project area envisages exploring and exploiting emerging technologies like structural biology, in-silico design and x-ray crystallography towards lead generation and optimization of drug like molecules, structural studies on small and macromolecules and identification of druggable targets.*


8.1 Studies on protein folding

8.2 X-ray crystallographic studies

8.3 Computational biology and bioinformatics in drug discovery

8.4 Structural genomics of Mycobacterium tuberculosis proteins using NMR spectroscopy

8.5 Structural function studies of proteins, antimicrobial peptides and design of peptide inhibitors

8.6 Understanding the mechanism of mitotic/spindle checkpoint using genetics approaches in fission yeast Schizosacchromyces pombe

8.7 Synthesis of combinatorial libraries

8.8 Novel methodologies for peptide design and synthesis.


## 8.1 Studies on protein folding

### 8.1.1 Structural and stability characteristics of a monothiol glutaredoxin: glutaredoxin-like protein 1 from *Plasmodium falciparum*

Recently discovered monothiol glutaredoxins with CXXS-active site sequence share a common structural motif and biochemical mechanism of action and are involved in multiple cellular functions. Here we report first studies on the structural and stability characterization of a monothiol glutaredoxin, in particular-PfGLP1. Our results demonstrate that in the native conformation, the enzyme has a compact core structure with a relatively flexible N-terminal portion having an open configuration. Comparative functional studies with the full-length and N-terminal truncated protein demonstrate that the flexible N-terminal portion does not play any significant role in functional activity of the protein. In contrast to other Grxs, PfGLP1 does not contain a Fe-S cluster. The pH dependent studies demonstrate that the protein is resistant to alkaline pH but highly sensitive to acidic pH and undergoes significant unfolding between pH 4 and 5. However, acidic conditions also do not induce complete unfolding of the enzyme. The protein is stabilized with a conformational free energy of about 3.2+/-0.1 kcal $mol^{-1}$. The protein is a highly cooperative molecule as during denaturant-induced equilibrium unfolding a simultaneous unfolding of the protein without stabilization of any partially folded intermediate is observed.

### 8.1.2 *Mycobacterium tuberculosis* isocitrate lyase (MtbIcl): role of divalent cations in modulation of functional and structural properties

Isocitrate lyase (Icl), an enzyme that plays an important role in the regulation of isocitrate flux and anaplerotic replenishment of pool of substrate required for biosynthetic process in *Mycobacterium tuberculosis* is a potential drug target for the antituberculosis drugs. Divalent cations induce differential effect of activation and inhibition of MtbIcl functional activity. The study for the first time demonstrates that interaction of cations with MtbIcl results in differential modulation of the enzyme structure which is probably the underlying mechanism for differential modulation of functional activity of enzyme by divalent cations. The $Mg^{2+}$ and $Mn^{2+}$ ions act as activators of the enzyme and in their absence no enzymatic activity was observed. These cations do not induce any significant structural alteration in the enzyme as observed by far-UV, CD and solvent denaturation studies using chaotropic salts. However, the thermal denaturation studies demonstrate that they do interact with the non-catalytic alpha/beta barrel core domain of the enzyme and destabilize it. The inhibitors $Zn^{2+}$ and $Cd^{2+}$ interact directly with the catalytic domain of the enzyme and unfold it as a result of which complete loss of the enzymatic activity is observed in their presence. The results obtained from the studies provide insight into the possible mechanism of divalent cation-induced changes in structure, function and stability of MtbIcl.

### 8.1.3 *Toxoplasma gondii* ferredoxin-$NADP^+$ reductase: Role of ionic interactions in stabilization of native conformation and structural cooperativity

The apicoplast and the proteins present therein are parasite-specific targets for chemotherapy of apicomplexan parasites. Ferredoxin-$NADP^+$ reductase (FNR) is an important enzyme present in the apicoplast of *Toxoplasma gondii* that operates as a general electron switch at the bifurcation step of many different electron transfer pathways. Inspite of its importance as drug target not much structural information on the enzyme is available. Using fluorescence and CD spectroscopy in combination with enzyme activity measurement and size exclusion chromatography, we studied the pH-dependent changes in structural and functional properties and inter-domain interactions in recombinant *Toxoplasma gondii* ferredoxin-$NADP^+$ reductase (TgFNR) to understand the interactions responsible for stabilization of native conformation and modulation of functional activity of the enzyme. Under physiological conditions, the recombinant TgFNR is stabilized in an open conformation. The open conformation of the enzyme was found to be essential for its optimum functioning, as induction of compactness/rigidity by modulation of pH, leads to decrease in the functional activity. In native conformation, strong interactions exist between the $NADP^+$- and FAD-binding domains thus making the enzyme a structurally cooperative molecule. Under acidic conditions (pH about 4), the inter-domain interactions present in native TgFNR were lost and the enzyme became structurally non-cooperative. The pH-induced structural alterations in the $NADP^+$ binding domain, more precisely compaction of the conformation lead to its stabilization against thermal denaturation. The studies demonstrate the significance of electrostatic interactions both in stabilization of native conformation and maintenance of structural cooperativity in TgFNR.

## 8.2 X-ray crystallographic studies: Small molecule crystallography

3-D X-ray intensity data collection/ processing and structure determinations of four compounds were performed. In addition, structural determination and analysis of eleven compounds were performed. The structure of Peganine hydrochloride dihydrate, a natural product possessing antileishmanial activity, shows that the molecule exists in hydrochloride salt form. Structural analysis of seven polycyclic aromatic hydrocarbons class of molecules showed helical conformation and atropism. Structural analysis of two pyrazolo [3,4-*d*]

pyrimidines class of molecules showed folded conformation having U-motif due to intramolecular - interactions. In addition, other weaker vanderWaals interactions were also found.

### 8.2.1 Macromolecule crystallography

Crystallographic and biochemical studies on proteins from pathogenic sources including use of in silico tools towards the development of novel therapeutics has been the major thrust of the project area. The progress achieved during this period includes:

(i) Crystal structures of proteins like L-alanine dehydrogenase and nucleoside diphosphate kinase have been elucidated. The former protein has been listed in the top-3 targets against persistence by the tuberculosis structural genomics consortium.

(ii) The functions of three hypothetical proteins from *M. tuberculosis* have been probed and resulted in novel insights into the respective functions.

(iii) The studies on the Sigma factor F and interacting proteins have revealed novel insights to the interactions and protein mechanism.

(iv) Development of IS-*IT*? A computational web server for evaluating ligand protein interactions through docking.

The results are being exploited in the design of novel inhibitors.

### 8.2.2 Structural studies on proteins from kinetoplastida

The focus of our group is the X-ray crystal structure determination of proteins from kinetoplastida and towards this, our focus is on proteins involved in the thiol based redox metabolism pathway. The redox metabolism in kinetoplastida is unique in that it is based on trypanothione as against the ubiquitous glutathione prevalent in other eukaryotes. Comparison of sequences of these enzymes with their corresponding human homologues shows suitable differences at the amino acid level. Altogether, five proteins involved in the synthesis of trypanothione in *Leishmania* spp. have been identified and targeted for their structural elucidation. For this, our approach is to clone the protein, express in a suitable bacterial expression vector followed by purification. All our initial targets have been cloned successfully, and two of them have been expressed and purified, suitable for crystallization attempts. Optimization of expression hosts, primer sequences, restriction enzymes, etc. are being carried out.

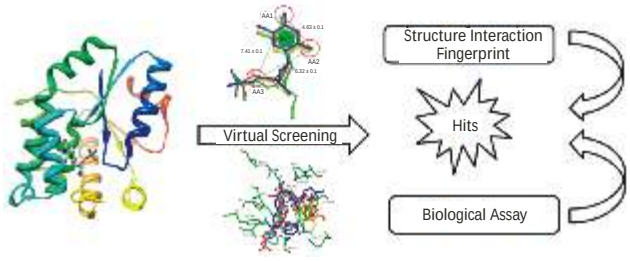
Of the two purified proteins, crystallization attempts have been successful in one, and X-ray diffraction data has been collected. Structure solution is in progress, and so far, molecular replacements calculations have not resulted in an unambiguous solution, most probably on account of the high number of copies present in the asymmetric unit. Efforts are on to crystallize with suitable heavy atoms, as these would enable us solve the structure using *ab initio* methods. For the other purified protein, preliminary crystallization attempts followed by iterative rounds of optimization have resulted in obtaining a weakly diffracting crystal. Further biochemical studies with its binding ligands are being carried out. We are also identifying other suitable target proteins from kinetoplastida for structural elucidation.

## 8.3 Computational biology and bioinformatics in drug discovery

### 8.3.1 Knowledge based identification of potent anti-tubercular compounds using structure based virtual screening and structure interaction fingerprints

In continuation of our efforts to identify new chemical entities endowed with activity against *Mycobacterium tuberculosis*, we have employed an

integrated approach to prioritize target specific anti-tubercular compounds using ligand and structure-based virtual screening and subsequently, we have employed structure interaction fingerprints to prioritize the leads. We have reported the identification of potent anti-tubercular compounds targeting TMPK*mt* using virtual screening methods. For this purpose, we have developed a pharmacophore hypothesis based on the substrate and known TMPK*mt* inhibitors and employed it to screen Maybridge small molecule database. The molecular docking was then performed in order to select the compounds on the basis of their ability to form favorable interactions with TMPK*mt* active site. In addition, we applied straight forward weighting using structure interaction fingerprints to include additional knowledge into structure based virtual screening. Eight compounds were acquired and evaluated for anti-tubercular activity against *M. tuberculosis* H37Rv *in vitro* and out of these 3 compounds showed MIC of 3.12 µg/ml whereas 2 compounds showed MIC of 12.5 µg/ml. All the active compounds were found to be non-toxic in Vero cell lines and mice bone marrow macrophages. All the identified hits highlighted a key hydrogen bonding interaction with Arg74. The observed p-stacking interaction with Phe70 was also produced by the identified hits. These hits represent promising starting points for structural optimization in hit-to-lead development (*J. Chem. Info. and model. http://pubs.acs.org/doi/abs/10.1021/ci8003607)*




### 8.3.2 Virtual screening against *Mycobacterium tuberculosis* dihydrofolate reductase

In this study, we suggested a new workflow for the identification and prioritization of potential compounds targeted against *Mycobacterium tuberculosis* dihydrofolate reductase, an important folate cycle enzyme and a validated target for the development of anti-tubercular agents. First, we have performed an integrated pharmacophore and structure based virtual screening using Maybridge small molecule database, subsequently interaction patterns from known activities to the receptor were applied for scoring and ranking the virtual screening hits using structure interaction fingerprint-based similarity approach. In addition, agglomerative hierarchical clustering of the structure interaction fingerprints permits the easy separation of active from inactive binding modes. Using this approach, we screened 59275 Maybridge compounds and 20 compounds were prioritized as promising virtual screening hits. Though using a receptor interaction scoring approach, the results were not biased toward the chemical classes of the known actives and the proposed compounds were structurally diverse with low molecular weights and structural complexities. Our results suggest that structure based virtual screening coupled with the SIFt should be a valuable tool for prioritization of virtual screening hits.

### 8.3.3 CoMFA based *de novo* design of pyrrolidine carboxamides as inhibitors of Enoyl Acyl Carrier Protein Reductase from *Mycobacterium tuberculosis*

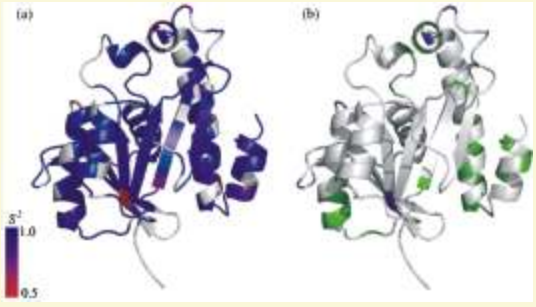
InhA, the enoyl acyl carrier protein reductase (EACP reductase) from *Mycobacterium tuberculosis*, is one of the key enzymes involved in the mycobacterial fatty acid elongation cycle and has been validated as an effective target for the development of anti-microbial agents. We report here, Comparative Molecular Field Analysis (CoMFA) studies and subsequent *de novo* ligand design using LeapFrog program on pyrrolidine carboxamides, which have been reported as selective inhibitors of EACP reductase from *Mycobacterium tuberculosis*. The CoMFA model, constructed from the inhibitors used in this study has been successfully used to rationalize the structure-activity relationship of pyrrolidine carboxamides. The CoMFA model produced statistically significant results with cross-validated and conventional correlation coefficients

of 0.626 and 0.953 respectively. Further, the predictive ability of CoMFA model was determined using a test set which gave predictive correlation coefficient $r^2_{pred}$ of 0.880, indicating good predictive power. Finally, Leapfrog was used to propose 13 new pyrrolidine carboxamide analogues, based on the information derived from the CoMFA contour maps. The designed molecules showed better predicted activity using the CoMFA model with respect to the already reported systems; hence suggesting that newly proposed molecules in this series of compounds may be more potent and selective towards EACP reductase inhibition.

## 8.4 Structural genomics of *Mycobacterium tuberculosis* - Proteins using NMR spectroscopy

During this period, a significant milestone has been achieved by solving structure of the potential drug target protein peptidyl-tRNA hydrolase from *M. tuberculosis* H37Rv (MtPth) in solution by NMR spectroscopy. The ensemble of 40 structures representing the solution structure of MtPth has been deposited in PDB under ID 2JRC. Parallely, we have measured the amide $^{15}$N T1, T2 and $^{15}$N {$^1$H}heteronuclear NOE and have used these parameters for detailed dynamic analysis of the protein. Our results highlight the dynamic interaction of the protein with its substrate peptidyl-tRNA.

The first solution structure of a bacterial Pth protein (MtPth) using NMR has been determined to enable designing of novel inhibitors by NMR based screening.




(a) The ribbon representation of MtPth structure shaded according to the $S^2$ values derived from model-free analysis. The color coding is from blue for $S^2$ = 1, to red for $S^2$ = 0.5. (b) MtPth structure shaded according to chemical exchange ($R_{ex}$) terms (green) and effective correlation ($t_e$) times (blue) from model-free analysis.

Additionally, complex formation for ESAT-6 like proteins of *M. tuberculosis* H37Rv/ Rv0288, Rv0287, Rv3619c and Rv3620c have been completed during this period.

Complete $^1$H, $^{13}$C, and $^{15}$N-NMR assignments for actin depolymerizing factor (ADF)/cofilin from *Leishmania donovani* has been carried out during this period and the assignments have been deposited with Bio Magnetic Resonance Bank, University of Wisconsin-Madison, USA under ID 15557.

## 8.5 Structural function studies of proteins, antimicrobial peptides and design of peptide inhibitors

Despite numerous studies, the molecular basis of antibacterial and cytotoxic activities of antimicrobial peptides is not fully understood. Melittin is a good model antimicrobial peptide to understand the basis of its lytic activities against bacteria and mammalian cells. Novel analogs of melittin were designed by substituting the leucine residue(s) at the'd' position(s) of the previously identified leucine zipper motif of melittin. The effect of these amino acid substitutions on the lytic activity of melittin on bacteria and human red blood cells (hRBCs) was examined as well as the direct interactions of these peptides with *E. coli* and hRBCs were carried out. It was observed that only the hemolytic activity but not the antibacterial activity of melittin progressively decreased as a result of the substitution of single and double leucine residues at the'd' positions by alanine. Interestingly melittin, but not its analogs, depolarized the hRBCs significantly though all three peptides permeabilized *E. coli* cells appreciably with almost equal efficiency. Both melittin and its analogs assembled similarly onto live *E. coli* cells while the extent of assembly of melittin progressively decreased with alanine substitution onto the live hRBCs.

We have recently designed short novel antimicrobial peptides based on heptad repeat sequence by placing either leucine or phenylalanine or valine or alanine in the 'a' and 'd' positions of the heptads. Contrasting results have been observed with

respect to their cytotoxic activity against human red blood cells and murine 3T3 cells. Detailed characterization of these peptides to understand the basis of their activity against microorganisms and mammalian cells is under progress.

The possible contribution of the putative transmembrane domain located in the tail region of the protein toxin hemolysin E was addressed by synthesizing peptides as well incorporating mutations in the corresponding region of the whole protein. The two mutant proteins with one having aspartic acid substituted for valine at 89$^{th}$ position and in the other glycine and valine at 88$^{th}$ and 89$^{th}$ positions substituted two aspartic acid residues were totally inactive in causing lysis and depolarization of hRBCs. Detailed studies with both wild type and mutated peptides and full-length protein suggested a possible role of the putative transmembrane domain in the tail region in the toxic activity of the toxin.

### 8.6 Understanding the mechanism of mitotic / spindle checkpoint using genetic approaches in fission yeast *Schizosacchromyces pombe*

The fission yeast *S. pombe* is a good model system for the studies of the cell cycle events. These events must be executed in correct order to ensure chromosome integrity which is monitored by checkpoint proteins. Defects in checkpoint proteins can lead to cancer in higher eukaryotes. DNA topoisomerases are required for altering DNA topology to facilitate replication, transcription and chromosome segregation. Topoisomerase II has been studied in detail in yeast and mammalian systems and suggested to be involved in cell proliferation and cell cycle regulation. Top2 dependent checkpoint has been shown are defective in some subset of human cancer. In budding and fission yeast, the essential role of Top2 in decatenating sister chromatids before anaphase has been demonstrated by means of temperature-sensitive *top2* mutants. But their involvement in DNA damage checkpoint is still elusive. We have isolated temperature sensitive mutant of *top2* which shows conditional synthetic lethality with DNA damage checkpoint kinase *chk1*. Activation of checkpoint kinase protein Chk1 takes place in the presence of defective *top2* activity. We have also have shown that *in the absence of DNA damage checkpoint response top2 mutant cells show severe chromosome segregation defects.*

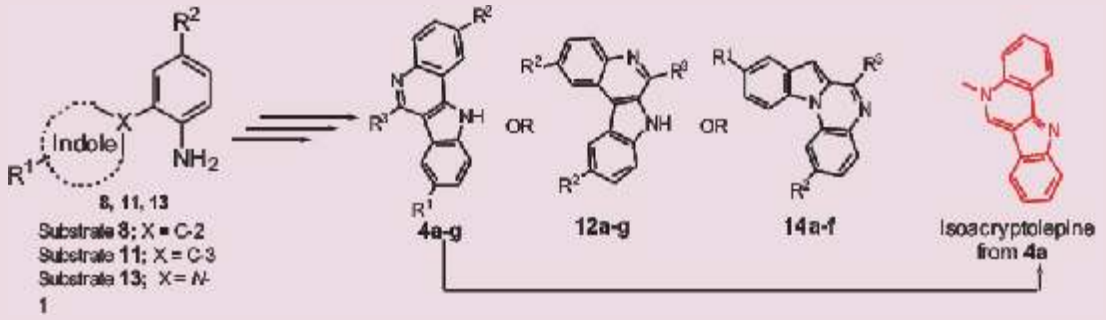
We have also isolated several conditional and non conditional mutants which are directly or indirectly involved in spindle checkpoint based on their sensitivity towards the microtubule destabilizing agent Benomyl and TBZ. These mutants have been extensively back-crossed and were confirmed for their sensitivity towards Benomyl. Conditional mutants were further confirmed for their inability to grow at non permissive temperature i.e. 37$^{o}$C. Currently we are trying to clone the gene coding for these mutants then we will try to identify how these genes are involved in spindle/mitotic checkpoint.

### 8.7 Synthesis of combinatorial libraries

#### 8.7.1 New route to the synthesis of isocryptolepine alkaloid and its related skeletons

A new synthetic routes to 1,2- and 2,3-fused indoles with six membered rings have been developed with the aim to generate libraries based on the three structural variants of an indole alkaloid isocryptolepine with antimalarial activity. Compounds based on the three variants in general were accessed in three steps with a modified Pictet-Spengler cyclization as the key step. The precursors *N*-1 or *C*-2 or *C*-3 linked aryl amine indoles required for cyclization were obtained by treating indoles with *o*-halonitrobenzene using either nucleophilic replacement or Pd-based chemistry (Heck/Suzuki reaction) followed by reduction of the aryl nitro functionality. The resulting structural variants of isocryptolepine, indoloquinolines and indoloquinoxalines with three-point diversity, were obtained in high yields and purities. Twenty compounds have been synthesized and submitted for antimalarial screening *in vitro*.

A new synthetic routes to 1,2- and 2,3-fused indoles with six membered rings have been developed with the aim to generate libraries based on the three structural variants (4, 12 and 14) of an indole alkaloid isocryptolepine with antimalarial activity. Isocryptolepine and its derivatives were accessed using a modified Pictet-Spengler cyclization as the key step from substrates 8, 11 and 13. (*Eur J Org Chem* 2009, 292-303.)




### 8.7.2 Novel application of the Pictet-Spengler reaction using the deactivated pyrimidine ring as nucleophilic partner

Recently, we developed a modified strategy wherein, aromatic amines originating from activated heterocyclic rings represented second generation substrates for the Pictet-Spengler reaction. The most interesting feature of our modified strategy is that 1) unlike traditional substrates, the -cyclizations with aryl amine substrates occurred with a wide variety of aldehydes having both electron-donating and withdrawing substituents and 2) the faster rate of reaction observed in substrates (Second generation) with aryl amine than the conventional Pictet-Spengler reaction substrates (First generation) having aliphatic amine. Based on these observations, we then successfully demonstrated that the Pictet-Spengler reaction could be affected even when aryl amine is linked to a deactivated (poorly nucleophilic triazoles, tetrazoles and quinoxalines) heterocyclic ring. In order to further strengthen the scope of our application of the modified Pictet-Spengler strategy to deactivated heterosystems, we decided to demonstrate its efficacy on pyrimidines, with established medicinal pedigree. Initially, three pyrimidine-based substrates were synthesized followed by *endo* cyclization. The strategy resulted in the synthesis of novel pyrimidoquinolines with strong structural analogy to banzonaphthyridines, a pharmacophore widely distributed in nature.

### 8.7.3 A new entry to the substituted cryptotackiene and related skeletons

A mild, efficient, and one-pot protocol for the intramolecular cyclization of 2-substituted nitroarenes via CN bond formation using $SnCl_2.2H_2O$ has been developed. The versatility of the method has been demonstrated by synthesizing two sets of polycyclic structures based on privileged structures of indole and pyrrole, and of an alkaloid cryptotackieine associated with antimalarial activity. Our new approach provides a powerful entry into polycyclic structures related to alkaloids.

## 8.8 Novel methodologies for peptide design and synthesis

Diabetes mellitus is a metabolic disorder marked by the insufficient secretion of insulin or development of resistance to insulin. Available treatment is inadequate and there is an urgent need for the search of novel chemotherapeutic agents for the treatment of diabetes. Therefore we have chosen protein tyrosine phosphatease-1B (PTP-1B), dipeptidyl peptidase (DPP-IV) and peroxisome proliferator-activated receptors-gamma (PPAR-gamma) as targets for the development of novel antidiabetic agents.

### 8.8.1 Peptidomimetcs as selective inhibitors of PTP1B

Inhibition of PTP1B which attenuates insulin signaling by catalyzing de-phosphorylation of insulin receptors (IRs) has gained significant attention as a

new target for diabetes therapy. We have been working on the lead optimization of a dipeptide by developing peptidomimetics to improve its potency and selectivity. During this period six new compounds have been synthesized. Compound S-008-752 has shown better inhibitory activity and selectivity compared to the reference compound.

### 8.8.2 DPP-IV inhibitors as antidiabetic agents

Dipeptidyl peptidase IV (DPP-IV) is a widely distributed serine protease acting on GLP-1 and cleaved it to its inactive form. Glp-1is incretin hormones are released from the L-cells in the intestine upon food intake and stimulate insulin secretion from the beta-cells in the pancreas. It is also reported that the GLP-1 helps to regenerate the degenerated beta cells in the pancreas. It has been demonstrated that blocking the action of DPP-IV on GLP-1 results in the protection of GLP-1 from its degradation thereby enhanced insulinotropic activity. DPP-IV is a highly specific aminopeptidase that cleaves Xaa-Pro and modification of proline to pyrolidine result in the powerful inhibitor. Therefore, we have chosen Xaa-prolidide as prototype for the lead optimization by designing several peptidomimetic. During this period nineteen new compounds were synthesized. The *in vitro* model for the DPP-IV enzyme assay has also been standardized. Among the new compounds tested for DPP-IV inhibitory activity compounds S-008-1606 and S-008-1608 exhibited $IC_{50}$ >10 µg/ml concentration.

### 8.8.3 Synthesis of PPAR-gamma agonist

Peroxisome proliferator-activated receptors-gamma is an important target for the development of antidiabetic agents. However, this class of compounds exhibit liver toxicity. We have designed several molecules with objective to minimize the toxicity and simultaneously improve the activity. During this period five new compounds have been synthesized. Some of the compounds synthesized have shown significant glucose lowering effect which is comparable to rosiglitazone.

### 8.8.4 3D-QSAR studies on NNRTIs

Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were performed on thiazolidin-4-one class of compounds as HIV-1 reverse transcriptase (HIV-1 RT) inhibitors using global minima and crystal structure conformations. Results obtained from the crystal structure-based model yielded superior statistical data (r2cv values of 0.683 for CoMFA and 0.678for CoMSIA) when compared to those obtained by the global minima-based model (r2cv values of 0.625 and 0.654 for CoMFA and CoMSIA, respectively). The models were validated using an external test set of 47 compounds. The predictive r2 values for the crystal-based CoMFA and CoMSIA models were 0.735 and 0.739, respectively, while the corresponding predictive r2 values for the global minima-based CoMFA and CoMSIA models were 0.654 and 0.635, respectively. 3D contour maps generated from these models provide the regions in space where interactive fields may influence the activity. The superimposition of contour maps on the active site of HIV-1 reverse transcriptase additionally helped in understanding the structural requirements of these inhibitors. The results provide insight for predictive and diagnostic aspects of this class of HIV-1 RT inhibitors for better activity.

# Reproductive Health Research 9

(Coordinator: Dr. Naibedya Chattopadhyay)

*Design and synthesize novel molecules/isolate from natural sources and bioevaluate them for generating new leads and to develop them as female or male contraceptives, spermicides with anti-STI properties, agents for the management of post-menopausal osteoporosis and other endocrine disorders; evaluate traditional remedies for fertility regulation and endocrine disorders; understand mode of action of promising agents and undertake basic research to generate new knowledge on female and male reproductive endocrinology relevant to fertility regulation.*


9.1 Development of vaginal spermicide (S-003-296)

9.2 Development of anti-implantation and early post-implantation pregnancy interceptive agents

9.3 Development of anti-osteoporosis agents

9.4 Development of agents for anti-cancer breast

9.5 Development of agents for the management of Benign Prostatic Hyperplasia (BPH)


## 9.1 Development of vaginal spermicide (S-003-296)

### 9.1.1 Specificity of spermicidal action

The study examined the action of the new compound on human sperm vs. human cervical (HeLa) cells to establish the specificity of spermicidal action and safety in comparison to the marketed compound nonoxynol-9 (N-9). At 20 µg/ml S-003-296 killed 100% sperm in <30 seconds but did not affect HeLa cell viability up to 24 hours. This concentration did not change annexin-V labeling pattern and mitochondrial transmembrane potential of HeLa in 24 hours, while $EC_{50}$ (~10 µg/ml) induced apoptosis in sperm in 3 hours. N-9 killed 100% sperm at 500 µg/ml while at 20 µg/ml it caused apoptosis of HeLa cells with a significant reduction in their viability. S-003-296 caused negligible induction of reactive oxygen species (ROS) and did not stimulate the expression of proinflammatory cytokine mRNAs (IL-1α, IL-6, IL-8, RANTES) while N-9 increased ROS and cytokines in HeLa. Scanning Electron Microscopy revealed an

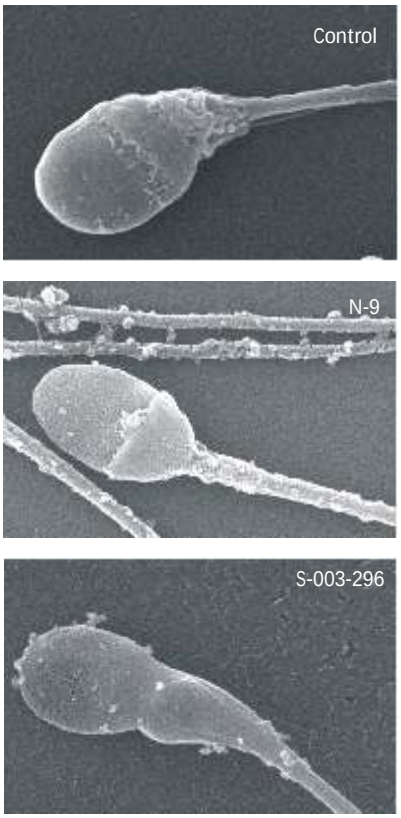
Control N-9 S-003-296

Scanning electron micrograph of *HeLa* cells treated with N-9 and S-003-296 at 20 µg/ml for 24 hours

apparently inert action of new spermicide on HeLa cell surface topography that was otherwise acutely damaged by N-9. Furthermore, S-003-296 did not inhibit *Lactobacillus* growth and exhibited a mild microbicidal activity against *Trichomonoas vaginalis* while N-9 significantly inhibited *Lactobacillus* and *Trichomonas* growth but had a lower prophylactic index. The unique ability of this novel spermicide to kill sperm almost instantaneously at innocuously low concentration indicates its worth as excellent active ingredient for safer vaginal contraceptive preparations, than N-9.

### 9.1.2 Targets of S-003-296 on human sperm

The various targets on the sperm cell for spermicidal action were studied in an attempt to define the mechanism of action of S-003-296. It included sperm membrane ultrastructure, physiology, membrane potential, intracellular pH, protein tyrosine phosphorylation, dynein ATPase, ROS, super-oxide dismutase, annexin-V/PI binding, mitochondrial transmembrane potential etc.




Scanning electron micrograph of human sperm killed with N-9 and S-003-296, *in vitro*

S-003-296 caused a very "physiological" and mechanism based sperm death by hyperpolarizing membrane potential, increasing intracellular pH, inhibition of tyrosine phosphorylation and induction of apoptosis without physically disrupting the plasma membrane integrity. Detergent (N-9) action was apparently non-specific and thus sustainable at much higher concentration than S-003-296. It involved complete breakdown of membrane structure, total loss of cellular physiology accompanied by neutralization of membrane potential, equilibration of intracellular and extracelluar pH, ROS generation and inhibition of superoxide dismutase.

### 9.1.3 Preclinical contraceptive efficacy of S-003-296

The *in vivo* contraceptive efficacy of pure compound was taken up in rabbits. S-003-296 dissolved in normal saline was instilled intravaginally at doses of 1.0 and 2.0 mg in young adult female rabbits (Belgian strain), which were then mated with fertile bucks. 100% contraceptive effect was seen in the rabbits receiving 2.0 mg intravaginal dose of S-003-296. Reference control animals were given intravaginal instillation of 2.0 mg nonoxynol-9 and mated similarly. These animals delivered normal pups after the gestation period. The *in vivo* efficacy of the compound in a vaginal formulation (suppository) is in progress.

## 9.2 Development of anti-implantation and early post-implantation pregnancy interceptive agents

### 9.2.1 Evaluation of RBA for estrogen receptors

In an ongoing programme on "Development of selective estrogen receptor modulator as anti-implantation/anticancer breast agents", the relative binding affinity of 30 compounds was evaluated for uterine estrogen receptor (ER).

The results revealed that compounds S-006-382, -384, -386, -717, -887, -888, S-007-147, -154, -156, -158 showed RBA of 0.01 to 0.1 % S-006-387, S-007-148, 152, -153, -155, -157, -159, S-008-162, -163, -164, -165 and -166 showed RBA of 0.1% to 0.2%

of estradiol whereas compound S-006-385, -712, -714, -715, -716, S-007-150, -151 and S-008-0167 could not compete with $^{3}$H-estradiol for binding to ER and hence were inactive.

### 9.2.2 Evaluation of anti-implantation activity of synthetic compounds/natural products

36 synthetic compounds and 29 extracts of natural products were tested for anti-implantation-cum-early post-implantation interceptive activity in adult female Sprague Dwaley rats when administered on days 1-7 post coitum by the oral route. Of these, only three synthetic compounds showed 50-67% activity. None of the compounds/extracts showed 100% efficacy.

### 9.2.3 Changes in expression of estrogen receptor co-regulators and their interaction with ER under the influence of ormeloxifene (Orm) a triphenylethylene derivative, in rat uterus

Studies were aimed to explore hormonal regulation of ER co-activator SRC-1, co-repressors RIP140 and NCoR and their interaction with ER under the influence of ormeloxifene and tamoxifen with a view to explore the mechanism of ER antagonism through cofactors. Findings revealed that uterine expression of SRC-1, RIP140 and NCoR was insensitive to E2, Orm and Tamoxifene (Tam) treatment. There was significant reduction in $E_2$-induced effect on ER-SRC-1 interaction in Orm treated rats in contrast to that observed with Tam. Both Orm and Tam antagonized $E_2$- induced inhibitory effect on RIP140 recruitment to ERa in rat uterus Interaction of corepressor NCoR with ER was found to be enhanced in Orm and Tam treated rats. Orm antagonized $E_2$-induced reduction in NCoR recruitment to ERa more efficiently that Tam, resulting in the inhibition of $E_2$induced expression of progesterone receptors mRNA levels. In conclusion, these findings suggest that Orm antagonizes ERa-mediated transcription through promoting the recruitment of NCoR and also by modulating the recruitment of SRC-1 to ERa.

## 9.3 Development of anti-osteoporosis agents

Anti-osteoporosis program of CDRI now has several leads and quite a few are currently under development. Anti-osteoporosis program essentially deals with osteogenic molecules although dual action (i.e. osteogenic and anti-resorptive) molecules are also being developed. Since Indians in general fail to achieve peak bone mass (PBM) during skeletal growth, osteogenic molecules may find use for this purpose.

### 9.3.1 Screening

Screening of osteogenic/bone anabolic agents is a major area of research. We focus on rational drug design targeting various osteogenic molecules. We use osteoblast mineralization as the assay for testing activity. We also study India's rich source of traditional and ethno-traditional knowledge base to screen natural extracts (terrestrial plants/marine flora and fauna) for the possible bone forming/healing actions. Our goals are to localize and identify the active ingredients of the active natural product and use those as leads in order to synthesize novel compounds by computer-aided drug design, chemi-informatics and combichem.

### 9.3.2 Studies with NP-1

NP-1 is a plant from which five compounds were identified for having osteogenic activity *in vitro* in the initial screening. Synthesis of 5 pure compounds K051, K052, K054, K080 and K095 is in progress.

#### 9.3.2.1 Mode of action studies

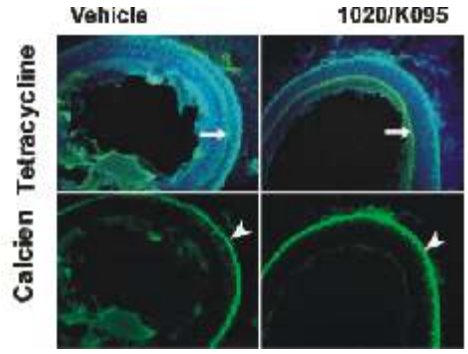
K051 had strong mitogenic as well as differentiation-promoting actions on osteoblasts via the activation of MEK-Erk followed by Akt pathway. K052 had strong anti-apoptotic effect in addition to promoting osteoblast differentiation by parallel activation of MEK-Erk and Akt pathways. K054 stimulated osteoblast proliferation and differentiation via MEK-Erk pathway. K080, a positional isomer of K052, stimulated osteoblast differentiation by stimulating p38 MAPK pathway. When human estrogen receptor (ER) a- and í were transfected to Cos-7 cells, none of these compounds from $10^{-11}$-$10^{-6}$M activated reporter gene, suggesting that their actions are ER-independent.

K095 promoted differentiation and

mineralization of rat calvarial osteoblasts at as low as $10^{-10}$M. K095 activated p38 MAPK pathway in osteoblasts to stimulate BMP-2 secretion, and the later, via autocrine fashion stimulated osteoblast differentiation. Docking studies using human estrogen receptor (ER)-a and -í revealed that K095 is an ER agonist. Consistent with this observation, antiestrogen ICI-182870 abrogated K095-induced osteoblast differentiation, BMP-2 production and p38 MAPK activation. K095 appears to act via both membrane and nuclear ER pathways.

9.3.2.2 Effects on the parameters of peak bone mass by pure compounds of NP-1

Single oral (by gavage) treatment for 30 consecutive days was given to recently weaned female *Sprague Dawley* rats with each of these compounds at 1.0- and 10.0 $mg^{-1}.kg^{-1}.day^{-1}$ doses. K051 increased BMD at all anatomic positions studied, femoral biomechanical strength, vertebral compression, mineral apposition rate (MAR) and bone formation rate (BFR), compared with control. BMD levels at various anatomic positions were also increased with K052 and K054, compared with control however, were less effective than K051. K052, K054 and K080 had no effect on biomechanical strength. K052 and K054 enhanced MAR and BFR compared with control, whereas K080 had no effect.




Sections of femur diaphysis were examined under fluorescent microscope. Representative photomicrographs of tertracyclin (arrow) and calcein (arrow head) labelings are shown. Increased bone formation in 1020/K095 treated rats revealed by greater distance between blue and green labeling compared with vehicle treated rats (upper panels).

K095 treatment resulted in robust increase in BMD, bone biomechanical strength and mineral apposition- and bone formation rates. Minimum effective dose (MED) for K052 and K095 were 1.0 mg/kg. Except for mild uterotrophic effect with K052, none had estrogen-'like' effect *in vivo*. Our data suggest that K051 and K095 are most effective among the five compounds in accelerating PBM achievement.

9.3.2.3 Bioavailability studies with K095

The absolute bioavailability of K095 was found to be 22.34% and did not produce equol as metabolite. Area under curve, elimination half-life and mean residence time were found to be 395±396 $ng^{-1}.ml^{-1}.h^{-1}$, 5.33 $h^{-1}$, 6.959 $h^{-1}$, respectively. The prolonged systemic exposure of medicarpin and plasma half life of 5.33 h indicate that once a dosage regimen is adequate enough to maintain the plasma concentration within the therapeutic range and this may be responsible for its osteogenic effect in experimental animals.

9.3.2.4 Effect of combining K052, K080 and K095 on achievement of PBM

Given their different modes of action and promotion of BMD at different skeletal sites, K052, K080 and K095 were thus speculated to have synergistic action in peak bone mass (PBM) attainment. *In vivo* efficacy of the combination of these three compounds were made in two ways; based on their ratios in the crude extract of the plant (stem bark) and minimum effective dose (MED) of these compounds. When K052, K080 and K095 were mixed on the basis of their ratios in the crude extract (1:100:3), synergistic action was observed in the cortical but not in trabecular bones. It is concluded that this combination could be useful for longitudinal bone growth and has potential cosmetic application in height gain. When K052, K080 and K095 were mixed (1:10:1) based on their MED, synergistic action was observed wherein all trabecular and cortical bones of the body exhibited increased BMD. It is concluded that this combination would be ideal for PBM attainment. A PCT patent claiming two different combinations and covering combination range has recently been filed.

9.3.2.5 Chemical analoging of K095 scaffold

Since K095 is a dual action (promoting bone formation and inhibiting bone resorption) molecule, and a known (chemically but not biologically) compound, chemical analoging of this molecule was

undertaken, using this scaffold. A series of 47 compounds were tested for osteogenic property. Four synthetic molecules, S-006-1709, S-007-1500, S-008-398 and S-008-399 were found to promote osteoblast functions. Subsequent determination of *in vivo* efficacy revealed promotion of PBM attainment by S-007-1500 at 5 and 10 mg/kg body weight doses.

### 9.3.3 OsteoJuvenate

A pharmaceutical composition designated as "OsteoJuvenate" for the management or prevention or treatment of bone disorders have been obtained from an Indian plant. Bone sparing and bone forming activities have been revealed *in vitro* and *in vivo* in the alcoholic extract and its fraction as well as in isolated pure compounds. Four pure compounds isolated from the plant exhibit differential modes of action in osteoblasts, all toward bone formation, making these compounds as potent anabolic agents for bone loss disorders. *In vivo* evaluation of two most abundant compounds K058 and K012 has been performed.

#### 9.3.3.1 Studies on K058

a. K058 reduces OVx-induced bone loss

BMD of excised bones measured by DEXA at 90 days of treatment of OVx rats with K058 (1.0- and 5.0 mg/kg b.w. doses) revealed that OVx rats treated with K058 at both doses had significantly higher BMD in the femur (global, neck and shaft regions) compared with OVx groups treated with vehicle. K058 at 1.0-& 5.0 mg/kg b.w. doses also exhibited higher BMD in the weight bearing, L4 vertebra and increased BMD in tibia head at only 5.0 mg/kg b.w. dose compared with OVx group treated with vehicle. From these results, it is concluded that K058 has bone sparing action under estrogen deficiency.

b. K058 is devoid of estrogen agonistic effect *in vivo*

OVx results in reduction of uterine weight and agents with estrogen-'like' action increase uterine weight of OVx animals. Ovx rats treated with K058 (1.0 and 5.0 mg/kg b.w. doses) for 90 days had uterine weight comparable to that of OVx rats treated with vehicle and both groups had significantly lower uterine weights than sham operated rats treated with vehicle. Therefore, it is concluded that K058 has no estrogen agonistic action at the uterine level.

c. Attainment of peak bone mass in immature rats by K058

*In vivo* efficacy of K058 was next tested in bone formation-dominant animal model using immature rats (female Sprague-Dawley rats at weaning) at 1.0- & 5.0 mg/kg body weight doses for 1 month with vehicle (gum acacia) control. BMD values were found to be significantly higher in the femur shaft of the rats treated with K058 at 5.0 mg/kg b.w. dose compared with control (vehicle). We conclude that K058 enhance attainment of PBM in developing female rats, particularly in the cortical bones.

*d. Stimulation of osteoprogenitor cells by K058*

Increase in osteoprogenitor cells in the bone marrow was assessed by ALP activity and mineralization of BMCs following K058 treatment (5.0 mg/kg b.w.) in growing rats, as described above. ALP activity, a measure of osteoblast differentiation was found to be significantly more in the BMCs obtained from K058 treated rats (5.0 mg/kg b.w.) compared with controls. Also, the number of mineralized nodules formed in BMCs of K058 treated rats was significantly more than controls (vehicle).

Sham + vehicle | OVx + vehicle | OVx+ K058 (1.0 mg. $kg^{-1}.day^{-1}$

Loss of trabecular bone in the femur epiphysis of OVx + vehicle (shown in yellow circle) has been maintained in OVx + K058 treated rats.

We conclude that K058 enhances attainment of PBM by increasing osteoprogenitor cells in the bone marrow.

9.3.3.2 Studies on K012

*a. K012 reduces OVx-induced bone loss*

BMD of excised bones measured by DEXA at 90 days of treatment of OVx rats with K012 (1.0 and 5.0 mg/kg b.w. doses) revealed that OVx rats treated with K012 at both doses had significantly higher BMD in the femur (global, neck and shaft regions) compared with OVx groups treated with vehicle. K012 at 1.0 and 5.0 mg/kg b.w. doses also exhibited higher BMD in the global and second lumbar compared with OVx group treated with vehicle. From these results, it is concluded that K058 has bone sparing action under estrogen deficiency. An increase in BMD was also seen in TFSP in rats treated with K012 at 1.0 mg/kg b.w. dose compared with OVx group treated with vehicle. From these results, it is concluded that K012 has bone sparing action under estrogen deficiency.

b. K012 is devoid of estrogen agonistic effect *in vivo*

OVx results in reduction of uterine weight and agents with estrogen-like action increase uterine weight of OVx animals. Ovx rats treated with K012 (1.0- & 5.0 mg/kg b.w. doses) for 90 days had uterine weight comparable to that of OVx rats treated with vehicle and both groups had significantly lower uterine weights than sham operated rats treated with vehicle. Therefore, it is concluded that K012 has no estrogen agonistic action at the uterine level.

## 9.4 Development of agents for anti-cancer breast

### 9.4.1 Screening

A total of 133 synthetic compounds were evaluated for anti-proliferative activity in MCF-7 cells *in vitro* using MTT assay. Of these, 5 synthetic compounds showed promising activity in initial screening with Tamoxifen (Tam) as standard.

### 9.4.2 Antiproliferative potentials of ormeloxifene (Orm) and its 7-hydroxy derivative in human breast cancer MCF-7 cells

Orm was found to promote proliferation in MCF-7 cells as efficiently as Tam. Orm functioned as an anti-estrogen at the level of transcriptional regulation in MCF-7 cells, effectively blocked the stimulatory effects of $E_2$ by antagonizing the $E_2$ - induced up-regulation of PR and pS2 genes. Our results also showed that 7-hydroxyormeloxifene has greater anti-estrogenic potency than the parent molecule, Orm, with an inhibitory potency comparable to that of 4-hydroxytamoxifen. Orm and 7-hydroxyormeloxifene downregulated the expression of coactivator SRC-1 while upregulated the expression of corepressor NCoR. Orm did not alter the expression of RIP140. Our results indicate that Orm partially and 7-hydroxyormeloxifene completely abolished the interaction between ERα and SRC-1; thereby evoked its antagonistic effects by inhibiting the recruitment of SRC-1 in MCF-7 cells. It appears that Orm and 7-hydroxyormeloxifene recruits NCoR to repress ERα-mediated effects. Orm at 1 µM reduced, while Tam at 1 µM induced the reporter gene activity in the presence of SRC-1 siRNA. These results suggest that Orm and Tam differentially modulate the ERE-mediated response at the level of SRC-1. In addition, Orm inhibited the ERE-Luc activity in the presence as well as in the absence of NCoR siRNA. However, the inhibitory effect of 7-hydroxyormeloxifene was abolished in the presence of NCoR siRNA suggesting the important role of NCoR in mediating the antagonistic effects of 7-hydroxyormeloxifene. We also found that Orm and Tam decreased $E_2$-induced protein expression of cyclin D1 and IGF-1 in MCF-7 cells suggesting that Orm also mediates its effects through non-classical pathway in MCF-7 cells. These studies would be helpful in deciding better use of Ormeloxifene as a selective estrogen receptor modulator, and also in designing the next generation SERMs with higher efficacy and minimal side effects.

### 9.4.3 Anti-cancer breast activity of substituted phenanthrenes with basic amino side chains

In continuation to development of anti-cancer breast agents with substituted phenanthrenes with basic amino side chains, fifteen new analogues were synthesized. Out of these, two (S-006-1485 & S-006-1655) showed significant anti-proliferative activity against ER-positive MCF-7 cell line with $IC_{50}$ in the range of 5-15µM. Compound S-006-1485 showed significant anti-cancer activity against MCF-7 xenograft tumor in nude mice model and the activity was comparable with that of tamoxifen.

### 9.4.4 New targeted compounds against p53-MDM2 interaction

A new series of synthetic compounds were designed *in silico* through molecular docking with crystal structure of p53-MDM2 interaction. The synthesized compounds showed anti-proliferative activity but also exhibited cytotoxicity. Further synthesis is underway to reduce non-specific cytotoxicity and improve cancer cell-specific inhibition of cell growth.

## 9.5 Development of agents for the management of Benign Prostatic Hyperplasia (BPH)

### 9.5.1 Selective Estrogen Receptor Modulators SERMs

It is now well established that both androgens and estrogens play a fundamental role in the growth and development of normal prostate as well as in the pathogenesis of BPH. However, while inhibition of androgen action by anti-androgen or 5a-reductase inhibitor (e.g. finasteride) was partially successful in the management of BPH, estrogen ablation by aromatase inhibitor had negligible effect. Recently some SERMs like tamoxifen, 4-hydroxytamoxifen and ICI-182780 have been shown to promote anti-proliferative and pro-apoptotic activity in human BPH stromal cells as well as in prostatic cancer cell lines, *in vitro*. Two CDRI SERMs (ormeloxifene and DL-2-[4-(2-piperidinoethoxy) phenyl]-3-phenyl-2H-1-benzopyran), which were originally designed for female contraception, specifically caused anti-proliferative/pro-apoptotic activity *in vitro* of stromal cells from human BPH tissue and rat prostate. When administered *in vivo*, these compounds were nearly equipotent to finasteride in reducing the prostate weights of adult mature rats. A combination therapy of finasteride and SERMs was however most effective in reducing rat prostatic weights. Tissue histology revealed that finasteride caused reduction in acinar area with moderate reduction in epithelial cell height while SERMs caused a very significant reduction in cell height along with dilation of acini. The combination therapy caused both (reduction of acinar area and epithelial cell height) and hence was most effective. A role of estrogen receptor beta, IGF-I and pro-apoptotic proteins in SERM action are indicated and being probed in detail. The studies indicate that some CDRI SERMs can be useful in management of BPH, either *per se* or in combination with 5a-reductase inhibitors.

# 10 Technology Development

(Coordinator: Dr. A.K. Saxena)

*The research activity pursued under this area includes Chemical Technology, Fermentation Technology and Pharmaceutical Technology. Development/improvement of processes for CDRI candidate drugs, synthetic compounds, natural products, development/improvement of the processes for known drugs/intermediates, search for novel bioactive agents, development of antibacterial compounds through microbial source, novel antifungal compounds from active microorganisms, bio-transformations, studies on enzymes such as ß-galactosidase, protease, lipase etc., bioreactors and biocatalysts processing, quality control and pre-formulation studies of candidate drugs and development of drug delivery systems are the major activity of this area.*


10.1 Sub Area: Chemical Technology
Coordinator: Dr. A.K. Saxena

10.2 Sub Area: Fermentation Technology
Coordinator: Dr. C.K.M. Tripathi

10.3 Sub Area: Pharmaceutical Technology
Coordinator: Dr. A.K. Dwivedi


## 10.1 Chemical Technology

### 10.1.1 Large scale preparation of CDRI candidate drugs

10.1.1.1 Synthetic compounds

(a) Compound 99-411 (Antimalarial)
140 g. of the compound was prepared and supplied to Pharmaceutics Division.

(b) Compound S-003-296 (Spermicidal)
5 g. of the compound was prepared and supplied to Pharmaceutics Division.

10.1.1.2 Natural Products

(a) Herbal Medicament (Brain stroke)
740 g. of the Herbal Medicament was prepared and supplied to Pharmaceutics Division. A process for the preparation of Herbal Medicament starting from 25 kg of plant material was successfully demonstrated to M/s Themis Medicare, Mumbai at their Wapi (Gujarat) plant.

(b) CDR-134 BS-479-C (Antidiabetic)
163 g Ethyl acetate fraction was prepared and supplied for further studies.

(c) Picroliv (Hepatoprotective)
The process of production of picroliv was demonstrated to M/s DIL Ltd., Mumbai on 10 kg scale and 716 g picroliv, prepared during demonstration, was handed over.

### 10.1.2 Synthesis of known drugs and intermediates

10.1.2.1 Centchroman (Antifertility)
An improved process for Centchroman has been standardized at bench scale. The process is ready for demonstration.

## 10.2 Fermentation Technology

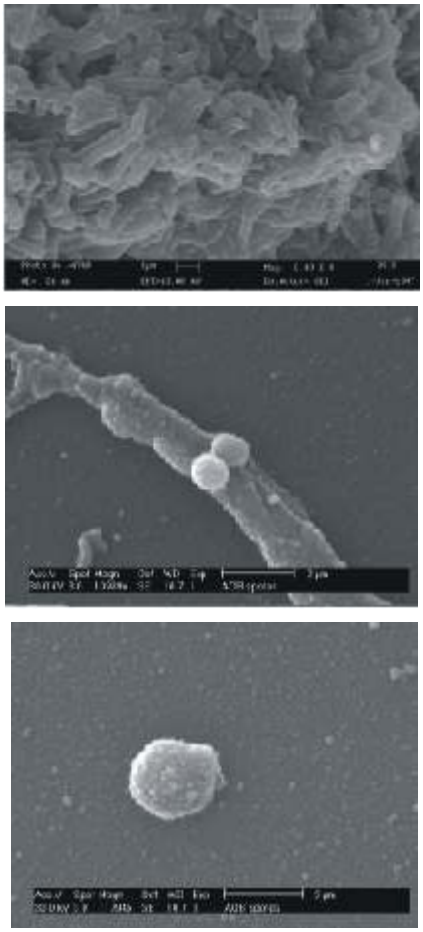
### 10.2.1 Screening of microbial cultures for antibacterial compounds

Search for novel antimicrobial agents are imperative as the existing antimicrobials remain ineffective for certain infectious diseases. Emergence of Multi Drug Resistant (MDR) pathogenic microbes compelling by needs the search for effective alternative antimicrobials. Microbial products, like other natural products, are rich source of drug lead molecules as they provide greater chemical / structural diversity showing activity against wide range of assay targets.

A strain of actinomycetes group, showing broad spectrum antibacterial activity, was isolated from the soil samples. 16S rRNA homology studies demonstrated that the strain showed maximum closeness with *Streptomyces triostinicus* NBRIC 13836 (98% gene sequence similarity). The presence of active compounds was detected in crude extracts of both fermented broth and cells of the culture. Four antibacterial compounds belonging to the same chemical class, as evidenced by the presence of phenoxazone ring, were purified. Two compounds were chemically characterized as Actinomycin V and D. Studies on the chemical characterization of other two compounds is progressing. Artificial neural network (ANN) and genetic algorithm was applied to optimize the medium components for the production of actinomycinV. Experiments were conducted using the central composite design (CCD), and the data generated was used to build an artificial neural network model. Using Genetic algorithm (GA), the input space of the neural network model was optimized to find out the optimum values for maximum antibiotic yield. Maximum antibiotic yield ranging to 452 $mgl^{-1}$ was obtained at the GA optimized concentrations of medium components. The antibiotic yield obtained by the ANN/GA was 38.6 % higher than the yield obtained with the response surface methodology (RSM). Work on the production and optimization of actinomycin V and D by a new source has been completed and the technology has been developed.

### 10.2.2 Screening of microbial cultures for antifungal compounds

Two antifungal compounds were isolated and purified from the fermented broth and cell extracts of *Streptomyces triostinicus*. Both the compounds are polyene in nature (molecular weight 1430 and 1331) and show activity against unicellular and filamentous fungi.



Electron Micrographs of Actinomycin Producing Cultures

*Streptomyces triostinicus* is not reported to produce antifungal antibiotics. However, *Streptomyces triostinicus*, produces antibacterial Quinoxaline antibiotics (chromolactones) which is of higher molecular weight. Work on structure elucidation of these compounds is in progress.

### 10.2.3 Cell wall active antifungals

Life-threatening fungal infections need broad-spectrum fungicidal agents that can be used for treatment and prophylaxis in immunocompromised patients. Microbial natural-product inhibitors of fungal cell wall components such as 1,3-í-d-glucan are the most extensively studied target for the development of novel antifungal agents. Chitin is the second most abundant carbohydrate polymer in nature and is a major structural polysaccharide of fungi. For the isolation of fungal cell wall active microorganisms, yeast cells were used as the only substrate and two actinomycete cultures, *Streptomyces halstedii* (accession no. MTCC 6817) and *Streptomyces annulatus* (accession no. MTCC 6818) were isolated. Fermented broth of these isolates showed antifungal and chtinase activity. Studies on purification and characterization of the chitinases and other active metabolites produced are in progress.

### 10.2.4 Studies on heparinase production

#### 10.2.4.1 Heparinase production and enzyme mediated depolymerization of heparin

Heparin, a linear highly sulfated glycosaminoglycan (HSGAGs) produced by mast cells, is a widely used clinical anticoagulant and is one of the few biopolymeric carbohydrate drugs. Low molecular weight heparins (LMWHs) are derived from unfractionated heparin via controlled chemical or enzymatic breakdown. LMWHs thus produced have enhanced efficacy (reduced rate of venous thromboembolism recurrence, thrombus extension and mortality) and safety (lower rates of major bleeding) as compared to heparin. Enzymatic depolymerization of heparin to generate LMWHs was done by commercially available heparinases obtained from *Pedobacter heparinus.* However, other microorganisms like *Bacillus circulans, Sphingobacterium* and *Bacteroides* are reported and patented for the production of heparinase. Heparinases are useful for a variety of purposes, including sequencing of heparin and heparin-like glycosaminoglycans (HLGAGs), neutralization of heparin/heparan sulfate/LMWHs, inhibition of angiogenesis and normalization of prothrombin and thromboplastin times of heparin containing plasma samples.

#### 10.2.4.2 Culture characterization and optimization of heparinase production

The heparinase producing bacterium, isolated from the soil samples collected from the hilly areas of India, was characterized biochemically and by 16S rRNA gene sequence homology study, at Microbial Type Culture Collection, Chandigarh. The culture was characterized as *Acinetobacter calcoaceticus* and deposited with an accession number MTCC-9488.

Statistical optimization of intracellular heparinase production by *Acinetobacter calcoaceticus* was investigated by using Plackett-Burman Design and Central composite design. The experimental results showed that the production of heparinase was significantly effected by heparin, glucose, $K_2HPO_4$, $NH_4HCO_3$ and casein acid hydrolysate.

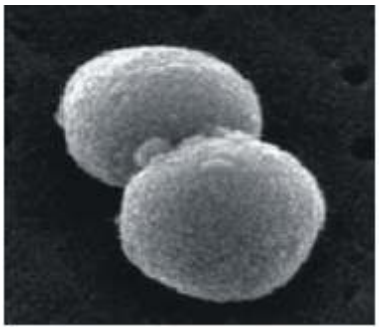
#### 10.2.4.3 Purification and characterization of heparinase

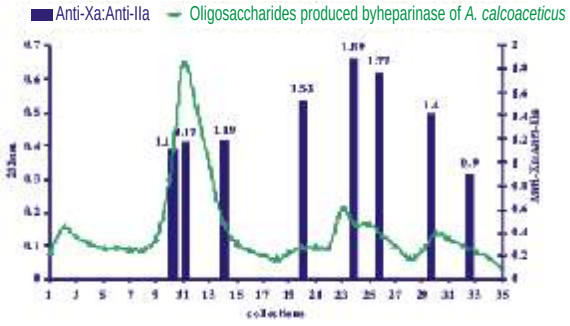
The intracellularly produced constitutive heparinase enzyme was isolated from the periplasmic space of *Acinetobacter calcoaceticus* by freeze fracturing and purified 51.2 fold by ion exchange and gel filtration chromatography. Specific activity of the purified enzyme was found to be 41 IU/µg protein with a 120 k Da molecular mass. The enzyme activity was maximum at 35°C in the presence of 250 mM NaCl at pH 7.5. The enzyme activity was inhibited in the presence of $Ba^{2+}$, $Pb^{2+}$, $Cd^{2+}$, IAA and DEPC, and enhanced by the presence of reducing agents such as í-mer captoethanol, DTT, and $Cu^{2+}$, $Fe^{2+}$ ions. The affinity of the enzyme for different glycosaminoglycans studied varied and showed high affinity for heparin, the substrate, with a $K_m$ value of 0.026 mM. In-situ gel digestion of the purified protein with trypsin did not show any

homology with heparinase I from *Pedobacter heparinus*, the commercially available enzyme.

#### 10.2.4.4 Generation of LMWHs and antithrombotic activity determination

Immobilization of cells/enzyme was done in different matrices and LMWHs generation was observed by gradient PAGE and by increase in optical density at 232 nm. The LMWHs generation by heparinase of *A. calcoaceticus* as well as by heparinase I (sigma) of *Pedobacter heparinus,* entrapped in cellulose membrane (12KDa cut off limit), was monitored by the continuous increase in uronic acid moiety as detected at 232nm. Fractionation of the LMWHs generated thereof was done by sephadex G-50 column chromatography with saline (0.85 % w/v NaCl) as mobile phase. The oligosaccharides fractionated were analyzed for anti-Xa and anti-IIa antithrombotic activities, and a comparison was done between antithrombotic activities of the unfractionated heparin, fractionated LMWHs and commercially available LMWHs.






Morphology and antithrombotic activity of LMWHs produced by heparinase from *A. calcoaceticus*.

## 10.3 Pharmaceutical Technology

### 10.3.1 Development of drug delivery systems

#### 10.3.1.2 Inhalable microparticles containing anti-tuberculosis drugs

For completion of pre-clinical studies, the pharmacokinetics, pharmacodynamics and partial biodistribution of drugs (isoniazid and rifabutin) incorporated in inhalable microparticles for treatment of pulmonary tuberculosis were experimentally determined in mice and monkeys at different dose levels spanning three orders of magnitude and at single-dose as well as steady-state (day 60 of repeated dosing) conditions. Dose-dependent pharmacokinetics were observed, but conventional compartment model fitting could not be carried out using the industry-standard software, WinNonLin ver 5.2. Non-compartmental pharmacokinetic models in WinNonlin allowed better fitting of the data, confirming that the drug delivery approach is novel enough to circumvent received wisdom. Inhalation safety/toxicity studies were carried out in non-human primates (*Macaca mulatta*) in the institutethe first ever experiment conducted in India in this fieldusing the inhalation apparatus reported as standardized during the previous year. The results were submitted to third-party analysis by a NABL/GLP-accredited extramural laboratory. No concerns regarding the safety of inhalable microparticles were raised, indicating suitability of the formulation for human trials

#### 10.3.1.3 Rational design of a male contraceptive / hormone supplementation delivery system of testosterone

A series of transdermal formulations was tested for contraceptive efficacy in rats, with testosterone (T) release profiles engineered for pulsatile release of T to inhibit endogenous T production. A computational model of the endocrine axis was refined and used to predict a parsimonious profile of T delivery that would inhibit sperm production/maturation while maintaining peripheral effects of T such as sexual motivation and performance.

10.3.1.4 Nanoparticles as self-administered contraception for women

Nanoparticles of biocompatible, biodegradable nature were prepared to test the hypothesis that these would cross the cervical barrier if instilled into the vagina and affect the uterine environment in such a manner that would be inimical to implantation of fertilized ovum. It was demonstrated that such nanoparticles cross the cervical barrier and generate proinflammatory cytokine responses, dominated by RANTES, from the fertile endometrium in mice.

10.3.1.5 Targeting erythrocytes for antimalarial delivery

The ubiquitous Glucose Transporter (GLUT-4) is upregulated in RBC infected with the malarial parasite. A delivery system was designed to target malaria-infected RBC through the GLUT-4 protein. Nanoparticles suitable for intravenous administration and containing CDRI compound 97-63 as a model drug were prepared and evaluated *in* vitro. It was observed that nanoparticles comprised of starch, bearing a free, terminal glucose moiety and engineered to have an average size 150 nm, target RBC, but are also susceptible to uptake by phagocytes present in the blood. Efforts are underway to refine targeting to RBC.

10.3.1.6 Delivery system for cyclosporine

The developed formulation showed almost 1.6 times increase in bioavailability compared to marketed formulation while it was 6 times compared to control when studied in rats. Further investigations are underway by preparing some more formulations and to study its efficacy in terms of bioavailability.

10.3.1.7 Delivery system for septic shock

Novel emulsion based formulations have been prepared by incorporating cationic charge with chitosan. Gel retardation studies indicated, there exist some direct interaction with LPS and nanoemulsion as there was change in electro mobility shift of LPS when it was incubated with nanoemulsion. The formulation provides experimental evidences that prototype formulations (chitosan based nanoemulsion) showed suppression of cellular binding of FITC-labeled LPS to the surface of cells. Further studies are underway to prove efficacy in animals.

### 10.3.2 Development of LBL based nanoreservoir

An layer-by-layer based ultrathin polyelectrolyte nanoreservoir has been developed in two combinations-synthetic/synthetic and synthetic/natural using spherical porous $CaCO_3$ core particles. The possibility has been explored for the delivery of macromolecule taking Bovine Serum Albumin as a model protein. Further studies are underway prove its suitability for protein administration.

### 10.3.3 Quality control and stability studies

Demonstrated the process of 'Herbal Medicament' at Themis Medicare Ltd. Vapi, Gujarat and the process for analysis of CDRI compound No. 97-78 during 13-14 October, 2008 at IPCA Laboratories Ltd. Mumbai. Quality control and stability studies on Herbal Medicament, CDR-134F194, and compound 99-411 have been continued. HPLC method for S-007-724 with proper resolution of the starting materials was developed.

# Research Output
# &
# Other Activities

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| 1. | Patents Filed Abroad | 07 |
| 2. | Patents Filed in India | 12 |
| 3. | Patents Granted Abroad | 14 |
| 4. | Patents Granted in India | 07 |


## 1. Patents Filed Abroad

1. PCT Patent Appl. No.: PCT/IN2008/000711 Filing Date: 31/10/2008

   Title: Novel hydroxy functionalized 1,2,4-trioxanes and their derivatives useful as antimalarial agents and a process for the preparation thereof

   Inventors: Chandan Singh, Ved Prakash Verma & Sunil Kumar Puri

   Supporting Staff: Ashok Kumar Sharma & Kamlesh Kumar

2. Chinese Patent Appl. No.: 200680041586.X Filing Date: 06/06/2008

   Title: A process for the isolation of an antidiabetic and antihyperlipidimic fraction from the fruits of *Xylocarpus granatum*, a mangrove plant

   Inventors: Vijai Lakshmi, Ajet Saxena, Rajesh Kumar, Raghwendra Pal, Satyawan Singh, Arvind Kumar Srivastava, Preeti Tiwari, Deepak Raina, Anil Kumar Rastogi, Sudhir Srivastava, Mahendra Nath Srivastava, Ramesh Chander, Anju Puri, Ram Raghubir, Poonam Gupta, Thadigoppula Narender & Brijendra Kumar Tripathi

   Supporting Staff: Naveen Prakash Misra, Hriday Ram Misra, Mukesh Srivastava, Suresh Chandra, Ganesh Shanker Sonker, Subhash Chandra Tripathi, Raja Krishna Purshottam, Ganga Ram Bhatt, Radhey Krishna, Madhuri Chaudhari, J.P. Chaturvedi, Teeka Ram, R.R. Gupta & Suresh Yadav

3. Russian Patent Appl. No.: 2008114302 Filing Date: 11/04/2008

   Title: A process for the isolation of an antidiabetic and antihyperlipidimic fraction from the fruits of *Xylocarpus granatum*, a mangrove plant

   Inventors: Vijai Lakshmi, Ajet Saxena, Rajesh Kumar, Raghwendra Pal, Satyawan Singh, Arvind Kumar Srivastava, Preeti Tiwari, Deepak Raina, Anil Kumar Rastogi, Sudhir Srivastava, Mahendra Nath Srivastava, Ramesh Chander, Anju Puri, Ram Raghubir, Poonam Gupta, Thadigoppula Narender & Brijendra Kumar Tripathi

   Supporting Staff: Naveen Prakash Misra, Hriday Ram Misra, Mukesh Srivastava, Suresh Chandra, Ganesh Shanker Sonker, Subhash Chandra Tripathi, Raja Krishna Purshottam, Ganga Ram Bhatt, Radhey Krishna, Madhuri Chaudhari, J.P. Chaturvedi, Teeka Ram, R.R. Gupta & Suresh Yadav

4. European Patent Appl. No.: 6808830.1 Filing Date: 10/04/2008

Title: A process for the isolation of an antidiabetic and antihyperlipidimic fraction from the fruits of *Xylocarpus granatum*, a mangrove plant

Inventors: Vijai Lakshmi, Ajet Saxena, Rajesh Kumar, Raghwendra Pal, Satyawan Singh, Arvind Kumar Srivastava, Preeti Tiwari, Deepak Raina, Anil Kumar Rastogi, Sudhir Srivastava, Mahendra Nath Srivastava, Ramesh Chander, Anju Puri, Ram Raghubir, Poonam Gupta, Thadigoppula Narender & Brijendra Kumar Tripathi

Supporting Staff: Naveen Prakash Misra, Hriday Ram Misra, Mukesh Srivastava, Suresh Chandra, Ganesh Shanker Sonker, Subhash Chandra Tripathi, Raja Krishna Purshottam, Ganga Ram Bhatt, Radhey Krishna, Madhuri Chaudhari, J.P. Chaturvedi, Teeka Ram, R.R. Gupta & Suresh Yadav

5. Indonesian Patent Appl. No.: W00200800804 Filing Date: 12/03/2008

Title: A process for the isolation of an antidiabetic and antihyperlipidimic fraction from the fruits of *Xylocarpus granatum*, a mangrove plant

Inventors: Vijai Lakshmi, Ajet Saxena, Rajesh Kumar, Raghwendra Pal, Satyawan Singh, Arvind Kumar Srivastava, Preeti Tiwari, Deepak Raina, Anil Kumar Rastogi, Sudhir Srivastava, Mahendra Nath Srivastava, Ramesh Chander, Anju Puri, Ram Raghubir, Poonam Gupta, Thadigoppula Narender & Brijendra Kumar Tripathi

Supporting Staff: Naveen Prakash Misra, Hriday Ram Misra, Mukesh Srivastava, Suresh Chandra, Ganesh Shanker Sonker, Subhash Chandra Tripathi, Raja Krishna Purshottam, Ganga Ram Bhatt, Radhey Krishna, Madhuri Chaudhari, J.P. Chaturvedi, Teeka Ram, R.R. Gupta & Suresh Yadav

6. PCT Patent Appl. No.: PCT/IN07/00618 Filing Date: 31/12/2007

Title: Novel substituted bis-1, 2, 4-trioxanes, useful as antimalarial agents, and a process for the preparation thereof

Inventors: Chandan Singh, Ved Prakash & Sunil Kumar Puri

Supporting Staff: Shashi Rastogi, Akhilesh Srivastava and Kamesh Singh.

7. PCT Patent Appl. No.: PCT/IN08/00083 Filing Date: 08/02/2007

Title: An improved process for preparation of trans-3,4-diarylchroman

Inventor: Devi Prasad Sahu

## 2. Patents Filed in India

1. Patent Appl. No.: 2647DEL2008 Filing Date: 24/11/2008
   Title: Novel substituted amino functionalized 6-(1-aryl vinyl)-1, 2, 4-trioxanes and a process for preparation thereof
   Inventors: Chandan Singh, Naikade Niraj Krishna, Sunil Kumar Puri & Ambuj Kumar Kushwaha
   Supporting Staff: Ashok Kumar Sharma

2. Patent Appl. No.: 2511DEL2008 Filing Date: 06/11/2008
   Title: Substituted benzfurochromenes and related compounds for the prevention and treatment of bone related disorders
   Inventors: Atul Goel, Amit Kumar, Sumit Chaurasia, Divya Singh, Abnish Kumar Gautam, Rashmi Pandey, Ritu Trivedi, Man Mohan Singh, Naibedya Chattopadhyay, Lakshmi Manickavasagam, Girish Kumar Jain & Anil Kumar Dwivedi
   Supporting Staff: Abdul Malik & Avinash Kumar

3. Patent Appl. No.: 1709DEL2008 Filing Date: 18/7/2008
   Title: A novel inhibitor which acts by disrupting HIV-1 NEF-PACS-I protein interactions maintained in HEK-293 cells identified by a new mammalian two-hybrid model
   Inventors: Raj Kamal Tripathi, Sudipti Gupta, Dharamsheela, Pankaj Singh, Richa Verma, Jimut Kanti Ghosh & Ravishankar Ramachandran

4. Patent Appl. No.: 0838DEL2008 Filing Date: 24/11/2008
   Title: Novel donor-accpetor fluorenes, fluorenones and their -conjugated systems: Scaffolds: A process and uses thereof
   Inventors: Atul Goel, Sumit Chaurasia, Vijay Kumar, Sundar Manoharan & Raghubir Singh Anand

5. Patent Appl. No.: 0773DEL2008 Filing Date: 19/11/2008
   Title: A process for isolation of 16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide from Polyalthia longifolia
   Inventors: Koneni Venkata Sashidhara, Anju Puri & Jammikuntla Naga Rosaiah
   Supporting Staff: Suriya Pratap Singh, Jai Kumar Joshi, Noor Jehan, K.K. Yadav, Devidutt & Ram Jivan

6. Patent Appl. No.: 0610DEL2008 Filing Date: 24/11/2008
   Title: Novel substituted benzocycloalkyl azoles
   Inventors: Kalpana Bhandari, Nagarapu Srinivas, Shraddha Palne, Nishi & Suman Gupta
   Supporting Staff: Anoop Kumar Srivastava

7. Patent Appl. No.: 0771DEL2008 Filing Date: 14/10/2008
   Title: Synthesis of new fluconazole analogues containing 1,2,3-triazole moiety and having better antifungal spectrum
   Inventors: Nilkhant Ganpat Aher, Vandana Sudhir Pore, Manoj Kumar Bhat, Gaddam Balakrishna Shiva Keshava, Awanit Kumar, Nripendra Nath Mishra & Praveen Kumar Shukla
   Supporting Staff: Anchal Sharma

8. Patent Appl. No.: 0768DEL2008 Filing Date: 26/03/2008
   Title: Novel hydroxy functionalized 1,2,4-trioxanes and a process for the preparation thereof
   Inventors: Chandan Singh, Ved Prakash Verma & Sunil Kumar Puri
   Supporting Staff: Ashok Kumar Sharma & Kamlesh Kumar

9. Patent Appl. No.: 0605DEL2008 Filing Date: 11/03/2008
   Title: 2,3-dideoxy hex-2-enopyranosid-4-uloses and their derivatives as antitubercular agents and a process for preparation thereof
   Inventors: Mohammad Saquib, Irfan Husain, Smriti Sharma, Arun Kumar Shaw, Manish Kumar Gupta, Yenamandra Subrahmanya Prabhakar, Brahm Shanker Srivastava & Ranjana Srivastava
   Supporting Staff: Arun K. Pandey & Sandeep Kumar Sharma

10. Patent Appl. No.: 0534DEL2008 Filing Date: 24/11/2008
    Title: A bioactive extract/fraction from *Ulmus wallichiana* and its compounds for prevention for treatment of osteo-health disorders
    Inventors: Rakesh Maurya, Preeti Rawat, Kunal Sharan, Jawed Akhtar Siddiqui, Gaurav Swarnkar, Geetanjali Mishra, Lakshmi Manickavasagam, Girish Kumar Jain, Kamal Ram Arya & Naibedya Chattopadhyay
    Supporting Staff: Satish Chandra Tiwari, Abdul Malik Tyagi, Devi Dutt & Amruta Kendurkar

11. Patent Appl. No.: 0770DEL2008 Filing Date: 26/03/2008
    Title: A kit for rapid diagnosis of *Mycobacterium tuberculosis*
    Inventors: Ranjana Srivastava, Parvez Akhtar & Brahm Shanker Srivastava

12. Patent Appl. No.: 0524DEL2008 Filing Date: 05/03/2008
    Title: Novel substituted pyrimidin-2-amines, derivatives and salts thereof and process for preparation thereof
    Inventors: Shivaji Narayanrao Suryavanshi, Suman Gupta & Susmita Pandey
    Supporting Staff: Manju & Shive Ram

## 3. Patents Granted Abroad

1. US Pat. No.: 7442393 Grant Date: 28/10/2008
   Patent Appl. No.: 11/819220 Filing Date: 26/06/2007
   Title: Herbal extracts of Salicornia species, process of preparation thereof, use thereof against tuberculosis
   Inventors: Meena Rajnikanth Rathod, Bhupendra Dhanvantrai Shethia, Jayant Batukrai Pandya, Pushpito Kumar Ghosh, Prakash Jagjivanbhai Dodia, Brahm Shanker Srivastava, Ranjana Srivastava, Anil Srivastava & Vinita Chaturvedi

2. US Pat. No: 7404962 Grant Date: 29/07/2008
   Patent Appl. No.: 10/296,215 Filing Date: 30/08/2000
   Title: Combination kit used in the treatment of malaria
   Inventors: Francis Joseph Pinto, Swati Ajay Piramal, Ram Pratap, Amiya Prasad Bhaduri, Harsh Pati Thapliyal, Sunil Kumar Puri, Guru Prasad Dutta, Anil Kumar Dwivedi, Satyawan Singh, Pratima Srivastava, Vikas Chandra Pandey, Sudhir Srivastava, Shio Kumar Singh, Ram Chandra Gupta, Jagdishwar Sahay Srivastava & Omkar Prasad Asthana

3. European Pat. No.: 1684778 Grant Date: 23/07/2008
   Patent Appl. No.: 818407.3 Filing Date: 29/03/2006
   Title: A novel use of herbal extracts of Salicornia species active against tuberculosis and process for the preparation thereof
   Inventors: Meena Rajnikanth Rathod, Bhupendra Dhanvantrai Shethia, Jayant Batukrai Pandya, Pushpito Kumar Ghosh, Prakash Jagjivanbhai Dodia, Brahm Shanker Srivastava, Ranjana Srivastava, Anil Srivastava & Vinita Chaturvedi

4. Australian Pat. No.: 2003259548 Grant Date: 26/6/2008
   Patent Appl. No.: 2003259548 Filing Date: 29/03/2006
   Title: Herbal extracts of Salicornia species, process of preparation thereof, use thereof against tuberculosis
   Inventors: Meena Rajnikanth Rathod, Bhupendra Dhanvantrai Shethia, Jayant Batukrai Pandya, Pushpito Kumar Ghosh, Prakash Jagjivanbhai Dodia, Brahm Shanker Srivastava, Ranjana Srivastava, Anil Srivastava & Vinita Chaturvedi

5. South African Pat. No.: 2007/6835 Grant Date: 28/05/2008
   Patent Appl. No.: 2007/06835 Filing Date: 29/05/2007
   Title: Synergistic combination kits of α, β-arteether, sulfadoxin and pyrimethamine for the treatment of severe/multi-drug resistant cerebral malaria
   Inventors: Renu Tripathi, Sunil Kumar Puri, Jagdishwar Sahai Srivastava, Satyawan Singh, Omkar Prasad Asthana & Anil Kumar Dwivedi

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| 6. | US Pat. No.: | 7365218 | Grant Date: 29/04/2008 |
| | Patent Appl. No.: | 10/393408 | Filing Date: 20/03/2003 |
| | Title: | Process for preparing guggulsterones | |
| | Inventors: | Ram Pratap, Dharmendra Pratap Singh, Raghwendra Pal & Satyawan Singh | |
| 7. | Uzbekistan Pat. No.: | 9878 | Grant Date: 28/04/2008 |
| | Patent Appl. No.: | IAP20060173 | Filing Date: 22/10/2003 |
| | Title: | Biodegradable, inhalable microparticles containing anti-tubercular drugs | |
| | Inventors: | Himadri Sen, Suryakumar Jayanthi, Rakesh Sinha, Rolee Sharma & Pawan Muttil | |
| 8. | Great Britain Pat. No.: | 2425310 | Grant Date: 16/04/2008 |
| | Patent Appl. No.: | 0614760.7 | Filing Date: 25/07/2006 |
| | Title: | Novel N-phenoxypropanolyl-N'-phenethyl-urea/thiourea derivatives as appetite suppressant | |
| | Inventors: | Kalpana Bhandari, Shipra Srivastava & Chandishwar Nath | |
| | Supporting Staff: | Anoop Kumar Srivastava, Ram Pati Maurya & Vishwambhar Nath | |
| 9. | Ukraine Pat. No.: | 82057 | Grant Date: 11/03/2008 |
| | Patent Appl. No.: | 2004705723 | Filing Date: 13/07/2004 |
| | Title: | Herbal medicaments for treatment of neurocerebrovascular disorders | |
| | Inventors: | Madhur Ray, Raghwendra Pal, Satyawan Singh & Nandoo Mal Khanna | |
| | Supporting Staff: | Jharna Arun & Madhuri Chaudhari | |
| 10. | Australian Pat. No.: | 2001234056 | Grant Date: 29/02/2008 |
| | Patent Appl. No.: | 34056/01 | Filing Date: 04/01/2001 |
| | Title: | Linker based solid support for peptide and small molecule organic synthesis | |
| | Inventors: | Wahajul Haq & Seturam Bandhacharya Katti | |
| 11. | Singapore Pat. No.: | 104797 | Grant Date: 31/01/2008 |
| | Patent Appl. No.: | 200403387-4 | Filing Date: 11/06/2004 |
| | Title: | Herbal medicaments for treatment of neurocerebrovascular disorders | |
| | Inventors: | Madhur Ray, Raghwendra Pal, Satyawan Singh & Nandoo Mal Khanna | |
| | Supporting Staff: | Jharna Arun & Madhuri Chaudhari | |

12. Singapore Pat. No.: 95729 Grant Date: 30/01/2008
Patent Appl. No.: 200301530-2 Filing Date: 30/03/2003
Title: Substituted 1,2,4-trioxanes useful as antimalarial agents and a process for the preparation thereof
Inventors: Chandan Singh, Pallvi Tiwari & Sunil Kumar Puri

13. European Pat. No.: 911330 Grant Date: 12/12/2007
Patent Appl. No.: 97308391.8 Filing Date: 22/10/1997
Title: 1–(4–Arylpiperazine–1–y1)–3–(2–oxopyrrolidin/piperidin–1–y1) propanes as therapeutic agents for hypertension, ischemia, cardiovascular and other adrenergic receptors related disorders
Inventors: Neelima Sinha, Sanjay Jain, Anil Kumar Saxena, Nitya Anand, Ram Mohan Saxena, Mangal Prasad Dubey, (Late) Gyanendra Kumar Patnaik & Madhur Ray

14. Philippines Pat. No.: 1-2003-500187 Grant Date: 19/10/2007
Patent Appl. No.: 1-2003-500187 Filing Date: 28/03/2003
Title: Substituted 1,2,4-trioxanes useful as antimalarial agents and a process for the preparation thereof
Inventors: Chandan Singh, Pallvi Tiwari & Sunil Kumar Puri

## 4. Patents Granted In India

1. Patent No.: 225322 Grant Date: 07/11/2008
   Patent Appl. No.: 0305DEL2003 Filing Date: 17/03/2003
   Title: (-)-3R, 4R-trans -2,2- dialkyl -3-substituted phenyl-4- (hydroxy substituted phenyl)- substituted chroman derivatives as useful intermediates for the synthesis of selective estrogen modulators
   Inventors: Atul Kumar, Sangita, Suprabhat Ray & Devi Prasad Sahu
   Supporting Staff: Vasi Ahmed

2. Patent No.: 223231 Grant Date: 08/09/2008
   Patent Appl. No.: 1367DEL2003 Filing Date: 06/11/2003
   Title: (3R,4R)- trans-3,4-diaryl chroman derivatives and a method for the prevention and/or treatment of estrogen dependent diseases
   Inventors: Sangita, Atul Kumar, Man Mohan Singh, Suprabhat Ray & Girish Kumar Jain

3. Patent No.: 222834 Grant Date: 26/08/2008
   Patent Appl. No.: 1533DEL2003 Filing Date: 11/12/2003
   Title: Novel N- phenoxypropanolyl-N'-phenethyl-urea/thiourea derivatives as appetite suppressant
   Inventors: Kalpana Bhandari, Shipra Srivastava & Chandishwar Nath
   Supporting Staff: Anoop Kumar Srivastava, Ram Pati Maurya & Vishwambhar Nath

4. Patent No.: 221610 Grant Date: 27/06/2008
   Patent Appl. No.: 1340DEL2003 Filing Date: 30/10/2003
   Title: Novel herbal composition for the treatment of gastric ulcer
   Inventors: Janaswamy Madhusudana Rao, Upparapalli Sampath Kumar, Boggavarapu Subrahmanya Sastry, Jhillu Singh Yadav, Kondapuram Vijaya Raghavan, Gautam Palit, Dwaraka Nath Bhalla, Deepak Rai, Panniyampally Madhavankutty Varier, Trikovil Sankaran Muraleedharan, Kollath Muraleedharan

5. Patent No.: 219883 Grant Date: 14/05/2008
   Patent Appl. No.: 1451DEL1999 Filing Date: 05/11/1999
   Title: A process for the preparation of novel 1-(4-aryl) heteroaryl piperazin/ piperidin -1-yl) -N- (quinaloxy- 6/7/8 -yl/4- (un) substituted pyrrolidin -2- oxo-l-yl) alkanes/ alkanones and their salts as potential therapeutic agents
   Inventors: Suresh Kumar Pandey, Alpana Srivastava, Keshav Kishor Awasthi, Ravish Chandra Tripathi, Shekhar Srivastava, Jharna Arun, Ram Mohan Saxena, Madhur Ray, Rakesh Shukla, Mangal Prasad Dubey & Anil Kumar Saxena

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| 6. | Patent No.: | 218348 | Grant Date: 31/03/2008 |
| | Patent Appl. No.: | 0228DEL2001 | Filing Date: 28/02/2001 |
| | Title: | An improved process for the preparation of 1-(4-arylpiperazin-1-yl) -W- (N-substituted amino) alkanes | |
| | Inventors: | V.K. Sharma | |
| 7. | Patent No.: | 217523 | Grant Date: 27/03/2008 |
| | Patent Appl. No.: | 0992DEL2003 | Filing Date: 12/08/2003 |
| | Title: | An improved process for racemization of [1S,2S-2-amino-1-(4- nitro phenyl)-1, 3-propanediol] | |
| | Inventor: | Devi Prasad Sahu | |

# 3 Papers Presented in Conferences

## 2007

### All India Seminar on Cyber Crime & Security Challenges, Lucknow (13-15 April)

Information security and patch management. R.K. Sharma & M. Abbas.

Protection of organizations sensitive information against insider attacks. M. Abbas & R.K. Sharma.

### 40th Annual Conference of Indian Pharmacological Society, Mohali (1-3 November)

Effect of guggulipid on the release of lipopolysaccharide (LPS) induced inflammatory mediators in rat glioma cell line. R. Niranjan & R. Shukla.

Effect of COX and NOS inhibitors on lipopolysacchride induced oxidative stress in rat brain. R. Shukla, E. Tyagi, R. Agrawal & C. Nath.

A pharmacological study on acetylcholinesterase activity and insulin receptors in brain areas of scopolamine amnesic mice. R. Agrawal, E. Tyagi, C. Nath & R. Shukla.

Streptozotocin induced dementia model in rats: histopathological evidences for neuronal degeneration. G. Saxena, S. Bharti, P.K. Kamat, S. Sharma & C. Nath.

### 25th Annual Conference of Indian Academy of Neurosciences, Varanasi (22-25 November)

Effect of intracerebroventricular lipopolysacchride on cholinergic activity and neuroinflammation. E. Tyagi, R. Agrawal, R. Shukla & C. Nath.

Effect of L-Histidine on scopolamine-induced memory deficits in passive avoidance test. G. Saxena, S.P. Singh & C. Nath.

### 2nd International Symposium on Translational Research: Natural Products & Cancer, Mumbai (9-12 December)

Novel human gingival fibroblast cell line as a vehicle for evaluating the cybernetics of curcumin action. A.K. Balapure, R. Sharma, Karamjeet, V. Ranjan, N. Singh, U.P. Verma & J. Dixit.

### 34th Annual Conference of Indian Immunology Society, Pune (16-18 December)

Malaria diagnosis based on detection of plasmodial lactate dehydrogenase using immundot enzyme assay and sandwich ELISA. D.C. Kaushal & N.A. Kaushal.

Cloning expression and characterization of filarial antigens having diagnostic potential. N.A. Kaushal & D.C. Kaushal.

## 2008

### Recent Trends of Research in Pharmaceuticals Sciences, Nathdwara (7-8 January)

Current concepts for the pharmacotherapy of Alzheimer's disease. C. Nath.

Augmentation of the nitric oxide synthase and free radical generation among infiltrated neutrophils during inflammation in the rat. R.S. Keshari, A. Jyoti, M.K. Barthwal & M. Dikshit.

Involvement of nitric oxide in monocyte differentiation and macrophage foam cell formation. R.L. Tiwari, M. Dikshit & M.K. Barthwal.

### 10th CRSI Symposium, Bangalore (1-3 February)

Application of allyl amines afforded from Baylis-Hillman adducts towards heterocyclic synthesis. S. Nag & S. Batra.

### International Conference on Nanomaterials Toxicology- ICONTOX, Lucknow (5-7 February)

Surface modified ultrathin polyelectrolyte nanoreservoir for delivery of proteins: Evaluation in terms of controlled release and biocompatibility. G.K. Gupta, V. Jain & P.R. Mishra.

Lipopolysaccharide induced J774.2 mouse macrophage activation using surface modified lipid nanoemulsion. V. Jain, P.R. Mishra & R. Pal.

### 27th Annual Convention of Indian Association for Cancer Research & International Symposium on Frontiers in Functional Genomics- IACRCON-2008, Ahmedabad (7-9 February)

Association of IL-4 and IL-1 receptor antagonist (IL-1Ra) gene polymorphisms with the risk of benign prostatic hyperplasia (BPH). R. Konwar, N.V. Lakshma, N. Chattopadhyay, V. Singh & H.K. Bid.

Genetic variation in IL-1 receptor antagonist (IL-1Ra) and IL-4 genes and breast cancer risk: study from northern India. H.*K. Bid, Preeti, N.* Chattopadhyay, *S. Kumar, S. Tiwari &* R. Konwar.

### Free Radicals & Natural Products in Health, Jaipur (14-16 February)

Mechanisms involved in nitric oxide induced free radical generation by human neutrophils. S. Patel, Anupam Jyoti, S. Kumar, A. Verma, M.K. Barthwal & M. Dikshit.

Hyperlipidemic hamster as a model to study atherothrombosis. V. Singh, M. Jain, W.R. Surin, M. Dikshit & M.K. Barthwal.

### 9th International Symposium on Vectors and Vector Borne Diseases, Puri (15 17 February)

Cloning and expression of 42 Kda fragment of *P. cynomolgi* B merozoite surface protein-1. N. Kumar, N.A. Kaushal & D.C. Kaushal.

### 9th International Symposium on Genetics, Health and Disease - OMICS in the 21st Century, Amritsar (17-19 February)

Genetic polymorphism of glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1) and susceptibility to type 2 diabetes patients: A northern India study. H.K. Bid, R. Konwar, C.G. Agrawal & M. Banerjee.

### 12th International Conference ISCBC - on The Interface of Chemistry-Biology in Biomedical Research, Pilani (22-24 February)

3D-QSAR studies of acetylcholine esterase (AChE) inhibitors for the treatment of alzheimer's disease. S.S. Chaudhaery, A. Dixit & A.K. Saxena.

A general strategy to substituted 3-methylene-2-pyridones and its synthetic applications. V. Singh & S. Batra.

Design and synthesis of peptidomimetics as DPP IV inhibitors. N. Sethi, W. Haq & S.B. Katti.

Design and synthesis of peptidomimetics as protein tyrosine phosphatase-1B (PTP-1B) inhibitors. A. Sharma, W. Haq, A. Srivastava, A. K. Tamarkar & S.B. Katti.

Design and synthesis of side chain modified 4-aminoquinolines as antimalarial agents. M. Sinha, W. Haq, K. Srivastava, S. K. Puri & S.B. Katti.

Exploitation of triose phosphate isomerase as a drug target in *Leishmania donovani*. K. Kumar & U. Roy.

Highly chemoselective S-methylation reactions using trimethyl orthoformate. R. Kumar, S. Khan & P.M.S. Chauhan.

Microwave assisted rapid synthesis of 1-alkylated-2-thiohydantoin and arylmethylene-2-thiohydantoins using easily accessible N-alkylglycine ethyl ester. S. Porwal, P.M.S. Chauhan.

Synthesis and biological evaluation of quinoline based piperazine derivatives as antimalarial agents. A. Kumar, K. Srivastava, S.K. Puri & P.M.S. Chauhan.

Synthesis of novel 2,4,6-trisubstituted triazine derivatives as antitubercular agents. N. Sunduru, V. Chaturvedi & P.M.S. Chauhan.

Synthesis of side chain modified 4-aminoquinolines as antimalarial agent. L. Gupta, M. Sharma, K. Srivastava, S.K. Puri & P.M.S. Chauhan.

Synthesis of substituted qinoline pyrimidines as new class of antimalarial agents. M. Sharma, K. Srivastava, S.K. Puri & P.M.S. Chauhan.

Topological descriptors in modelling antimalarial activity: N1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine as prototype. S.K Deshpande, V.R. Solomon, S.B. Katti & Y.S. Prabhakar.

# 3 Papers Presented in Conferences

## Microbicides 2008, New Delhi (24-27 February)

Ingeniously designed molecules target sperm precisely at concentrations that are inert to heLa and lactobacillus *in vitro*. Striving towards HIV prevention. R.K. Jain, J.P. Maikhuri, V.L. Sharma, A.K. Dwivedi, K.K. Srivastava & G. Gupta.

Novel formulations for dual protection. Striving towards HIV prevention. A. Jain, R.K. Jain, J.P. Maikhuri, V.L. Sharma & G. Gupta.

## National Symposium on an Update of Male Reproduction and Infertility, Lucknow (13-14 March)

Clinical aspects of male infertility. A.K. Dwivedi.

## 8th International Conference on Mathematical Chemistry and 5th Indo-US Workshop on Mathematical Chemistry, USA (2227 June)

Is feature selection essential for ANN modeling? M. Goodarzi, S.K. Deshpande, V. Murugesan, S.B. Katti & Y.S. Prabhakar.

Pharmacophore model for HIV-reverse transcriptase inhibitors. V. Balaramnavar & A.K. Saxena.

## Euro-India First International Conference on Holistic Medicine, Kottayam (21-23 August)

Synthesis and antitubercular activity of 5-benzyl-3-phenyl dihydroisoxazole. S.S. Bisht, V.P. Pandey & R.P.Tripathi.

## 16th International Conference on Bioencapsulation, Dublin (4-6 September)

Pharmacokinetic evaluation of inhalable microparticles in rhesus monkeys. J. Kaur, R.K. Verma, A.B. Yadav, K. Kumar & A. Misra.

PLA microparticles for pulmonary delivery of anti-TB drugs - biodistribution study. R.K. Verma, J. Kaur, A.B. Yadav, K. Kumar & A. Misra.

Paclitaxel delivery by micro-nano encapsulation using layer-by-layer assembly. G.K. Gupta, V. Jain & P.R. Mishra.

## 13th Human Genome Meeting, Hyderabad (27-30 September)

A proteomic-based approach for the identification of potential Th-1 stimulatory novel proteins in a subunit vaccine (68-97.4kDa) of soluble antigens of *Leishmania donovani* promastigotes that protects against fatal visceral leishmaniasis in HUGO'S. A. Dube, S. Kumari, M. Samant, P. Misra, P. Khare, B. Sisodia & A.K. Shasany

Cloning, overexpression and purification of *Leishmania donovani* Enolase in HUGO'S. R. Gupta, P.K. Kushawaha, M. Samant & A. Dube.

Studies on genetic variation among *Leishmania major*, *L. tropica* and *L. donovani* by AFLP analysis in HUGO'S. A. Kumar, V.R. Boggula, S. Sundar & A.K. Shasany.

## Conference on Genomics, Model Organisms and Disease, Bangalore (1-2 October)

Silencing effects of ATP dependent RNA Helicase in human filarial parasite *Brugia malayi* using RNAi. M. Singh & S.M. Bhattacharya.

## National Conference on Genomics, Proteomics and Systems Biology, Bangalore (1-3 October)

Apigenin induced hepatotoxicity in mice is oxidative stress mediated. P. Singh, S.K. Mishra, S. Noel, S. Sharma & S.K. Rath.

Association of the single nucleotide polymorphism (SNP) rs1052133 in human 8-oxoguanine glycosylase I (hOGG1) gene with the risk of squamous cell carcinomas of the head and neck (SCCHN). A.K. Mitra, S.V. Singh, V.K. Garg, M. Sharma, R. Chaturvedi & S.K. Rath.

## Human Genome Variation Meeting, Toronto (15-17 October)

Single nucleotide polymorphisms in DNA ligase 1: The Indian scenario and risk of head and neck cancer. A. K. Mitra, A. Singh, V. K. Garg, M. Sharma, R. Chaturvedi & S. K. Rath.

### 20th National Congress of Parasitology, Shillong (3-5 November)

Antileishmanial activity of novel bis and mono imidazoles of cyclohexane. Nishi, N. Sriniwas, Palne, Shraddha, K. Bhandari & S. Gupta.

Arginase: A potential antileishmanial drug target. K. Kumar & U. Roy.

Biochemical characterization of dipeptidyl carboxypeptidase of *Leishmania donovani*. S. Gangwar, M.S. Baig & N. Goyal.

Biochemical mechanism of Drug resistance in rodent Malaria. A. Rizvi, S.K. Pandey, J.K.Saxena & R. Tripathi.

Characterization of a 27kDa protein from rodent malaria parasite *Plasmodium vinckei* to elucidate its role in arteether resistance. R. Chandra, S. Kumar & S.K. Puri.

Cloning, expression and purification of *Leishmania donovani* nucleoside diphosphate kinase b. Expression and purification of calreticulin from *Leishmania donovani* clinical isolate. R. Gupta.

Expression and purification of SAG resistant gene II of *Leishmania donovani* in *E. coli*. N. Kumari, Ashutosh & N. Goyal.

Functional characterization of *P. falciparum* transketolase. S. Joshi, A.R. Singh, P.C. Misra & J.K. Saxena.

Hydroxamates: As broad spectrum anti-protozoals. S.K. Pandey, M. Mishra, R.P. Tripathi, A. Rizvi, S. Gunjan & R. Tripathi.

Immunimodulatory activity of analog of muramyl dipeptide and their use as adjunct to chemotherapy of *Leishmania donavani* in hamster. A. Puri & W. Haq.

Isolation, cloning and over-expression of Glutathione-S-Transferase from *Plasmodium vinckei.* A. Tripathi, R. Chandra & S.K. Puri.

Multiple intermediates in the equilibrium unfolding of trypanothione reductase from the human pathogen *Leishmania donovani*. S. Rai, U.N. Dwivedi, N. Goyal, P.K. Kushawaha, M. Samant & A. Dube.

### International Conference of Junior National Organic Symposium Trust, Madurai (6-9 November)

Applications of 1,3-dipolar cycloaddition for the synthesis of spiro-derivatives from the Baylis-Hillman derivatives. V. Singh & S. Batra.

### 26th Annual National Conference of Indian Society for Medical Statistics, Nainital (7-9 November)

Biclustering for high dimensional data: Approaches and applications in gene expression data. M. Abbas & M. Srivastava.

Fuzzy extension of partitional clustering: Algorithms and applications to microarray data. M. Abbas & M. Srivastava.

Data mining in optical coherence tomography data for retinal disease diagnosis. M. Srivastava, K.D. Singh, Vijjan, M. Abbas & S. Saxena.

A diagnostic criteria for osteoporosis in animals. M. Srivastava, M. Abbas & M.M. Singh.

### Conference on Plant Life Through the Ages, Lucknow (16-17 November)

Notes on *Frerea Indica* Dalz.(Asclepiadaceae): A palaeoendemic plant of Maharashtra state. D.K. Mishra, K.R. Arya.

### 45th Annual Convention of Chemistry and International Conference on Recent Advances in Chemistry, Dharwad (23-27 November)

Synthesis and *in vitro* evaluation of leishmanicidal activity of novel bis-tetraryl quinazolines. S. Kumar, D.P. Sahu & S. Gupta.

### National Conference on Traditional Knowledge Systems, IPR and Their Relevance for Sustainable Development, New Delhi (24-26 November)

Sacred Grooves: An alternate aid for conservation of plants at the species level status report. S .M. Rajendran.

### 4th International Symposium on Emerging Trends in Tuberculosis Research: Biomarkers, Drugs & Vaccine, New Delhi (1-3 December)

Cloning, expression & purification of *M. luteus* Rpf and its role in the resuscitation of non culturable cells of mycobacteria. R.K. Gupta, A. Yadav & R. Srivastava.

Role of Rv3219c in persistence and its expression profiling. S. Kaur, B.S. Srivastava & R. Srivastava.

Expression of acetyohydroxyacid synthase (AHAS) from M*ycobacterium tuberculosis.* V. Singh & R. Srivastava.

Serine thronine kinase, PknK (Rv3080c) is responsible for the slow growth of Mycobacteria. R. Kumari, S.K. Chaurasiya & K.K. Srivastava.

Inhibition of protein kinsase C-a increases the survival of Mycobacteria within macrophages. S.K. Chaurasiya & K.K. Srivastava.

Differential expression of sigma factor H paralogs during growth and upon exposure to different stress conditions in *Mycobacterium smegmatis.* A.K. Singh & B.N. Singh.

A double recombinant *Mycobacterium aurum* strain for the screening of primary and rationale based antimycobacterial compounds. R.K. Biswas & B.N. Singh.

Inhalable microparticles containing isoniazid and rifabutin target macrophages and "Stimulate the phagocyte" to achieve high efficacy. A. Misra.

### International Seminar on Role of Plant Taxonomy in Biodiversity Management and Human Welfare, Dehradun (1-3 December)

Pharmacognostical investigation and validation of an ethno-medicinal remedy for piles. K .R. Arya, D.K. Mishra and Sayyada Khatoon.

### Integrating Physics, Chemistry, Mathematics and Biology to Understand Living Systems IPCMB, Kolkata (4-6 December)

Timing feedback-inhibition of the male reproductive hormone axis. R. Malik, S. Tondwal, K.S. Venkatesh, G. Gupta & Amit Misra.

### 35th Annual Meeting of the Indian Immunology Society, Bhubaneswar (12-14 December)

Immunization with *Brugia malayi* parasite fraction BmAFI facilitates infective larval survival and development in the hostile peritoneal cavity of *Mastomys coucha*. S.K. Joseph, M.K. Sahoo, S.K. Verma, A.K. Verma & P.K. Murthy.

### 60th Indian Pharmaceutical Congress, New Delhi (12-14 December)

HPLC method for determination of kaempferol in rat serum and tissues. A.K. Dwivedi, V. Gupta, J. Madan, S. Kumar, A. Kumar, R. Trivedi & N. Chattopadhyay.

T-cell proliferation, IFN-, IL-2 and nitric oxide release after administration of a single shot hepatitis B vaccine formulation with PLGA microsphere. V. Saini, V. Jain, P.K. Murthy & D.V. Kohli.

### International Symposium on Advances in Neurosciences & 26th Annual Conference of Indian Academy of Neurosciences, Cochin (12-14 December)

Melatonin alleviates memory deficits and neuronal degeneration induced by intracerebroventricular administration of streptozotocin in rats. G. Saxena. S. Bharti, K.P. Kamat, S. Sharma & C. Nath.

Inhibition of pro-inflammatory cytokines via a7 nicotinic acetylcholine receptor in LPS induced neuoinflammation. E. Tyagi, R. Agrawal, C. Nath & R. Shukla.

Correlation of oxidative stress with acetylcholinesterase activity in ICV STZ induced dementia model in rat. R. Agrawal, E. Tyagi, R. Shukla & C. Nath.

Protective effect of donepezil on LPS-induced neuroinflammation in rat. R. Shukla, E. Tyagi, R. Agrawal & C. Nath.

Candesartan improves memory decline in mice: Involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin. S.K. Tota, H. Awasthi, K.P. Kamat, N.Singh, R. Raghubir, K. Hanif & C. Nath.

Anti-inflammatory and anti-proliferative effect of guggulipid on LPS induced human astrocytoma cell line U373MG. R. Niranjan & R. Shukla.

Role of glutamate transporters and their modulation following cerebral ischemia/reperfusion injury in rats. R.K. Verma, V. Mishra, D. Sasmal & R. Raghubir.

Possible role of ASIC in cerebral ischemia / reperfusion injury. V. Mishra, R.K. Verma & R. Raghubir.

### NBNI Workshop on Neurobiology and Neuroinformatics, Cochin University of Science & Technology, Cochin (9-10 December)

Does Inhibition of Endoplasmic reticulum stress is neuroprotective after cerebral ischemia. N.V. Prasuja & R. Raghubir.

### International Symposium on Prognostic and Predictive Factors in Cancer Management: Clinical Applications, Lucknow (15-16 December)

Significant association of the single nucleotide polymorphism (SNP) rs13181 in the gene ERCC2 with the risk of squamous cell carcinomas of the head and neck (SCCHN) and breast cancer among north Indians. A. K. Mitra, S. V. Singh, S. Agarwal, A. Zaidi, V.K. Garg, R. Chaturvedi, M. Sharma, S. K. Rath .

### 41st Annual Conference of the Indian Pharmacological Society, New Delhi (18-20 December)

Role of nitric oxide in Parkinson's disease. S. Singh & M. Dikshit.

Neuropharmacological effects of *Ocimum sanctum.* M. Chatterjee & G. Palit.

Gastroprotective effect of *Tectona grandis*: possible involvement of $H^+ K^+$ ATPase inhibition. N. Singh, R. Maurya & G. Palit.

Role of oxidative stress and inflammation in experimentally induced reflux esophagitis in rats. P. Singh & G. Palit.

Evolving process towards drug development from Indian medicinal plants for peptic ulcer disease. G. *Palit.*

Perindopril improves memory decline in rat: involvement of angiotensin converting enzyme in memory deficit induced by ICV streptozotocin. S. Tota, K.P. Kamat, C. Nath & K. Hanif.

Potent antiallergic / antiasthmatic activity of plant extracts 4397. A. Nath, R. Maurya & R. Raghubir.

### International Symposium on Novel Startegies for Targeted Prevention and Treatment of Cancer, New Delhi (19-20 December )

Selective estrogen receptor modulator ormeloxifene induces apoptosis in ovarian cancer cells through the mitochondrial pathway. R.K. Garg, A.K. Tripathi , M. Das & D.P. Mishra.

Selective estrogen receptor modulator ormoloxifene inhibits STAT-3 activation and induces apoptosis in head and neck cancer cells. V.K. Srivastava, S. Tewari, M.L.B. Bhatt, M. Das & D.P. Mishra.

## 2009

### National Symposium on Cellular and Molecular Biophysics, Hyderabad, (2224 January)

Characterization of binding sites of apoptosis inducing factor (AIF) by molecular docking. K. Gupta & R. Raghubir.

# 4 Inter Agency Linkages

| Title of the Project | Funding Agency | Principal Investigator |
|---|---|---|
| An approach towards exploration of mechanism of drug non-responsiveness to Sb (V) in field isolates of *Leishmania donovani* | Department of Biotechnology, Govt. of India | Dr. Neena Goyal |
| Leishmania target antigens from promastigotes and amastigotes: Identification on experiential visceral leishmaniasis | -do- | Dr. Anuradha Dubey |
| Solution structure of *Mycobacterium tuberculosis,* E. coli and Homo sapiens peptidyl-tRNA hydrolase by NMR spectroscopy | -do- | Dr. Ashish Arora |
| Structure based drug design of inhibitors targeting recombinant pteridine reductase 1 from *Leishmania donovani* clinical isolate | -do- | Dr. Neeloo Singh |
| Cloning , expression and characterization of filarial acetylcholine esterase | -do- | Dr. N.A. Kaushal |
| Correlation of single nucleotide polymorphism in gene encoding cytokines and adhesion and immune regulatory molecules with severity of *P. falciparum* malaria in Uttar Pradesh | -do- | Dr. Saman Habib |
| Evaluation of Mycobacterium as an immunomodulator for the management of visceral leishmaniasis and as an adjunct to antileishmanial vaccine/drug | -do- | Dr. Anuradha Dubey |
| Up-gradation of the CDRI project on design, synthesis and development of new molecules against MDR tuberculosis to a DBT centre of excellence of TB drug discovery | -do- | Dr. Sudhir Sinha |
| Studies on neutrophil nitric oxide synthase: Isolation, molecular characterization and identification of interacting proteins | -do- | Dr. Madhu Dikshit |
| New inhibitor design/drug development using novel protein targets: NAD+ dependent DNA ligases and feast/famine regulatory proteins from *M. tuberculosis* | -do- | Dr. R. Ravishankar |
| Studies on the structure and functions of actin cytoskeletal network in *Leishmania donovani* | -do- | Dr. C.M. Gupta |

| Title of the Project | Funding Agency | Principal Investigator |
|---|---|---|
| Understanding the mechanism of mitotic/spindle checkpoint using genetics approaches in fission yeast *Schizosachromyces pombe* | -do- | Dr. S. Ahmed |
| Anti-osteoclastogenic effect of 99/373 and its mode of action | -do- | Dr. N. Chattopadhyay |
| National project on development of potential drugs from the ocean | Ministry of Earth Sciences, Govt. of India | Director, CDRI |
| Sophisticated Analytical Instrument Facility (SAIF) | Department of Science &Technology, Govt. of India | Director, CDRI |
| Synthesis and characterization of novel polysilane high polymers for UV and NUV LEDs materials | -do- | Dr. S.K. Shukla |
| Structural characterization of γ-glutamylcysteine synthetase and glutathione synthetase from Leishmania sp. | -do- | Dr. J.V. Pratap |
| Studies on the modulation of neutrophil free radical generation and nitro oxide synthesis by calcium, reactive nitro and oxygen species | -do- | Dr. Madhu Dikshit |
| Diversity oriented organic synthesis of small but smart molecules in drug discovery research | -do- | Dr. G. Panda |
| Isolation and characterization of proteo-phosphoglycans of Leishmania donovani | -do- | Dr. Anuradha Dubey |
| Studies on synthesis of cyclic compounds using Baylis-Hillman chemistry | -do- | Dr. Sanjay Batra |
| Expansion of facilities in national centre for pharmacokinetic and metabolic studies | -do- | Dr. G.K. Jain |
| Identification and elucidation of novel signaling pathways involved in monocyte /macrophage activation, migration, differentiation, proliferation and death during dyslipidemia and atherosclerosis | -do- | Dr. M.K. Barthwal |
| Establishing national facility for regulatory pharmacology and toxicology | DST (PRDSF) | Director, CDRI |

## 4 Inter Agency Linkages

| Title of the Project | Funding Agency | Principal Investigator |
|---|---|---|
| Synthesis of hepta-saccharide motifs found in the cell wall of *Mycobacterium gordonae* towards the preparation of carbohydrate vaccine against Mycobacteria | DST (Ramanna Fellowship) – transferred to Bose Institute, Kolkata | Dr. Anup Kumar Misra |
| Molecular diversity oriented synthesis of aromatic scaffolds through ring transformation strategy | DST (Ramanna Fellowship) | Dr. Atul Goel |
| A mechanistic approach towards improvement in oral bioavailability with special reference to cyclosporine | DST (SERC Fast Track) | Dr. P.R. Mishra |
| Osteoporosis in Indian women and men: Diagnosis using bone mineral density and biochemical markers of bone turnover | DST (Women Scientist Scheme) | Dr. Anu Makker |
| Genome wide approaches to assess the involvement of Cyp1A1 polymorphism in Indian breast cancer patients and the effect of resveratol on cyclophosphamide induced gene expression profile of MCF-7 cell line | -do- | Dr. Neetu Singh |
| Identification and development of novel anticancer agents: Extended work plan for lead optimization and drug candidate selection | DST/DABUR | Dr. Sudhir Sinha |
| Lead optimization and development of new orally active antimalarial peroxides | DST/IPCA | Dr. Chandan Singh |
| Search for the cell wall and membrane protein(s) of *Candida albicans* to be used as target molecule | Indian Council of Medical Research, Govt. of India | Dr. P.K. Shukla |
| Development of new chemotherapeutic agents and drug combinations for the multi-drug resistant/service malaria treatment | -do- | Dr. Renu Tripathi |
| Development of antiulcer drug from Indian medicinal plant *Tectona grandis* | -do- | Dr. G. Palit |
| Synthesis of monosaccharide derivatives as potential antimycobacterial agents | -do- | Dr. A.K. Shaw |

| Title of the Project | Funding Agency | Principal Investigator |
|---|---|---|
| Design and synthesis of novel SERMs for the management of osteoporosis and other estrogen related disorders | -do- | Dr. G. Panda |
| Target based design and synthesis of novel compounds for treating diabetes and dyslipidemia | -do- | Dr. Atul Goel |
| Cytokine gene polymorphism in breast cancer patients | -do- | Dr. R. Konwar |
| Syntheses of antimalarial agents and their combinatorial chemistry | -do- | Dr. P.M.S. Chauhan |
| Technological innovations for commercial exploitation of *Morinda citrifolia* (noni) as livelihood option for island farmers | Ministry of Health & Family Welfare, Govt. of India | Dr. J.K. Saxena |
| Mass spectrum fingerprinting of Indian medicinal plants (antidiabetic aspect) | -do- | Dr. B. Kumar |
| Reproductive health research program | -do- | Director, CDRI |
| Pharmacological and genomic investigations on *Withania somnifera* - an Indian medicinal plant | NMITLI (CSIR) | Dr. Shailja Bhattacharya |
| Mode of action of Artemisinin based antimalarial drugs | UPCST | Dr. J.K. Saxena |
| 14 Days irritancy study on SMA coated copper T in animals | Indian Institute of Technology, Kharagpur | Dr. O.P. Asthana |
| Targetting protein synthesis in apicoplast and cytoplasm of Plasmodium | European Commission, Brussels, Belgium | Dr. Saman Habib |
| Development of new macrofilaricidal and/or embryostatic agents | WHO, Geneva, Switzerland | Dr. Shailja Bhattacharya |
| Development of new chemotherapeutic agents for Human African Tripanosomiasis (HAT) | DNDi, Geneva | Dr. Renu Tripathi |
| Lead identification for antileishmanial compounds | -do- | Dr. S.K. Puri |

# 5 R&D/Technical Facilities & Services

## 1. Sophisticated Analytical Instrument Facility

During the year, 632 users utilized the analytical services of the Division. There were 592 users from Colleges/Universities while 32 and 8 users were from research laboratories and industries respectively while 345 users were from the Institute itself. Total number of internal samples amounted to 22695 while 8070 external samples were analyzed this year. .

## 2. National Laboratory Animal Center

The center ensured the supply of healthy animals for research (rat, mouse, hamster, gerbil, cotton rat, mastomys rat, guinea pig and rabbit); supply of quarantined and tested rhesus monkeys obtained from recognized sources; supply of tissues, organs, blood or sera samples of laboratory animals for research; health monitoring of laboratory animals through microbiological, pathological and parasitological screening; nutritional monitoring of laboratory animal feed; radiological monitoring of animals; feed trial studies on experimental animals; production and monitoring of CDRI in-house/ commercial laboratory animal feed; etc. The center was also involved with human resource development and training in laboratory animal science through conducting training courses in Laboratory Animal Science including care, breeding and management; their health monitoring and quality control; nutritional monitoring; diagnosis and management of laboratory animal diseases.

The center supplied research animals for institutional use and to outside agencies as per following details:

Tissue and Cell Culture Unit provided 155 T-25 Cell Culture Flasks of various cell lines to the scientists at Eastern Medikit Ltd., Gurgaon and Shriram Institute of Industrial Research, Delhi.

## 3. Biological Screening of Outside Samples

During the year 2008-09, the Institute continued to provide *in vitro* and *in vivo* biological screening facilities to R&D institutions, academic organizations, Universities, industrial houses, etc. on payment basis. Botany Division continued to be the nodal center for receiving and distributing the marine bio-materials to different biologists for bio-assay and communicating the screening results to the concerned centers under MoES project operating at the Institute

## 4. Documentation and Library Services

CDRI has a well equipped library and it continued to receive recognition from different national and international agencies. Its present

| Month | Mice | Rat | Hamster | Mastomys | Gerbil | G. pig | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|
| January | 1859 | 1686 | 452 | 140 | 75 | 30 | 2 | 4244 |
| February | 1573 | 1347 | 375 | 85 | 50 | 30 | 25 | 3485 |
| March | 1187 | 1382 | 244 | 140 | 85 | 24 | 28 | 3090 |
| April | 1054 | 1336 | 305 | 75 | 40 | 15 | 6 | 2831 |
| May | 1190 | 1478 | 73 | 73 | 65 | - | 4 | 2883 |
| June | 1175 | 1401 | 186 | 20 | 55 | 15 | 27 | 2879 |
| July | 1684 | 1817 | 510 | 40 | 40 | - | 22 | 4113 |
| August | 1016 | 1118 | 375 | 40 | 40 | 15 | 15 | 2619 |
| September | 1500 | 1877 | 227 | 55 | 70 | 15 | 3 | 3747 |
| October | 1030 | 1340 | 197 | 65 | 60 | - | 8 | 2700 |
| November | 1159 | 1375 | 427 | 75 | 50 | 19 | 2 | 3107 |
| G. Total | 14427 | 16157 | 3371 | 808 | 630 | 163 | 142 | 35698 |

collection comprises of 21928 books and 69890 bound volumes of journals. The department continued to provide computerized information services to its users and a total of 45 users utilized these services during the year. All activities of the division are fully computerized and confirm to the norms of e-governance. Publication of three periodicals viz. Drugs and Pharmaceuticals Industry Highlights (monthly), Drugs and Pharmaceuticals Current R&D Highlights and Ocean Drugs Alert (quarterly) continued and the subscribers largely appreciated the contents of these bulletins. Technical queries related to the publications were promptly attended to. Library manages, maintains and updates the website of the Institute.

## 5. Laboratory Engineering Services

The Laboratory Engineering Services division of CDRI provided engineering services for infrastructural needs and support services of various divisions towards setting up of new facilities, scientific instruments, renovation, up-gradation and repair/maintenance of laboratories, infrastructures, buildings and services. The division is also involved in construction of new CDRI on ~61 acres of land at Sitapur Road, Lucknow. The division is acting as an interface between the users and implementing agency viz. Engineering Projects India Ltd. Completed project is expected to have state of art laboratories with excellent working environment at par with international standards and will include a hostel, housing units, cafeteria, bank, dispensary, open air theatre, sport complex, guest house, etc.

## 6. IT Unit

The activities of IT Unit during the year include: systems and network administration; operation and maintenance of internet and mail services; operation and maintenance of software (for Stores & Purchase system, online compounds code register); LAN Infrastructure; development of MoES Database application software; Web Hosting for Intranet Web Site; Secured Web Access within CSIR; digital knowledge repository of DLS Division; R&D database application of MSB Division; network planning for New CDRI; up-gradation of ICT infrastructure; and Planning & Implementation of IT Setup for NIPER, Rae-Bareli.

# 6 Human Resource Development

## 1. Training Programmes/Workshops Attended by CDRI Staff

| Name of the Scientist | Title of the Training Programme/Workshop | Place | Date |
|---|---|---|---|
| Dr. (Mrs.) Anila Dwivedi | Training Programme on Mentoring as a Management Tool | HRDC, Ghaziabad | 21-22 Jan., 2008 |
| Dr. J. Lal | Reservation of Persons with Special Abilities and Others | HRDC, Ghaziabad | 17-18 Jan., 2008 |
| Dr. S.K. Puri | Training Programme on Enhancement of Managerial Efficiency for Scientists | HRDC, Ghaziabad | 4-8 Feb., 2008 |
| Dr. A.K. Srivastava | Training Programme on Enhancement of Managerial Efficiency for Scientists | HRDC, Ghaziabad | 4-8 Feb., 2008 |
| Dr. S.M. Rajendran | INDO-US Training on Good Laboratory Practice | Sree Chitra Tirunal Instituite for Medical Sciences & Technology Thiruvananthapuram, India | 5-7 Mar., 2008 |
| Dr. D.N. Upadhayay | Training Programme on Scientific Project Formulation Implementation and Evaluation | ASCI, Hyderabad | 17-28, Mar., 2008 |
| Mr. Naseem A. Siddiqui | Training Programme of Valorization of R&D in Association with APCTT | HRDC, Ghaziabad | 12-14 Mar., 2008 |
| Dr. Ram Raghubir | Training Programme on Strategic R&D Management | HRDC, Ghaziabad | 26-28 Mar., 2008 |
| Dr. Gautam Palit | Training Programme on Strategic R&D Management | HRDC, Ghaziabad | 26-28 Mar., 2008 |
| Dr. A.K. Dwivedi | Training Programme on Strategic R&D Management | HRDC, Ghaziabad | 26-28 Mar., 2008 |
| Mr. Wahajuddin | International Biomedical Modeling School and Workshop | NCBS, Bengalooru | 27 Feb.-2 Mar., 2008 |
| Mr. Wahajuddin | Quality Measurements | CDRI, Lucknow | 11-12 Mar., 2008 |
| Mr R.S.P. Singh | International Biomedical Modeling School and Workshop | NCBS, Bengalooru | 27 Feb.-2 Mar., 2008 |
| Dr. Ananad P. Kulkarni | Training Programme on Scientific Project Formulation : Implementation and Evaluation | ASCI, Hyderabad | 21 Apr. - 2 May, 2008 |

| Name of the Scientist | Title of the Training Programme/Workshop | Place | Date |
|---|---|---|---|
| Dr. Ram Raghubir | Programme on Decision Support Tools & Techniques for Senior Scientists | ASCI, Hyderabad | 28 Apr. - 2 May, 2008 |
| Dr. C. Nath | Programme on Decision Support Tools & Techniques for Senior Scientists | ASCI, Hyderabad | 28 Apr. - 2 May, 2008 |
| Dr. J. Lal | Reservation in Services Maintenance of Rosters | HRDC, Ghaziabad | 9-12 June, 2008 |
| Dr. S.C. Nigam | Training Programme on Planning for Life after Retirement | HRDC, Ghaziabad | 16-18 June, 2008 |
| Mr. G.P. Singh | Training Programme on Planning for Life after Retirement | HRDC, Ghaziabad | 16-18 June, 2008 |
| Dr. A.K. Khanna | Training Programme on Planning for Life after Retirement | HRDC, Ghaziabad | 16-18 June, 2008 |
| Mrs. Madhuri Chaudhary | Training Programme on Planning for Life after Retirement | HRDC, Ghaziabad | 16-18 June, 2008 |
| Dr. M.N. Srivastava | Programme on Project Management Techniques and Practices | HRDC, Ghaziabad | 4-7 Aug., 2008 |
| Dr. Gautam Palit | Training Programme on Mentoring as Management Tool | HRDC, Ghaziabad | 11-12 Aug., 2008 |
| Mr. A.K. Srivastava | Training Programme on Mentoring as Management Tool | Ghaziabad | 11-12 Aug., 2008 |
| Dr. A.K. Dwivedi | Training Programme on Mentoring as Management Tool | HRDC, Ghaziabad | 11-12 Aug., 2008 |
| Dr. Gitika Bhatia | Training Programme on Mentoring as Management Tool | HRDC, Ghaziabad | 11-12 Aug., 2008 |
| Dr. Anand P. Kulkarni | Leadership Development Programme for Young & Middle Level Scientists | LBSNAA, Musoorie | 4-8 Aug., 2008 |
| Dr. S. K. Puri | Theory of Inventive Problem Solving Methods (TRIZ) | HRDC, Ghaziabad | 25-27 Aug., 2008. |

| Name of the Scientist | Title of the Training Programme/Workshop | Place | Date |
|---|---|---|---|
| Dr. B. Kundu | Training Programme on Knowledge Management | HRDC, Ghaziabad | 10-12 Sep., 2008 |
| Dr. Neeraj Sinha | Training Programme on Knowledge Management | HRDC, Ghaziabad | 10-12 Sep., 2008 |
| Mr. Kashif Hanif | Programme on Induction Training | HRDC, Ghaziabad | 12-14 Sep., 2008 |
| Mr. Wahajuddin | Programme on Induction Training | HRDC, Ghaziabad | 12-14 Sep., 2008 |
| Dr. A.K. Trivedi | Programme on Induction Training | HRDC, Ghaziabad | 12-14 Sep., 2008 |
| Dr. R. Shukla | Prospective Trainers at WHO-TDR GLP Training Workshop | IITR, Lucknow | 13-18 Oct., 2008 |
| Mr. Wahajuddin | Good Laboratory Practice | NCBS, Bangalore | 29-31 Oct., 2008 |
| Dr. S.K. Rath | Leadership Development Programme | HRDC, Ghaziabad | 2-14 Nov., 2008 |
| Dr. Neena Goyal | Leadership Development Programme | HRDC, Ghaziabad | 23 Nov. - 5 Dec., 2008 |
| Dr R.S. Bhatta | LC MS/MS User's Meet | Applied Bio-systems, Jaipur | 28 Nov.-1 Dec., 2008 |
| Mr. Wahajuddin | LC MS/MS User's Meet | Applied Bio-systems, Jaipur | 28 Nov.-01 Dec. 2008 |
| Dr. K.R. Arya | Training Programme on Crafting Effective Communication | HRDC, Ghaziabad | 22-24 Dec., 2008 |
| Dr. D.K. Misra | Training Programme on Crafting Effective Communication | HRDC, Ghaziabad | 22-24 Dec., 2008 |
| Dr. Smrati Bhadauria | Training Programme on Crafting Effective Communication | HRDC, Ghaziabad | 22-24 Dec., 2008 |
| Dr. Sarika | Training Programme on Crafting Effective Communication | HRDC, Ghaziabad | 22-24 Dec., 2008 |

2. Ph.D. thesis submitted during 2008

| Name of the Research Fellow | Title of the Ph.D. Thesis/Supervisor | Name of the University awarding Ph.D. Degree |
|---|---|---|
| Antima Goel | Mechanism of isoniazid drug resistance in *Mycobacterium aurum*/ *Dr. Ranjana Srivastava* | BHU, Varanasi |
| Shilpi Pandey | Synthesis and chemistry of potential antiparasitic cyclic peroxides/*Dr. Chandan Singh* | Lucknow University |
| Rishi Kumar | Structural studies of synthetic molecules of biological importance/*Dr. P.R. Maulik* | JNU, New Delhi |
| Manish Kumar Gupta | QSAR and modeling studies on antimalarial and antitubercular agents/*Dr. Y.S. Prabhakar* | JNU, New Delhi |
| Sudipti Gupta | Characterization and modulation of the interaction between HIV-1 accessory protein NEF and its cellular host protein/ *Dr. R.K. Tripathi* | JNU, New Delhi |
| Neeta Asthana | Understanding the structure-function relationships in some naturally occurring antimicrobial peptides and design of novel peptides with antimicrobial activity/ *Dr. J.K. Ghosh* | JNU, New Delhi |
| Maya Datt Joshi | Target sites of novel antidiabetic agents for the treatment of type-2 *Diabetes mellitus*/*Dr. Arvind Srivastava* | JNU, New Delhi |
| Rishi Sharma | Molecular and cellular mechanisms involved in the neurotrophins following cerebral ischemia/reperfusion injury/*Dr. Ram Raghubir* | JNU, New Delhi |
| Vijay Singh | Studies on the synthetic and medicinal applications of Baylis-Hillman Chemistry/ *Dr. Sanjay Batra* | Kanpur University, Kanpur |
| Richa Pathak | Design and synthesis of novel guanidine derivatives as antiparasitic agents/*Dr. Sanjay Batra* | Kanpur University, Kanpur |
| Sarvind Mani Tripathi | Structural studies on latent phase metabolic pathway protein(s) from *Mycobacterium tuberculosis* H37Rv/ *Dr. Ravishankar R*. | JNU, New Delhi |

| Name of the Research Fellow | Title of the Ph.D. Thesis/Supervisor | Name of the University awarding Ph.D. Degree |
|---|---|---|
| Satish Vedi | Prophylactic evaluation and immunological characterization of recombinant heavy chain myosin of adult female *Brugia malayi*/ *Dr. Shailja Bhattacharya* | Devi Ahilya Vishwavidyalaya, Indore |
| Ved Prakash Verma | Synthesis and antimalarial assessment of novel organic peroxides/ *Dr. Chandan Singh* | JNU, New Delhi |
| Ajit Shankar Singh | Synthesis, biology and chemistry of novel synthetic 1,2,3-trioxanes/*Dr. Chandan Singh* | JNU, New Delhi |
| Swapnil Sinha | Analysis of single nucleotide polymorphisms in genes related to resistance or susceptibility to *Plasmodium falciparum* malaria in the Indian population/*Dr. Saman Habib* | JNU, New Delhi |
| Rohit Saluja | Molecular and biochemical studies on nitric oxide synthase in polymorphonuclear leukocytes under normal and pathological conditions/ *Dr. Madhu Dikshit* | JNU, New Delhi |
| Suresh L. Mehta | Studies on cellular and molecular mechanisms of stroke damage in diabetes/ *Dr. Ram Raghubir* | JNU, New Delhi |
| Ramesh | Chemotherapy of visceral Leishmaniasis: Molecular and biochemical approach/*Dr. Suman Gupta* | JNU, New Delhi |
| Sudershan Madapa | Design and synthesis of urea derivatives of heterocycles as possible antimalarial agents/ *Dr Sanjay Batra* | Kanpur University |
| Biswajit Kumar Singh | Synthetic studies in antimycobacterial agents based on carbohydrates and heterocycles/ *Dr. R.P. Tripathi* | Hyderabad University |
| Sajal Kumar Das | Diversity oriented organic synthesis of biologically important molecules/ *Dr. G. Panda* | JNU, New Delhi |
| Sudhir Kumar | Development of novel agents for the management of osteoporosis/*Dr. M.M. Singh* | Dr. Bhim Rao Ambedkar University, Agra |
| Rahul Srivastava | Identification of mycobacterial regulatory sequences affecting virulence/*Dr. Ranjana Srivastava* | JNU, New Delhi |

| Name of the Research Fellow | Title of the Ph.D. Thesis/Supervisor | Name of the University awarding Ph.D. Degree |
|---|---|---|
| Ajay Kumar Srivastava | An approach to target and diversity oriented synthesis/*Dr. Gautam Panda* | Jadavpur University, Jadavpur |
| Mirza Saqib Baig | Identification and characterization of stage specific gene(s) in amastigote form of *Leishmania donovani* through microarray/*Dr. Neena Goyal* | JNU, New Delhi |
| Preeti Dohare | Neuroprotection by inhibiting the neuronal death pathways in focal cerebral ischemia/*Dr. Madhur Ray* | JNU, New Delhi |
| Shuja Shafi Malik | Structural studies on transcriptional regulatory protein(s) from *Mycobacterium tuberculosis* H37Rv/*Dr. R. Ravishankar* | JNU, New Delhi |
| Amit Luthra | Structural studies on hypothetical protein(s) from *Mycobacterium tuberculosis* H37Rv/*Dr. R. Ravishankar* | JNU, New Delhi |
| Deepti Verma | 2-Pyronones derived arenes and heteroarenes as biodynamic agents/*Dr. Atul Goel* | JNU, New Delhi |
| Devesh Sawant | Novel application of Pictet-Spengler reaction leading to the synthesis of heterocycles of medicinal interest/ *Dr. Bijoy Kundu* | Dr. Bhim Rao Ambedkar University, Agra |
| Tripti Shrivastava | Structural studies on transcriptional regulatory and metabolic proteins from *Mycobacterium tuberculosis* H37Rv/*Dr. R. Ravishankar* | JNU, New Delhi |
| Pratibha Tiwari | Development of novel anti-trichomonas agents/*Dr. M.M. Singh & Dr. Divya Singh* | Dr. Bhim Rao Ambedkar University, Agra |
| Mukesh Sawant | Studies on expression and characterization of proteophosphoglycans (PPGs) of *Leishmania donovani*/*Dr. Anuradha Dube* | JNU, New Delhi |
| Ajay Sharma | Design and synthesis of CCK-8 derived peptidomimetics as selective inhibitors of protein tyrosine phosphotase1B (PTP1B) for the development of antidiabetic agents/*Dr. S.B. Katti* | JNU, New Delhi |

| Name of the Research Fellow | Title of the Ph.D. Thesis/Supervisor | Name of the University awarding Ph.D. Degree |
|---|---|---|
| EVSR Ram | Analysis of nuclear encoded proteins putatively involved in replication of Plasmodium falciparum apicoplast DNA/*Dr. Saman Habib* | JNU, New Delhi |
| Shubhra Singh | Growth profile and chemosensitivity of *Plasmodium falciparum* in a modified medium and the characterization of the genetic factors involved in artemisinine mediated resistance in parasites/ *Dr. KumKum Srivastava* | JNU, New Delhi |
| L. Vijaya Raghava Reddy | Synthesis of carbohydrate molecules of biological importance/*Dr. A.K. Shaw* | Sri Krishnadevaraya University, Anantpur |
| Ritu Malik | Rational design of a drug delivery system for intervention in episodic endocrine phenomena in hypothalamo-pitutary-testicular axis/ *Dr. Amit Misra* | JNU, New Delhi |
| Tanaya De | Development of anti-resorptive compound in the management of osteoporosis/ *Dr. M.M. Singh* | JNU, New Delhi |
| Amita Daverey | Molecular mechanism of action of ormeloxifene – A selective estrogen receptor modulating agent/ *Dr. Anila Dwivedi* | JNU, New Delhi |
| Kulwant Singh | Structural and stability characteristics of an apicoplast ferridoxin reductase / *Dr. Vinod Bhakuni* | JNU, New Delhi |
| Biju B | Development of novel bone forming agents from natural sources/*Dr. M.M. Singh* | Dr. Bhim Rao Ambedkar University, Agra |
| Amit Kumar Mitra | Analysis of polymorphisms within some DNA repair genes in selected Indian sub-populations/*Dr. S.K. Rath* | JNU, New Delhi |
| Ramesh Chandra | Chemotherapy and biochemical studies with arteether resistant *Plasmodium vinckei*, a rodent malaria parasite/*Dr. S.K. Puri* | Dr. Bhim Rao Ambedkar University, Agra |

### 3. M.D. Thesis submitted/awarded

| Name of the Research Fellow | Title of the Thesis/Supervisor | Name of the University awarding Degree |
|---|---|---|
| Dr. Hina Narayan | *In vitro* study on the role of curcumin in human jingival fibroblast cell line / *Dr. Anil K. Balapure & Dr. Ramesh Sharma* | C.S.M. Medical University, Lucknow |
| Dr. Rajesh Kumar | Role of platelet – rich plasma in the regulation of jingival fibroblast – an *in vitro study/ Dr. Anil K. Balapure & Dr. Ramesh Sharma* | C.S.M. Medical University, Lucknow |
| Dr. Somya Govil | A comparative evaluation of microleakage of different tooth coloured restorative materials: An *in vitro study/Dr. J.K. Saxena* | Dr Ram Manohar Lohia Avadh University, Faizabad |
| Dr. Rituraj Tripathi | Toxicological study of vaikranta bhasma prepared with sulphur (Gandhaka) media and its effect as vrishya w.s.r. to seminal parameters/*Dr. Ram Raghubir* | H.P. University, Shimla |

## 4 Training

### 4.1 Training to sponsored personnel

Under this programme, the Institute conducted the "Advance Technology Training Programme", for scientists and technical persons, mainly from industry; training to foreigners under bilateral cooperation with different countries and international agencies; training to sponsored students from academic institutions and ad-hoc short-term training for academia and industry.

### 4.2 International training under bilateral cooperation

Long-term/short term training was provided to the following persons sponsored by their institutions:

CSIR-TWAS Fellowship

Mr. Akinsomisoye Olimide Stephen
Physiological Sciences
Obafemi University Lle-Ife, Osun State, Nigeria

Reproductive & Endocraniological affect of some synthetic antimalarial drugs

Dr. S.A. Onasanwo
Lecturer
Department of Physiology Sciences
University of Ibadane, Ibadane, Nigeria

Anti-nociceptive, anti-inflammatory and anti-allergic properties of some Nigerian plants

CCSTDS/RTFDCS Fellowship

Mr. A.C. Akinomoladin
Lecturer
Federal University of Technology
Ankure, Nigeria

Identification & characterization of the active constituents of some medicinal plants and neuro and cardio protective functions of the extracts.

### 4.3 Training under cooperation with Indian universities

Under the training programme, 7 students from Birla Institute of Technology and Science, Pilani, were provided six months training on monthly stipend.

### 4.4 Training under cooperation with Indian Academy of Science

Under the programme, 3 students were provided two months training under the cooperation with Indian Academy of Science, Bangalore.

### 4.5 Adhoc training

Following industry and academia sponsored personnel were trained in Toxicology and Pharmacology divisions of the Institute.

| | |
|---|---|
| Dr. Nilesh Bhadja<br>Cadila Pharmaceuticals Ltd.<br>Ahmedabad, Gujarat | Toxicology |
| Mr. Vishal Khandelwal<br>G.L.A. Institute of Professional Studies<br>Mathura, U.P. | Pharmacology |
| Dr. B.K. Sharma<br>S.K. Government College<br>Sikar, Rajasthan | Pharmacology |
| Mr. Lokesh Deb<br>Institute of Bioresources &<br>Sustainable Development<br>Imphal, Manipur. | Pharmacology |

### 4.6 Following university/colleges sponsored students were imparted training in different division of the Institute. Tenure of the training ranged from 2 to 12 months.

| Name of University/College | No. of Students |
|---|---|
| Allahabad Agricultural Institute, Allahabad | 4 |
| Allahabad Univeristy, Allahabad | 5 |
| Amity University, Noida | 13 |
| Andhra University, Visakhapatnam | 2 |
| Annamalai University, Annamalainagar | 2 |
| Appasaheb Birnale College of Pharmacy, Sangli | 1 |
| Banasthali University, Banasthali | 26 |
| Bhupan Nobles College of Pharmacy, Udaipur | 2 |
| B. R. D. School of Biosciences, Sardar Patel University, Vallabh Vidyanagar | 2 |
| Bundelkhand University, Jhansi | 10 |
| C. S. J. M. University, Kanpur | 6 |

| Name of University/College | No. of Students |
|---|---|
| Centre for Microbiological Research, Tiruchengode | 1 |
| Ch. Charan Singh University, Meerut | 1 |
| Cochin University, Cochin | 3 |
| D. A. V. College, Kanpur | 1 |
| D. A. V.(PG) College, Muzaffarnagar | 3 |
| Dayanand Girls College, Kanpur | 3 |
| Dolphin (PG) Institute of Biomedical Natural Sciences, Dehradun | 2 |
| Doon (PG) College of Agriculture Science & Technology, Dehradun | 7 |
| Dr. B. R. Ambedkar University, Agra | 1 |
| Dr. G. R. Damodaran College of Science, Coimbatore | 1 |
| Dr. R. M. L. Avadh University, Faizabad | 4 |
| Feroze Gandhi College, Raibareli | 3 |
| G. Pulla Reddy College, Hyderabad | 1 |
| Gaytari College of Pharmacy, Sambalpur | 1 |
| G. I. C. T. College, Gwalior | 1 |
| Gorakhpur University, Gorakhpur | 1 |
| Government Post Graduate College, Guna | 1 |
| Gupta College of Technological Science, Asansol | 1 |
| Guru Ghasidas University, Bilaspur | 5 |
| Gyan Vihar University, Jaipur | 1 |
| H. N. B. Garhwal University, Garhwal | 3 |
| Harcourt Butler Technology Institute, Kanpur | 1 |
| Hindu P. G. College, Ghazipur | 1 |
| I. E. T. Biotechnology Institute, Alwar | 2 |
| Banaras Hindu University, Varanasi | 1 |
| Institute of Engineering & Technology, Lucknow | 1 |
| Institute of Pharmacy & Technology, Salipur, | 1 |
| Integral University, Lucknow | 11 |
| I. T. S. Paramedical College (Pharmacy), Ghaziabad | 6 |
| Jain College, Gwalior | 2 |
| Jaipur National University, Jaipur | 1 |
| Jamia Milia Islamia, New Delhi | 2 |
| Jawahar Lal Nehru Centre for Advance Scientific Research, Bangalore | 1 |
| Jaypee Institute of Information Technology University, Noida | 1 |

| Name of University/College | No. of Students |
|---|---|
| J. K. K. M. Medical College Research Foundation College of Pharmacy, Komarapalayam | 1 |
| Kanya Gurukul Mahavidyalaya, Haridwar | 3 |
| Kongu Arts & Sciences College, Nanjanapuram, Erode | 1 |
| Krupanidhi College of Pharmacy, Bangalore | 2 |
| Kumaun University, Nainital | 1 |
| Kurukshetra University, Kurukshetra | 1 |
| Lucknow University, Lucknow | 4 |
| Maharishi Arvind Institute of Engineering & Technology, Jaipur | 1 |
| Mahatama Gandhi Institute of Applied Sciences, Jaipur | 2 |
| Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Satna | 1 |
| Mahatma Gandhi University, Ettumanoor | 1 |
| Mahatma Jyoti Rao Phoole P.G. Mahila Mahavidyalaya, Jaipur | 1 |
| Meerut Institute of Engineering & Technology, Meerut | 3 |
| Modi Institute of Management & Technology, Kota | 4 |
| National Centre for Cell Sciences, Pune | 1 |
| Northern India Engineering College, Lucknow | 6 |
| Orissa University, Bhubaneshwar | 1 |
| R. R. P. G. College, Amethi | 1 |
| Rajendra Institute of Science & Technology, Sirsa | 3 |
| Rajiv Gandhi Biotechnology Centre, Nagpur University, Nagpur | 1 |
| Rajiv Gandhi Technological University, Bhopal | 2 |
| Rani Durgavati University, Jabalpur | 2 |
| R. K. D. F. College of Pharmacy, Bhopal | 1 |
| Roland Institute of Pharmaceutical Science, Orissa | 1 |
| Sai Institute of Paramedical & Allied Science, Dehradun | 3 |
| Sardar Patel University, Vallabh Vidyanagar | 2 |
| Sastra University, Thanjavur, Tamil Nadu | 1 |
| Saurasthtra College, Madurai | 1 |
| School of Studies in Zoology & Biotechnology, Ujjain | 1 |
| Seedling Academy, Jaipur | 1 |
| Sri Krishna Arts & Science College, Coiambatore | 1 |
| S. R. M. University, Kattankulathur | 1 |
| St. John's College, Agra | 1 |

| Name of University/College | No. of Students |
|---|---|
| Tagore Biotech College, Jaipur | 1 |
| University of Rajasthan, Jaipur | 3 |
| V.B.S. Purvanchal University, Jaunpur | 1 |
| Vels' College of Pharmacy, Chennai | 2 |
| Vikram University, Ujjain | 1 |
| Vinayaka Missions College of Pharmacy, Salem | 3 |
| Visvabharati, Santiniketan, Bholpur | 1 |
| VIT University, Vellore | 6 |

# 7 Lectures Delivered

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. A.K. Saxena | 2D & 3D QSAR and molecular modeling: Basics. SERC Summer School in modeling and informatics in drug design; | NIPER, Chandigarh (30.06.08 to 18.07.08) |
| | Molecular modeling studies on AChE inhibitor carbamates to design and synthesize anti-alzheimer agents; | United Nations Industrial Development Organisation, Trieste, Italy (04.07.08) |
| | 2D and 3D QSARs: Basics and applications; | Lucknow University, Lucknow (25.04.08) |
| | QSARs and molecular modeling studies on substituted pyrazino-pyridoindoles and related compounds as potential antihistamines; | ABCT, The Hong Kong Polytechnic University, Kowloon, Hongkong (02.04.08) |
| | Design and synthesis of octa / decahydropyrazino-pyridoindoles as potential antipsychotic agents; | ABCT, The Hong Kong Polytechnic University, Kowloon, Hongkong (02.04.08) |
| | Drug R&D at Central Drug Research Institute, Lucknow; | Pear L Materia Medica Development (Shenzhen) Ltd., Shenzhen, China (01.04.08) |
| | Bioinformatics and molecular modeling in drug design: A case study of B3-adrenergic receptor agonist; | Dr. B.R. Ambedkar Center for Biomedical Research, New Delhi (29.01.08) |
| | Drug discovery research: Current scenario. Recent trends of research in pharmaceutical sciences. | Shrinathji Institute of Pharmacy, Rajasthan (07.01.08) |
| Dr. (Mrs.) Ranjana Srivastava | A compreshensive murine infection model of *Mycobacterium fortuitum* to dilineate latency, reactivation, and screening of mutants and inhibitory compounds; | ICGEB, New Delhi (03.12.08) |
| | Rapid *in vivo* model for screening of antimycobacterial compounds. | Indian Institute of Technology, Kanpur (08.07.08) |
| Dr. Ram Raghubir | Bio-actives from the ocean; | AAI-DU, Allahabad (03.01.08) |
| | Neuroprotective mechanisms in diabetic cerebral stroke; | CIIMS, Nagpur (22.08.08) |
| | Marine environment as a novel source of pharmacotherapeutics; | ISVPT , Mathura (06-08.11.08) |

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| | Does inhibition of E-R stress is neuroprotective following cerebral ischemia; | IAN , Kochi (12-14.12.08) |
| | Molecular mechanisms in diabetic cerebral stroke; | IAN , Kochi (12-14.12.08) |
| | Current status on drugs from ocean. | IPS, New Delhi (18-20.12.08) |
| Dr. G. Palit | Evolving process of drug designing: a perspective; | IMS, Ghaziabad (18.12.08) |
| | Evolving process of drug designing for psychiatric disorders; | CSMCRI, Bhavnagar (01.08.08) |
| | Identification and evaluation of potential anti-ulcer agents from Indian medicinal plants: A perspective. | IICT, Hyderabad (04.011.08) |
| | Prospects in drug development of functional leads from Indian medicinal plants against peptic ulcer disease; | NIPER, Mohali (19-20.11.08) |
| Dr. C. Nath | Current concepts for the pharmacotherapy of Alzheimer's disease; | Shri Nathji Institute of Pharmacy, Nathdwara (07.01.08) |
| | Neurotransmitters, adrenergic & cholinergic systems; | Amity Institute of Biotechnology, Lucknow (17.11.08) |
| | Psychopharmacology: drugs affecting behavioral responses. | Amity Institute of Biotechnology, Lucknow (18.11.08) |
| Dr. S.B. Katti | Thiazolidinones as HIV-RT inhibitors; | Wockhardt Research Centre, Aurangabad (24.03.08) |
| | Chemical synthesis of oligonucleotides: An overview; | Dr. Reddy's Research Centre, Hyderabad (20.06.08) |
| | Design, synthesis and 3D-QSAR of 4-thiazolidinones as HIV-RT inhibitors. | Indian Pharmaceutical Congress, New Delhi (14.12.08) |
| Dr. S. K. Puri | Indian initiatives towards developing synthetic artemisinine derivatives. | AIIMS, New Delhi (18.12.08) |
| Dr. J. K. Saxena | Can glycolytic pathway be utilized for development of antifilarials; | North East Hill University, Shillong (04.11.08) |
| | The interface of chemistry and biology in biomedical research. | BITS, Pilani (23.02.08) |

## 7 Lectures Delivered

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. Kanchan Hajela | Selective estrogen receptor modulators: A novel class of non steroidal estrogen agonists and antagonists as multifunctional designer molecules. | Dayalbagh Educational Institute, Agra (02.02.08) |
| Dr. A. K. Dwivedi | Principles and applications of HPLC: Concept and application of advance instrumentation monitoring GC-MS, HPLC, AAS, ICP, TCLP and their monitoring instruments. | IITR, Lucknow (22.07.08) |
| Dr. Madhu Dikshit | Functional assays using Flow Cytometry; | CDRI, Lucknow (13.11.08) |
| | Molecular characterization and distribution of nitric oxide synthase isoforms in rat and human polymorphonuclear leukocytes; | Bhabha Atomic Research Center, Mumbai (23.10.08) |
| | Cell function assays using Flow Cytometry; | CDRI, Lucknow (18.09.08) |
| | Cell function assessment by Flow Cytometry | NCBS, Bangalore (04.05.08) |
| | Cell Function Assays: Assessment by Flow Cytometry; | NCBS, Bangalore (23.01.08) |
| | Assessment of Cell cycle S phase by Brdu labeling; | NCBS, Bangalore (22.01.08) |
| | Molecular, Biochemical and pharmacological approaches for new anti-thrombotic drug development. | Punjab University, (15.03.08) |
| Dr. Rakesh Shukla | Guggulipid as potential therapeutic agent against neuro-inflammation and memory impairment | NIPER, Mohali (16-20.11.08) |
| | Curcumin: A Hope for millions. | Shri Nathji Institute of Pharmacy, Nathdwara (07.01.08) |
| Dr. M. Abbas | Mathematics and its applications in molecular biology; | JMI, New Delhi (04.12.08) |
| | Statistical analysis and modeling of data in post genomic era; | CDRI, Lucknow (16.09.08) |
| | Theoretical and computational aspects of bioinformatics. | CDRI, Lucknow (01.09.08) |

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. Naibedya Chattopadhyay | Kaempferol a triple action molecule for the treatment of menopausal osteoporosis: A method for further enhancing its action; | New Delhi (20.12.08) |
| | Phytochemicals for bone loss disorders: What needs to be done; | MD Bioalpha, Seoul, Korea (20.05.08) |
| | Role of calcium-sensing receptor in bone. | Annual Meeting of Korean Endocrine Society, Seoul, Korea, (17.05.08) |
| Dr. D. C. Kaushal | DNA cloning and expression; | MPCST, Bhopal (03.06.08) |
| | Gene cloning 1: Basic techniques; | MPCST, Bhopal (01.06.08) |
| | Gene cloning 2: Cloning vectors. | MPCST, Bhopal (02.06.08) |
| Dr. Bijoy Kundu | New developments in drug discovery and natural products and traditional medicines. | NIPER, Chandigarh (17.11.08) |
| Dr. Neeraj Sinha | NMR based metabonomics in toxicology; | B.N. Degree College, Kanpur (13.12.08) |
| | NMR based metabonomics applied for the investigation of nephrotoxicity in rats; | Taj Residency, Lucknow (21.11.08) |
| | eVkckukfeDl ij vk/kkfjr ,u·,e·vkj· }kjk oDd fo"kkDrrk dk ijh{k.k; | vkb·oh·vkj·vkb·, cjyh (25.03.08) |
| | Testing teratogenicity at the platform of metabonomics by using NMR. | Berlin, Germany (18.01.08) |
| Dr. D.S. Upadhyay | Public health issues in management and supervision of research animal facility; | Hotel Taj Residency, Lucknow (20.11.08) |
| | Welfare issues in management of experimental animals at research institutions. | Annamalai University, Chidambaram (29.03.08) |
| Dr. R. P. Tripathi | Our effort to develop new antitubercular agents. | Kottayam, Kerala (21.08.08) |
| Dr. (Mrs.) Uma Roy | Cloning, over-expression and characterization of *L. donovani* squalene synthase. | North East Hill University, Shillong (04.11.08) |
| Mr. Vinay Tripathi | Intellectual property rights and related issues. | CDRI, Lucknow (27.11.08) |
| Dr. A. K. Shaw | Stereoselective synthesis of densely substituted tetrahydrofurans and their application. | BITS, Pilani (24.02.08) |

## 7 Lectures Delivered

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. Neena Goyal | Molecular mechanisms of antimony resistance in fields isolates of *Leishmania donovani;* | North East Hill University, Shillong (04.11.08) |
| | Drug resistance: Functional analysis using flow cytometry; | CDRI, Lucknow (16.09.08 and 13.11.08) |
| Dr. P. K. Shukla | Natural products and drug discovery; | Institute of Advanced Study in Science and Technology, Guwahati (08.04.08) |
| | Drugs from the sea: Finding new drugs in ocean. | Prof Dhanpalan College for Women, Chennai (28.01.08) |
| Dr. Gopal Gupta | Selectively targeting sperm in vagina for safe contraception using novel, non-detergent molecules. | CDRI, Lucknow (14.03.08) |
| Dr. Saman Habib | Regulating DNA replication in the apicoplast; | IIT, Chennai (18.12.08) |
| | DNA organization and replication in the *Plasmodium falciparum* apicoplast; | TIFR, Mumbai (27.11.08) |
| | Human genetic variation and susceptibility to *P. falciparum* malaria; | St. Xavier's College, Mumbai (26.11.08) |
| | Patriarchal attitudes among scientists: the need for gender sensitization; | IIT, New Delhi (01.11.08) |
| | Understanding DNA organization and replication in the *Plasmodium* apicoplast; | Gangtok (18.10.08) |
| | Genomics of microbial pathogens and host-pathogen interactions; | Hi-Tech City Convention Centre, Hyderabad (28.09.08) |
| | Human genetic factors and susceptibility to *Plasmodium falciparum* malaria in India; | Cebu, The Philippines (03.04.08) |
| | DNA protein interactions involved in replication and organization of the Plasmodium falciparum apicoplast genome. | Vigyan Bhawan, New Delhi (09.03.08) |
| Dr. Sanjay Batra | Applications of the Baylis-Hillman derivatives for the synthesis of heterocycles; | Loughborough University, United Kingdom (29.10.08) |
| | Applications of the Baylis-Hillman derivatives for the synthesis of heterocycles. | University of Leicester, United Kingdom (03.11.08) |
| Dr. Jimut K. Ghosh | Design of cell-selective antimicrobial peptides and understanding their mode of action. | Karunya University, Coimbatore (23.08.08) |

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. Nuzhat A. Kaushal | Colony PCR for screening of transformants; | MPCST, Bhopal (03.06.08) |
| | Polymerase Chain Reaction (PCR) and its applications; | MPCST, Bhopal (02.06.08) |
| | RAPD-PCR Markers. | MPCST, Bhopal (02.06.08) |
| Dr. S. K. Rath | Chromosomal aberration assay; | IITR, Lucknow (16.12.08) |
| | Micronucleus assay; | IITR, Lucknow (15.12.08) |
| | SNP and Cancer. | Veer Kunwar Singh University, Ara (27.09.08) |
| Dr. Atul Goel | Versatile precursors with unlimited synthetic potential. | University of Wuerzburg, Germany (16.10.2008) |
| Dr. Gautam Panda | Synthesis of α-amino acid based chiral privileged polycycles and their pharmacological evaluation. | Indian Institute of Technology, Kharagpur (19.11.08) |
| Dr. Anila Dwivedi | Selective estrogen receptor modulators in female reproductive health research. | CDRI, Lucknow (12.12.08) |
| Dr. S.K. Singh | Important aspects of animal and human pharmacokinetics. | CDRI, Lucknow (01.10.08) |
| Dr. J. Lal | Role of pharmacokinetics in drug development. | CDRI, Lucknow (10.10.08) |
| Dr. P. R. Mishra Pharmaceutical | Rational approach towards LPS neutralization | Institute of Pharmacy and |
| | using nano-structured formulations. | Technology, Berlin, Germany (24.09.08) |
| Dr. S.M. Rajendran | Importance of medicinal plants, making herbal garden and its scaping and herbal methods and its management. | Regional Science Centre, Lucknow (15.12.08) |
| Dr. Mukesh Srivastava | Experimental design, testing and regression in drug research. | CDRI, Lucknow (15.09.08) |
| Dr. Rajender Singh | Genetic defects in cellular protein turnover system correlate with male infertility. | CDRI, Lucknow (13.03.08) |
| Dr. D.P. Mishra | Sphingosine-1-Phosphate: A Regulator of Male Germ Cell Apoptosis | CDRI, Lucknow (13.03.08) |
| Dr. R.K. Singh | 14- Days irritancy study of SMA coated copper T in rats. | CDRI, Lucknow (11.12.08) |

## 7 Lectures Delivered

| Name of Scientist | Title of the Lecture | Place / Date |
|---|---|---|
| Dr. Rituraj Konwar | Future scenarios with nanotechnology, stem cell therapy and pharmacogenomics - Part I; | CSMMU, Lucknow (05.08.08) |
| | Future Scenarios with Nanotechnology, Stem cell therapy and Pharmacogenomics - Part II. | CSMMU, Lucknow (06.08.08) |
| Dr. Poonam Singh | Decolorization and degradation of azo dyes by bacterial cultures | Biobrainz, Lucknow (28.05.08) |
| Dr. R.S. Bhatta | Series of lectures in bio-pharmaceutics and pharmacokinetics; | NIPER, Rae Bareli (From 17.11.08 to 11.01.09) |
| | General laboratory and practical classes. | NIPER, Rae Bareli (From 17.11.08 to 11.01.09) |
| Mr. Wahajuddin | Quantitation of active pharmaceutical substance in biological samples by LC-MS/MS; | CDRI, Lucknow (02.12.08) |
| | Series of lectures on fundamentals of intellectual property and technology | NIPER, Rae Bareli (from 18.11.08 to 09.01.09) |
| Dr. Sripathi Rao Kulkarni | Intellectual property rights and related issues. | CDRI, Lucknow (28.11.08) |
| Mr. Shreekant Deshpande | Is feature selection essential for ANN modeling? | University of Minnesota Duluth, Duluth, Minnesota,USA (22.01.08) |

# Conference/Symposia/Training/Workshops Organised 8

| Sl No. | Titel of the Programme | Date |
|---|---|---|
| 1 | An update of Male Reproduction and Infertility [Sponsored by MOH] | 13-14 March, 2008. |
| 2 | Four Weeks Training Program on Laboratory Animal Sciences | 28 April – 23 May, 2008 |
| 3 | Third Basic Flow Cytometry Workshop [Sponsored by the Cytometry Society, India] | 15-18 September, 2008. |
| 4 | Fourth Basic Flow Cytometry Workshop [Sponsored by the Cytometry Society, India] | 10-13 November, 2008. |
| 5 | Inaugural FACS-Asia Workshop [Sponsored by Becton Dickinson, India] | 25-28 November, 2008 |
| 6 | Workshop cum Users Meeting, Sophisticated Analytical Instrument Facility. | 1-2 December, 2008 |
| 7 | Recent Advances in Female Reproductive Health Research [Sponsored by MOH] | 11-12 December, 2008 |


Dr. Naibedya Chattopadhyay, Deputy Director, CDRI addressing the audience during National Symposium. Also seen on the dais Prof. Vinita Das, CSMMU, Lucknow, Dr. A.K. Saxena, Senior Deputy Director, CDRI and Dr. Anila Dwivedi, Scientist, CDRI.

# 9 Distinguished Visitors and Lectures

| Name of the Visitor | Title of the Lecture | Date |
|---|---|---|
| Prof. Joseph D. Puglisi<br>Director<br>Magnetic Resonance Laboratory<br>Stanford University<br>USA. | RNA and Infection | 06.02.08 |
| Prof. Johann Gasteiger<br>University of Erlangen<br>Nurenberg Germany. | (i) Explorations into Biochemical Pathways for Drug Design<br>(ii) How to Assist the Organic Chemist by Computer Methods | 17.02.08<br>19.02.08 |
| Prof. Xi Chen<br>University of California<br>USA. | Chemo-enzymatic Approaches for Chemical Glyco-biology | 19.02.08 |
| Prof. P. George Wang<br>Ohio State University<br>USA. | Glyco-pharmaceuticals: A Sponge of Sugar Makes the Medicine Go Down | 20.02.08 |
| Dr. Abbas Ali Mahdi<br>Chhatrapati Sahuji Maharaj<br>University Lucknow. | Oxidative Insult in Male Infertility and Role of Indian Herbs | 13.03.08 |
| Dr. S.N. Sankhwar<br>Chhatrapati Sahuji Maharaj<br>University Lucknow. | Surgical Options in Treatment of Male Infertility | 13.03.08 |
| Dr. K. Thangaraj<br>Centre for Cellular and<br>Molecular Biology Hyderabad. | Genetic Causes of Male Infertility | 13.03.08 |
| Dr. Rima Dada<br>All Indian Institute of<br>Medical Sciences, New Delhi. | Genetic Studies in Infertility and Recurrent Art Failure | 13.03.08 |
| Dr. S. K. Gupta<br>National Institute of Immunology<br>New Delhi. | Molecular Basis of Fertilization in Humans | 13.03.08 |
| Dr. Vrinda Khole<br>National Institute for<br>Research in Reproductive Health<br>Mumbai. | Novel High Throughput Combinatorial Approach for Identification of Immunodominant Domain Specific Epididymal Sperm Proteins | 13.03.08 |
| Dr. Niraj Pant<br>Indian Institute of<br>Toxicology Research, Lucknow. | Analysis of Mitochondrial Membrane Potential in Fertile, Asthenospermic, and Oligoasthenospermic by Flow Cytometry: Correlation with Semen Quality | 13.03.08 |

# 9 Distinguished Visitors and Lectures

| Name of the Visitor | Title of the Lecture | Date |
|---|---|---|
| Dr. Anand Kumar<br>All Indian Institute of Medical Sciences<br>New Delhi. | Thyroid Hormone and Male Reproductive Health | 14.03.08 |
| Dr. G. C. Makker<br>Makker Medical Centre<br>Lucknow. | Life Style and Male Infertility | 14.03.08 |
| Dr. A. Nath<br>Mahavir Cancer Sansthan<br>Patna. | Personalised Cell Organelles Injury Due to Endosulfan Causes Male Infertility | 14.03.08 |
| Dr. Geeta Vanage<br>National Institute for Research in Reproductive Health<br>Mumbai. | Exposure of Endocrine Disrupter, Bisphenol in Neonatal Rats: Effect on Reproduction and Steroid Receptor Expression Pattern at the Testicular Level | 14.03.08 |
| Mr. Kaleem Ahmad<br>Chhatrapati Sahuji Maharaj University<br>Lucknow. | Effect of *Mucuna pruriens* on Hormonal Profile and Oxidative Biomarker in Seminal Plasma of Infertile Men | 14.03.08 |
| Dr. S. Shivaji<br>Centre for Cellular and Molecular Biology<br>Hyderabad. | Molecular Basis of Sperm Capacitation: Role of Protein Tyrosine Phosphorylation | 14.03.08 |
| Dr. P. B. Seshagiri<br>Indian Institute of Science<br>Bangalore. | Regulation of Sperm Capacitation: Role of Protein Phosphorylation Signaling | 14.03.08 |
| Dr. S. K. Guha<br>Indian Institute of Technology<br>Kharagpur. | Advances in Vas Deferens Based Male Contraception | 14.03.08 |
| | Transcervical delivered fallopian tube drug implant for reversible female contraception | 11.12.08 |
| Prof. M.K. Chandrashekaran<br>Honorary Professor<br>Jawaharlal Nehru Center for Advanced Scientific Research<br>Bangalore. | Biological Clocks in Bats, Mice and Humans | 19.03.08 |
| Dr. Girish C. Makker<br>Laparoscopic Surgeon<br>Makker Medical Center<br>Lucknow. | Andropause | 26.03.08 |

# 9 Distinguished Visitors and Lectures

| Name of the Visitor | Title of the Lecture | Date |
|---|---|---|
| Prof. Mahadi Hasan<br>Dean and Principal<br>Jawaharlal Nehru Medical College<br>Aligarh. | Nanotoxicology | 25.04.08 |
| Mr. H.E. David M. Malone<br>High Commissioner<br>Canada. | International Economic Cooperation | 09.05.08 |
| Prof. M.K. Mitra<br>Chhatrapati Shahuji Maharaj<br>University<br>Lucknow. | Management of Hypertension in the Elderly | 15.05.08 |
| Prof. Ashok Chandra<br>Chhatrapati Shahuji Maharaj<br>University<br>Lucknow. | Hypertension Plus – A Clinicians Approach | 15.05.08 |
| Dr. Rajavashisth B. Tripathi<br>Professor of Medicine<br>Charles R. Drew University of<br>Medicine & Science<br>Los Angeles CA 90059, USA. | Inflammation and Atherosclerosis:<br>The Role of M-CSF using Mutant Mice. | 13.06.08 |
| Prof. Samir Bhattacharya<br>INSA Senior Scientist<br>Department of Zoology<br>School of Life Sciences<br>Visva Bharti Shantiniketan (West Bengal). | Molecular Mechanism of Insulin Resistance<br>and Type 2 Diabetes | 26.09.08 |
| Dr. Rajan Shah<br>International Consortium on<br>Antivirals<br>Canada. | (i) Introducing ICAV in India<br>(ii) Small Molecule as Drug | 31.10.08 |
| Dr. Sheo Singh<br>Director<br>Merck Research Laboratories<br>Rahway, New Jersy, USA | Novel Approaches for Antibiotic Discovery:<br>Story of Platens and Platencin. | 24.11. 08 |
| Dr. Subhash Pandey<br>Professor & Director<br>Department of Psychiatry<br>University of Illinois, Chicago | Histone Deacetylases as a Therapeutic<br>Targets for Alcoholism | |

| Name of the Visitor | Title of the Lecture | Date |
|---|---|---|
| Dr. S.K. Gupta<br>National Institute of<br>Immunology New Delhi. | Role of Interleukin-6 Group of Cytokines<br>in Trophoblast Invasion and Proliferation | 11.12.08 |
| Prof. Roya Rozati<br>Deccan College of<br>Medical Sciences, Hyderabad. | Molecular and Toxicology Evaluation of<br>Patients with Endometriosis | 11.12.08 |
| Dr. Deepa Bhartiya<br>National Institute for<br>Research in Reproductive Health<br>Mumbai. | Derivation of Embryonic Stem Cell Lines Using<br>Spare Human Embryos and Eggs | 11.12.08 |
| Dr. Deepak Modi and<br>Dr. Geeta Godbole<br>National Institute for Research<br>in Reproductive Health Mumbai. | Homeodomain Gene HOXA10 Regulates Multiple<br>Pathways in Human Endometrial Decidual Cells | 11.12.08 |
| Dr. Geetanjali Sachdeva<br>National Institute for Research<br>in Reproductive Health<br>Mumbai. | Endometrial Receptivity and its Superimposition<br>by Embryonic Stimuli | 11.12.08 |
| Dr. Malini Laloraya<br>Rajiv Gandhi Centre for<br>Biotechnology<br>Trivandrum. | Distinguishing Molecular Signatures During<br>Successful Embryo Implantation | 11.12.08 |
| Prof. Polani B. Seshagiri<br>Indian Institute of Sciences<br>Bangalore. | Molecular Regulation of the Phenomenon<br>of Blastocyst Hatching | 11.12.08 |
| Prof. Suneeta Mittal<br>All India Institute of<br>Medical Sciences, New Delhi. | Endometriosis – Recent Trends | 12.12.08 |
| Dr. Mausumi Ganguly<br>Cotton College<br>Guwahati, Assam. | Evaluation of Antifertility Effects of some<br>Traditionally Used Medicinal Plants | 12.12.08 |
| Dr. Smita D. Mahale<br>National Institute for<br>Research in Reproductive Health<br>Mumbai. | An Update on FSH-FSH Receptor Interaction:<br>Implications in Female Reproductive Health | 12.12.08 |

# 9 Distinguished Visitors and Lectures

| Name of the Visitor | Title of the Lecture | Date |
|---|---|---|
| Dr. S.N. Kabir<br>Indian Institute of Chemical Biology<br>Kolkata. | A Close Look into the Pathogenesis of Premature Ovarian Failure under Experimental Galactosemia | 12.12.08 |
| Prof. Vinita Das<br>CSM Medical University<br>Lucknow. | Endometrial Receptivity in Relation to Female Fertility, Infertility | 12.12.08 |
| Dr. Vijay K. Yadav<br>Division of Genetic and Development<br>Columbia University<br>New York, USA. | Serotonergic Bone Formation | 17.12.08 |
| Dr. Rama Jayasundar<br>Associate Professor<br>Dept. of NMR<br>All India Institute of Medical Sciences<br>New Delhi. | Quantum Logic in Ayurveda | 09.01.09 |
| Dr. Prashant Sharma<br>National Institute of Health<br>Bethesda<br>USA. | Small Ubiquitin Like Modifier (SUMO) Post Translational Modification in Embryonic Development and Cancer | 15.01.09 |

# Membership of Committees/Boards 10

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. T.K. Chakraborty | Member, Americal Chemical Society;<br>Life Member, Chemical Research Society of India;<br>Life Member, Indian Chemical Society;<br>Life Member, Indian Peptide Society;<br>Member, Chemical Sciences Sectional Committee, Indian Academy of Sciences;<br>Member, India – Taiwan Joint S&T Committee;<br>Member, Program Advisory Committee (Organic Chemistry), DST;<br>Member, Chemical Sciences Research Committee, CSIR;<br>Member, Steering Committee, National Bioresource Development Board, DBT;<br>Member, Research Advisory Committee, Indian Association for the cultivation of Science;<br>Member, Council of the National Organic Symposium Trust;<br>Member, Council of the Chemical Research Society of India;<br>Member, Editorial Board, Indian Journal of Chemistry-B and Journal of Chemical Sciences. |
| Dr. Rakesh Tuli | Advisor Consultant, Unichem Laboratories Ltd., Mumbai; Kemwell Laboratories Ltd., Bangalore; Khandelwal Laboratories Ltd.,<br>Mumbai; Gene Craft Ltd., Noida;<br>Member, Research Advisory Committee, Sugarcane Breeding Institute, Coimbatore; Indian Institute of Sugarcane Research, Lucknow; Indian Council of Agricultural Research; Member, Editorial Board, Proc. (Biological Sciences) of the National Academy of Sciences, India;<br>Expert Member, Career Advancement, Paper Setter and Examiner, NET-CSIR, Ph.D. and postgraduate degrees in Biotechnology and Botany in Indian Agricultural Research Institute, Jawahar Lal Nehru University, Banaras Hindu University, Lucknow University, Guwahati University, Punjab University, Meerut University, S.G.P.G.I., Indian Institute of Technology (Kharagpur);<br>Chairman/Member, Assessment/Selection/Screening Committees CSIR, ICAR, ASRB, DBT & DST;<br>Member, Ad-hoc Committees, DST, DBT, DAE, CSIR, ICAR, Indo-French Centre, European Communities, Rajiv Gandhi Institute of Contemporary Studies;<br>Chairman, Advisory Committee/Peer Group of Experts, Central Research Institute of Unani Medicine, Lucknow; Member, Task Force, Agricultural Biotechnology, Bio-pesticide and Crop Management, Improvement of Fiber Crops, Department of Biotechnology;<br>Member, Joint Working Group, Department of Biotechnology, New Delhi; Chairman, Local Advisory Committee, Regional Science Centre, Lucknow;<br>Member, Executive Council, The National Academy of Sciences, Allahabad;<br>Vice President, Executive Committee, Uttar Pradesh Association for Science & Technology Advancement; |

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| | Member, Uttar Pradesh State Biodiversity Board;<br>Member, GEAC, RCGM, MECs, Bureau of Indian Standards (GMOs) etc., Government of India. |
| Dr. A.K. Saxena | Member, Board of International Charitable Foundations, Russia;<br>UGC Nominee, Advisory Committee, Saurastra University, Rajkot and A. P. S University, Rewa; Patent Evaluator, Current Drugs Ltd., U.K.; Secretary, QSAR Society of India;<br>Member, American Chemical Society, USA;<br>Life Member, Indian Chemical Society, Indian Association of Medicinal Chemists, and UP Association for Science & Technology Advancement;<br>Member, Board of Directors, American Bibliography Inc., USA;<br>Reviewer, Journal of Medicinal Chemistry, Bioorganic Medicinal Chemistry, Bioorganic Medicinal Chemistry Letters, International Journal of QSAR;<br>Chairman, Safety Committee, CDRI;<br>Chairman, Security and Sensitivity Committee, CDRI;<br>Convener, CNS-CVS and Related Disorders Project, CDRI;<br>Member, Performance Parameter Reviewer Committee, CDRI;<br>Member, Ph.D. Registration Screening Committee, CDRI;<br>Member, Editorial Board, Medicinal Chemistry Research, SAR and QSAR in Environmental Research, Online International Journals viz. ARKIVOC, ARKAT, The Open Toxicology Journal. |
| Dr. Ranjana Srivastava | Member, Task Force, Biotech Products and Process Development, DBT;<br>Editor, Indian Journal of Microbiology;<br>Member , Microbial Prospecting, National Bio-resource Development Board, DBT;<br>Member, IBSC, IITR, Lucknow;<br>DBT Nominee, IBSC, IITR, Lucknow;<br>DBT Nominee, IBSC, IIT, Kanpur;<br>Chairman, IBSC, CDRI;<br>Member, Doctoral Committee, SGPGI, Lucknow;<br>Member, Editorial Board, Current R&D Highlights. |
| Dr. S.K. Puri | Member, Editorial Board, Journal of Parasitic Diseases;<br>Member, Executive Committee, Indian Society for Parasitology;<br>Member, Steering Committee, DNDi sponsored Pan Asian Network for Drugs for Neglected Diseases from Natural Sources;<br>Member, Institutional Animal Ethics Committee, Indian Animal Suppliers, Lucknow. |

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. Zaka Imam | Member, Editorial Board, International Journal of Health Technology & Management, Inder Science Enterprises Ltd., UK.;<br>Member, Management Council, CDRI. |
| Dr. O.P. Asthana | Member, Panel of Project Reviewers, UPCST;<br>Member, Selection Committee, CDRI, Lucknow;<br>Medical Ethics Committee, CDRI, Lucknow;<br>Member, Medical Ethics Committee, U.P. Biotechpark, Lucknow;<br>Member, Medical Ethics Committee, Era Medical College, Lucknow;<br>Member, Medical Ethics Committee, IITR, Lucknow;<br>Member, Management Council, CDRI;<br>Member, IND Committee, DCG(I), Ministry of HFW, Govt. of India. |
| Dr. Ram Raghubir | Member, National Steering Committee, MoES Project: Drugs from the Sea, New Delhi<br>Chairman, Ocean Drug Alert, CDRI, Lucknow;<br>Member, Discovery Group on Anti-hypertension, CSIR Network Project on Bioactives from Plant Sources;<br>Member, Assessment Committee, IITR, Lucknow;<br>Member, Master's & Doctoral Committee, SGPGIMS, Lucknow, IVRI, Izatnagar, Delhi University, Delhi, Jiwaji University, Gwalior, BITS, Ranchi and NDAUST, Faizabad;<br>Member, Editorial Board, Drugs & Pharmaceuticals, Current R&D Highlights; Member, Editorial Board, Annals of Neurosciences;<br>Member, Animal Ethics Committee, CDRI, Lucknow;<br>Reviewer, Brain Research, J. Neurochemistry & Molecular Brain Research;<br>Reviewer, DST, DBT, ICMR, MoES Research projects;<br>Secretary, Indian Pharmacological Society (Lucknow Branch). |
| Dr. S.B. Katti | Member, Editorial Advisory Board, The Open Natural Products Journal, Bentham Science Publishers. |
| Dr. Gautam Palit | Member, Project Review Committee, DSIR, DST, New Delhi;<br>Member, Fellowship Expert Group Committee, ICMR, New Delhi;<br>Member, Ethics Advisory Committee, CDRI;<br>Member, Institutional Ethics Committee, Vivekananda Polyclinic & Institute of Medical Sciences, Lucknow;<br>Member, Task Force, CSIR Coordinated Program on Bioactive Substances from Plant Sources – Anti-ulcer and Anti-anxiety Activity;<br>Member, Board of Examiners, Jadavpur University, Kolkata., University of Calcutta, Kolkata and Aligarh Muslim University, Aligarh. |

# 10 Membership of Committes/Boards

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. C. Nath | Member, GLP Core Committee, CDRI;<br>Member, Institutional Ethics Committee (Human Research), CDRI;<br>Chairman, Assessment Committee, IITR;<br>Member, Task Force, CSIR project FAC-008. |
| Dr. Shailja Bhattacharya | Member, Scientific Advisory Committee, VCRC, Pondicherry;<br>Member, Academic Council, JNU, Delhi;<br>Honorary Advisor, German Academic Exchange Services (DAAD);<br>Member, Management Council, CDRI;<br>Member, Advisory Board, J. Immunology and Immunopathology. |
| Dr. J. S. Srivastava | Member, Ethics Committee, SGPGI, Lucknow;<br>Member, Ethics Committee, CSMMU, Lucknow;<br>Member Secretary, Medical Ethics Committee, CDRI, Lucknow. |
| Dr. R.K. Sharma | Member, Research Advisory Committee of Govt Homeopathic Medical Colleges |
| Dr. G. K. Jain | Chairman, CDRI Security Committee;<br>Chairman, CDRI Adhoc Committee;<br>Member, Official Side of the Local Council of CSIR for Adoption of Central Civil Services (Recognition of Service Association) Rules, 1993 & for Establishment of Joint Consultative Machinery; Life Member, UP Association for Advancement of Science & Technology;<br>Life Member, Indian Pharmaceutical Association;<br>Member, ISTAG & ISTAD CSIR;<br>Member, Editorial Board, Drugs & Pharmaceuticals, Industry Highlights;<br>Member, Rajbhasha Karyanvayan Samiti, CDRI, Lucknow; Member, Management Committee, SAIF, CDRI, Lucknow;<br>Member, Academic Committee for CDRI-JNU Ph.D. Programme;<br>Course Coordinator, MS (Pharm), Pharmaceutics, NIPER Rai Bareli;<br>Member, Management Committee, NIPER Rai Bareli. |
| Dr. Rajendra Prasad | Life Member, UP Association for Advancement of Science & Technology;<br>Member, Editorial Board, CDRI Annual Report. |
| Dr. A. K. Dwivedi | Member, Drugs Panel for New Drugs Manufacturing Licenses Directorate of Medical & Health Services, U.P.;<br>Life Member, UP Association for Advancement of Science & Technology, Indian Pharmaceutical Association; |

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| | Member, Expert Committee, Dr. B. R. Ambedkar University, Agra;<br>Joint Secretary, Indian Society of Chemists and Biologists, Lucknow. |
| Dr. Madhu Dikshit | Member, Editorial Board, Indian Journal of Pharmacology; Drugs and Pharmaceuticals Industry Highlights, Annals of Neurosciences, Proceedings of the National Academy of Sciences India (Section B: Biological Sciences). |
| Dr. A.K. Goel | Executive Editor, Ocean Drugs Alert Bulletin and CDRI Annual Report. |
| Dr. M. Abbas | Member, Institutional Animal Ethics Committee, CDRI;<br>Member, Editorial Board, Drugs and Pharmaceutical Industry Highlights. |
| Dr. Rakesh Shukla | Member, Expert Committee, Chemical and Pharmaceutical Science, Council of Science & Technology, U.P.;<br>Member, Task Force Committee, Pharmacological Studies of Homeopathic Drugs, Central Council for Research in Homoeopathy, New Delhi;<br>Treasurer, Indian Academy of Neurosciences, Lucknow Branch. |
| Dr. J.K. Saxena | Member, Expert Committee, B. Tech., IIT, Roorkee;<br>Member, Agriculture Research Service Examination Board;<br>Expert, Department of Biochemistry, Lucknow University, Lucknow;<br>Expert, School of Biotechnology, BHU, Varanasi;<br>Member, Expert Committee, IVRI, Izatnagar;<br>Member, Expert Committee, UPCST, Lucknow;<br>Member, Expert Committee Sai Institute of Paramedical Research Institute, Dehradun;<br>Secretary, The Indian Society for Parasitology;<br>Vice President, Society of Biologists and Chemists;<br>Head, Quality Assurance Unit, CDRI;<br>Member, Maintenance and Works Committee, Bio-safety Committee, and Animal House Committee, CDRI. |
| Dr. Naibedya Chattopadhyay | Member, Editorial Board, American Journal of Physiology and Biochemical Pharmacology. |
| Dr. D.C. Kaushal | Member, Editorial Board, Journal of Parasitic Diseases and Current R & D Highlights,<br>Member, Research Degree Committee, Ram Manohar Lohia Avadh University, Faizabad;<br>Member, CDRI Biomedical Safety Committee. |

## 10 Membership of Committes/Boards

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. Vinod Bhakuni | Member, Program Advisory Committee, Life Sciences, DST; Member, NIMITLI Screening Committee, CSIR;<br>Member, Technical Screening Committee, SBIRI, DBT, New Delhi;<br>Member, Department of Biotechnology, PDF Fellowship ;<br>Member, Research Advisory Council, Sriram Institute of Industrial Research, New Delhi;<br>Member, Editorial Board, International Journal of Integrative Biology. |
| Dr. R.P. Tripathi | Excutive Member, ACCTI(I);<br>Member, Editorial Board, ARKIVOC;<br>Associate Editor, Carbohydrate News Letter;<br>Member, Editorial Board, Trends in Carbohydrate Chemistry. |
| Mr. Vinay Tripathi | Member, Editorial Board, Ocean Drugs Alert Bulletin and CDRI Annual Report. |
| Dr. D.S. Upadhyay | Member, CPCSEA Sub-Committee for Rehabilitation of Laboratory Animals;<br>Member, Livestock Feeds, Equipment & System, Sectional Committee, FAD, Bureau of Indian Standards, New Delhi;<br>Member, Veterinary Council of India;<br>Member, Institutional Animal Ethics Committee, IVRI, Izatnagar, CIMAP, Animal Husbandry Department, Uttar Pradesh, Lucknow, CDRI, IITR, Integral University, Lucknow;<br>Member, CSIR Nominee, National Institute of Animal Welfare (AWBI) U.P. Veterinary Council. |
| Dr. Neeraj Sinha | Life Member, Society of Toxicology, India, ISCA, Laboratory Animal Science Association of India, Indian Society of Cell Biology, New Delhi, National Academy of Science, Allahabad, Society of Toxicologists of India, Izatnagar and Indian Science Congress Association, Calcutta;<br>Founder Life Member, Laboratory Animal Science Association of India, Lucknow. |
| Name of Scientist | Membership of Committees/Boards |
| Dr. R.C. Tripathi | Member, Editorial Board, CDRI Annual Report;<br>Member, Research Board of Advisors, American Bibliographical Institute. |
| Dr. P.K. Shukla | Member, CDRI-GLP Core Committee, CDRI, Lucknow;<br>Member, Course Coordination Committee, NIPER, Rae Bareli;<br>Associate Editor, Asian Journal of Biochemistry, Academic Journals Inc., USA;<br>Member, Editorial Board, Research Journal of Biological Sciences, Medwell Online and Journal of Applied Bioscience, India;<br>Member, Dissertation Review Committee, Chatrapati Shahuji Maharaj Medical University, Lucknow. |

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. D. N. Upadhyay | Life Member, Society for Advancement of Electrochemical Science & Technology;<br>Member, Editorial Board, CDRI Annual Report. |
| Dr. N. A. Kaushal | Reviewer, Journal of Experimental Parasitology. |
| Dr. A. K. Srivastava | Life Member, UP Association of Science and Technology and Indian Society of Parasitology. |
| Dr. Jawahar Lal | Life Member, Indian Society of Chemists and Biologists. |
| Mr. S.M. Rajendran | Member, Executive Council Society of Ethnobotanists, NBRI, Lucknow;<br>Member, Editorial Board, Phytotaxonomy, NBRI, Lucknow, Journal Biopesticide, St.Xaviers College, Palayamkottai. |
| Mr. Prem Prakash | Expert Member, Board of Studies, B.Pharm., V.B.S. Purvanchal University, Jaunpur;<br>Life Member, UP Association for Advancement of Science & Technology and Indian Pharmaceutical Association;<br>Member, Editorial Board, CDRI Annual Report. |
| Dr. S.K. Rath | Life Member, ADNAT;<br>Member, Indian Genome Variation Data Base;<br>Member, Board of Examiners for D.Phil. in Biotechnology, Allhabad University;<br>Member, Board of Examiners for Ph.D. in Biotechnology Kanpur University;<br>Member, Board of Examiners for Ph.D. in Zoology, Veer Kunwar Singh University;<br>Member, Assessment Committees, IITR;<br>Member, Agricultural Committee, Organic Farming Awareness Programme, Mahima Research Foundation and Social Welfare, Varanasi;<br>Life Member, Genome Foundation, Indian Society of Cell Biology and Environmental Mutagen Society of India. |
| Dr. Amit Misra | Life Member, Indian Pharmaceutical Association;<br>Member, Controlled Release Society, Indian Chapter;<br>Founder Member, Indian Nanoscience Society;<br>Member, Consultative Committee on Drug Discovery and Delivery. |
| Dr. (Mrs.) Kumkum Srivasatava | Life Member, Society of Biological Chemists, India, Bangalore. |

# 10 Membership of Committes/Boards

| Name of Scientist | Membership of Committees/Boards |
|---|---|
| Dr. R. K. Singh | Life Member, Society of Toxicology, India; Indian Society for the Study of Reproduction and Fertility, Mumbai; International Society of Applied Biology, India; Society for Reproductive Biology and Comparative Endocrinology, Chennai; Laboratory Animal Science Association of India, CDRI, Lucknow; National Academy of Science, Allahabad; International Society for Environmental Protection, Society of Bio Sciences and Society of Embryology, India. |
| Dr. P. R. Mishra | Member, Advisory Board, IIPC, Bilaspur University;<br>Life Member, Indian Pharmaceutical Association;<br>Founder Member, Indian Nano-science Society;<br>Member, Expert Committee, Jamia Hamdard, New Delhi; Member Editorial Board, Journal "Recent Patents in Drug Delivery and Formulations (Bentahm Sciences)". |
| Dr. Amogh Sahasrabuddhe | Member, Management Council, CDRI. |
| Dr. Aamir Nazir | Life Member, Indian Society of Cell Biology;<br>Member, American Society of Genetics. |
| Dr. Akhilesh Tamrakar | Life Member, Society for Biological Chemists. |
| Dr. Dhananjay Hansda | Life Member, Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases;<br>Life Member, West Bengal Veterinary Council;<br>Life Member, Rajayoga Education and Research Foundation, Mt. Abu, Rajasthan. |
| Dr. Anand P.Kulkarni | Associate Editor, Editorial Board, CDRI Annual Report. |
| Dr. Sripathi Rao Kulkarni | Life Member, Association of Microbiologists of India;<br>Member, Editorial Board, CDRI Annual Report. |
| Mr. Naseem Ahmed Siddiqui | Member, All India Management Association, New Delhi. |
| Mr. Janki Prasad | Associate Member, Institution of Engineers (India);<br>Member, Indian Institute of Chemical Engineers, Kolkata. |

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|---|---|
| Dr. Neeraj Sinha | *Germany*, To learn advance work going on in the field of Reproductive Toxicology and also to explore possibilities for joint research programme (14 January - 02 February, 2008). |
| Dr. A.K. Saxena | *China*, To deliver an invited lecture on Drug R&D at CDRI, (01 April, 2008); |
| | *Hong Kong*, To deliver invited lecture on Drug R&D at CDRI in ABCT, The Hong Kong Polytechnic University ( 03-05 April, 2008); |
| | *Italy*, To deliver an invited lecture "Molecular modeling studies on AChE inhibitor carbonates to design and synthesize antialzheimer agents" (02 - 06 July, 2008); |
| | *Itlay*, To attend international conference on "Drug design and discovery for developing countries" (03 - 05 July, 2008). |
| Dr. Atul Kumar | *France*, INSA-France Exchange Programme to learn the techniques of application of organocatalysed synthesis of hetrocycles as bioactive agents (25 February - 24 March, 2008). |
| Dr. Jayanta Sarkar | *Singapore*, To attend high control cellular screening workshop (12 - 14 November, 2008). |
| Dr. Sanjay Batra | *U.K.*, INSA Bilateral Exchange Programme (15 October - 13 November, 2008). |
| Dr. Mohammad Imran Siddiqui | *Italy,* To participate and deliver a presentation in international conference on drug design and discovery for developing countries (03 - 05 July, 2008); Italy, To learn the state of the art in the field of computer aided molecular designing and establish collaboration on molecular modeling (20 September - 19 November, 2008). |
| Dr. (Mrs.) Vinita Chaturvedi | *USA*, To participate in training programme on controlling laboratory biorisks (10 November - 16 November, 2008). |
| Dr. (Mrs.) Kalpana Murthy | *Switzerland*, To attend the meeting on screeners and medicinal chemistry network meeting (27 - 29 August, 2008). |
| Dr. Neeloo Singh | *Tehran*, To attend international workshop on Leishmaniasis (01 - 07 March, 2008). |
| Dr. Sudhir Kumar Sinha | *USA,* To attend Workshop on high content screening system (04-07 November, 2008). |
| Dr. Kalyan Mitra | *USA,* INDO-US Research Fellowship (01 July, 2008 - 30 June, 2009). |
| Dr. (Mrs.) Saman Habib | *Philippines*, To attend a conference on 7th HUGO Pacific Meeting and the 8th Asia Pacific Human Genetics (02 - 05 April, 2008). |
| Dr. K.V. Sashidhara | *USA*, BOYSCAST Fellowship (27 March, 2008 - 26 March, 2009). |
| Dr. Anil Gaikwad | *Germany*, BOYSCAST Fellowship (31 March, 2008 - 30 March, 2009). |

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|---|---|
| Dr. Prabhat Ranjan Misra | *Germany*, Bilateral Exchange Programme of INSA (01 July - 30 September, 2008) |
| Dr. Atul Goel | *Germany*, Humbolt Fellowship (01 July, 2008 - 31 March, 2009). |
| Dr. S.K. Puri | *Japan*, To attend meeting of DNDi Pan Asian screening network for new drugs for neglected diseases for natural substances (09 - 11 June, 2008). |
| Mr. Prem Prakash Yadav | *Germany*, DAAD Fellowship (09 June 2008 - 30 September, 2009). |
| Dr. N. Chattopadhyay | *Korea*, To deliver a talk in Korean Endocrine Society (16 - 17 May, 2008). |
| Dr. Shailja Bhattacharya | *Switzerland*, To participate in the Screeners and medicinal chemistry networking meeting (27 - 29 August, 2008). |
| Dr. Vinod Bhakuni | *Hong Kong*, To attend 6th Asian Biophysics Association Symposium (11 - 14 January, 2009) |

# Honours and Awards 12

| Name of Scientist | Honours and Awards |
|---|---|
| CDRI, Lucknow | CSIR Technology Award (2008) for innovation and discovery of guggulsterones and development of analogs with novel mechanism of action, (26 September, 2008). |
| Dr. S.B. Katti | Award for Most Cited Paper 2005-08. [Bioorganic & Medicinal Chemistry, Elsevier Ltd. UK], (18 August, 2008). |
| Dr. G. Palit | CDRI Incentive Award – 2008; |
| | Certificate of Appreciation Award for the paper "Gastroprotective effect of *Tectonia grandis*: Possible involvement of H+ K+ ATPase inhibition" (presented at International Conference on Advances in Neurosciences, Cochin). |
| Dr. S.K. Puri | Best Poster Award for the paper "Characterization of a 27 kDa protein from rodent malaria parasite *Plasmodium vinckei* to elucidate its role in arteether resistance" (presented at 20th National Congress of Parasitology, Shillong). |
| Dr. Ram Pratap | CDRI Incentive Award for Great Britain Patent "Process for preparing guggulsterones" (2007). |
| Dr. Anuradha Dube | Best Poster Award for the paper "Expression and purification of Calreticulin from *Leishmania donovani* clinical isolate" (presented at 20th National Congress of Parasitology, Shillong). |
| Dr. G.K. Jain | Appointed Course Coordinator, MS (Pharm.), Pharmaceutics, NIPER Rai Bareli. |
| Dr. Rakesh Shukla & R. Niranjan | Best Paper Award for the paper "Antiproliferative and anti-inflammatory effect of Gugulipid on LPS stimulated human astrocytoma cell line, U373MG" (presented at International Conference on Advances in Neurosciences, Cochin). |
| Dr. J.K. Saxena | Zoological Society of India Award, 2008. |
| Dr. (Mrs.) P.K. Murthy | Best Poster Award in 'Biotechnology & Bio-therapeutics' (for paper presented in 60$^{th}$ Indian Pharmaceutical Congress, New Delhi, December 12-14, 2008). |
| Dr. R.P. Tripathi | Second Best Poster Presentation (Euro-India First International Conference on Holistic Medicine, 2008). |
| Dr. Atul Kumar | OPPI-2008 Award (2008). |
| Dr. (Mrs.) Vinita Chaturvedi | Immunology Foundation Prize [2007] (given by Immunology Foundation of India during 25th Conference of Indian Association of Leprologists at Kanpur held on November 19- 21, 2007). |
| Dr. Saman Habib | Prof. B.K. Bachhawat Memorial Award in Young Scientist Lecture by National Academy of Sciences, India, 2008. |
| Dr. S.K. Rath | Genomic Pioneer Award (Ocimum Biosolutions, 2008, 13 International Human Genome Meeting at Hyderabad in September, 2008). |

# 12 Honours and Awards

| Name of Scientist | Honours and Awards |
|---|---|
| Dr. Atul Goel | Alexander von Humboldt Fellowship by Federal Republic of Germany, AvH Stiftung, Bonn, Germany in 2008; |
| | CDRI Incentive Award for one of the best papers (2008). |
| Dr. Kashif Hanif | Certificate of Appreciation Award for the paper "Perinopril improves memory decline in rat: Involvement of angiotensin converting enzyme in memory deficit induced by ICV streptozotocin" (presented at International Conference on Advances in Neurosciences, Cochin). |
| Dr. P.R. Mishra | INSA-DFG Fellowship (under Bilateral Exchange Programme to carry out research in Germany); |
| | Best Paper Award (for the paper "Surface modified ultrathin polyelectrolyte nanoreservior for delivery of proteins: Evaluation in terms of controlled release and biocompatibility" at International Conference on Nanomaterial Toxicology held at IITR, Lucknow). |
| Mr. Alok Ranjan Singh | M. B. Mirza Award, 2008 (by The Indian Society for Parasitology during 20th National Congress of Parasitology at NEHU, Shillong, November 3-5, 2008). |
| Ms. Shweta Joshi | Young Scientist Award, 2008 (20th National Congress of Parasitology held at NEHU, Shillong, November 3-5, 2008). |
| Mr. Vishal Ranjan | P.C. Dandiya Prize (awarded by Indian Pharmaceutical Society held at AIIMS, New Delhi, December 18-20, 2008). |
| Mr. Surendra S. Bisht | ACCT (I) Young Scientist Award by Association of Carbohydrate Chemists & Technologists (India) |

## 2008-2009 (Sanctioned Estimates)

| HEADS | | (Rs. in Lakhs) |
|---|---|---|
| *(a) Recurring* | | |
| Pay & Allowances | | 1762.500 |
| Contingencies | | 190.000 |
| HRD | | 4.000 |
| Maintenance | | 140.000 |
| Staff Quarter Maintenance | | 12.000 |
| Chemicals & Consumables | | 350.000 |
| | Sub-Total | 2458.500 |
| *(b) Capital* | | |
| Works & Services | | 59.000 |
| Equipments and Office Equipments | | 605.000 |
| Furniture and Fittings | | 4.000 |
| Library Books & Journals | | 207.000 |
| Staff Quarters | | 27.000 |
| | Sub-Total | 902.00 |
| *(c) SIP/NWP/IAP/FAC/CMM/SMM/COR Projects* | | 3167.327 |
| | Grand Total | 6527.827 |

## 2007-2008 (Actual Expenditure)

| HEADS | Against CSIR Grant (Rs. in Lakhs) | Against L.R.F. (Rs. in Lakhs) |
|---|---|---|
| (a) Recurring | | |
| Pay & Allowances | 1787.176 | |
| Contingencies | 203.882 | |
| HRD | 2.890 | |
| Maintenance | 153.722 | |
| Staff Quarter Maintenance | 10.976 | |
| Chemicals & Consumables | 500.195 | |
| Sub-Total | 2658.841 | |
| (b) Capital | | |
| Works & Services | 25.320 | 21.499 |
| Equipments and Office Equipments | 917.218 | 81.496 |
| Furniture and Fittings | 5.646 | 0.105 |
| Library Books & Journals | 217.545 | |
| Model & Exhibits | 0.884 | |
| Staff Quarters (Construction) | 17.524 | 0.048 |
| Infrastructure, Renovation & Refurbishing (ICT) | 41.184 | 8.378 |
| Infrastructure, Renovation & Refurbishing (Construction) | -5.084 | 5.084 |
| Sub-Total | 1220.237 | 116.610 |
| (c) SIP/NWP/IAP/FAC/CMM/SMM/COR Projects | 5814.887 | |
| Grand Total | 9693.965 | 116.610 |

# Research Council

(April 2007 - March 2010)

*Chairman*

Prof. N.K. Ganguly
Former Director-General ICMR
National Institute of Immunology
Aruna Asaf Ali Marg
New Delhi - 110 067.

*Members*

Dr. A. Surolia
Director
National Institute of Immunology
Aruna Asaf Ali Marg
New Delhi - 110 067.

Dr. T.P. Singh
Professor and Head
Department of Biophysics
All India Institute of Medical Sciences
Ansari Nagar
New Delhi 110 029.

Dr. Y.K. Gupta
Professor and Head
Department of Pharmacology
All India Institute of Medical Sciences
Ansari Nagar
New Delhi 110 029.

Dr. M.D. Nair
Formerly Vice President, SPIC Pharmaceuticals
A-11, Sagarika, No. 15, 3rd Seaward Road
Valmiki Nagar, Thiruvanmiyur
Chennai - 600 041.

Prof. K. Muniyappa
Professor & Chairman
Head, Department of Biochemistry
Indian Institute of Science
Bangalore 560 012.

Dr. Surinder Singh
Drug Controller General (India)
Directorate General of Health Sciences
Ministry of Health and Family Welfare
Nirman Bhawan
New Delhi 110 011.

Dr. K.P. Mohankumar
Scientist F
Head, Division of Cell biology
Indian Institute of Chemical Biology
4, Raja S.C. Mullick Road, Jadavpur
Kolkatta 700 032.

Dr. Rakesh Tuli
Director
National Botanical Research Institute
Rana Pratap Marg
Lucknow 226 001

Dr. T.K. Chakraborty
Director
Central Drug Research Institute
Lucknow 226 001.

Dr. Naresh Kumar
Head
R&D Planning Division
Council of Scientific & Industrial Research
Rafi Marg
New Delhi - 110 001.

*Secretary*

Dr. S.B. Katti
Scientist G
Central Drug Research Institute
Lucknow - 226 001.

# Management Council

(July 2007 - June 2009)

*Chairman*

Dr. T.K. Chakraborty
Director
Central Drug Research Institute
Lucknow 226 001.

*Members*

Dr. Rakesh Tuli
Director
National Botanical Research Institute
Lucknow.

Dr. Zaka Imam
Scientist G
Central Drug Research Institute
Lucknow - 226001

Dr. O.P. Asthana
Scientist G
Central Drug Research Institute
Lucknow - 226001

Dr. (Smt.) Shailja Bhattacharya
Scientist G
Central Drug Research Institute
Lucknow - 226001

Dr. (Smt.) Saman Habib
Scientist EII
Central Drug Research Institute
Lucknow - 226001

Dr. Amogh Sahasrabuddhe
Scientist C
Central Drug Research Institute
Lucknow - 226001

Sh. R.K. Srivastava
Technical Officer Gr. III (4)
Central Drug Research Institute
Lucknow - 226001

Controller of Finance & Accounts
Central Drug Research Institute
Lucknow - 226001

*Member Secretary*
Controller of Administration
Central Drug Research Institute
Lucknow - 226001

# The Staff

*Director*
Dr. Tushar Kanti Chakraborty, M.Sc. (IIT, Kanpur), Ph.D. (IIT, Kanpur), FNA, FASc, FNASc, J.C. Bose Fellow
Dr. Rakesh Tuli, M.Sc. (Pantnagar), Ph.D. (Gujarat) (01/08/2007 to 18/12/2008)

## R & D DIVISIONS/UNITS

### BIOCHEMISTRY

*Scientists Group IV (5)*
J.K. Saxena, M.Sc. (Lucknow), Ph.D. (Kanpur), *In-Charge*
Uma Roy, M.Sc., Ph.D. (Kanpur)
Gitika Bhatia, M.Sc., Ph.D. (Agra)

*Scientists Group IV (4)*
A.K. Srivastava, M.Sc. (Lucknow), Ph.D. (Kanpur)
Neena Goyal, M.Sc. (Lucknow), Ph.D. (Agra)

*Scientist Group IV (3)*
Anju Puri, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Scientist Group IV (1)*
A.K. Tamrakar, M.Sc., Ph.D. (Jiwaji)

*Technical Officer Group III (7)*
M.M. Khan, M.Sc., Ph.D. (Kanpur) [Retired on 31/08/2008]

*Technical Officer Group III (6)*
A.K. Khanna, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Technical Officer Group III (4)*
B. Maity, M.Sc. (Kanpur), Ph.D. (Rohilkhand)

*Technical Assistants Group III (1)*
Rima Ray Sarkar
Ishbal Ahmad

*Group II (4)*
Suresh Yadav

*Group II (3)*
B.R. Yadav
Ram Pal Rawat

*Group I (4)*
Ramesh Chandra
Noor Jehan

### BOTANY

*Scientist Group IV (5)*
R.K. Sharma, M.Sc., Ph.D. (Agra), *In-Charge*

*Scientist Group IV (4)*
M.N. Srivastava, M.Sc. (Kanpur), Ph.D. (Lucknow)

*Scientist Group IV (3)*
S.M. Rajendran, M.Sc. (Madurai Kamaraj), Ph.D. (Lucknow)

*Scientists Group IV (2)*
K.R. Arya, M.Sc. (Kumaon), Ph.D. (Kanpur)
D.K. Mishra, M.Sc. (Vidyasagar), Ph.D. (Pune)

*Technical Assistant Group III (1)*
Savita Tripathi

*Group II (4)*
J.K. Joshi

*Group I (4)*
Jeewan Ram
K. K. Yadav
Devi Dutt
Maiku Lal Lodh
Makhan Lal
Gopi
Satya Narain

*Group I (2)*
R.C. Maurya

*Group I (1)*
Lakhana Devi
N.K. Khanduri

*Sr. Steno (ACP)*
Gehani J. [Retired on 30/04/2008]

## CLINICAL & EXPERIMENTAL MEDICINE

*Scientist Group IV (6)*
O.P. Asthana, M.B.B.S., D.C.H., M.D. (Lucknow), FNASc. [Retired on 31.01.2009]

*Scientists Group IV (5)*
S.P.S. Gaur, M.B.B.S., M.D. (Lucknow), *In-Charge*
J.S. Srivastava, M.B.B.S., M.D. (Lucknow), D.M. (PGIMER), M.H.Sc. (Toronto)
A. Ghatak, M.B.B.S., M.D. (Lucknow), FICP

*Technical Officer Group III (7)*
A.K. Nigam, M.Sc. (Kanpur)

*Technical Assistant Group III (1)*
Shail Singh, M.Sc. (Jabalpur)

*Group II (4)*
J.R. Gupta
H.S. Dubey
Kishori Lal

*Group I (3)*
Umesh Kumar

## DRUG TARGET DISCOVERY AND DEVELOPMENT

*Scientist Group IV (5)*
Sudhir K. Sinha, M.Sc. (Lucknow), Ph.D. (Kanpur), *In-Charge*

*Scientists Group IV (4)*
Neeloo Singh, M.Sc. (Lucknow), Ph.D. (Kanpur)
Vinita Chaturvedi, M.Sc., Ph.D. (Agra)

*Scientist Group IV (3)*
Sabyasachi Sanyal, M.Sc. (Viswabharati), Ph.D. (CNU, South Korea)

*Scientists Group IV (2)*
Anil N. Gaikwad, M.S. (Pharm.) (NIPER, Chandigarh), Ph.D. (JNU)
Arun Kumar Trivedi, M.Sc. (Varanasi), Ph.D. (Ludwik Maximillians)

*Scientists Group IV (1)*
Y.K. Manju, M.Sc. (Calicut), Ph.D. (Thiruvananthapuram) [on EOL from 16/07/2007]
Jayant Sarkar, M.V.Sc., Ph.D. (IVRI)

*Technical Officer Group III (5)*
S.L. Verma, B.Sc.

*Technical Assistants Group III (1)*
Ajay Singh Verma, M.Sc. (Aligarh)
Shyam Singh, M.Sc. (Agra)
Sanjeev Meena, M.Sc. (Rajasthan)

*Group II (4)*
Chandramool
B.P. Yadav [Retired on 31/12/2008]

*Group II (3)*
Lal Hori

## ENDOCRINOLOGY

*Scientists Group IV (5)*
Naibedya Chattopadhyay, M.Sc. (Calcutta), Ph.D. (SGPGIMS), *In-Charge*
Archana Srivastav, M.Sc., Ph.D. (Lucknow)

*Scientists Group IV (4)*
Govind Keshri, M.Sc. (Lucknow), Ph.D. (Agra) [Retired on 30/06/2008]
Anila Dwivedi, M.Sc. (Lucknow), Ph.D. (Kanpur)
Gopal Gupta, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Scientists Group IV (3)*
F.W. Bansode, M.Sc. (Nagpur), Ph.D. (Udaipur)
Durga Prasad Mishra, M.Sc. (Karnal), Ph.D. (Delhi)

*Scientists Group IV (2)*
Divya Singh, M.Sc. (Lucknow), Ph.D. (JNU)
Rajender Singh, M.Sc. (Amritsar), Ph.D. (JNU)

*Scientists Group IV (1)*
Ritu Trivedi, M.Sc. (Lucknow), Ph.D. (SGPGIMS)
Hemant Kumar Bid, M.Sc. (Avadh), Ph.D. (Kanpur)
Konwar Rituraj, M.V.Sc., Ph.D. (IVRI)

*Technical Officer Group III (6)*
J.P. Maikhuri, M.Sc. (Garhwal), Ph.D. (Jamia Hamdard)

*Technical Officers Group III (5)*
P.K. Dasgupta, B.Sc. [Retired on 30/11/2008]
Mohini Chhabra, B.Sc., CLSc.

*Technical Officers Group III (4)*
Shakti Kitchlu, M.Sc. (Kanpur)
Balvir Singh, M.Sc. (Rohilkhand)

*Technical Assistants Group III (1)*
Lakshma Nayak V.
Preeti

*Group II (4)*
A.P. Dev
T. Firdaus
Kanak Lata
P.C. Patni [Retired on 30/06/2008]

*Group II (3)*
P.K. Bhattacharya
Chattar Pal
Geet Kumar Nagar

*Group I (4)*
Prakash [Retired on 30/09/2008]
N.P. Misra
B.P. Mirsa
R.G. Pandey

*Group I (2)*
Mahesh Chandra Tewari

*Group I (1)*
Nabbulal Kori
Ram Karan
Jagdish Prasad

## FERMENTATION TECHNOLOGY

*Scientist Group IV (5)*
C.K.M. Tripathi, M.Sc., Ph.D. (BHU), *In-Charge*

*Scientist Group IV (4)*
P.K. Shukla, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Technical Officer Group III (7)*
A.K. Joshi, M.Sc. (Kumaon)

*Technical Officers Group III (5)*
Shyamendra Mehrotra, B.Sc.
Bikram Banerjee, B.Sc.
M.K. Srivastava, M.Sc. (Sagar)
Malkhan Singh, B.Sc.
Agney Lal, B.Sc.

*Group II (4)*
A.K. Pandey
Kishan Singh

*Group II (3)*
O.P. Gupta

*Group I (4)*
Lakshmi Prasad
A.N. Dixit

*Private Secretary*
H.K. Khulve

## MEDICINAL AND PROCESS CHEMISTRY DIVISION

*Scientists Group IV (6)*
A.K. Saxena, M.Sc., Ph.D. (Meerut), *In-Charge*
D.P. Sahu, M.E. Chem. Engg. (S.I.T., USA), Ph.D. (IIT, Kharagpur)
S.B. Katti, M. Pharm., Ph.D. (Mysore)

*Scientists Group IV (5)*
Kanwal Raj, M.Sc., Ph.D. (Lucknow) [Retired on 31/12/2008]
Bijoy Kundu, M.Sc., Ph.D. (Kanpur)
Ram Pratap, M.Sc., Ph.D. (BHU)
K.C. Agarwal, M.Sc., Ph.D. (Lucknow)
S.N. Suryawanshi, M.Sc., Ph.D. (Pune)
Kamlakar Avasthi, M.Sc., Ph.D. (Lucknow)
Rakesh Maurya, M.Sc., Ph.D. (Varanasi)
Kalpana Bhandari, M.Sc., Ph.D. (Lucknow)
R.P. Tripathi, M.Sc. (Gorakhpur), M. Phil, Ph.D. (Delhi)
Vijay Lakshmi, M.Sc., Ph.D. (Allahabad)
Kanchan Hajela, M.Sc., Ph.D. (Lucknow)

*Scientists Group IV (4)*
W. Haq, M.Sc., Ph.D. (Lucknow)
Y.S. Prabhakar, M.Sc. (Vishakhapatnam), Ph.D. (Pilani)
Arun K. Shaw, M.Sc., Ph.D. (Calcutta)
P.M.S. Chauhan, M.Sc., Ph.D. (Agra)
V.L. Sharma, M.Sc., Ph.D. (Lucknow)
Pradeep Kumar, M.Sc. (Kanpur)
Atul Kumar, M.Sc., Ph.D. (Lucknow)

*Scientists Group IV (3)*
Sanjay Batra, M.Sc., Ph.D. (Meerut)
Anup K. Misra, M.Sc. (Calcutta), Ph.D. (Jadavpur) [Resigned on 01/10/2008]
Atul Goel, M.Sc., Ph.D. (Lucknow)
Gautam Panda, M.Sc. (IIT, Khargpur), Ph.D. (Hyderabad)

*Scientists Group IV (2)*
T.G. Narender, M.Sc., Ph.D. (Kakatiya)
Sashidhara K.V., M.Sc. (MS Univ.), Ph.D. (Avadh)

*Scientist Group IV (1)*
Prem Prakash Yadav, M.Sc. (Allahabad), Ph.D. (Avadh)

*Technical Officer Group III (7)*
R.K. Asthana, M.Sc. (Agra)

*Technical Officers Group III (6)*
S.P. Vishnoi, M.Sc., Ph.D. (Meerut)
A.K. Mandwal, M.Sc., Ph.D. (Avadh)
S.C. Tripathi, B.Sc.
Janki Prasad, M. Tech. (BHU)
Keshav Prasad, AMIE, M. Tech. (BHU)

*Technical Officers Group III (5)*
Suresh Chandra, B.Sc., L.L.B.
S.P.S. Bhandari, M.Sc. Ph.D. (Avadh)
P.N. Rai, Dip. Mech. Engg.
S.K. Kakaji, B.Sc.
Vasi Ahmed, B.Sc.
Zahid Ali, B.Sc., L.LB.
Tara Rawat, B.Sc.
Deepali Pandey, B.Sc.

*Technical Officer Group III (4)*
A.S. Kushwaha, B.Sc.

*Technical Assistant Group III (2)*
Ashok Kumar Sharma, B.Sc., D.Ch.E., A.M.I.E.

*Technical Assistants Group III (1)*
Atma Prakash Dwivedi
Vidisha Sharma
K. S. Anil Kumar
Tahseen Akhtar
Surya Pratap Singh

*Group II (4)*
Preeti Rastogi
Ahmad Zaheer (Glass Blowing)
Radha Rani Gupta
Raju Arora
Ram Sant [Retired on 31/12/2008]
Ramjeet

*Group II (3)*
V.K. Maurya
A.K. Srivastava
Shashi Rastogi
Mithilesh Sharma
Tika Ram
Veena Mehrotra
Kumar Rajesh
Rajesh Kumar
Shukla K M
Akhilesh Kumar Srivastava
D.N. Vishwakarma
Manju

*Group II (2)*
Ram Lakhan

*Group II (1)*
H.R. Misra
N.P. Misra
Krishna Kumar

*Group I (4)*
Ram Sanehi
M.S. Bhol
G.S. Sonkar
J.C. Rajan

*Group I (2)*
Satish Chandra

*Sr. Steno*
Renuka Mushran
*Sr. Steno (H)*
Avadhesh Kumar

## MICROBIOLOGY

*Scientist Group IV (6)*
Ranjana Srivastava, M.Sc., Ph.D. (Kanpur), *In-Charge*
*Scientists Group IV (4)*
D.C. Kaushal, M.Sc. (Pantnagar), Ph.D. (Kanpur)
K.K. Srivastava, M.Sc., Ph.D. (Kanpur)

*Scientist Group IV (3)*
B.N. Singh, M.Sc., Ph.D. (BHU)

*Scientist Group IV (1)*
Sudhir Kumar Singh, M.Sc., M.Tech., Ph.D. (Purvanchal)

*Technical Officers Group III (7)*
A.P. Singh, M.Sc. (Lucknow)
M.N. Joshi, M.Sc., Ph.D. (Agra)

*Technical Officer Group III (5)*
Reeta Singh, M.Sc., Ph.D. (Kanpur)

*Technical Assistant Group III (1)*
Sandeep Kumar Sharma, M.Sc. (Barkatullah)

*Group II (3)*
P.D. Misra
Nuzhat Kamal
D.K. Tripathi, M.Sc. (Avadh)

*Group I (4)*
U.C. Pandey
J.C. Pant

*Group I (1)*
Ravi Shankar Misra
Ram Prakash
Shyam Sunder Yadav

## MOLECULAR & STRUCTURAL BIOLOGY

*Scientists Group IV (5)*
Vinod Bhakuni, M.Sc., Ph.D. (Lucknow), FNA, FASc, FNASc, *In-Charge*
P.R. Maulik, M.Sc., Ph.D. (Calcutta)

*Scientists Group IV (4)*
Saman Habib, M.Sc. (Delhi), Ph.D. (NII, Delhi)
Ravishankar, R., M.Sc., Ph.D. (IISC, Bangalore)

*Scientists Group IV (3)*
Ashish Arora, M.Sc. (Jaipur), Ph.D. (Chandigarh)
Jimut Kanti Ghosh, M.Sc., Ph.D. (Kalyani)
J. Venkatesh Pratap, M.Sc., Ph.D. (IISc, Bangalore)

*Scientists Group IV (2)*
Mohammad Imran Siddiqi, M.Sc., Ph.D. (AIIMS)
Amogh Anant Sahasrabuddhe, M.Sc. (Kanpur), Ph.D. (JNU)
Shakil Ahmed, M.Sc. (Aligarh), Ph.D. (Punjab)

*Scientist Group IV (1)*
Mohammad Sohail Akhtar, M.Sc. (Calicut), Ph.D. (JNU) [on EOL from 16/07/2007]

*Technical Officers Group III (4)*
R.K. Srivastava, B.Sc.
J.P. Srivastava, B.Sc., LL.B.

*Technical Assistants Group III (1)*
Ruchir Kant, M.Sc. (Lucknow)
Anupam Jain, M.Sc. (Agra)
Sarita Tripathi, M.Sc. (Lucknow)

*Group II (3)*
Ram Radhey Shyam

## PARASITOLOGY

*Scientists Group IV (6)*
S.K. Puri, M.Sc., Ph.D. (Punjab), *In-Charge*
Shailja Bhattacharya, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Scientists Group IV (5)*
P.K. Murthy, M.Sc. (Lucknow), Ph.D. (Kanpur)
Anuradha Dube, M.Sc. (Lucknow), Ph.D. (Kanpur)
Suman Gupta, M.Sc. (Lucknow), Ph.D. (Kanpur)
*Scientist Group IV (4)*
Renu Tripathi, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Scientists Group IV (3)*
N.A. Kaushal, M.Sc. (Lucknow), Ph.D. (Kanpur)
Kumkum Srivastava, M.Sc. (Lucknow), Ph.D. (Kanpur)
S. Rajakumar, M.Sc. (Madras)

*Technical Officer Group III (7)*
S.C. Nigam, M.Sc., Ph.D. (Kanpur)

*Technical Officers Group III (5)*
A.K. Roy, M.Sc. (Kanpur)
R.N. Lal, M.Sc. (Agra)

*Group II (4)*
V.K. Bose
R.S. Dubey
Ram Dayal
Ravi Kumar Mehra
K.K. Singh

*Group I (4)*
Saheb Prasad

*Group I (1)*
Prem Babu

*Sr. Steno (ACP)*
T.S. Sashi Kumar

## PHARMACEUTICS

*Scientist Group IV (5)*
A.K. Dwivedi, M.Sc., Ph.D. (Agra), *In-Charge*
*Scientist Group IV (3)*
Amit Misra, M. Pharm. (Delhi), Ph.D. (JNU)

*Scientists Group IV (2)*
Prabhat Ranjan Mishra, M.Pharm., Ph.D. (Sagar)
Manish Kumar Chourasia, M.Pharm., Ph.D. (Sagar) [On EOL from 27/08/2007 to 26/08/09]
Akhilesh Kumar Jain, M.Pharm. (Sagar), Ph. D. (Jamia Hamdard) [Resigned on 14/10/2008]

*Technical Officer Group III (6)*
Madhuri Chaudhry, M.Sc. (Lucknow)

*Group II (3)*
Bhatnagar S. K.

*Group I (4)*
Ghanshyam

*Group I (1)*
Ram Kumar

## PHARMACOKINETICS & METABOLISM

*Scientist Group IV (5)*
G.K. Jain, M.Sc. (Rewa), Ph.D. (Kanpur), *In-Charge*

*Scientists Group IV (4)*
S.K. Singh, M.Sc. (Patna), Ph.D. (IIT, Kanpur)
Jawahar Lal, M. Pharm., Ph.D. (BHU)

*Scientists Group IV (1)*
R.S. Bhatta, M. Pharm. (Nagpur)
Wahajuddin, M.S. Pharm. (NIPER)
R.S.P. Singh, M. Pharm. (BITS Pilani) [Resigned on 22/08/2008]

*Technical Officer Group III (6)*
S.K. Pandey, M.Sc. (Kanpur)

*Group II (3)*
Narendra Kumar

*Group II (1)*
Akhilesh Kumar
*Group I (4)*
Shiv Lal

*Helpers Group I (1)*
Ram Bhajan Shukla
Ram Sunder Lal
Chandramani

*Sr. Steno*
Nandita Pandey

## PHARMACOLOGY

*Scientists Group IV (6)*
Ram Raghubir, M.V.Sc., Ph.D. (Agra), *In-Charge*
G. Palit, M.B.B.S., M.D. (Lucknow), (*Unit In-charge, Neuropharmacology Unit*)
*Scientists Group IV (5)*
Madhu Dikshit, M.Sc., Ph.D. (Kanpur) *(Unit In-charge, Cardiovascular Pharmacology Unit)*
Rakesh Shukla, M.Sc., Ph.D. (Lucknow)

*Scientist Group IV (4)*
M. Ray, M.Sc., Ph.D. (Lucknow) [Retired on 31/07/2008]

*Scientists Group IV (3)*
Amar Nath, M.Sc. (Lucknow)
K.G. Raghu, M.Sc. (Calicut), Ph.D. (Saurashtra) [Transferred to NIIST on 11/04/2008]

*Scientist Group IV (2)*
Manoj K. Barthwal, M.Sc., Ph.D. (Lucknow)

*Scientist Group IV (1)*
Kashif Hanif, M.Sc. (Hamdard), Ph.D. (Delhi)

*Technical Officer Group III (7)*
G.P. Singh, M.Sc. (Kanpur)

*Technical Officers Group III (6)*
Kanta Bhutani, M.Sc. (Kanpur) [Retired on 30/04/2008]
M.S. Ansari, B.Sc. [Resigned on 30/09/2008]
*Technical Officers Group III (5)*
S. Sengupta, B.Sc.
T.L. Seth, B.Sc.
Jharna Arun, B.Sc.
M.L. Bhatnagar, B.Sc.
V.S. Nigam, B.Sc.
C.P. Pandey, M.Sc. (Chandigarh)

*Technical Assistants Group III (1)*
Sultana Jawaid, B.Sc.
Sheeba Saji Samuel, M.Sc. (M.G. Univ.)
Sachi Bharti, M.Sc. (Kanpur)

*Group II (4)*
O.P. Pandey, B.A.

*Group II (3)*
Bharti Bhushan, B.Sc.
H.C. Verma, B.A.
Shailendra Mohan, M.Sc. (Kanpur)
Ramesh Chandra, M.Sc. (Kanpur)

*Group II (2)*
Anil Kumar Verma, B.Sc.

*Group II (1)*
Surendra Singh, M.Sc., Ph.D. (Kanpur)

*Group I (1)*
Pankaj Sengupta

*Jr. Steno*
Varun Kumar Pathak

TOXICOLOGY
*Scientists Group IV (6)*
C. Nath, M.B.B.S., M.D. (Lucknow) , In-charge

*Scientists Group IV (4)*
Neeraj Sinha, M.Sc., Ph.D., D.Sc. (Kanpur)
Sharad Sharma, M.B.B.S., M.D. (Kanpur)

*Scientist Group IV (3)*
S.K. Rath, M.Sc. (Utkal), Ph.D. (BHU)
*Scientists Group IV (2)*
R.K. Singh, M.Sc., Ph.D., D.Sc. (Lucknow)
R.K. Tripathi, M.Sc., Ph.D. (Kanpur)
Aamir Nazir, M.Sc., Ph.D. (Jamia Hamdard)

*Scientists Group IV (1)*
Smrati Bhadauria, M.Sc., Ph.D. (Jiwaji)
Sarika Singh, M.Sc., Ph.D. (Lucknow)
Poonam Singh, M.Sc., Ph.D. (Kanpur)

*Technical Officer Group III (7)*
S.K. Srivastava, M.Sc. (Bombay) [Retired on 31/07/2008]

*Technical Officers Group III (5)*
S.M. Verma, B.Sc.
Sadan Kumar, M.Sc. (Bihar)
P.K. Agnihotri, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Technical Assistants Group III (1)*
Neeti Sagar, M.Sc. (Lucknow)
Anurag Kumar Srivastava, B.Sc.

*Group II (3)*
Anupma

*Group I (4)*
Mahabir
V.K. Samant
Shree Krishan
R.K. Sarkar

*Group I (1)*
Ram Kumar
Nand Lal Yadav
Ganesh Prasad

CLINICAL PHARMACOLOGY UNIT (CDRI), SETH G.S. MEDICAL COLLEGE, MUMBAI
*Technical Assistant Group III (1)*
N.A. Rajwade

*Technical Assistant Group II (4)*
P.S. Acharya
*Group II (3)*
Vijal J. Ashar, M.Sc.

*Group I (4)*
R.B. Pawar

## TECHNICAL INFRASTRUCTURE DIVISIONS / UNITS

### BIOMETRY AND STATISTICS

*Scientist Group IV (5)*
M. Abbas, M.Sc. (IIT, Kanpur), Ph.D. (IIT, Bombay), *In-Charge*

*Technical Officer Group III (6)*
Mukesh Srivastava, M.Sc. (Lucknow), Ph.D. (Kanpur)

*Group II (4)*
Negi M.P.S.
*Group I (2)*
Savitri Devi

### CSIR DISPENSARY

*Medical Officers Group III (7)*
D.K. Bhateja, M.B.B.S., M.D. *In-Charge,*
K.K. Arora, M.B.B.S., M.D., [Voluntary retirement on 10/04/2008]

*Medical Officer Group III (6)*
Asha Negi, M.B.B.S., M.D.

*Medical Officer Group III (4)*
N.K. Srivastava, M.B.B.S., M.D.

*Group II (4)*
Nandita Dhar
H.U. Khan
Subramaniam M. [Retired on 31/05/2008]

*Jr. Steno*
*Ajay Kumar*

*Group I (4)*
S.K. Paswan

*Gp-'C' Cdr-D*
Sundari

### DOCUMENTATION & LIBRARY

*Scientists Group IV (5)*
Sheela Tandon, M.Sc., Ph.D. (Agra), *B.L.I.Sc. (IGNOU), In-Charge*
P.K. Roy, M.Sc., Ph.D. (Gauhati), [Retired on 31/05/2008]
A.K. Srivastava, B. Tech. (Bangalore)
Shyamala Saxena, M.Sc. (Tirupati), B.L.Sc. (Lucknow)
S.K. Mallik, M.A. (JNU), B.L.I.Sc. (IGNOU)

*Scientist Group IV (4)*
N.N. Mehrotra, M.Sc. (Pantnagar), Ph.D. (AIIMS) [On deputation from 17/04/2007]

*Technical Officer Group III (7)*
Seema Mehrotra, M.Sc. (Lucknow)

*Technical Officers Group III (6)*
J.A. Zaidi, M.Sc. (Aligarh), M.L.I.Sc. (IGNOU)
Sanjay Kumar, M.L.I.Sc (IGNOU)
V.K. Vohra, B.Sc.

*Technical Officers Group III (5)*
W.F. Rahman, M.A. (Rohailkhand), M.L.I.Sc. (Alagappa)
A.K. Verma, M.A. (Kanpur), L.L.B. (Lucknow)

*Technical Assistant Group III (2)*
Ramesh Chandra Gupta, M.L.I.Sc. (Lucknow)
*Group II (4)*
B. K. Sethi
*Group II (3)*
Nazir Akbar
Y.C. Pandey

*Group I (4)*
Mohd Moen
Rasheed Ahmad
S. Islam

*Group I (1)*
Deepayan

*Asst. (G) GR. I*
M.K. Thapar

### DRAWING AND PHOTOMICROGRAPHY

*Technical Officer Group III (7)*
Ali Kausar, B.F.A. (Lucknow), *In-Charge*

*Technical Officer Group III (6)*
G.C. Gupta, B.Sc.

*Technical Officer Group III (5)*
R.M. Pathak, B.F.A. (Lucknow)

*Technical Officer Group III (4)*
R.N.S. Londhe, GD Art (Comm.), Art Teachers Dip.

*Group I (3)*
Basanti Mukherjee

### INSTRUMENTATION

*Scientist Group IV (5)*
Ravinder Singh, B.E. (Allahabad)

*Scientist Group IV (4)*
N.K. Agarwal, M.Sc. (Calcutta)

*Technical Officer Group III (7)*
Usha Kapil, I.Sc., Dip Electronic Engg.
*Technical Assistant Group III (2)*
Sanjay Kumar
*Group II (4)*
Kamal Singh

*Group II (3)*
Laxmi Narain

## DIVISION OF LABORATORY ANIMALS

*Scientists Group IV (4)*
D.S. Upadhyay, M.V.Sc. (Pantnagar), Ph.D. (Izatnagar), *In-Charge*
A.K. Srivastava, M.Sc., Ph.D. (Lucknow)

*Scientist Group IV (2)*
Dhananjoy Hansda, M.V.Sc. (IVRI)

*Scientist Group IV (1)*
Dr. P. Nagarajan, B.V.Sc., M.Sc. (Manipal) [Resigned on 13/06/2008]

*Technical Officers Group III (5)*
S.N.A. Rizvi, M.Sc. (Lucknow)
A.K. Bhargava, B.Sc.
Karunesh Rai, M.Sc. (Lucknow)

*Technical Assistants Group II (4)*
Baldev Singh
A.K. Dubey

*Technical Assistants Group II (3)*
Ravinder Singh
Ram Avatar
S.R. Yadav
Deep Mala Misra
Ravi Kumar Shukla
Sanjeev Kumar Saxena

*Group II (2)*
Narendra Kumar
Dinesh Kumar
Pradeep Tirkey

*Group II (1)*
*Arun Sharma, B.Sc.*

*Group I (4)*
Babu [Expired on 15/06/2008]
Ahrar
Asharfi Lal
Singh Vikram
M.H. Khan [Retired on 31/07/2008]
Wazahtullah
Gaffar Ali
Hari Lal
M.D. Kushwaha
V.B.L. Srivastava
T. B. Thapa
P.B. Thapa
Shiv Pal Singh
O.P. Verma
S.K. Varma
Mohd. Saleem
G.K. Sharma
Dilip Kumar
R.P. Maurya
Singh Bhim

*Group I (1)*
Changa Lal
Jameel Beg

*Sr. Steno (H)*
Raj Kumar

## SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY

*Scientist Group IV (6)*
D.K. Dikshit, M.Sc., Ph.D. (Lucknow), *In-charge*

*Scientist Group IV (5)*
Raja Roy, M.Sc. (Lucknow), Ph.D. (Meerut), FNASc, [On deputation from 16/04/2007]

*Scientist Group IV (4)*
Brijesh Kumar, M.Sc., Ph.D. (Awadh)

*Scientists Group IV (1)*
Sanjeev Kanojiya, M.Sc. (Jabalpur)
Sanjeev Kumar Shukla, M.Sc., Ph.D. (Kanpur)

*Technical Officer Group III (7)*
Prakash Narain, M.Sc. (Lucknow)
*Technical Officers Group III (6)*
H.M. Gauniyal, M.Sc. (Garhwal)
A.L. Vishwakarma, M.Sc. (Kanpur)
Rakesh Khanna, B.Sc., A.I.C. (Calcutta)
A.K. Sinha, M.Sc. (Kanpur)

*Technical Officers Group III (5)*
A. Vohra, B.Sc., M.A. (Lucknow)
A.K. Sircar, B.Sc., B.A. (Lucknow)
Sunil Kumar, B.Sc. (Lucknow)
Pramod Kumar, M.Sc. (Bundelkhand)

*Technical Officer Group III (4)*
R.K. Purushottam, B.Sc. (Lucknow)

*Technical Assistant Group III (1)*
Binod Kumar Saw, M.Sc. (Hazaribag)

*Group II (4)*
R. K. Varma
Sandeep Sengupta, B.Sc.
Ashok Pandey, B.Sc.

*Group II (3)*
Madhu Chaturvedi
Abdul Haleem
Radhey Krishna, B.Sc., L.T., C.Lib.Sc.
Vashundhara Madhwar, B.A.
S.A. Singh, B.Sc.
*Group I (4)*
S.K. Manjhi [Retired on 31/08/2008]

*Group I (1)*
Mansoor Ali
J.S. Singh

*Asst. (G) Grade I*
V.K. Kanal

## TECHNICAL INFORMATION, INDUSTRIAL LIAISON & PLANNING

*Scientist Group IV (6)*
Zaka Imam, M.Sc., M.Phil., Ph.D. (Aligarh), *In-Charge*

*Scientist Group IV (5)*
A.K. Goel, M.Sc., Ph.D. (Lucknow)
*Scientists Group IV (4)*
Vinay Tripathi, M.Sc., M.B.A. (AMU), P.G. Dip. in S & T (Pilani)
R.C. Tripathi, M.Sc. (Kanpur), Ph.D. (Lucknow) [Retired on 31/12/2008]
N.S. Rana, M.Sc. (Kumoun)
D.N. Upadhyay, M.Sc., Ph.D. (Gorakhpur)

*Scientist Group IV (3)*
Prem Prakash, M. Pharm. (BHU)

*Scientists Group IV (1)*
Anand P. Kulkarni, M.Sc. (Karnatak), Ph.D. (Mysore)
Sripathi Rao S. Kulkarni, M.Sc. (SRTMU, Nanded), Ph.D. (JNTU, Hyderabad), P.G. Dip. in Patents Law (NALSAR, Hyderabad)

*Technical Officer Group III (7)*
Shri Ram, B.Sc., LL.B.

*Group II (4)*
Krishna Prasad, B.Sc.

*Group II (3)*
Chandrika Singh, B.Sc., LL.B.

*Group I (4)*
V.P. Srivastava [Retired on 29/02/2008]
Madho Singh
Kamlesh

*Sr. Steno (ACP)*
Manoshi Chatterjee, B.A., B.Lib.I.Sc.

*Sr. Steno (H)*
Jitendra Patel

## ACADEMIC AFFAIRS UNIT

*Scientist Group IV (5)*
Alka Singh, M.Sc., Ph.D. (Rajasthan)

*Scientist Group IV (4)*
Sheela Ghoshal, M.Sc. (Burdwan), Ph.D. (Kanpur)

## BUSINESS MANAGEMENT UNIT

*Scientist Group IV (5)*
Rajendra Prasad, M.Sc., Ph.D. (Lucknow), *Unit In-Charge*

*Scientist Group IV (1)*
Naseem Ahmed Siddiqui, M.B.A. (Rohilkhand)

## ELECTRON MICROSCOPY UNIT

*Scientist Group IV (5)*
V.K. Bajpai, M.Sc., Ph.D. (Kanpur), *Unit In-Charge*

*Scientist Group IV (1)*
Kalyan Mitra, M.Sc. (Culcutta) [on deputation to USA]

*Technical Officer Group III (6)*
Abha Arya, B.Sc., B.Ed. (Kumaun)

*Technical Assistants Group III (1)*
Kavita Singh, M.Sc., Ph.D. (Lucknow)
Manish Singh, M.Sc. (Allahabad)

*Group II (3)*
Madhuli Srivastava

## INFORMATION TECHNOLOGY UNIT

*Scientist Group IV (4)*
Kural, BE (BIT, MESRA), *Unit In-Charge*
*Technical Assistant Group III (2)*
Ajay Kumar Maurya, MCA (Purvanchal)

## TISSUE AND CELL CULTURE UNIT

*Scientist Group IV (5)*
A.K. Balapure, M.Sc., Ph.D. (Lucknow), *Unit In-Charge*

*Technical Officer Group III (5)*
Ramesh Sharma, M.Sc., Ph.D. (Kanpur)

## LABORATORY ENGINEERING SERVICES

*Senior Superintending Engineer Group III (7)*
Parvez Mahmood, B.Sc. Engineering (Civil)

*Executive Engineers Group III (5)*
Manoj Kumar, B.Sc. Engineering (Civil)
Kamal Jain, B.E. (Electrical), MBA (Marketing)

*Technical Officer Group III (4)*
A. Dayal, Diploma (Mechanical)

*Technical Officers Group III (3)*
Mohit Kumar Shukla
Jai Prakash
Sidho Hembrom

*Technical Assistant Group III (2)*
D.K. Vishwakarma

*Group II (4)*
Khan Abdul Jabbar
Sayeed Mohammad
A.K. Tewari
B.P. Sunwar
S.R. Shukla
E.A. Bhatti
Om Prakash
K.K. Kaul
A.K. Sonkar
S.K. Biswas
V.K. Mishra
Radhey Lal
Mahindra Singh
Radhey Shyam
*Group II (3)*
Ramakant Ram
M.S. Verma
S.K. Kar
Naseem Mohammad
Harish Kumar
Vijay Kumar
Pradhan Basudev
Verma Kamal Kishore
Ramesh Kunwar
G.C. Roy
Arun Kumar Srivastava
Swapan Karmi
S.S. Bhakuni
Ram Karan Ram
Rajesh Chand Dwivedi
R.C. Samanta

*Group II (1)*
Bhagwan Singh Pokhariya

*Group I (4)*
A.N. Rabbani
Popinder Singh
Ramanuj
Ram Anjore
Hussain Taqui
Kandhai Lal
S.K. Bhattacharya
Munna Lal
T.P. Pathak
S.K. Yadav
A.K. Misra
Lallu
R. K. Yadav
Raju
Mahabir Prasad
N.K. Mudgal
Shiv Giri
Bishan Singh
Om Prakash
Rama
Iftikhar Ahmad
Ganeshi Prasad
Garibe
Ram Lal
Shankar Roy
Tan Sen
Om Prakash
Phool Chand

*Group I (3)*
Z.U. Beg
Ramesh Chandra

*Group I (2)*
Tara Chand
*Group I (1)*
Dhirendra Misra
Mohd. Irfan
Ram Autar
Raju Vishwakarma
Hari Om Garg
Sandeep Roy
Ram Samujh
Darshan Lal

*Asstt. (G) Grade I*
N.K. Checker
A.G. Khan [Retired on 31/12/2008]

ADMINISTRATION
*Controller of Administration*
B.D. Vashisth, M.A. (Kurukshetra)

*Administrative Officer*
L.R. Arya

COA OFFICE
*Private Secretary*
G.M. Dayal
Sumit Srivastava

*Jr. Steno*
Kamla Kandpal

*Group I (4)*
Maiku Lal
Sohan Lal

DIRECTOR's OFFICE
*Private Secretary*
Kanhaiya Lal

*Sr. Steno (ACP)*
Sunita Chopra

*Group I (1)*
Nand Kishore

*Group D*
Ramswarth Prasad Rai

ESTABLISHMENT I
*Section Officer (G)*
Sunil Kumar

*Asstt. (G) Grade I*
Sachin Mehrotra
Krishna Raj Singh
B. K. Shukla

*Asstt. (G) Grade II*
Smriti Srivastava
Saju P. Nair
Reena Bisaria

*Jr. Steno (H)*
Mohd. Sufiyan

*Group I (4)*
Vinod Kumar

*Gp-'C' Cdr-D*
Manju Yadav

ESTABLISHMENT II

*Section Officer (G)*
*Biranchi Sarang*
Ramesh Singh

*Asstt. (G) Grade I*
B. K. Pillai
Rashmi Srivastava
*Sr. Steno*
Vinod Kumar Yadav

*Asstt. (G) Grade II*
Aparna Bajpai
Dilip Kumr Sen
Lata Bhatia
Rani
Neena Raizada
Madan Chandra

*Asstt. (G) Grade III*
Mohd. Irfan

*Group I (4)*
Shanti Devi

*Group D*
Ram Kumar

GENERAL SECTION

*Asstt. (G) Grade I (ACP)*
Masood Sahab

*Asstt. (G) Grade I*
Birendra Singh
Kailash Chandra

*Sr. Steno*
Seema Rani Srivastava

*Asstt. (G) Grade II*
Gangadin Yadav
Rajendra Prasad
Ajay Shukla

*Asstt. (G) Grade III*
Shakuntala Singh

*Technical Assistant Group II (2)*
K.K. Kashyap
Shakeel Ahmad Khan

*Drivers*
Chote Lal
Prem Chand
Daya Shankar Singh

*Group I (4)*
Kishori Kumari
Mohd Islam

*Group D*
Kalpanath Sharma

*Gp-'C' Cdr-D*
Munna

BILL SECTION

*Section Officer (G)*
Madhuranjan Pandey

*Asstt. (G) Grade I*
H.K. Jauhar
Vatlsala G. Nair
Hem Chandra
Rama Dhawan
Harsh Bahadur
Vivek Bajpai
Dilip Kumar (Cash)

*Asstt. (G) Grade II*
Naseem Imam

*Group I (2)*
Vinod Kumar Sharma

*Group I (1)*
Lalji Prasad

## VIGILANCE

*Asstt. (G) Grade I*
C.P. Nawani
Chandra Kant Kaushik

*Asstt. (G) Grade II*
Tez Singh

*Sr. Steno*
P.S. Padmini

*Group I (3)*
Bhagwanti Devi

## RECORDS

*Asstt. (G) Grade II*
S.K. Pandey
*Group I (4)*
Ved Prakash Misra

## HINDI SECTION

*Senior Hindi Officer*
V.N. Tiwari, M.A., Ph.D. (BHU)

*Senior Translator (Hindi)*
Mrs. Neelam Srivastava, M.A., L.L.B. (Lucknow)

*Sr. Steno (Hindi)*
Anil Kumar

*Group D*
Mohd. Saleem

## FINANCE & ACCOUNTS

*Controller of Finance & Accounts*
Padam Singh

*Finance & Accounts Officer*
A.K. Dwivedi

*Section Officers (F&A)*
I.B. Dixit
A.K. Chauhan
Ankeshwar Misra
Kailash Singh

*Private Secretary*
V.P. Singh

*Asstt. (F&A) Grade I*
R.P. Tripathi
S.L. Gupta
Nitu Kumari
Viresh
Mahesh Babu
R.C. Bisht
Ajitha Nair

*Asstt. (F&A) Grade II (ACP)*
Sashidharan Radha
U.K. Tewari

*Asstt. (F&A) Grade II*
D.K. Khare
Mahendra Kumar
Sanjay Kumar
Tahseen Talat

*Asstt. (F&A) Grade III*
S.A. Siddiqui
Chandrashekhar

*Jr. Steno*
*Rekha Tripathi*

*Group I (1)*
Vikramaditya
Angad Prasad

*Group D*
Mohd. Firoz

STORES & PURCHASE

*Stores & Purchase Officer*
Thomas T. Kuriakose

*Section Officers (Stores & Purchase)*
Shekhar Sarcar
Prafful Kumar
Prasenjeet Mitra
*Asstt. (S&P) Grade I*
P.S. Chauhan
G.C. Dwivedi [Retired on 31/12/2008]
Arun Wadhera
A.K. Misra
A.K. Govil
*Asstt. (S&P) Grade II (ACP)*
K.K. Mishra
*Asstt. (S&P) Grade II*
H.B. Neolia
R.C. Dwivedi
M.C. Verma

*Asstt. (S&P) Grade III*
Srikant Mishra
Kanchan Bala
Vandana Parwani
G.P. Tripathi
Shail Tewari

*Sr. Steno (ACP)*
K.P. Balani

*Group I (4)*
Kishan Kumar
Rama Shukla

*Attendant*
Hardwari

SECURITY

*Senior Security Officer*
R.S. Deswal, B.Sc., LL.B.

*Security Guard Group D*
Chakrasen Singh

CDRI CANTEEN

*Manager*
J.P. Satti

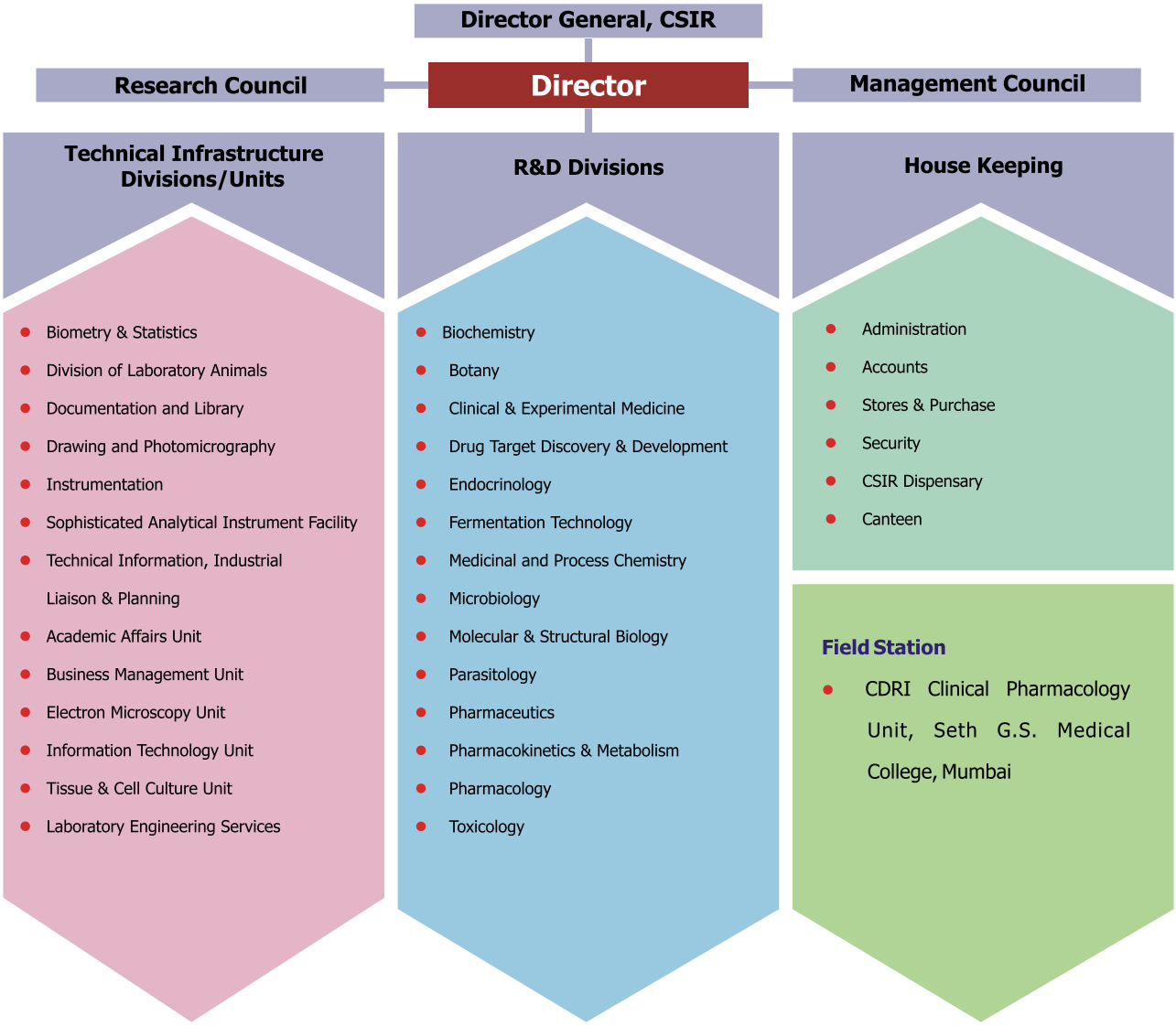
*Asstt. Manager*
R.S. Tewari
*Count Clerk (ACP)*
Ram Jiyawan Tewari
Y.K. Singh

*Cook (ACP)*
Man Bahadur
*Asstt. Halwai*
Uma Shanker Tewari

*Bearer*
Dil Bahadur
Ganga Ram Yadav
Rajender
Kripa Shanker
Sukhdev Prasad
*S/Man*
Raj Kumar

*Wash Boy*
Ram Murat
Dinesh Pal Singh

# ORGANISATIONAL STRUCTURE



# वार्षिक प्रतिवेदन

# ANNUAL REPORT

## 2008-09