



Haffkine Institute

For Training Research and Testing

An Autonomous Institute of Govt. of Maharashtra Registered under Societies Registration Act 1860
Recognised as "Scientific & Industrial Research Organisation" (SIRO) by Govt. Of India, Ministry of Science & Technology.



ANNUAL REPORT

2015-16

ANNUAL REPORT

2015-2016



**Haffkine Institute for Training, Research & Testing,
Acharya Donde Marg,
Parel, Mumbai 400 012.**

An ISO 9001:2008 Certified Organization

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Director's Desk

Greetings from Haffkine Institute !!

I am happy to present the significant achievements and activities of the Haffkine Institute for Training, Research & Testing for the year 2015-2016.



Haffkine Institute is one of the oldest biomedical research institutes in the country and over the years, it has evolved as a center for multidisciplinary research with public health at its focus.

I am confident that the staff of Haffkine Institute will be ready to face new challenges in the next year and that their whole hearted participation in the Institute's research, training & testing activities will help in achieving the Institute's goal to become one of the finest research Institutes in the country and in the world.

Mrs Seema Vyas (IAS)

Governing Council of Haffkine Institute for Training, Research & Testing

1.	Shri. Vinod Tawde. Hon. Minister, Medical Education, Government of Maharashtra, Mantralaya, Mumbai – 400 032.	President
2.	Hon. Minister of State, Medical Education, Government of Maharashtra, Mantralaya, Mumbai – 400 032.	Vice – President
3.	Smt. Medha Gadgil, IAS Addl.Chief Secretary, Medical Education & Drugs Department, Government of Maharashtra, Mantralaya, Mumbai – 400 032.	Member
4.	Dr. Harshadeep Kamble, IAS Commissioner, Food & Drugs Administration, Bandra-Kurla Complex, Bandra (E), Mumbai – 400 051.	Member
5.	Dr. Pravin Shingare, Director, Medical Education & Research, Government of Maharashtra, St. George's Hospital Campus, Mumbai – 400 001.	Member
6.	Dr. Jagdish Prasad, Directorate General of Health Services, Government of India, Room No. 444, 'A' Wing, Nirman Bhawan, New Delhi – 110108.	Member
7.	Smt. Seema Vyas, IAS Director, Haffkine Institute for Training, Research & Testing, Parel, Mumbai – 400 012.	Member – Secretary

Executive Council of Haffkine Institute for Training, Research & Testing

1.	Smt. Seema Vyas, IAS Director, Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Chairperson
2.	Dr. Shashikant P. Vaidya Asst. Director, Department of Clinical Pathology Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member
3.	Dr. Nishigandha Naik Asst. Director, Department of Biochemistry and Toxicology, Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member
4.	Dr. Shweta Kothari Senior Scientific Officer, Department of Virology Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member
5.	Dr. Mrunal Ghag-Sawant Scientific Officer, Venomous Animal Unit, Zoonosis Dept. Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member
6.	Shri. Sandeepan Mukharjee Scientific Officer, Department of Virology Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member
7.	President, Shramik Utkarsha Sabha (Workers' Union Representative)	Member
8.	Shri. Dinesh. B. Malekar Chief Administrative Officer Haffkine Institute for Training, Research & Testing, Parel, Mumbai-12	Member Secretary

Advisory Council for Research of Haffkine Institute for Training, Research & Testing

1.	Padma Bhushan Dr. R.D. Lele	Chairperson
2.	Dr. (Mrs). Mrududla A. Phadke Consultant, WHO, Vaccine committee	Member
3.	Dr. Pravin H. Shingare Director, Medical Education & Research	Member
4.	Dr. Mohan Jadhav Director, Health Services	Member
5.	Vd. Kuldeep Raj Kohli Director, Ayush	Member
6.	Dr. Shubhada V Chiplunkar Director, ACTREC.	Member
7.	Dr. (Mrs.) Nilima Khsirsagar Dean, ESI-PGIMSR, M.G.M. Hospital	Member
8.	Dr. H. S. Palep	Member
9.	Mrs. Seema Vyas IAS Director, Haffkine Institute for Training, Research & Testing.	Member
10.	Dr. S.P. Vaidya, Asst. Director, Haffkine Institute for Training, Research & Testing.	Convener

The Council met on 5th Feb 2016 to review the projects in the various departments

Academic Council of Haffkine Institute for Training, Research & Testing

1.	Dr. (Mrs). Mrududla A. Phadke Consultant, WHO, Vaccine committee	Chairperson
2.	Dr. Pravin H. Shingare Director, Medical Education & Research	Member
3.	Dr. A. S. Khemnar Director, Institute of Science	Member
4.	Dr. G. D. Yadav Director, Institute of Chemical Technology	Member
5.	Padmahsree Dr. T.P. Lahane Dean, Grant Government Medical College & Sir J.J. Group of Hospitals	Member
6.	Dr. (Mrs.) Nilima Khsirsagar Dean, ESI-PGIMSR, M.G.M. Hospital	Member
7.	Dr. Avinash Supe Dean, Seth G.S. Medical College & K.E.M. Hospital	Member
8.	Dr. A. M. Paturkar Dean, Bombay Veterinary College	Member
9.	Mrs. Seema Vyas IAS Director, Haffkine Institute for Training, Research & Testing	Member
10.	Dr. S.P. Vaidya, Asst. Director, Haffkine Institute for Training, Research & Testing	Convener

The RAC met on

Administration

Chief Administrative Officer

As per the Memorandum of Association, the Minister, Ministry of Medical Education, Government of Maharashtra is the ex-officio President of the Governing Council. The Governing Council meets once/twice a year and gives directions for the smooth functioning and progress of the Institute. The Governing Council also consists of other members who are ex-officio members of Government of Maharashtra and a representative from the Health department, Government of India. The Governing Council is considered to be the think-tank of the Institute.

The last Governing Council meeting was held on 28th November 2015. It was recommended that a project proposal be submitted to Indian council of Medical Research / Department of Biotechnology to secure funding for the Institute.

Apart from this supreme body of the Institute, the Executive Committee (Administration) meets regularly to execute the decisions of Governing Council and to take decisions on local issues. All decisions with financial implications are discussed in the Executive Committee. Said decisions are then ratified by the Governing Council, if deemed fit.

Dinesh. B. Malekar

Chief Administrative Officer

Research

Department of Virology

Head of Department's Overview

Year 2015-16 for research at Virology has been the year of RNA viruses. In the years gone by, we have tried to understand the pathogenesis, immune responses and interventions for Rabies virus. We have undertaken proteomic profiling and host response study using cytokines, as well as studied the utility of alternative medicine (herbal) formulations for this understanding. We have set up an in-vitro model for understanding the processes of primary and secondary Dengue infections and have tried to evaluate the potential of single and super infections in breaching the blood brain barrier and signalling towards cell death. Influenza has been one of the most important targets for the year 2015-16. We have analysed 1500 plus samples for the detection of pdm H1N1 2009 Influenza A virus under the National Program for Laboratory Surveillance for Influenza like Illness. We have initiated studies on the epidemiology, isolate characterization and novel neuraminidase inhibitors for intervention of the Influenza virus. We have also studied the role of interferon induced proteins as an indicator for host response.

The departmental training program entitled Hands on Training in Techniques in Virology and Immunology was well received with the enrolment of 32 UG/PG students. The staff of the Department also took up training programs to align with both technical and administrative work processes in the Institute.

Testing activities included testing of post-mortem and ante-mortem samples for detection of Rabies virus (antigen) from matrices like corneal smear, nuchal biopsy, Cerebrospinal fluid and brain tissue. We also offered contract research services to premier companies for evaluation of their products for virucidal activity as per EN 14476 (European Standard)

Presently, the department is aiming at completing two extramurally funded projects one each from the Department of Biotechnology (DBT) and Department of Science & Technology (DST), both in the domains of neurobiology. We have also initiated work on the collaborative project funded by the ICMR, which involves drug designing and in-vitro activity analyses for intervention of pandemic influenza virus.

In the years to come, the Department of Virology plans at having three major sections under its umbrella, viz. Conventional Research Unit (CRU), Molecular Systems Unit (MSU) and Diagnostics Unit (DU) catering in both Virology as well as Immunology. Under MSU, we aim at

using novel methods like NGS/WGS, Flow Cytometry and Microarrays to answer our queries on pathogenesis and host response. This unit would be the Applied Research arm of the Department. The CRU would undertake gold standard methods like viral propagation and isolation, enzyme assays and basic Immunology. This unit would be the training-in-research and contractual service provider front of the Department. Most importantly, the DU would be providing with super specialized testing facilities, e.g., immune disorders, untypable infections, unusual matrices, etc. that would require a research bend for interpretation.

Dr. Sweta T Kothari

Research projects

1. Laboratory surveillance of Influenza like Illness and severe acute respiratory illness (ILISARI) in India

Technical team

1. Ms. Devanshi Gohil,
2. Dr. Sweta T Kothari,
3. Mr. Pavankumar Todkar,
4. Dr. Abhay Chowdhary.

Period

Apr 2015 - Mar 2016

Introduction

Influenza viruses are highly contagious, causing acute respiratory illness and afflicting humans since ancient times. World Health Organization (WHO) declared first pandemic of the 21st century caused by Influenza A (H1N1) pdm 09 virus. Department of Virology at Haffkine Institute has been identified as a network laboratory for the national project on surveillance of Influenza like illness under the NCDC-IDSP. Understanding the prevalence and epidemiology of influenza virus infections will not only help in control of the disease as well as aid in the development of effective vaccines.

Under this program, we receive nasopharyngeal/throat swabs from hospitals in and around Mumbai, Thane, and Raigad region. These swabs are subjected for the extraction of viral RNA and the positivity is detected using qRT-PCR.

Objectives

1. To provide epidemiology and seasonality data of influenza virus.
2. To understand the groups at high risk of severe outcomes, including hospitalization and deaths.

Results and Conclusions

The inflow of samples for detection of pdm H1N1 2009 Influenza A virus was higher in 2015-16 as compared to earlier years.

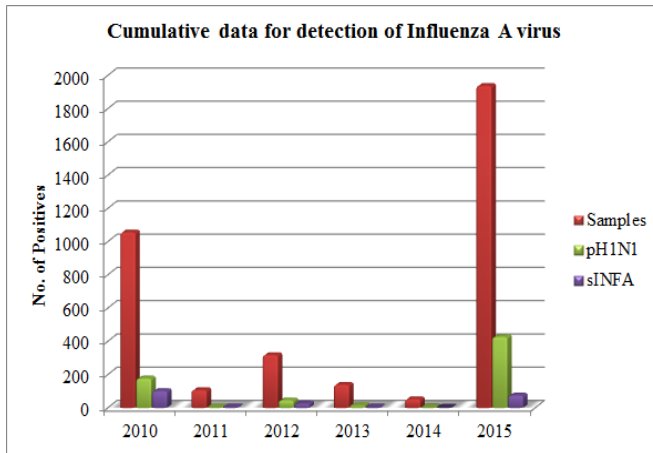


Figure 1: Sample inflow and positivity for pdm H1N1 2009 influenza A from 2010 to 2015.

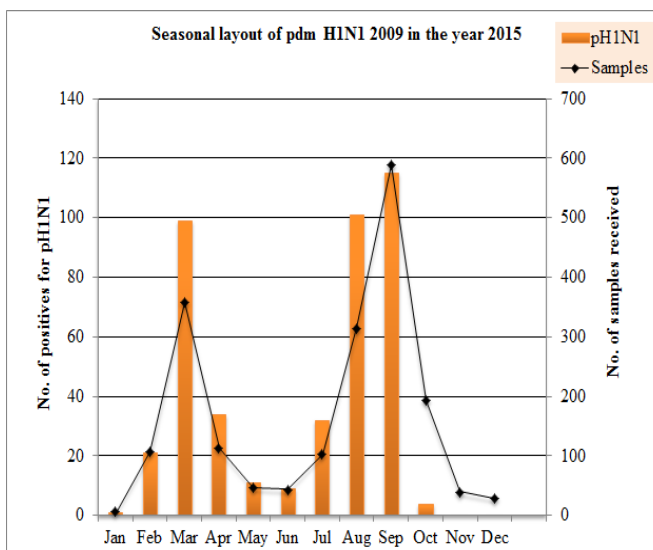


Figure 2: Seasonal layout of pdm H1N1 2009 Influenza A in the year 2015.

From April 2015 till March 2016, we received 1586 nasopharyngeal/throat swabs, of which almost 70% samples were received in the post monsoon period July-September 2015. A total of 330 samples were detected positive for the virus, giving a seasonal positivity of 20.8%.

2. Molecular characterization of the circulating 2014-2016 strains of H1N1 viruses for genomic mutations conferring resistance to neuraminidase inhibitors (NA) and adamantanes (M2) and virulence (with respect to HA gene)

Technical team

1. Dr. Sweta T Kothari,
2. Surg. Cdr. (Dr.) Kavita Anand,
3. Ms. Devanshi Gohil,
4. Dr. Abhay Chowdhary

Period

Oct 2015 - Oct 2017

Introduction

It has been observed that antiviral resistance in influenza not only develops during treatment but also sometimes replaces susceptible strains in the absence of drug pressure. Various mutations have also been implicated towards resistance to oseltamivir and/ or zanamavir. The transmission is exemplified by the global spread adamantane-resistant pandemic A (H1N1) viruses in 2009. Samples being evaluated for the present study are the isolates from the Influenza season of 2014-15-16. This phylogenetic evaluation is an off-shoot of the ongoing National ILI Surveillance project

The availability of PCR has made it possible to detect viral nucleic acid sequences and thereby made rapid diagnosis of influenza possible. However due to the uncertainty of variations in viral genes namely HA and NA have made it difficult to monitor and diagnose new emerging variable H1N1 infections. This has given rise to a huge lag between the ongoing prevention and outbreak control measures. Molecular characterization of influenza strains is therefore the need of the hour to develop pre- as well as post-prophylaxis measures to curb the spread of the infection and contain the resistance.

Objectives

1. To carry out molecular characterization of HA and NA gene of H1N1 Pandemic 2009
2. To study the drug susceptibility pattern of influenza H1N1
3. To study phylogenetic relationship of pandemic H1N1 2009 strains of Mumbai with the strain circulating worldwide.

Results and Conclusions

Around 40 pdm 2009 H1N1 Influenza A positive samples of the 2014-2015 season and 300 pdm 2009 H1N1 Influenza A positive samples of the 2015-2016 season respectively have been inoculated on MDCK cells and have completed one round of passaging, these are being passaged in the second round to obtain isolates. They will be now titrated using Hemagglutination assay or quantified using an H1N1 Influenza virus quantification kit.

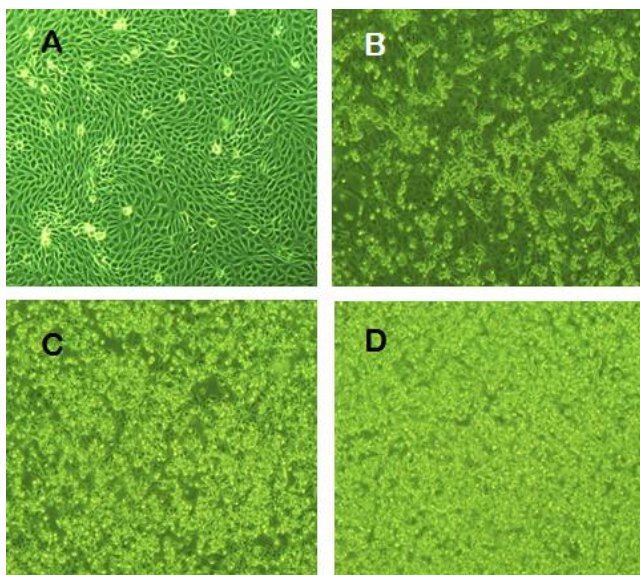


Figure 1: MDCK cells infected with pdm H1N1 2009 Influenza A virus: (A) Uninfected cells (B) Day 4 post infection (C) Day 6 post infection (D) Day 7 post infection

3. Structure Aided Drug Design, Synthesis and Evaluation of Newer Neuraminidase Inhibitors in Treatment of Pandemic Influenza

Technical team

1. Dr. Meena Kanyalkar,
2. Dr. Sweta T Kothari,
3. Ms. Khushboo Malbari,
4. Ms. Devanshi Gohil,
5. Dr. Abhay Chowdhary

Period

Jan 2015 – Dec 2018

Introduction

The avian influenza, caused due to H5N1 subtype, and swine flu, caused due to H1N1, has heightened awareness of threat of a pandemic. Both subtypes are highly pathogenic (284,500 deaths in 2009) and have attracted global attention, and this has spurred efforts to develop plans for its control. Since influenza virus has a high potential to develop resistance to the commercially available drugs such as Oseltamivir and Zanamivir which are competitive inhibitors of neuraminidase, it becomes necessary to modify the existing drugs. This highlights the need for effective vaccine or antiviral drug which can control the pandemic disaster. The vaccine development is greatly hindered by high mutability of the virus.

Among several potential targets for drug intervention, Neuraminidase is of particular importance as it is responsible for viral release and progression of the disease. Interestingly, the residues within active site of neuraminidase are conserved which promotes it as an excellent target. Structure based drug design methods have played critical role in discovery of its inhibitors such as Oseltamivir and Zanamivir which are commercially in use for the treatment of swine as well as avian flu.

Objectives

1. Structure Aided Drug Design and QSAR: to explore and further quantify the active site of the Neuraminidase enzyme of influenza virus (H1N1) based on our recent outcomes
2. Synthesis: to screen and synthesize the potential promising candidates
3. Evaluation: To target the enzyme selectivity to lead to a molecule which will be less susceptible to drug resistance
 - a. Anti-Influenza activity against pandemic and mutant viral strains using cell based technique

- b. Neuraminidase inhibitor activity of synthesized molecules using reported in-vitro methods in order to explore the mechanism and the type of inhibition using enzyme kinetics study
- c. Comparison of the activity with Oseltamivir as standard, against pandemic and mutant viral strains to address the issue of drug resistance

Results and Conclusions

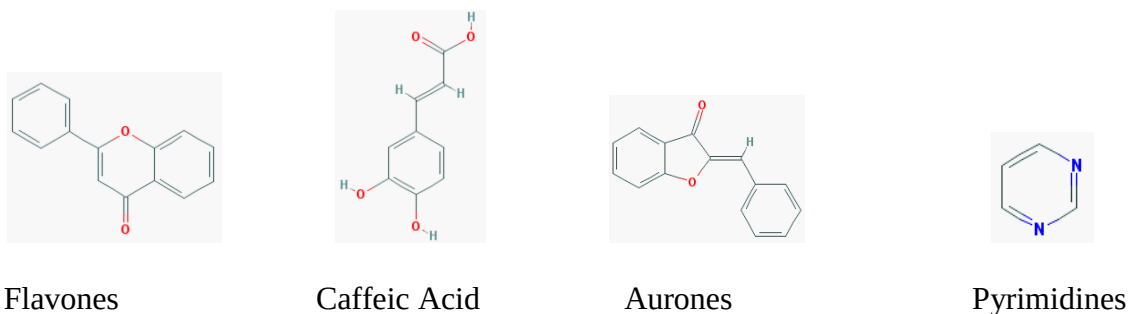


Figure 1: Scaffolding core rings selected for evaluation of anti-Influenza in-silico evaluation

Presently, selected scaffolds are screened for positive interaction with neuraminidase and synthesis using bioinformatics tools at Princ. K. M Kundnani College, our collaborator on this project. These candidates belong to the classes of pyrimidine, aurones, flavones and caffeic acid. At Haffkine Institute, MDCK cells and H1N1 virus are being propagated for activity studies.

4. Molecular characterization of 2009 H1N1 pandemic influenza and seasonal influenza viruses from patients in Mumbai region (completed Ph.D project)

Investigators

1. Ms. Devanshi Gohil
2. Dr. Ranjana A. Deshmukh
3. Dr. Abhay Chowdhary

Period

Nov 2010 – Aug 2014

Introduction

Influenza (commonly referred to as “flu”) is one of the most commonly known viral diseases that represent a health and economic threat to both humans and animals worldwide. An outbreak of Influenza A commonly referred to as “Swine flu”, was officially declared by WHO to be the first influenza pandemic of the 21st century. Since the first case reported for pandemic (pdm) H1N1 influenza in April 2009, the virus had created havoc all around the world. Subsequently, the virus soon became endemic and was reported in different parts of India, including Maharashtra. Haffkine Institute for Training, Research and Testing is a National Centre under WHO for Influenza surveillance in and around Mumbai region. Mumbai being a major migration hub of people nationally and globally, the infections transferred herein and the circulating strains become critically important to be identified and reported. The present study investigated molecular characterization of co-circulating strains in Mumbai which would help to identify similarities/differences between them. With antigenic drift and or shift occurring at irregular and unpredictable intervals the virus develops mutations that alter its susceptibility to antiviral drugs, thereby making its treatment difficult. Hence, determination of drug susceptibility/resistance pattern would also help in understanding possible trends underlying the emergence of resistant variants in humans.

Objectives

1. To isolate pandemic H1N1 influenza and seasonal influenza virus using cell culture methods.
2. To carry out molecular characterization of HA, NA and M gene of the pandemic H1N1 influenza and seasonal influenza virus strains.
3. To study the similarity/differences of pandemic H1N1 influenza virus with seasonal influenza virus.

4. To determine the genotypic and phenotypic drug susceptibility of influenza viruses against oseltamivir.

Results and Conclusions

1. A total of 47 (31.33%) isolates (out of 150 samples cultured) of influenza A (H1N1) pdm 09 virus were obtained while 17 (17%) isolates (out of 100 samples cultured) of seasonal influenza A virus were obtained.

2. Sub-typing of seasonal influenza A viruses revealed that 16 isolates belonged to Influenza A sub-type H3 and were antigenically related to A/Perth/16/2009(H3N2)-like virus while one isolate belonged to Influenza A sub-type H1 and was antigenically related to A/Brisbane/54/2007 seasonal (H1N1)-like virus.

3. Molecular analyses revealed substantial sequence variations in both pandemic and seasonal influenza A viruses.

4. HA1 sequences of pandemic isolates were conserved at the receptor-binding site, while variations in the amino acid substitutions at the antigenic sites resulted in the changes at the N-linked glycosylation sequon.

5. In pandemic isolates, amino acid substitutions in the NA gene were dissociated with the catalytic or framework sites.

6. Seasonal Influenza A virus exhibited antigenic drift.

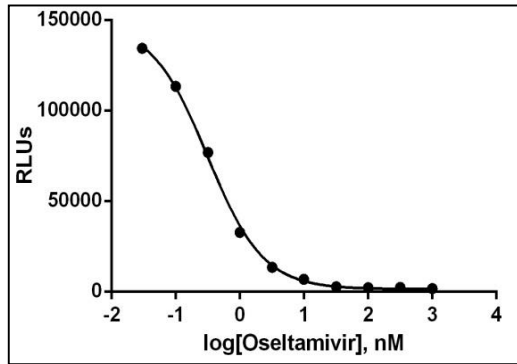
7. Phylogenetic analyses of the HA and NA genes of pandemic and seasonal isolates illustrated their evolutionary closeness or drift with their concomitant prototype vaccine strains respectively.

8. Genotypically, all influenza A (H1N1) pdm 09 isolates were observed to harbor mutations known to confer resistance to adamantane. However, no known mutations conferring resistance to NA inhibitors were detected.

9. Resistance to oseltamivir was observed in the seasonal influenza A (H1N1) isolate with H275Y mutation in the NA gene and an extremely high IC₅₀ value of 1261nM (Figure 1). Conversely, the isolate was sensitive to adamantane.

10. Seasonal influenza A (H3N2) isolates were observed to harbor mutations known to confer resistance to amantadine. However, no known mutations conferring resistance to NA inhibitors were detected.

(A)



(B)

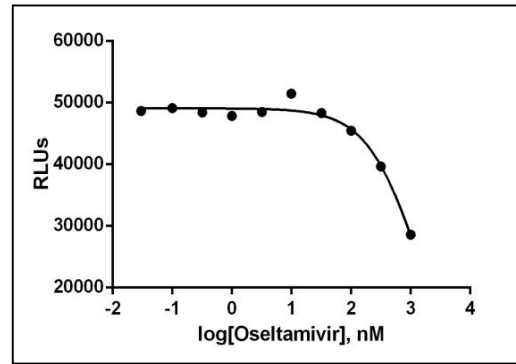


Figure 1: Assessment of the IC₅₀ for oseltamivir for influenza A (H1N1) pdm 09 and seasonal influenza A (H1N1) using chemiluminescent neuraminidase inhibitor assay. (A) Oseltamivir-sensitive A/Mumbai/3011/2009 (H1N1) virus; (B) Oseltamivir-resistant A/Mumbai/3212/2009 (H1N1) virus

5. Assessment of IFITM gene & protein expression in human influenza infection (completed Ph.D project)

Technical team

1. Ms. Paly Chatterjee,
2. Dr. Abhay Chowdhary

Period

Aug 2013 – Sept 2015

Introduction

Influenza epidemics exact a formidable toll on world health. Moreover, viral super-infections can produce antigenic shifting, resulting in more virulent pathogens. Interferon-induced transmembrane (IFITM) genes are transcribed in most tissues and are with the exception of IFITM5 interferon inducible. The interferon induced transmembrane (IFITM) genes IFITM1, IFITM2 and IFITM3 (IFITM1-3) belong to the family of small ISGs. Its members are induced in response to viral infections. The IFITM gene family was first identified in a cDNA screen from IFN-treated neuroblastoma cells back in 1984. Their chromosomal arrangement is not conserved between human, mouse and rat both in length and in number of genes present in the chromosomal locus. IFITM1, 2 and 3 are ubiquitously expressed. These proteins have been ascribed roles in immune cell signaling, cell adhesion, oncogenesis, and germ cell homing and maturation and bone mineralization. Variations in the basal and inducible levels of these factors as well as the dependency factors within a population might predict the severity of influenza infections among individuals, or across species. The present study shall assess the gene and protein expressions of IFITM protein in influenza patients.

Objectives

Collection of samples from individuals suspected of human influenza infection.

1. Screening of collected samples for influenza (influenza A, influenza B, pH1N1) infection.
2. Determination of interferon (α & γ) & IFITM (1 & 3) gene expression in the screened human influenza samples.
3. Determination of interferon (α & γ) & IFITM (1 & 3) protein expression in the screened human influenza samples.
4. Comparative analysis of gene & protein expression levels of interferon & IFITM.

Results and Conclusion

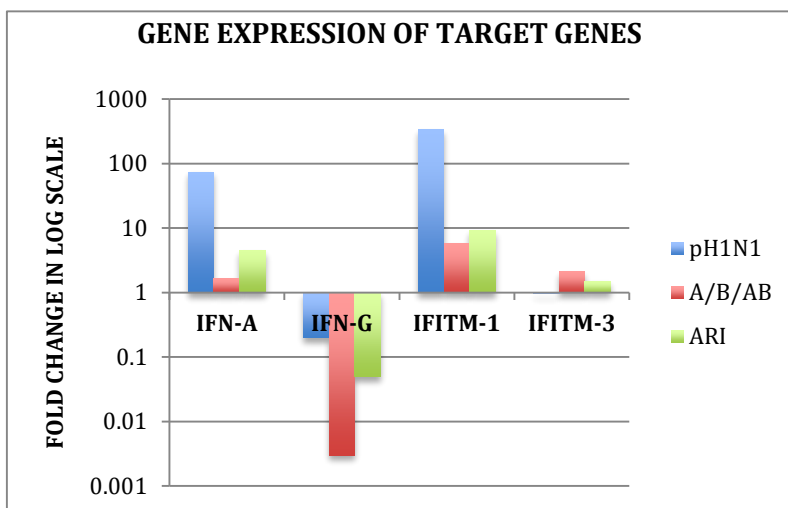


Figure 1: Gene Expression levels of the target Genes in the Study Cohort. Fold change is calculated using $\Delta\Delta C_t$ method.

Gene expression of Interferon A is upregulated in all the three groups, whereas Interferon G was downregulated in the study groups. Gene expression of IFITM 1 and 3 were up regulated in the study groups. Downregulation of Interferon gamma in the active stage of the disease enhances the pathogenicity of the disease, as IFN-G is needed to induce Th1 immune responses. Similarly gene expression of IFITM-3 is mildly upregulated (below 2 fold). This may also contribute to the active infection as IFITM-3 has been found to possess potent anti-viral activity against respiratory viruses.

6. Detection of TORCH infections in children and its possible correlation with birth defects

Technical team

1. Ms. Payal Panchal,
2. Dr. Sweta T Kothari,
3. Dr. Usha Dave,
4. Dr. Vandana Kumavat,
5. Dr. Abhay Chowdhary

Period

Aug 2015 - Dec 2017

Introduction

Early diagnosis, evaluation and adequate therapy in mothers and their newborns help in prevention of diseases and long term disabilities in children. One of the most important causes of bad obstetric history in women and/or congenital disorders in children is TORCH infection. TORCH is the acronym for group of organisms viz; Toxoplasma gondii, Rubella, Cytomegalovirus, Herpes simplex virus 1 and 2.

TORCH is the group of perinatal infections that cause congenital anomalies to the fetus which include mental retardation, cataracts, Intra Uterine Growth Retardation (IUGR), SGA etc. They also cause They also lead to a bad obstetric history in mothers such as sterility, multiple abortions etc. These infections are grouped together because they can cause a cluster of symptomatic birth defects in newborns, collectively called the TORCH syndrome.

TORCH infections in the mother are transmissible to fetus in the womb or during the birth process and cause a cluster of symptomatic birth defects. Approximately 2%– 3% of neonates in India are found to harbor congenital malformations, which lead to morbidity, mortality and disability in such children, wherein the prevalence varies as per geographical area.

As the classical diagnosis of TORCH infections based on serological tests, there is an ambiguity in data, which may be consequent to false positivity or cross reactivity in serological estimation. Therefore, a confirmatory diagnosis based on the direct demonstration of the etiological agents in tissues or biological fluids becomes imperative. PCR is sensitive and the diagnosis can be confirmed within 24-48 hours. It has simplified the applications for prenatal diagnosis, making it faster, more sensitive, and thus, helping decrease the morbidity and mortality due to these

infections. However, no PCR data is available from Indian literature on infected children. Our present study aims to bridge this gap.

Objectives

1. To detect the presence of nucleic acid of *Toxoplasma gondii*, Rubella, CMV, HSV1 and HSV2 from whole blood samples from children.
2. To correlate the results of the study with the clinical presentation or birth defects of the subjects under investigation.
3. To correlate the clinical & laboratory findings with maternal infections, if any.

Results and Conclusions

The study has been approved by the Mumbai University as a thesis topic for MSc (by research). Ethics Committee Clearance has been obtained from both Haffkine Institute and CSM Kalva Hospital from where the samples are being collected. qPCR and qRT-PCR have been standardized using appropriate internal and endogenous controls. Sample collection is in process.

While amplification of the target gene and the endogenous control (that indicates the sample quality) is detected on the FAM channel, the internal/extraction control amplification is detected on the VIC channel on a qPCR system.

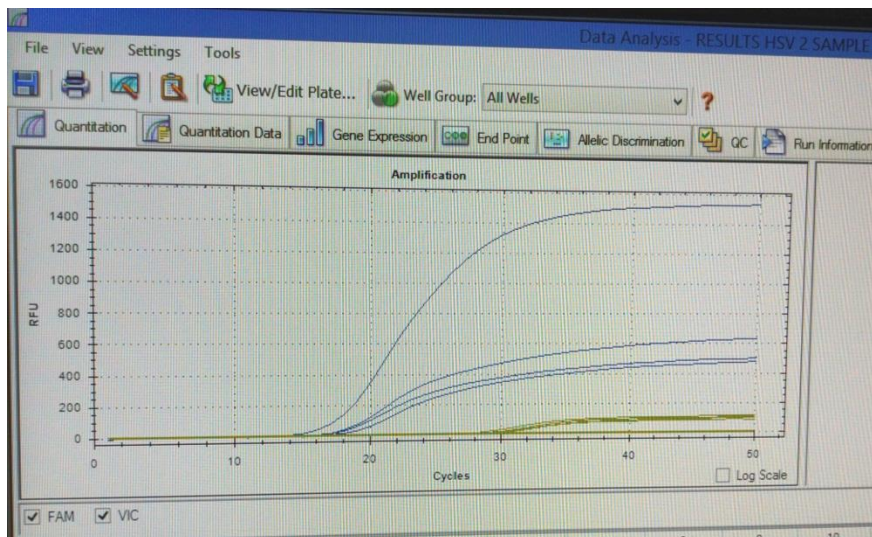


Figure 1: Representative picture of amplification of HSV-2 using qRT-PCR. The (■) represents the FAM Channel wherein the target gene is amplified along with (■) internal control/extraction control, whose amplification is detected on the VIC channel.

7. To study siRNA associated suppression of Rabies Virus infection and assess its therapeutic potential (Ph. D project)

Technical team

1. Ms. Perna Charan,
2. Dr. Abhay Chowdhary

Period

Jun 2011 - Dec 2016

Introduction

Rabies virus belonging to genus Lyssavirus of family Rhabdoviridae causes one of the oldest neurodegenerative diseases known to mankind. Rabies virus is an enveloped, non-segmented, negative-stranded RNA virus. As per the WHO studies about 55,000 deaths are still an attribute of rabies infection. Rabies virus has approximately 12 kb long single stranded RNA genome consisting of a leader region followed by genes encoding Nucleoprotein (N), Larger Polymerase (L), Matrix Protein (M), Glycoprotein (G) and Phosphoprotein (P). Antivirals currently used against rabies include ketamine, midazolam, ribavirin and amantadine, but only one successful case of human treatment happened in 2004. RNA interference (RNAi) is a system within living cells that takes part in controlling the genes which are active and how active they are. Short RNAs can direct repression of genes with homology to those short RNAs. This repression is called RNAi. The purpose of this study is to understand the RNAi process and its application in suppressing rabies virus infection in humans. In this study, we will target the viral proteins like Nucleoprotein and Large Polymerase with siRNA designed specifically to be homologous to their mRNAs and observe the effect on the viral replication as the targeted proteins and enzymes are crucial for viral replication.

Objectives

1. To design the siRNA sequence specific for the target gene.
2. To perform *in vitro* study of RNA Interference (RNAi) associated suppression of Rabies Virus by targeting viral genes.
3. To perform *in vivo* study of RNA Interference (RNAi) associated suppression of Rabies Virus.
4. To assess the potential of siRNA-RVG-9dR Complex as a therapeutic tool for combating Rabies Infection

Results and Conclusions

Immuno-Fluorescence Assay was performed for detecting the rabies virus Nucleoprotein antigen and we observed reduced or no fluorescence in N2a cells infected with RABV CVS-11 strain and treated with siRNA for viral genes.

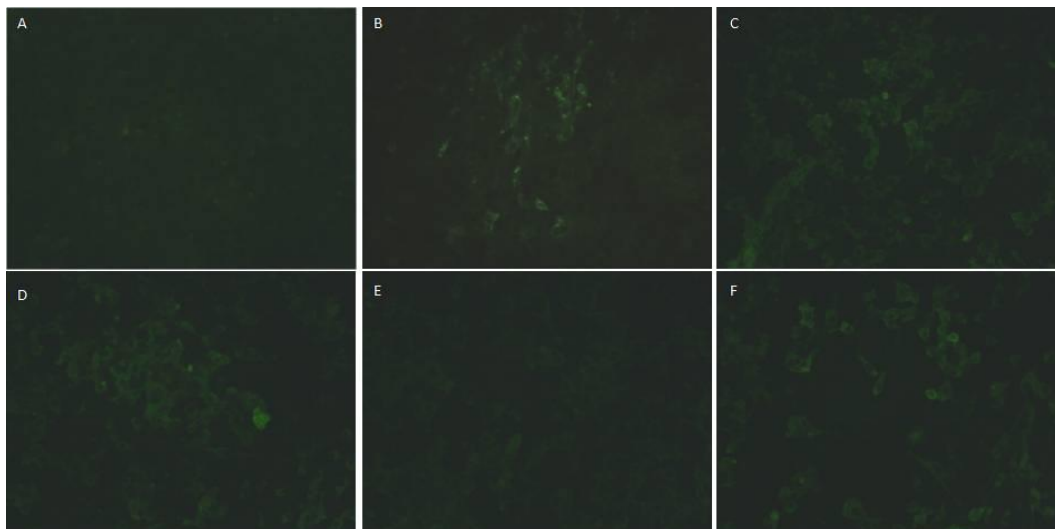


Figure 1: Detection of RABV-CVS infection using FAT. A-B: FAT analysis of Non-infected and Infected Cell Control control groups CC &RABV CVS used in the study; [C-F]: FAT analysis of study groups siN Treated, siP Treated, siL Treated and siCombinatorial Treated respectively in CVS infected brain tissue using (1:20) diluted FITC-tagged antibody specific against N antigen.

8. Assessment of in-vitro Immunomodulatory activity of Anti-inflammatory agents

Technical team (part of Ph. D project)

1. Mr. Siddhant Gawli,
2. Ms. Perna Charan,
3. Mr. Sandeepan Mukherjee

Period

Nov 2015 - ongoing

Introduction

Anti-inflammatory agents are irrationally used for treatment of different types of indications including fever and pain. Given the fact that most of these are available as over-the-counter drugs, makes their judicious usage difficult. Administration of anti-inflammatory agents may modulate the immune system and affect the course/prognosis of a disease/disorder. Therefore understanding the immunomodulatory agents shall be pivotal to their application in clinical set-ups. The present study attempts to understand the gene expression levels of several genes with immunomodulatory roles on administration of anti-inflammatory agents.

Objectives

1. To culture RAW CELLS (murine macrophage)
2. To expose RAW cells to anti-inflammatory drugs
3. To assess cytokine gene expression levels in the cells

Results and Conclusions

Preliminary studies indicated TNF-A and IL-1 Beta gene is down-regulated in cells exposed to Paracetamol, Diclofenac and Dexamethasone. Il-10 gene is up-regulated in these cells. Cells exposed to Tramadol do not exhibit much immunomodulation.

9. Interplay of Gaso-transmitters in in-vitro infection of Rabies Virus

Investigators

1. Mr. Sandeepan Mukherjee
2. Ms. Prerna Charan
3. Mr. Rohit Beher

Period

Nov 2015 - ongoing

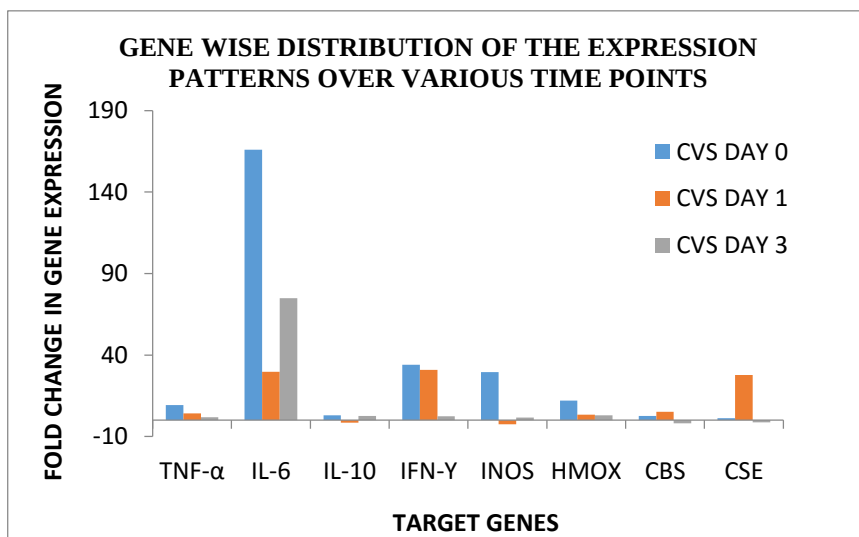
Introduction

The mechanisms behind the neuronal dysfunction seen in Rabies are far from being understood. Many an important endogenous systems have been indicated as potential underlying causes of this yet cryptic pathology. Gaso-transmitters (NO, CO and H₂S) have been reported to have important immunomodulatory and neuromodulatory activities. They interact with a various components of the nervous system and are also used as neurotransmitters. Understanding the role of these endogenous gases will be critical in the understanding of the overall pathophysiology of the disease.

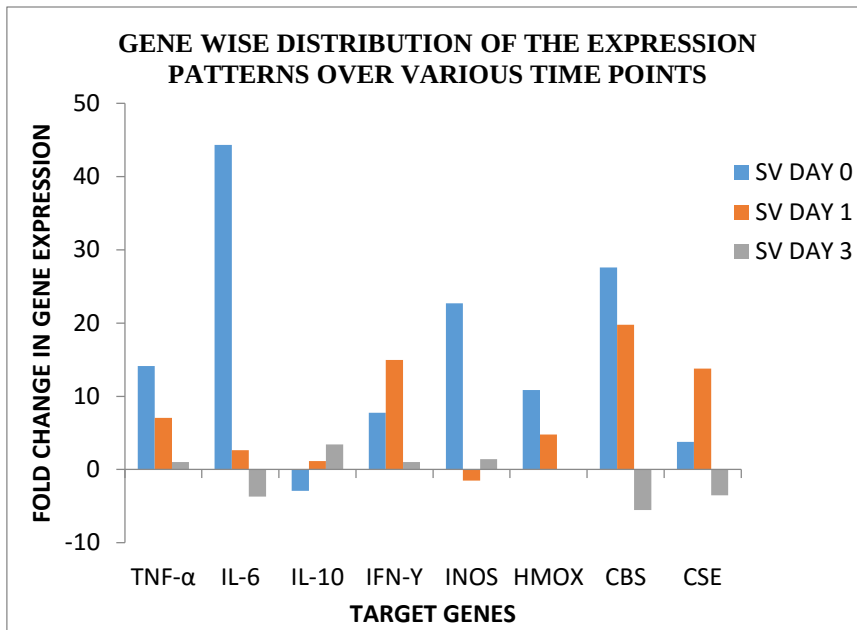
Objectives

1. To establish Rabies infection in N2a cells with CVS-11 and Street Virus (SV) strains of the Rabies Virus.
2. To assess the gene expression levels of pivotal genes involved in gaso-transmitter physiology

Results and Conclusions



Rabies infection was successfully established in the N2a cells. Gens involved in gasotransmitter synthesis and regulation are upregulated at all time points in the CVS-11 infection, however the levels kept decreasing over the time points. SV infection



upregulated the gene expression at 4 hrs and 24 hrs post infection. However the iNOS, HMOX-1, CBS and CSE genes were downregulated at 72 hrs post infection.

10. Studies on Neuronal Proteomics, Virus-Host Interaction and Immune-Pathological Alterations in Rabies Encephalitis in Murine Model.

Technical team

1. Dr. Abhay S. Chowdhary,
2. Dr. S. Madhusudhana,
3. Dr. Keshava Prasad,
4. Mr. Sandeepan Mukherjee,
5. Ms. Prerna Charan,
6. Mr. Paras Mahale

Period

Mar 2013 – Mar 2015

Introduction

The study is based on enhancing the knowledge on pathophysiological basis of neurological infections causing encephalitis with emphasis on rabies. It is intended to fill the gaps that are left in the overall comprehension of the immunopathogenesis of rabies and the current therapeutic options available for the management of rabies. Although vaccine preventable, rabies is left with no treatment once the clinical signs and symptoms of the disease appear. The mechanism of classical symptoms and inevitable death is still poorly understood. As there is no marked neuronal lysis and apparent pathological findings, focus is now mainly towards understanding the mechanisms that lead to profound neuronal dysfunction that may result from alteration in neuronal metabolism involving synthesis of neuronal proteins including neurotransmitters. Some earlier studies suggested probable deficiency of acetyl choline, serotonin and over production of GABA. Our study hopes to glean better understanding of rabies virus infection focusing on these aspects coupled with host factors and immune response elicited during disease progression. We hope that understanding these mechanisms may give some leads to formulate specific therapeutic measures to otherwise a fatal disease. We propose to carry out quantitative proteomic profiling to identify gene expression levels associated with rabies in rabies virus infected mouse brain. We also aim to identify proteins, which are associated with the viral infection by quantitative proteomics. In order to accomplish this, we will carry out a quantitative proteomic profiling of rabies infected mouse brain and normal mouse brain. We will also perform proteomic profiling of mouse brain tissues, which are challenged with various rabies strains. This proteomic approach will be achieved by in

vitro labeling using iTRAQ technology followed by liquid chromatography and high resolution tandem mass spectrometry (LC-MS/MS). An important goal of this proposal is to identify proteins that are differentially regulated in rabies.

Objectives

1. Establishment of Rabies infected Murine Model & Antigen Detection
2. Immunophenotyping & Assessment of role of effector molecules in permeability of BBB in relation to the progression of Rabies.
3. To understand the underlying mechanisms of rabies virus clearance in a vaccine challenge study
4. Quantitative proteomic profiling of CNS to monitor progression of rabies in infected mice (provided by Haffkine Institute)

Results and Conclusions

A. Work under HAFFKINE INSTITUTE:

1. Establishment of Rabies Infected Murine model and Antigen Detection:

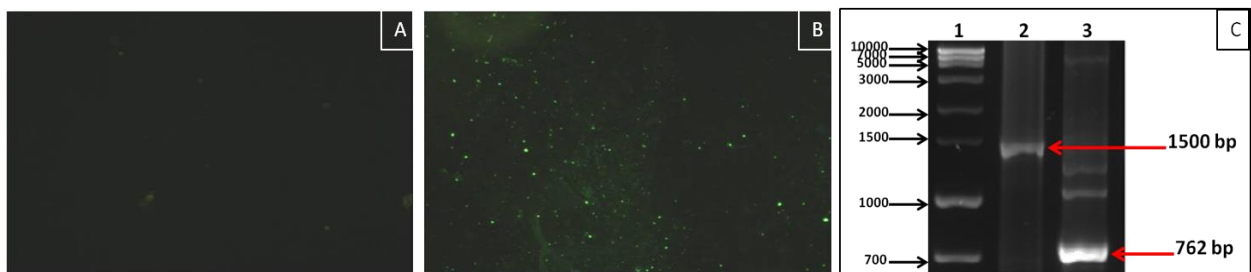
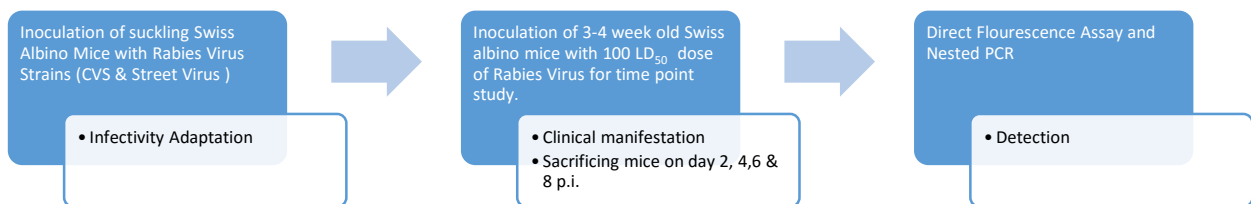


Figure 1 [A-B]: Detection of rabies nucleoprotein antigen by FAT using FITC tagged antibody in normal mouse brain and rabies infected mouse brain respectively; 2 [C]: Detection of rabies N gene using nested RT-PCR- Lane1:Ladder, Lane 2: 1500 bp product of the first round (outer), Lane 3: 762 bp product of the second round (inner) of PCR

- To perform immunophenotyping & assessment of the role effector molecules play in the permeability of BBB in relation to the progression of rabies infection

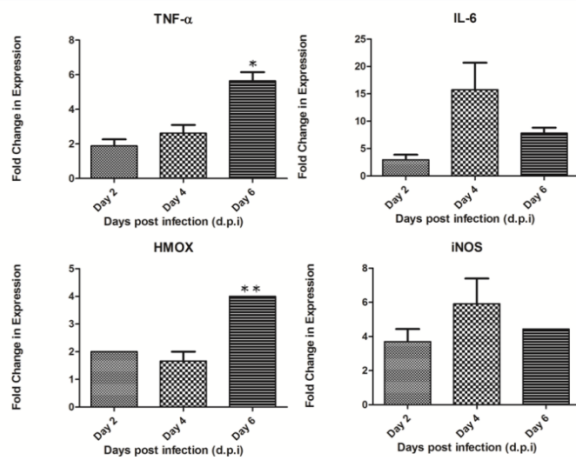


Figure 2: Results of fold change in expression for each of the target genes are expressed as the mean $\pm$ SEM for each group. Evaluation of the significance of differences between the means of parameters was performed using the One way ANOVA and Tukey test. In all cases $P < 0.05$ was considered significant. [*- $P < 0.05$; **- $P < 0.01$, ***- $P < 0.0001$]

B. Work under NIMHANS:

The main aim of the project was to understand the underlying mechanisms of rabies virus clearance in a vaccine challenge study. This was achieved by performing flow cytometry as well as cytokine/chemokine studies in mice brain. ELISpot assay to study the immune responses after vaccine challenge is also being standardized.

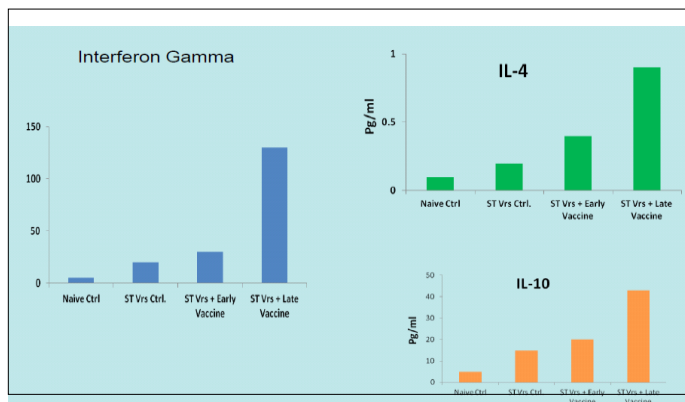


Figure 3: Mouse Cytokine / Chemokine by magnetic bead panel. Graphs showing IFN-g, IL-4 and IL-10 in the 4 groups of mice; naive, street virus (symptomatic), street virus with early vaccination and street virus with delayed vaccination. Naïve mice showed low levels of IFN-g, IL-4 and IL-10.

All groups of mice showed almost equal percentage of CD3 lymphocytes. It was also interesting to note that the mice with early vaccination/asymptomatic displayed double negative ($CD3^+$, $CD4^-$, $CD8^-$) cells which was comparable to that of the naïve mice group and street virus control mice and infected mice that received delayed vaccination / symptomatic displayed low levels of double negative population of cells

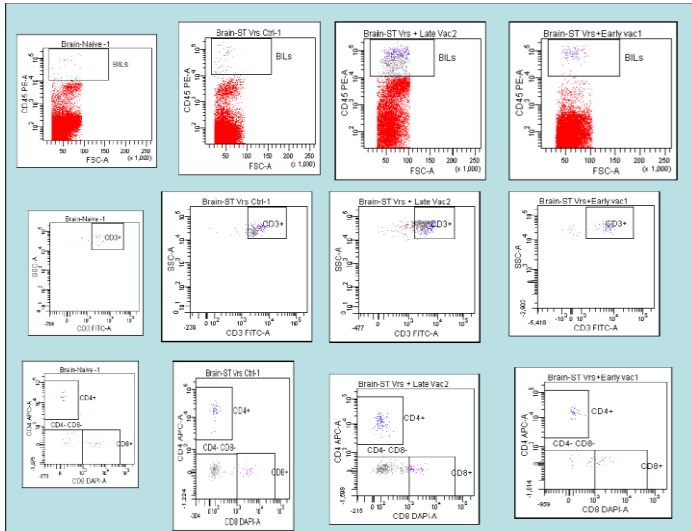


Figure 4: Flow cytometry data for all groups of mice; naive, street virus (symptomatic), street virus with early vaccination and street virus with delayed vaccination

Flow cytometry

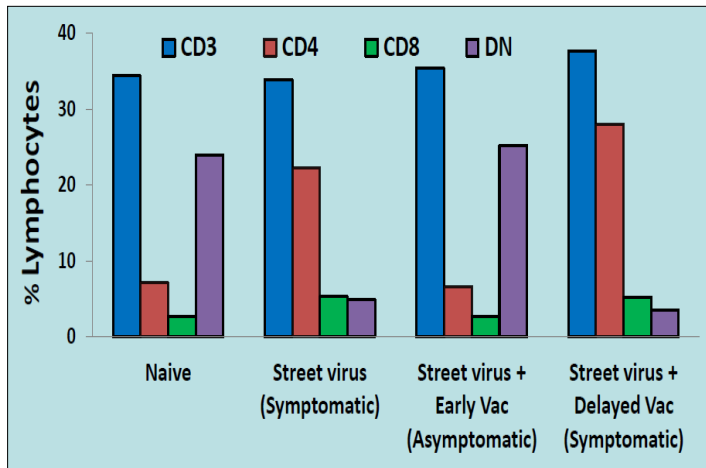


Figure 5: Graph showing percentage of lymphocytes CD3, CD4, CD8 and Double negative (DN) cells in the four groups of mice; naive, street virus (symptomatic), street virus with early vaccination and street virus with delayed vaccination.

C. Work Under Institute of Bioinformatics

Identification of total 1,155 proteins in mouse brain infected with rabies CVS and street virus strain as compared to the uninfected control. Out of the total proteins identified, most of the altered proteins were observed on day 4 post-infection of CVS rabies virus while in street virus day 2 post-infected mouse brain tissue showed more number of altered expression of protein. We found that altered proteins were involved in processes such as immune response, interleukin signalling disruption of blood brain barrier which signify the sequential progression of infection in brain tissue infected with CVS and street strain of rabies virus.

Table 1: Partial list of differentially expressed protein in mouse brain tissue infected with rabies virus CVS as compared to uninfected control

Sr no.	Gene Symbol	Protein name	Fold change		
			Day2	Day4	Day6
1	<i>Hbb-bt</i>	hemoglobin, beta adult t chain	0.14	0.31	0.77
2	<i>Lphn1</i>	latrophilin-1 isoform X10	3.83	3.50	4.18
3	<i>Itpr1</i>	inositol 1,4,5-trisphosphate receptor type 1 isoform X13	2.19	1.94	1.38
4	<i>Cox6c</i>	cytochrome c oxidase subunit 6C	1.91	0.67	1.20
5	<i>Ahsg</i>	alpha-2-HS-glycoprotein isoform 3 precursor	0.78	0.37	0.39
6	<i>Ap1g1</i>	AP-1 complex subunit gamma-1 isoform X1	1.86	2.27	2.27
7	<i>Eif5a</i>	eukaryotic translation initiation factor 5A-1 isoform X1	1.74	2.23	1.73
8	<i>Pspc1</i>	paraspeckle component 1	1.79	0.89	2.09
9	<i>Atxn10</i>	ataxin-10	1.09	1.46	1.90
10	<i>Snap47</i>	synaptosomal-associated protein 47 isoform X1	1.66	1.06	0.42

Table 2: Partial list of differentially expressed protein in mouse brain tissue infected with rabies street virus as compared to uninfected control

Sr no.	Gene Symbol	Protein name	Fold change			
			Day 2	Day 4	Day 6	Day 8
1	<i>Car2</i>	Carbonic anhydrase 2 isoform X1	0.22	0.77	0.81	0.69
2	<i>Pvalb</i>	Parvalbumin alpha isoform X1	0.34	0.65	0.56	0.60
3	<i>Gripap1</i>	GRIP1-associated protein 1 isoform X16	1.69	1.18	1.12	1.19
4	<i>Hnrnph3</i>	Heterogeneous nuclear ribonucleoprotein H3 isoform X4	0.82	1.18	0.42	1.17
5	<i>Reep2</i>	Receptor expression-enhancing protein 2 isoform 2	-	1.69	2.24	1.22

6	<i>Armc10</i>	Armadillo repeat-containing protein 10	0.78	0.78	2.01	1.18
7	<i>Atp5k</i>	ATP synthase subunit e, mitochondrial	0.50	0.47	0.70	0.40
8	<i>Ipo5</i>	importin-5 isoform X4	1.33	3.26	1.37	1.67
9	<i>Pcp4</i>	Purkinje cell protein 4	1.36	2.42	1.87	2.70
10	<i>Ppfia4</i>	liprin-alpha-4 isoform X5	0.99	2.27	1.03	2.40

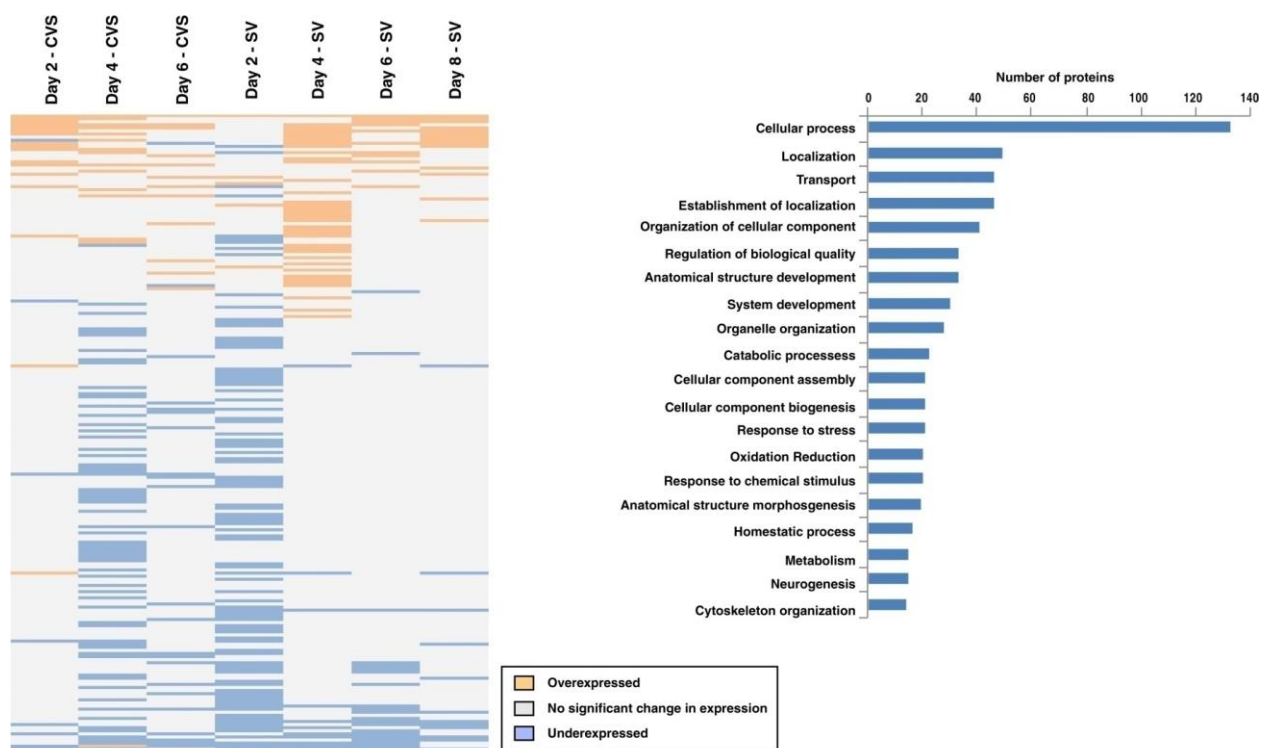


Figure 6 A. Heatmap of differentially expressed proteins (1.5 fold) in various stages of infection when compared with non-infected sample. **B.** Bar graph depicting the various biological processes affected due to change in protein expression in infected samples when compared with normal.

11. To study siRNA associated suppression of Rabies Virus infection in vitro

Investigators

1. Dr. Abhay Chowdhary
2. Mr. Sandeepan Mukherjee
3. Ms. Prerna Charan
4. Mr. Rohit Beher

Period

Jun 2012 - ongoing

Introduction

siRNA based methods are widely used for arresting viral infection in in-vitro and in-vivo systems. The study is designed to analyze the role of Rabies Virus specific siRNA in the in vitro infection of Rabies Virus.

Objectives

1. To establish Rabies infection in N2a cells
2. To administer Rabies Virus specific siRNAs
3. To assess the gene expression levels of pivotal viral genes and assess degree of protection.

Results and Conclusions

Immunofluorescence assay exhibited reduction of Rabies Virus Infection in siRNA treated cells as compared to virally infected cells. Gene expression studies are currently underway.

12. Study of neuronal proteome in prognosis of Rabies in murine model (completed Ph. D project)

Technical team

1. Ms. Shraddha Mehta,
2. Dr. Abhay Chowdhary

Period

Jan 2011 – Jan 2015

Introduction

Rabies is a zoonotic viral disease that invariably leads to fatal encephalitis. In contrast to some other infectious diseases, a highly effective treatment already exists. Despite this, rabies continues to pose a significant health problem particularly in rural communities. In addition, once the clinical signs and symptoms appear, vaccination and immunoglobulin are ineffective and the disease invariably proves to be fatal. A few antè-mortem laboratory tests are available however a negative result does not rule out infection. It is important to overcome the dilemma of antè-mortem diagnosis and to develop novel treatment strategies. A study was therefore designed to analyze the role of differential host proteins that are produced in response to street rabies virus (SRV) and laboratory strain— Challenge Virus Standard (CVS) upon infection in mouse model using two dimensional gel electrophoresis (2-DE) and mass spectrometry (MS).

Objectives

1. To establish a murine model for the assessment of proteomic changes in the neurons of rabies virus infected animals
2. To perform the protein profiling and characterization of rabies infected CNS proteome
3. To compare the above events in infection with wild-type (wt) and laboratory adapted strains of rabies virus

Results and Conclusion

A total of 37 and 8 (altogether 45) spots were found to be differentially expressed in SRV and CVS infected groups respectively with a vast majority of proteins being altered on day 4 pi. One hundred forty three proteins corresponding to 40 spots were identified with major alterations observed in cytoskeletal proteins (22.37%), metabolic proteins (14%) and those belonging to synapse, neurotransmission and neurophysiology (10%) category. Data analysis using bioinformatics tools such as Ingenuity Pathway Analysis and STRING provided overlapping as

well as complementary information that may be explored further to develop biomarker based assays.

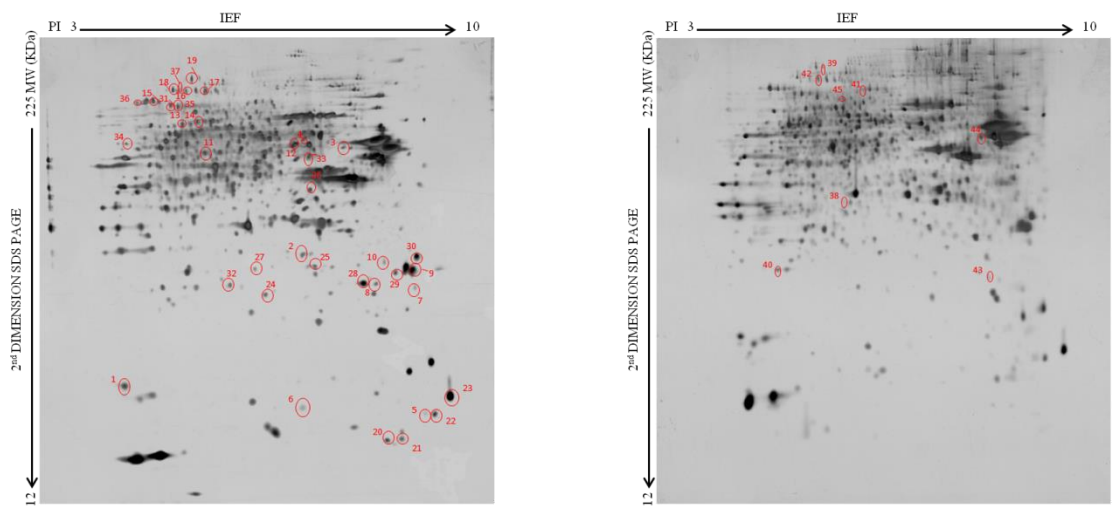


Figure 1: Representative high-resolution annotated 2-DE image of SRV (left) and CVS (right). 0 around the protein spot indicates spots with differential expression i.e. upregulation or downregulation with respect to uninfected controls. Spots with only fold change ≥ 2.0 and ANOVA <0.05 were selected.

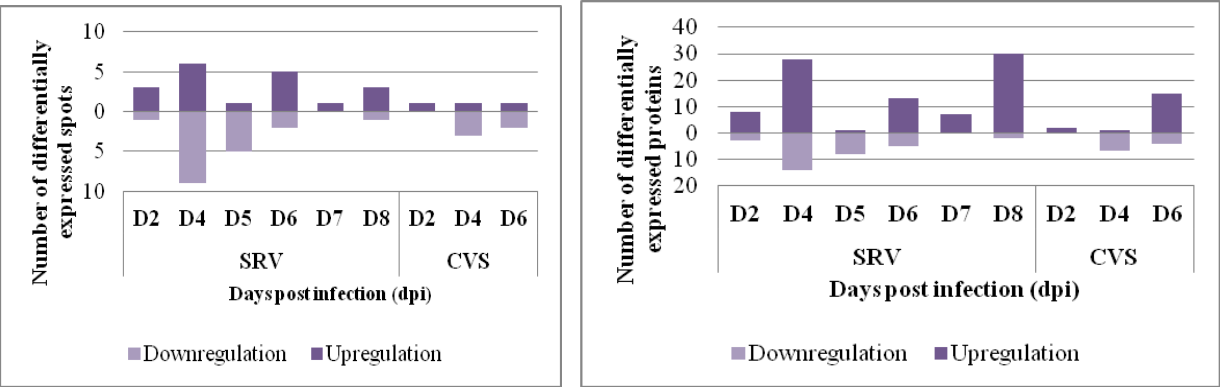


Figure 2: Expression pattern of differentially expressed spots (above) and proteins (below)

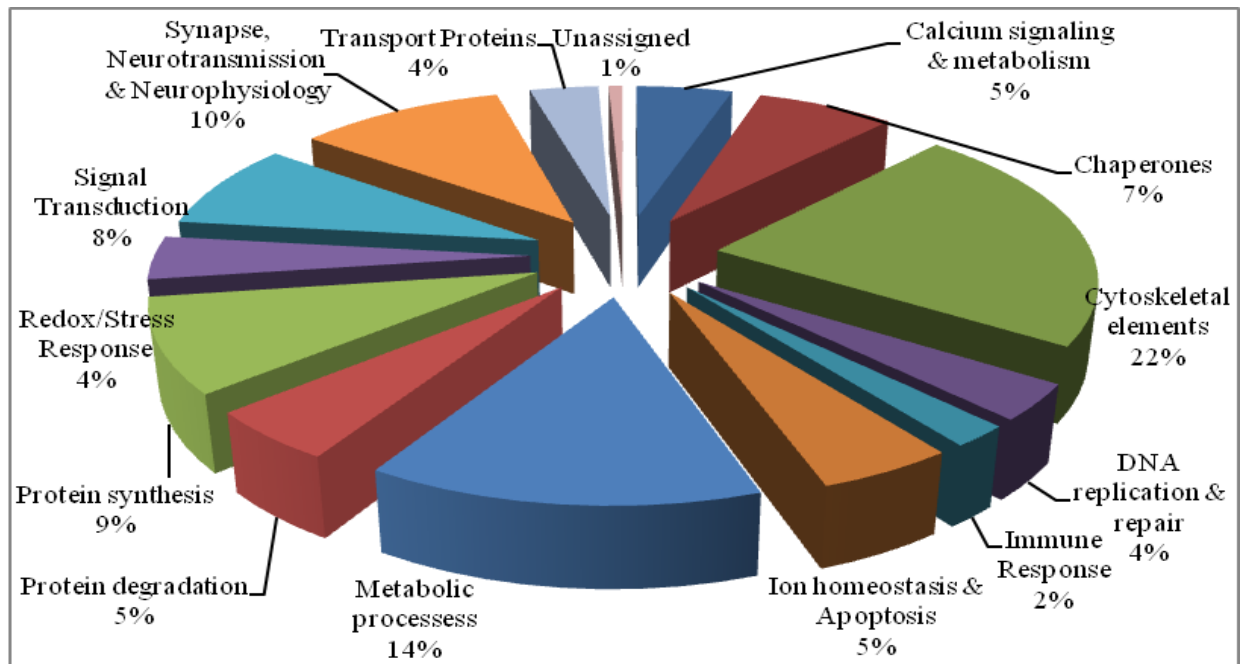


Figure 3: Percentage distribution of functionally classified differentially expressed proteins in RABV infection

13. Interplay of Gaso-transmitters in in-vitro infection of Japanese Encephalitis Virus

Investigators

1. Mr. Sandeepan Mukherjee
2. Ms. Perna Charan
3. Ms. Amruta Anvekar

Period

Nov 2015 - ongoing

Introduction

Gaso-transmitters (NO, CO and H₂S) have been reported to have important immunomodulatory and neuromodulatory activities. They interact with a large number of Pro and Anti inflammatory cytokine genes and modulate their activities in turn. Their interactions are critical to many a viral diseases. Japanese Encephalitis Virus causes extensive inflammation in the CNS. Understanding the interplay of these endogenous gases will be pivotal in the understanding of the overall pathophysiology of the disease.

Objectives

1. To establish JEV infection in RAW cells
2. To study the levels of endogenous gases (NO, CO and H₂S)
3. To assess the gene expression levels of pivotal genes involved in gaso-transmitter physiology

Results and Conclusions

RAW cells infected with JEV exhibited an overall down regulation of pro-inflammatory cytokine genes. Gene expression levels of HMOX-1, CBS and CSE were also down-regulated.

14. Immunogenicity study of recombinant Dengue gene construct in mice. (completed Ph. D project)

Technical Team

1. Mr. Ajit Kulkarni,
2. Dr. Ranjana A. Deshmukh

Period

Jun 2011 - Jan 2015

Introduction

Dengue infections are rising in recent years causing high morbidity and mortality. Annually, there are around 390 million infections reported globally. Since, there is no specific anti-viral drug available for dengue and most of the mosquito eradication programs are not effective; there is an urgent need of effective vaccine against dengue which can prevent infections from all four dengue virus serotypes. The present study reports the construction of novel tetravalent recombinant dengue vaccine and investigated its effectiveness in mice.

Objectives

1. To construct tetravalent recombinant dengue DNA vaccine.
2. To determine humoral and cellular immune response in mice.
3. To determine efficacy in mice.

Results and Conclusions

The tetravalent recombinant dengue DNA vaccine was successfully constructed and was found to be effective against dengue virus serotypes 1, 3 and 4. However, vaccine was less effective against dengue virus serotype 2. Thus, there is need of improvement in vaccine formulation in terms of increasing immunogenicity particularly against serotype 2.

Figure 1 (A)

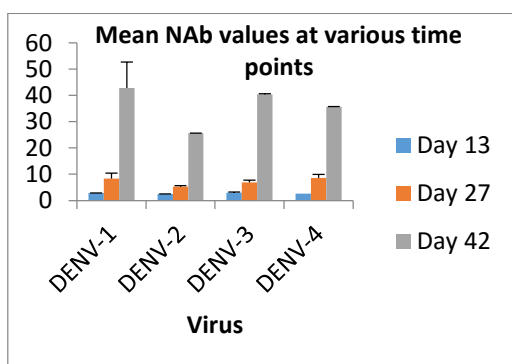


Figure 1 (B)

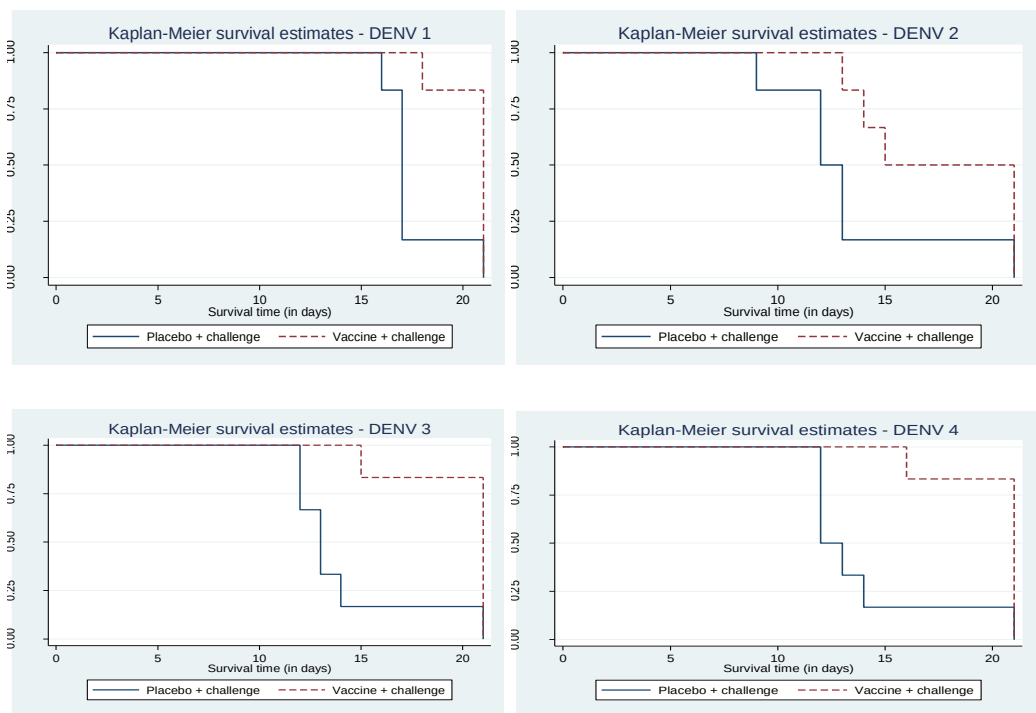


Figure 1: Vaccine efficacy. A: Induction of neutralizing antibodies against dengue virus serotypes at various time points; **B:** Survival estimates of dengue virus serotypes 1 to 4.

15. Mechanism Based Screening and Validation of Herbal Drugs Using Radiotracer Techniques

Technical team

1. Dr. Abhay Chowdhary
2. Dr. R. D. Lele
3. Dr. Tarala Nandedkar
4. Dr. H. S. Palep
5. Mr. Harish Chander
6. Mr. Sandeepan Mukherjee
7. Ms. Purna Charan,
8. Ms. Pallavi Choudhary

Please see details under Research Projects based on Ayurveda

16. In-vitro evaluation of the dermato - protective potential of *Hibiscus rosasinesis* flower extract

Technical team

1. Dr. Abhay Chowdhary
2. Dr. Anil Bobade
3. Dr. Sweta T Kothari
4. Mrs. Suvarna Pachpore
5. Mrs. Amita Patil

Please see details under Research Projects based on Ayurveda

17. Assessment of Plant Proteins/Peptides as Potential Anti-Viral Agents.

Technical team

1. Dr. Abhay Chowdhary
2. Mr. Sandeepan Mukherjee

Please see details under Research Projects based on Ayurveda

Publications

1. Novel evidence of microglial immune response in impairment of Dengue infection of CNS. Bhatt R, Kothari S, Gohil D, D'Souza M, Chowdhary A. Immunobiology, 2015, 220(10), 1170–1176.
2. Chauhan G, Kesarkar R, Kothari S, Rath G, Chowdhary A, Goyal A. In-situ stabilized AgNPs and (Cu-Cur)CD dispersed gel. A topical contraceptive anti-retroviral (ARV) microbicide. RSC Advances, 2015, 5, 83013-83028.
3. Oseltamivir Resistant Influenza A (H1N1) Virus Infection in Mumbai, India. Gohil D, Kothari S, Shinde P, Chintakrindi A, Meharunkar R, Warke R, Kanyalkar M, Chowdhary A and Deshmukh R, J Antivir Antiretrovir 2015, 7(4), 108-114.
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5. Postgenomics biomarkers for rabies-the next decade of proteomics. Mehta SM, Banerjee SM, Chowdhary AS. OMICS, 2015, 19(2), 67-79.
6. Unique Clinical and Imaging Findings in a First Ever Documented PCR Positive Rabies Survival Patient: A Case Report. Netravathi M, Udani V, Mani R, Gadad V, Ashwini M, Bhat M, Mehta S, Chowdhary A, Pal P, Madhusudana S. J Clin Virol. 2015, 70, 83-88.
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11. Rabies Virus Severely Influences Host Protein during Terminal Stages of Infection. Mehta S, Chowdhary A. Int J Curr Microbiol App Sci, 2015, 4(10), 129-137.
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14. Molecular characterization of nucleoprotein gene of rabies virus from Maharashtra, India. Mehta S, Charan P, Dahake R, Mukherjee S, Chowdhary A. J Postgrad Med, 2016, 62(2), 105-108.
15. Pathway Analysis of proteomics profiles in Rabies infection: Towards future biomarkers? Mehta S, Sreenivasamurthy S, Banerjee S, Mukherjee S, Prasad K, Chowdhary A. OMICS. 2016, 20(2), 97-109.
16. Evaluation of in vitro cytotoxic and antioxidant activity of *Datura metel* Linn. and *Cynodon dactylon* Linn. Extracts. Pharmacognosy Res. Soumen Roy, Sandip Pawar, Abhay Chowdhary. 2016; 8(2), 123-127.

Zoonosis Department

Head of Department's Overview

Department of Zoonosis is committed for training, research and testing in the field of Vector and vector borne diseases.

History The present Zoonosis department was started in the year 1938 as Department of Entomology under the leadership of Dr. M. Sharif, DSc. PhD, to work mainly of entomology with plague and its transmission. Dr. P. J. Deoras, Assistant Director has succeeded after that and continued to work on plague and established venomous animal unit along with insect colony of Public Health Importance. In 1970 the department was renamed as Department of Zoonosis under the guidance of Dr. M. V. Sant, Deputy Director to concentrate more on communicable diseases of Zoonotic importance.

After that Dr. D. M Renapurkar had taken the charge of the department as Assistant Director and flourished the activity of the department to study the prevalence of the disease, serological diagnosis, and immunology of parasitic diseases and control measure of important vector borne diseases like malaria, filariasis, hookworm, schistosomiasis, anestrongliasis and dracunculiasis. He also established the testing activity of important pests of public health importance to know the bio-efficacy against insecticide in laboratory and during his period the short term training program on Parasitology and medical entomology was started for those who are working with public health authorities. Dr. N.G. Chandekar, Assistant Director held the charge and continued the same activity till 1999. After him Dr. B. D. Sathe, served the department as Assistant Director. Nodal Laboratories for Re-emerging Disease such as Leptospirosis, Dengue and Chickungunya were established in due course. Mr. L.S. Patil was incharge in 2006. After this Dr. Mira Ramaiya took the charge of the department and besides earlier activities new training and testing methodologies were introduced with full zeal & devotion.

Present Status In research, the department is contributing to existing knowledge in Public health and Infectious diseases. The various projects in the department are aimed to understand-vector behaviour, Vector Control, Contribution of the genetic mutations towards insecticide resistance, Diagnosis of the disease, investigating the role of trace metals in disease pathophysiology, Screening of various natural compounds for therapeutic use, Novel drug delivery system, Development of new and improving existing vaccines. Zoonosis department has a sub-division of Venomous Animals named as 'Snake Farm' (Serpentarium) established in 1953 and housed in a separate building. This unit maintains various kinds of a poisonous,

non-poisonous snakes and scorpions. Snake farm supplies snake venom & scorpion venom for research purpose. The unit regularly conducts programs to create awareness in people, students for identification of snakes& snake bites. The snake farm is renamed as Venomous animal unit. The Department is a **Central Insecticide Board**, Ghaziabad, New Delhi approved centre and is actively engaged in testing for Bio efficacy, persistency, repellence, testing against crawling & flying insects, mosquito repellence test on human skin, Water resistance study and study of products for testing of Anti- Ant and Anti Wasp activity as per WHO guidelines. Ex-Director, Dr. Abhay Chowdhary (2008-2015) supported and added new facilitates to the department for research and testing.

The department has successfully organized various Training Programs, Workshops, Seminars and Lecture Series on the topics of public health interest from time to time. The workshop on “mission to control mosquito menace” aimed at training and teaching the college students and other interested professionals to create public health awareness in the control of Mosquito and mosquito borne diseases. A new training program on “Medical Entomology” is going to start shortly for students and professionals working in public health departments in view of health and socioeconomic burden created by vector borne diseases in India. I am sure; this Training Program will act as a milestone in the public education program. We are thankful to Mrs. Seema Vyas for her tremendous interest and vision to rejuvenate the research potential of the department.

Dr. Mira Ramaiya

Research Projects

1. Assessment of Dendritic cell vaccines as a potential Immunotherapeutic agent in Malaria (Ph.D project)

Technical Team

1. Dr (Mrs.) Ranjana A. Deshmukh
2. Dr. Abhay S. Chowdhary
3. Dr. Mira Ramaiya
4. Mr. Rahul A. Gosavi

Period

Oct 2010- Nov 2015

Introduction

In most tropical and many sub-tropical developing countries, malaria is a major cause of morbidity and mortality, especially among infants, young children and pregnant women. Dendritic cells based immunotherapy may be highly promising and novel method for the development of malaria vaccines. The present study mainly focuses on the role of pulsed and activated Dendritic cells response *in vivo* against *Plasmodium berghei* and *Plasmodium yoelii* in murine model.

Objectives

- 1) To **sensitize** murine bone marrow-derived Dendritic cells with parasitized RBCs.
- 2) To **check the efficacy** of Dendritic cells processed with parasitized RBCs (pRBCs)
- 3) To **transfer mature** murine **bone marrow-derived Dendritic cells** into the malaria infected mice
- 4) To check ***in vivo* immune response** elicited against *Plasmodium yoelii* (NL) and *Plasmodium berghei* (L) strain in murine model.

Results and Conclusions (in brief)

Cultures set up for the proliferation of Bone marrow derived Immature and Mature Dendritic cells: The principle method for generating Bone Marrow Derived Dendritic cell were carried out with different protocols such as by both GM-CSF and IL-4 (fig1a), 35.4% by IL-4 alone (fig1b), 75.0 % by GM-CSF alone (fig1c), 98% by adherent cell population (fig 1d) and 91% by non-adherent suspended cell population (fig 1e). The yield and purity of Dendritic Cells were checked by Flow cytometry.

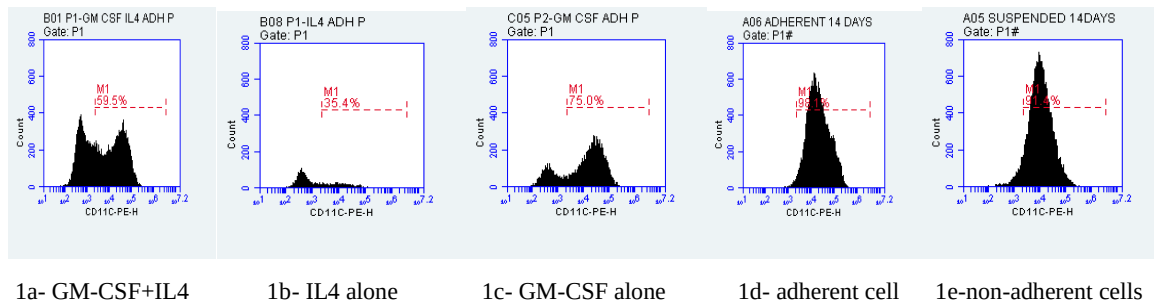


Fig. 1: BMDC generation with different protocols

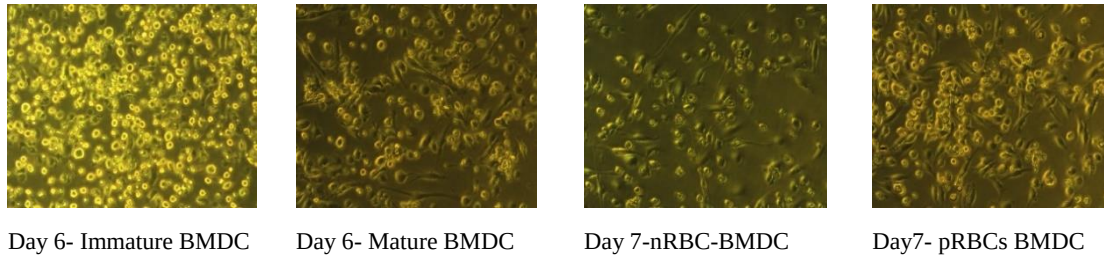


Fig. 2: Morphology of BMDCs

Here we show that whole erythrocytes isolated from mice infected with *Plasmodium* spp. can be taken up by DC, *ex vivo*, and stimulate powerful protective blood-stage malaria immunity *in vivo*. A novel and potentially useful pattern of protection was observed, with induction of Th1- and Th2-type cytokines, antibodies and long-term protection (90 days) after a single immunization. These results suggest that DC may be involved in the induction of natural immunity against blood-stage malaria parasites and further indicate that targeting of parasite antigens to DC may provide an attractive new approach for the development of blood-stage malaria vaccines. A better understanding of the early interactions of DCs with *Plasmodium* parasites will not only provide insights into the immunobiology of malaria infection but may also identify key target for the future development of vaccines and immunotherapy.

2. To Assess the Contribution of Sweat in Parasite Induced Attractiveness to Insect Host in Malaria Patients (Ph.D project)

Technical Team

1. Dr. Abhay S. Chowdhary
2. Dr. Mira Ramaiya
3. Mr. Chinmayanand Oroskar

Period

Dec 2011 -Aug 2017

Introduction

Protozoan parasites of the genus *Plasmodium* infect a wide variety of vertebrates, and are responsible for the devastating impact of malaria in humans. Parasites' twofold evolutionary concern is to multiply and be transmitted between hosts. So in order to facilitate its transmission from human host to mosquito, the prime intention of the parasite will be to attract the mosquito towards its human host. As it has been observed in the previous studies, that malaria infection increases attractiveness of humans to mosquitoes and taking into consideration that humans are potent attractants to mosquito, the parasite must be inducing some changes in its host, such that it becomes more attractive to the mosquitoes. Human sweat contains components that are attractive to anthropophilic mosquito species, and variation in sweat composition causes differential attractiveness to mosquitoes within and between individuals and also between humans and other mammals. Thus there lies a scope for the parasite; whose ultimate aim is to disseminate to the insect host i.e. mosquito (in order complete its lifecycle); to alter the composition of sweat in such a way that it would attract the mosquitoes. Thus this study will help to understand if the parasite is inflicting some change in the composition of sweat in such a way that it helps its transmission to the insect host. The understanding of how exactly does the parasite make the modifications in the sweat to cause the attractiveness; would be the outcome of this study.

Objectives

1. The first objective of this project is
 - a. To identify components of sweat in patients who are smear positive for malaria at Day 0 (baseline)
 - b. To identify components of sweat the same patients followed up at Day 60
2. To compare these patients with normal healthy participants without malaria.
3. To carry out experiments in *Anopheles* mosquitoes using the components identified.

4. To perform *in vivo* experiments on Anopheles mosquitoes to study the effect of the components of sweat.
5. To perform Molecular Docking studies for the selected compounds of sweat against Odorant Binding Proteins of Anopheles Mosquito using bioinformatics software. It will be performed to study the effect and binding pattern of odorant molecules with the Odorant Binding proteins.

Results and Conclusion

1. Sweat sample collection method using glass beads was successfully carried out. Glass beads were cleared off the impurities using an in house developed technique and were made suitable for use in sample collection.
2. Collection of sweat components on glass beads was successfully achieved and confirmed using Gas Chromatography.
3. Solvent extraction method for sample collection was successfully using Ethanol (analytical grade)
4. Hardware modification were successfully done in the GC-MS instrument to facilitate use of chromatoprobe for analyzing sweat samples collected using glass beads.

3. Interplay of Trace Elements in the pathophysiology of a Murine Model of Malaria (Ph. D project)

Technical Team

1. Dr. Abhay S. Chowdhary
2. Dr. Mira Ramaiya
3. Ms. Mansi Malik

Period

May 2014 – May 2017

Introduction

Malaria still constitutes one of the most important infectious diseases, affecting 300–500 million people and causing the death of 1.5–2.5 million people annually, many of whom are Young children. Although several reports have implicated elevated levels of ROS in the pathogenesis of malaria, the early and stage specific and tissue specific detection is still not attainable. Transition metals have a very important role to play in the oxidative stress damage and the disease pathophysiology. The up-regulation of many phase II proteins, including ferritin, heat shock proteins, MTs (metallothioneins) and thioredoxin, protects against oxidative stress induced cellular injury. Metals such as iron or copper cannot be free under physiological conditions (pH, biological components with high affinity toward transition metals) and are always bound to biological sites such as proteins and membranes. Metallothioneins are also induced and are reported to harbour antioxidant function. MT genes are ubiquitously expressed in many tissues of basal levels, and their expressions are induced readily by many factors, especially by metal ions such as cadmium, zinc, and copper. Metals Iron(Fe), Copper(Cu), Selenium(Se), Zinc(Zn), Magnesium(Mg) are released into the cellular milieu with Oxidative stress conditions and thus the transcription and subsequent translation of these metal binding proteins viz Transferrin, Ceruloplasmin, Metallothionein I, II & III, Gamma glutamyltransferase, Glutathione peroxidase I needs to be investigated.

Objectives

1. To establish parasitaemia in experimental murine models (C57BL & BALB/C) & harvest the organs Liver, Brain & Spleen at subsequent stages of parasitaemia.
2. To quantitatively estimate trace elements such as Fe, Se, Cu, Zn, Se and Cr from the Liver, Brain and Spleen tissue homogenates.
3. To assess the Gene and protein expression of the Transferrin, Ceruloplasmin, Metallothionein I, II and III, Glutathione Peroxidase I, Gamma Glutamyl Transferase, metallothionein isoform (I, II and III) in the Liver, Brain and Spleen tissues

4. To quantify and to localize the specific metalloproteinase such as Transferrin, Ceruloplasmin, Metallothionein I, II and III, Glutathione Peroxidase I, Gamma Glutamyl Transferase in the Liver, Brain and Spleen tissues and establish its correlation with the disease progression.

Results & Conclusions

1. The expression of MT1 and MT2 in the liver spleen and brain tissues was found to be up regulated in intermediate and severe parasitaemia as compared to that of Mild parasitaemia.
2. MT3 gene expression was slightly up regulated in the brain tissue however an increase in the protein expression was observed in brain and specifically in severe parasitized brain.
3. The gene expression and further the increased protein expression of MTI, II,, III & MT1/2 were observed to be in tandem thus confirming the role of oxidative stress increasing with the increase in the malaria infection. The up regulation of these isoforms can also be explained as a result of the manifestation of pathology caused due to the parasite, as the liver, spleen and brain are the major affected organs through which disease progresses.
4. The free zinc measured by means of ICP-AES depicts decrease in the severe parasitized liver and brain, which explains the up regulation of the translated MT isoforms to chelate&/sequester the increased zinc in the cellular microenvironment.

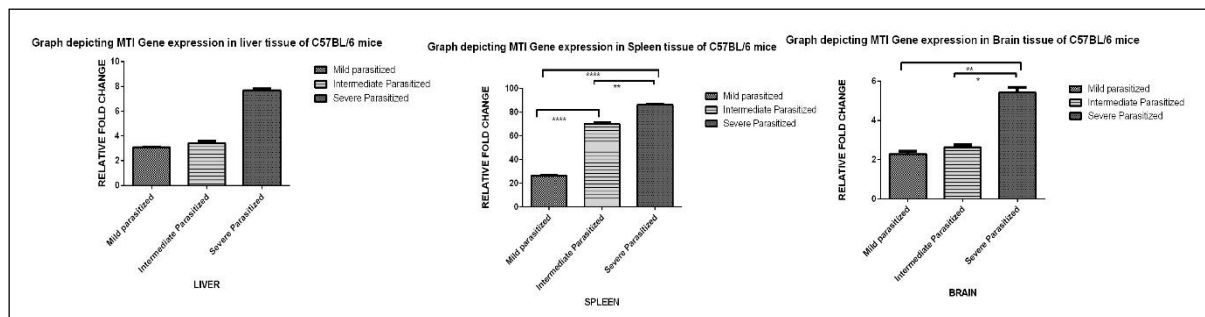


Figure 1A. MT I gene expression in Liver, Brain & Spleen

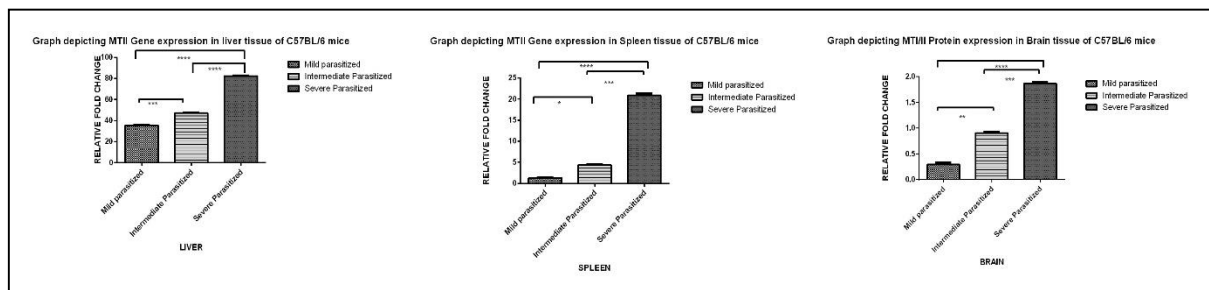


Figure 1B. MT II Gene expression in Liver, Brain & Spleen

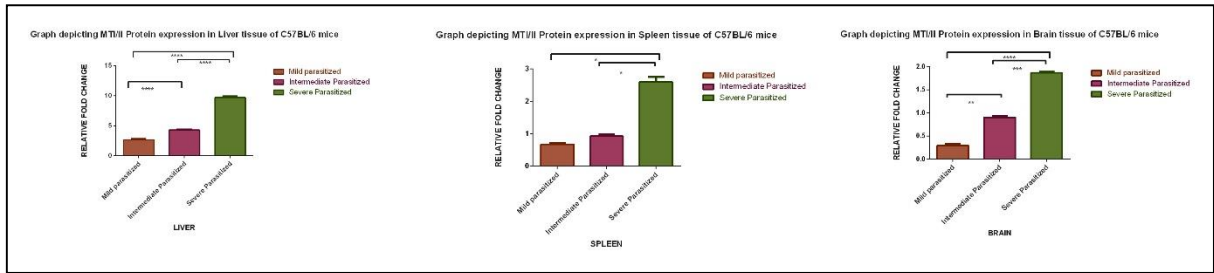


Figure 1C: MTI/II Protein Expression in Liver, Spleen & Brain

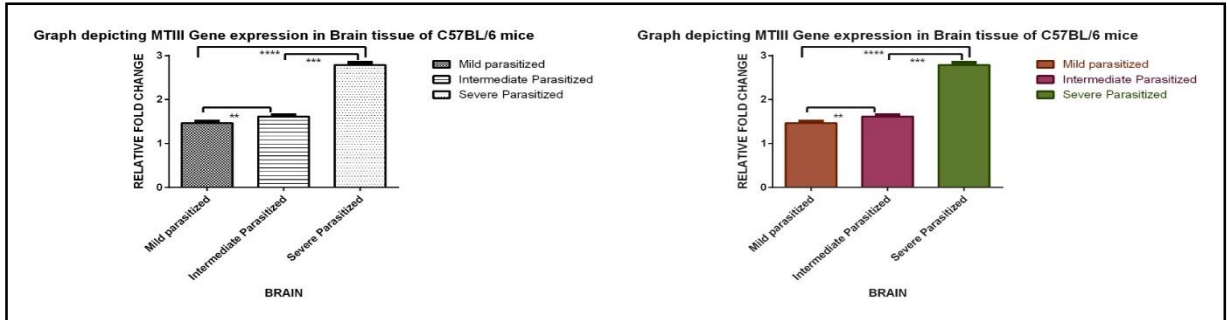


Figure 1D: MTIII Gene Protein Expression in Brain

4. In vitro production of plasmodium falciparum gametocytes and screening of agents blocking malaria transmission

Technical Team

1. Dr. Saroj Bapna
2. Dr. Mira Ramaiya
3. Mrs. Trupti Satvekar

Period

Nov 2015 – Dec 2017

Introduction

Gametocytes, the sole stage of malaria parasites capable of infecting mosquitoes, represent a key link in human/vector malaria transmission. Development of new antimalarials have traditionally been focused on asexual blood stages which are responsible for the proliferation of the parasites in human host and disease symptoms. Sexual stages has not been studied in details as asexual stages due to lack of standardized in vitro culture and identification of sexual developmental stages which are important gametocidal drug targets. The present project designed to develop a standardized in vitro production of gametocytes. Considering the key factors involved in gametocytogenesis, such as the reduction of asexual stages by heparin and addition of lysed uninfected erythrocytes to manipulate culture conditions. The pure gametocyte culture free from asexual stages will be used for screening of new compounds effective against gametocytes to prevent transmission of malaria.

Objectives

1. To setup an optimized and improved method for production of sexual stages (gametocytes) of *Plasmodium falciparum* in vitro
2. Screening of natural compounds for gametocidal activity to identify new malaria transmission blocking drug molecule.

Results and Discussion

Continuous culture of *Plasmodium falciparum* 3D7 (Chloroquine sensitive) and (Dd2 (CQ resistant) are maintained in vitro. Induction of gametocytes production with manipulation in culture conditions are achieved by reduction in sexual stages using heparin and addition of lysed uninfected erythrocytes initiated gametocyte production by day 7 and increased until day 15 (Fig. 1). Identification of different developmental stages of gametocytes (I-V) are in progress.

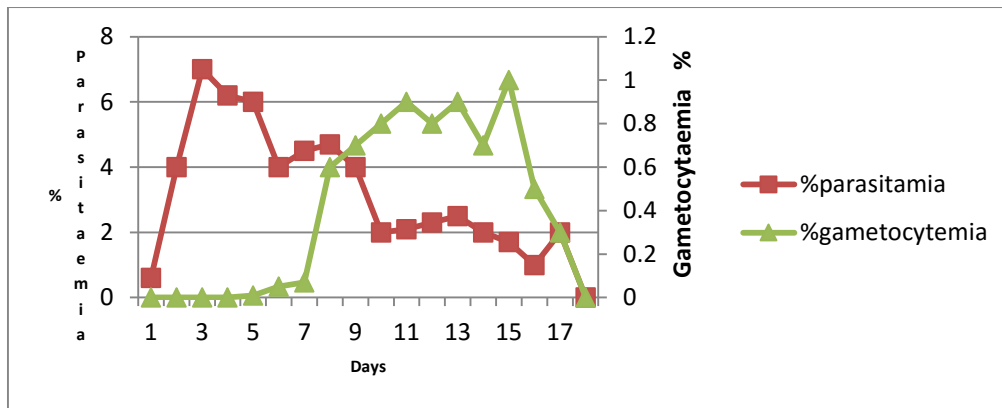


Figure 1. Progression of *Plasmodium falciparum* 3D7 gametocyte with time course

Conclusion

Considering key factors involved in gametocytogenesis, by manipulation in culture condition, we propose an optimized and efficient strategy for the in vitro production of *P. falciparum* gametocytes based on the comparison of different protocols. The method developed will allow the accurate determination of the gametocidal activity of new antimalarial drugs against each developmental phase of the sexual blood stage to prevent transmission of malaria.

5. Evaluation of herbal, biological and common household disinfectants against mosquito larvae.

Technical Team

1. Dr. Saroj Bapna
2. Dr. Mira Ramaiya
3. Mrs. Trupti Satvekar

Period

Jan 2016 – Dec 2016

Introduction

Mosquitoes are important pests that affect human health and well-being throughout the world¹. The problems of mosquito transmitted diseases are quite severe worldwide resulted in high morbidity and mortality. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. The mosquito *Aedes aegypti* is responsible for transmitting dengue virus. These mosquitoes are associated with the living spaces of humans. They generally spend their entire lives in and around the houses where their eggs hatched. Difficulty of accessing indoor larval habitats of *Ae. Aegypti* (e.g. water-storage containers, plant vases, saucers) to apply larvicides is a major limitation in many urban contexts. Keeping this in mind the present study was aims

Objectives

To evaluate the potential of different household disinfectants, herbal and biological agents to check *Aedes aegypti* mosquito larvae in domestic environment.

Results and Discussion

The results of five disinfectants (Mopz™, Lizol, Dettol, Savlon, Sterillium® hand Sanitizer) evaluated against third stage larvae of *Aedes aegypti*, only mopz™ and Lizol demonstrated promising larvicidal activity with 100% mortality below 0.04 % concentration followed by Dettol the same activity at 0.08% concentration. Savlon and hand sanitizer Sterillium®, were not found effective against larvae.

Table I: Larvicidal activity of household disinfectants against *Aedes aegypti* larvae

Sr.No	Disinfectants	% mortality Of larvae after 24 hrs*					
		% Concentration					
		0.1	0.08	0.06	0.04	0.02	0.01
1	Mopz TM	100	100	100	100	55.64±2.61	8.17±0.92
2	Lizol	100	100	100	100	31.37±1.92	2.03±1.05
3	Dettol	100	99.01±0.65	31.94±8.16	07.38±3.12	00.00	00.00
4	Savlon	2.91±1.19	-	-	-	-	-
5	Sterillium®	1.08±0.73	-	-	-	-	-
6	Water control	0.00					

Conclusion

The present investigation was carried out to know the potentials of different house hold disinfectants under different trade names against *Aedes aegypti* mosquito larvae. Outbreak of dengue makes government agencies to enforce in-house survey and survey of residential colony. Searching each and every house is not practically possible and faces lots of practical problems. In such situation the promising larvicidal activity of disinfectants makes them very important measures for *Aedes* control. The results are particularly interesting because household disinfectants are ready to use larvicide in domestic environment. Further studies are in progress to identify more such compounds from herbal and biological source to develop handy larvicide for practical use. The findings of household disinfectants are sent for publication in Indian Journal of medical research (under review).

6. Isolation & Identification of the novel bacterium having mosquito Larvicidal activity

Technical Team

1. Dr. Avinash Kale
2. Dr. Abhay Chowdhary
3. Dr. Mira Ramaiya

Period

Jan 2014 - Jan 2017

Introduction

There are millions of people all around the world losing their life due to various mosquito borne diseases like malaria, dengue, chickungunya, etc. The different methods employed to control these lethal diseases are through vaccination, medication or precaution against the vector but still human deaths due to the above mentioned diseases are tremendously increasing. Most of the biologists are more interested in studying the mechanism of invasion of the parasite in human body but very few of them actually focus on the control of the vector- (mosquito). Therefore, in an attempt to take one step ahead in the field of controlling the number of vectors instead of the actual parasite, this research work mainly includes identification of some bacteria having mosquito larvae-cidal activity. A lot of studies have been done on larvicidal toxins produced by *Bacillus* spp. like *B. thuringiensis* & *B. sphaericus* known to produce crystal toxins like cry, bin8 , sphaericolysins and so on. The aim is to collect different species of dead mosquito larvae from the wild environment and to isolate & identify their gut microflora which might be responsible for their larvicidal action. The isolates will be used to check their toxicity level on lab cultured larvae of different species and the one showing maximum toxic potency will be used for further proteomic studies.

Objectives

1. Collection of different species of dead mosquito larvae from all around the city
2. Isolation of a novel bacterium from their midgut responsible for their toxicity.
3. The structural & biophysical study of the toxins at their atomic & molecular level.
4. Elucidating various ways to use these toxins against larval population.

Result and Conclusion

Collection of mosquito larvae of different species from all the three divisions of Mumbai (Western, Central & Harbour line).

Isolation of their gut micro-flora on the three media selective for gram positive bacteria.

Currently carrying out high-throughput toxicity assay of the individual bacterial strain on the mosquito larvae of *Culex*, *Aedes* & *Anopheles* species.

So far 25 strains have been tested and repeated for mosquito larvae-cidal activity out of which 10 strains have been selected for showing a range of mortality between 5% to 60% Identification of these 10 strains by 16S rDNA sequencing is in process.

7. Assessment of BCG as an Adjuvant in Preparation of Japanese Encephalitis Vaccine

Technical Team (Ph.D project)

1. Dr. Abhay S. Chowdhary
2. Dr. Mira Ramaiya
3. Mr. Sahil Kulkarni

Period

Oct 2013 - Sept 2016

Introduction

Japanese encephalitis (JE) is the leading cause of Acute Encephalitis syndrome (AES) and Other neurological impairment in the tropics. SA-14-14-2 live attenuated JE vaccine. However there are several concerns with the use of these vaccines as addition multiple Booster doses are required and to maintain immunity. Hence there is a prime concern and need of developing a safe and effective vaccine with an adjuvant to enhance the immune response in order to provide long term immunity with reduced booster doses. The aim of our study is to evaluate the efficacy of SA-14- 142 virus vaccine using BCG as an adjuvant. BCG itself is a known potent adjuvant as a single inoculate can elicit long-term immunity. It has been demonstrated that relatively low doses of BCG lead to an almost exclusive cell-mediated, Th1 response, while higher doses can induce a mixed Th1/Th2 response. To carry out the work 40 (13 days old) BALB/c suckling mice were inoculated with live attenuated vaccine virus (SA-14- 14-2) subcutaneously and BCG vaccine intra-peritoneally. The study groups were as follows Group I: SA-14- 14-2 with BCG, Group II:SA-14- 14-2, Group III: Vaccine Diluent , Group IV: BCG vaccine control. Blood was collected from each group retro-orbitally at an interval of 15 days post inoculation. Expression of different pro inflammatory and anti-inflammatory cytokines and chemokines - TNF- α , IL-1 Beta, IL-4, IL-5, IL-10, IFN- γ and IL-6 (GAPDH as housekeeping gene) was studied by one step qRT-PCR using RNA extracted from blood. IgG1, IgG2a levels have been measured to determine the TH1 and TH2 responses. JEV neutralising antibody titre from serum will be quantitatively measured using Neutralisation assay. These immunized mice were further challenged with an appropriate multiple of LD 50 dose of Japanese encephalitis virus intravenously.

Preliminary results indicate that BCG as an adjuvant to the SA-14-14-2 vaccine is capable of eliciting an increased antibody response than the vaccine alone.

Objectives

1. To Assess neuro protection conferred by BCG if any to JE Virus Infection
2. To increase efficacy of the vaccine against JEV, by using BCG as an adjuvant.

3. To make the vaccine more effective in pediatrics and geriatrics. Study shall be established in suckling mice and aged mice.

Result & Conclusion

1. The Clinical symptoms and cytokine data of IL6, iNOS, TNF alpha obtained indicates a neuroprotective role of BCG when administered prophylactically in murine model of Japanese Encephalitis Virus.
2. IL4, IL5, IL6, TNF alpha, IFN gamma, IL1, IL10, etc. Levels were studied and they indicate that when the vaccine is adjuvinated with BCG we have a stable and long lasting immune response.
3. The levels of Antibody that were estimated with Indirect ELISA showed a more balanced TH1/ TH2 response in the group adjuvinated with BCG as compared to just the Vaccine and This may lead to a more stable long term Humoral Immunity
4. Our study shows that BCG augments SA-14-14-2 immune response.

8 Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations (completed)

Technical team

1. Ramaiya M
2. Patil V N
3. Bobade AS
4. Ghag- Sawant M S,
5. NarayakkadanA
6. Asrondkar A L

Please see details under Research Projects based on Ayurveda

9. Comparative Assessment of immunomodulatory activities of three plant extracts and their synthesized nanoparticles. (Ph.D project)

Technical team

1. Mrs. Pallavi Kurkure Choudhary
2. Dr. Mira Ramaiya
3. Dr. Abhay Chowdhary

Please see details under Nanoparticle Research

Publications

1. Antimalarial activity of Argemone Mexicana: an in vivo and in vitro study. Bapna S, Choudhary K. P, Ramaiya M, Chowdhary A. World journal of Pharmaceutical Research, 2015, 4(11), 1653-63.
2. Larvicide and growth retarding activity of alkaloidal extract of two plants towards *Aedes aegypti* (Dipteria: culicidae) Bapna S, Shinde. R. Satvekar T, Ramaiya M. World journal of Pharmaceutical Research: 2015, 5(01), 998-1004

Sent for publication

1. A novel environmental friendly mosquito control strategy without chemical use Bapna S. International Journal of Mosquito Research (In press) May 2016.
2. Control of dengue vector *Aedes aegypti* using house hold disinfectants. Bapna S, Satvaker T, Ramaiya M, Indian Journal of Medical Research May 2016.
3. Surveillance of JE virus infection in *Culex* mosquitos' larvae in and around Mumbai. Kulkarni S, Sheikh A, Sheikh R, Sinde R, Mahale P, Bapna S, Ramaiya M, Chowdhary A. Journal of Arthropod- borne Diseases May 2016.

Department of Clinical Pathology

Head of Department's Overview

The Department of Clinical Pathology was established in the year 1905. From its very inception the department undertook studies on plague and diagnostic and investigational studies on almost the entire range of communicable diseases like cholera, Malta fever (Brucellosis), typhoid, malaria, diphtheria, and relapsing fever. At present the department is actively involved in Training, Research and Testing activities.

The major thrust areas of the department includes Tuberculosis, Hospital infections and Nanotechnology. In particular, the department is actively involved in research on various infectious diseases like Tuberculosis, Methicillin Resistant Staphylococcus Aureus (MRSA), Extended Spectrum β - Lactamase (ESBL) and Metallo β - Lactamase (MBLs). The department is interested in the development of diagnostic tests for Tuberculosis and herbal anti tuberculosis compounds, also research in autoimmune disorder (Scleroderma) in collaboration with National Institute of Immunohaematology. The department is also focussing on diagnosis and drug development, including conventional and molecular techniques for diagnosis of various infections like Tuberculosis and other hospital infections like MRSA, ESBL etc. Analysis of drug resistance in these agents and drug development with herbal and contemporary chemical synthesis along with aspects of nanotechnology is also part of the departmental activities. The department is also involved in biosynthesis of nano particles, checking anti-bacterial activity of Kombucha (Chinese tea) and Caffeine. The department also carries out anti-bacterial and antifungal activities of different fruit juices. This year, the department could publish ten original research articles in the topics of mycology, mycobacteriology and antimicrobial activities in national and international scientific journals.

The department is involved in testing activities in the areas of hematology, serology, bacteriology, mycology and mycobacteriology. It receives clinical samples from E.S.I.S. hospitals, govt. hospitals and public health dispensaries. The department also carries out serological tests for diagnosis of auto-immune diseases and other infectious diseases. It conducts anti-microbial, anti-mycobacterial and antifungal testing of synthetic compounds developed by the research centres, colleges and pharmaceuticals. The department conducts bacteriological investigation of Operation theatre post fumigation. Operation theatre swabs received from various primary Health care centres and rural hospitals.

The department participates in training programmes like Annual Biotechnology Training and Viro-vitals. Department also train students for M.Sc. and Ph.D. degree in Microbiology and

applied Biology affiliated to Mumbai University and Ph.D in Medical Microbiology from Maharashtra University of Health Sciences (MUHS) Nasik.

The department is also at the forefront in maintaining record and conducting Institutional Ethics Committee and Institutional Animal Ethics Committee meetings. This year clinical pathology department played an instrumental role in organizing and successfully conducting the Research Advisory Council and Academic council research meeting. These meetings have led to appreciation of research work carried out in the Institute by the notables in the field. Various students from other departments also work in the department and avail the facilities, with due acknowledgement to the department.

The department has future plans of conducting training program on “Scientific Writing” and “Pharmaceutical Microbiology”. The department has also planned training for health care workers on “Operation Theatre Infection Control and Prevention”. The department has future plan to reintroduce various histopathological and cytological examination of biopsy specimens. Long term goals, for research are extensive studies in diagnostic tool for Tuberculosis and hospital infections. And genomic understanding of mutation and drug resistance in such infections. For “testing”, are recognized and accredited laboratory, to become a surveillance centre for testing of infectious diseases in general and in case of epidemic situations. And for “training”, are to impart training to healthcare professionals in fields of molecular biology, pathology and infection control. And conduct customized training programmes in microbiology, mycology, mycobacteriology, tissue culture, pharmaceutical microbiology and molecular biology.

Dr. Shashikant P. Vaidya

1. Comparative Analysis of Different Molecular Methods for Diagnosis of Multi-Drug Resistant Tuberculosis (M.Sc Project)

Technical Team

1. Dr. Shashikant P Vaidya
2. Mr. Jaishankar S Sharma

Period

Dec 2012 - Apr 2016

Introduction

Tuberculosis (TB) is an infection with *Mycobacterium tuberculosis* (*M.tuberculosis*) which can occur in any organ of the body but is most well known in the lung. TB declined after the late 19th century but remained a major public health issue as it still is today. It is a major global health problem. In 2014, there were an estimated 9.6 million new TB cases and 1.5 million TB deaths. It is an infectious disease caused by the bacillus *M.tuberculosis*. TB is the only disease ever declared a global emergency by the WHO. Diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture. Signs and symptoms in Extra Pulmonary TB are variable and may depend on the organ/system involved. Treatment is difficult and requires long courses of multiple antibiotics. Antibiotic resistance is a growing problem in Multi-Drug-Resistant tuberculosis (MDR-TB). Prevention relies mainly on screening programs and vaccination, usually with *Bacillus Calmette-Guérin* (BCG) vaccine. The challenge now is to combat the increasing MDR-TB. However a significant obstacle in controlling TB is the amount of time required to reach a diagnosis. Due to the slow growth rate of *M. tuberculosis*, the culture methods can take up to 6 weeks. This can cause significant delays in identifying MDR cases, adjusting treatment regimens, and initiating epidemiological investigations. In view of these limitations, there is a need for rapid and accurate tests for diagnosis of drug resistant TB. In the present study we shall perform a comparative analysis of the different molecular diagnostic procedure available for the diagnosis of TB. The study shall be useful to identify the best possible methodology in the detection and diagnosis of MDR-TB.

Objectives

The aim of this study is to compare different molecular methods for detection of TB with the following objectives:

- To detect TB by phenotypic methods like microscopy and culture based method.
- To carry out DST with RIF and INH.
- To detect MDR-TB by molecular methods like PCR, hybridization techniques, etc.
- To identify MDR-TB from the suspected cases of tuberculosis.

Results and Conclusions

1. Table 1: AFB staining No. of samples: 105

Sr No.	AFB status	Number of samples (%)
1	Positive	82
2	Negative	18

82% of the sputum samples were found to be positive for AFB by ZNCF Staining.

2. Table 2: Culturing on LJ Medium (TB results) No. of samples: 105

Sr No.	Culture	Number of samples (%)
1	Positive	80
2	Negative	20

80% of the sputum samples were found to be Culture positive.

3. Table 3: Para-Nitro Benzoic acid Test: (Differentiation between MTB Complex and MOTT) No. of samples: 105

Sr No.	Group	Number of samples (%)
1	MTB Complex	53
2	MOTT	27
3	Negative	20

53% of the all the sputum samples were from the Mycobacterium tuberculosis Complex whereas 27% were MOTT.

4. Table 4: Drug Susceptibility Test (Drug resistance)

DST was carried out for those samples which were found to be of MTB Complex Group. No. of samples: 56

Sr No.	Status	Number of samples (%)
1	MDR-TB	34%
2	Drug Susceptible	50%
3	RIF Mono-resistant	4%
4	INH Mono-resistant	12%

34% of the all the sputum samples from the MTB Complex group were MDR-TB.

5. Table 5: Line Probe Assay (TB results) No. of samples: 105

Sr No.	TB	Number of samples (%)
1	Positive	95
2	Negative	05

95% of the sputum samples were found to be TB positive by LPA.

6. Table 6: Line Probe Assay (MTB Complex and MOTT results) No. of samples: 105

Sr No.	Group	Number of samples (%)
1	Mycobacterium tuberculosis Complex	61
2	Mycobacterium Other Than Tuberculosis (MOTT)	34
3	Negative	05

61% of the all the sputum samples were from the Mycobacterium tuberculosis Complex whereas 34% were MOTT.

7. Table 7: Line Probe Assay (Drug resistance)

Results of those samples which were found to be of MTB Complex Group. No. of samples: 64

Sr No.	Status	Number of samples (%)
1	MDR-TB	39%
2	Drug Susceptible	40%
3	RIF Mono-resistant	6%
4	INH Mono-resistant	14%

39% of the all the sputum samples from the MTB Complex group were MDR-TB.

8. Table 8: Multiplex PCR (Differentiation between MTB Complex and MOTT)

No. of samples: 105

Sr No.	Status	Number of samples (%)
1	Mycobacterium tuberculosis Complex	59
Out of 59% of all the sputum samples 61% were <i>Mycobacterium tuberculosis</i>		

9. Table 9: Real Time PCR

All the samples which were found to be positive for MTB Complex by Multiplex PCR were subjected for Real Time PCR. High Resolution Melt (HRM) Curve Analysis was done.

No. of samples: 69

Sr. No.	Mutation Targeted	No. of Variants found
1	rpoB 531 and rpoB 526	15
2	rpoB 516	3
3	katG 315	3

All the different variants were sent for sequencing analysis. Results awaited.

10. Table 10: Comparison of conventional method, LPA and Multiplex PCR for detection of Mycobacterium tuberculosis Complex (MTB complex):

Sr No.	Item	Conventional method (PNB Test)	LPA	Multiplex PCR
1	MTB complex	53 %	61 %	59%

Conventional method showed 53%, in LPA 61% and by Multiplex PCR 59% of the sputum samples were MTB Complex.

11. Table 11: Comparison of All the methods for Diagnosis of MDR-TB

	DST	LPA	REAL TIME PCR
TIME (Results) (Approx.)	42 Days	12 Hours	20 Hours
COST (Approx.)	Rs. 150 to 200/-	Rs. 1200/-	Rs. 800 to 1000/-
INFRA-STRUCTURE	Bio-safety	Bio-safety, Centrifuge, Conventional PCR, Twincubater	Bio-safety, Centrifuge, Conventional PCR, Gel Electrophoresis, Real Time PCR,
MAN-POWER	Skilled laboratory Personnel required	Skilled laboratory Personnel required	Skilled laboratory Personnel required

Laboratory diagnosis of TB still involves the smear examination and culture confirmation for MTB. Conventional culture confirmation using LJ medium has the disadvantage as the time required to observe the growth is high. Since the TB bacilli takes around 4 weeks to grow. This contributes in delay of treatment, spread of the disease and leads to unnecessary treatment of non-specific pulmonary infections. Nevertheless, conventional method still remains the gold standard for culture and DST of TB. It is useful for early confirmation of viable TB bacilli, observing response to treatment in MDR-TB. Significant advances have been made in the rapid and accurate diagnosis of MDR-TB. The use of molecular tests can reduce the need for invasive diagnostic procedures, which are both costly and pose an added risk to the patient. These tests are reliable, rapid and easy to perform for the simultaneous detection of RIF and INH resistance in TB. With high sensitivity and high specificity obtained in this study with respect to detection of MDR-TB, we can conclude that this test strongly assists in adequate treatment of MDR-TB, long before the results of conventional DST are available. Such molecular methods contributes in many ways; significant reduction in the time to diagnose MDR-TB, the earlier initiation of

appropriate therapy and the potential to prevent transmission of drug-resistant strains constitutes the major advantages of such methods. These methods require skilled operators, good infrastructure and relatively high expenses. The molecular tests serve as an early guidance of therapy, which can be followed by a phenotypic DST confirmation for all suspected MDR-TB patients.

2. The study of differential expression of matrix metalloproteinase genes in macrophage infected with *Mycobacterium tuberculosis* (M. Sc project)

1. Dr. Shashikant Vaidya
2. Mr. Jinesh Maniar

Period

Mar 2014- Dec 2016

Introduction

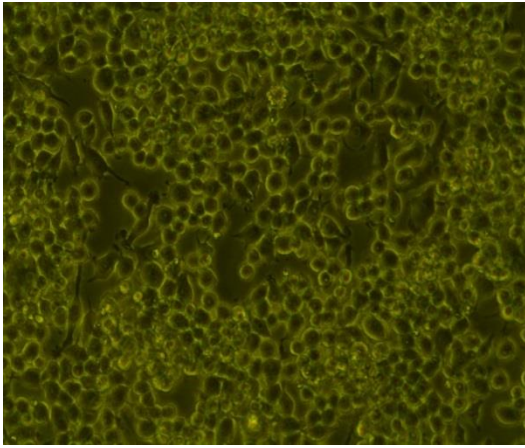
In order to initiate a successful infection, *Mycobacterium tuberculosis* must drive destruction of the lung extracellular matrix. Cavity formation must involve the action of proteases, specifically matrix metalloproteinases (MMPs), which are able to degrade the structural fibrils of the lung. Matrix metalloproteinases (MMPs, matrixins) are a family of secreted and membrane- bound zinc-dependent endopeptidases that have the combined capacity to degrade all the components of the extracellular matrix. From the recent studies it has become clear that MMPs are involved in pathogenesis of *M. Tb*

Objectives

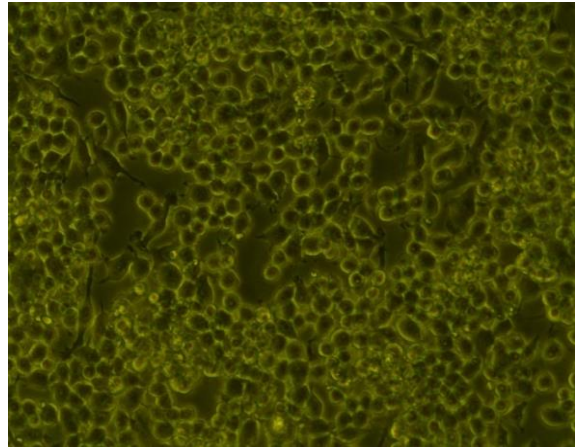
1. To establish tuberculosis infection in all of murine macrophage cell line.
2. To determine the effect of infection on regulation of genes (MMP genes) responsible for development and severity of infection.
3. To compare the expression of MMP genes between the above mentioned cell line.

Results and Conclusion

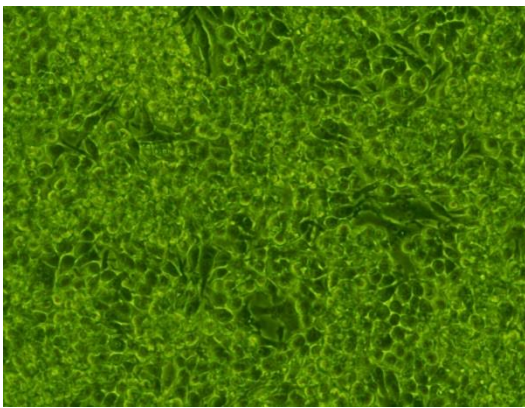
1. MOI 1:10 generated a stable infection for 10 days and the cytopathic effect of the infection was clearly visible at 4th day of the infection. MOI of 1:100 produced an infection of 4 days while MOI of 1:250 severely altered the cell morphology after 6 hours of the infection.



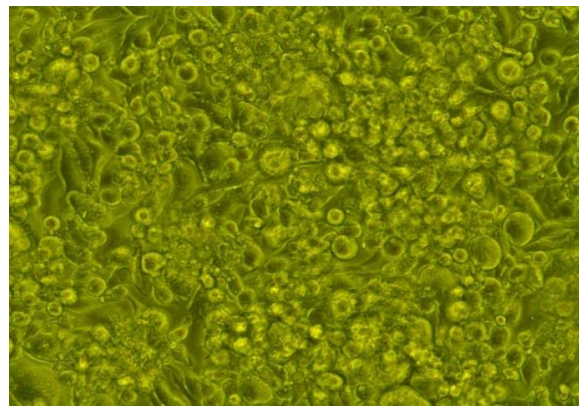
Infection at Day 0 at MOI 1:10



Infection at Day 1 at MOI 1:10



Infection at Day 4 at MOI 1:10



Infection at Day 8 at MOI 1:10

2. **MMP and IFN-gamma gene expression at Day 0**

MMP-3 was chiefly upregulated followed MMP-9 and then MMP-12. IFN-gamma was also upregulated after 6 hr of infection but its upregulation was less compared to MMP-3. Strain to strain variation was observed in regulation of these genes.

3. **MMP and IFN-gamma gene expression at Day 1**

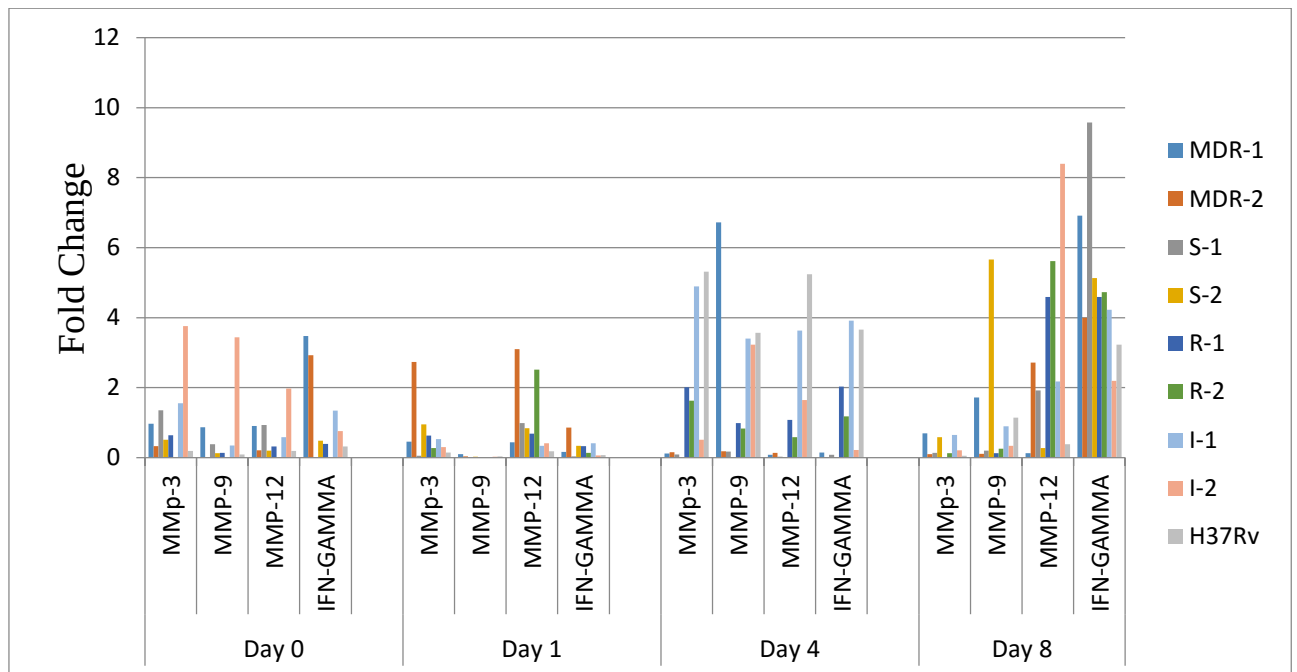
Among the four genes, expression of MMP-9 was virtually undetected at this time point in infection of most of the strain. MMP-12 expression level were much higher compared to expression of others gene at Day1 followed by IFN-gamma and then MMP-3. Similar to 6 hours infection, strain to strain variation was observed in expression of particular gene.

4. **MMP and IFN-gamma gene expression at Day 4**

After four days of infection the chiefly upregulated gene was MMP-9 followed MMP-3 and MMP-12 and the least upregulated gene at day 4 was IFN-gamma.

5. MMP and IFN-gamma gene expression at Day 8

After 8 days of infection IFN-gamma and MMP-12 were highly upregulated and expression of MMP-3 and MMP-9 was at much lower level than the other two genes.



6. In Tuberculosis infection of cell line the infection is divided to three different phases depending upon various physiological and morphological changes in cell line due to infection. Display of cytopathic effect marks the beginning of middle phase and phase before it is generally considered as early phase of infection. The point, during the course of infection at which the cells began to deadhere marks the beginning of late phase.

7. At Day 1 the expression of MMP-3 was reduced then it again peaked at day 4. At Day 4 cytopathic effect due to TB infection was visible. During late phase of infection expression of MMP-3 was reduced to much greater extent which demonstrate that MMP-3 is not much needed during this phase which marks the beginning of detachment of infected cell.

8. During the course of infection MMP-9 was highly upregulated at Day 4 and Day 8 compared to other two time points. This demonstrates that MMP-9 may not be crucial for

early phase of infection since it was not detected at after 24 hours of infection of most of the strains. Studies have correlated the presence of MMP-9 with disease severity in case of tuberculosis. Thus, with the progression of TB its severity increases, this explains the upregulation of MMP-9 at Day 4 and day 8.

9. MMP-12 was highly upregulated during the course of infection of most of the strain than other MMP. Thus, this MMP plays a critical role on TB infection as the activation of infected macrophages depends on this presence.

10. After 6 hours of infection IFN-gamma was extensively upregulated compared to other genes under study because IFN-gamma is one of the gene which is primarily upregulated in case of MTB infection. High level of IFN-gamma transcripts were also detected at day 4 and 8 but day 8 had the highest level of upregulation of this gene compared to other time point. Thus, as the severity of disease increases the expression of IFN-gamma is also increases.

11. The growth curve of *M.tuberculosis* isolates is to be performed to correlate the effect of MTB isolates with the expression of MMP genes.

3. Confirmation and Characterization of Extended Spectrum β -Lactamases (ESBL) in Multidrug resistance Gram Negative Bacteriam (part of Ph.D project)

Technical Team

1. Dr. Shashikant P. Vaidya
2. Mr. Jaishankar Sharma
3. Mr. Anuruddha Barua

Period

Nov 2015- Apr 2016

Introduction

Resistance to β -lactam antimicrobial agents is on the rise worldwide. Extended Spectrum β -Lactamases are mutant, plasmid mediated beta-lactamases derived from older broad-spectrum beta-lactamases which have an extended profile that permits hydrolysis of penicillins, extended spectrum cephalosporins and monobactams. These enzymes can be chromosomal or plasmid-mediated and are encoded by genes such as TEM, SHV, CTX-M, VEB, PER and GES (3). In addition, ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and cotrimoxazole resulting in limitation of therapeutic options. Understanding the molecular foundations of resistance can play an important role in developing new strategies to fight against these infections (4). The study thereby aims to detect and characterize the ESBL producing clinical isolates using conventional and molecular methods.

Objectives

1. To evaluate the antibiogram pattern and assess the MDR status of the clinical isolates.
2. To confirm ESBL production by phenotypic method among the clinical isolates.
3. To detect *bla*TEM, *bla*SHV and *bla*CTX-M genes in the isolates by PCR.
4. To correlate the antibiotic susceptibility pattern of ESBL producers with ESBL gene carried by them.

Results and Conclusions

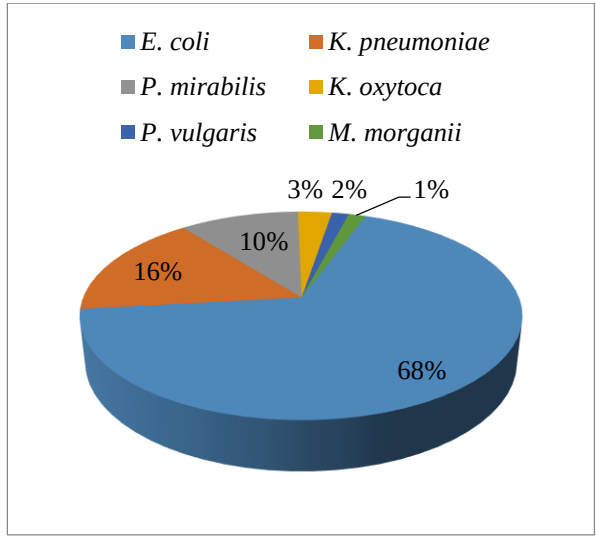


Figure 1. Distribution of clinical isolates

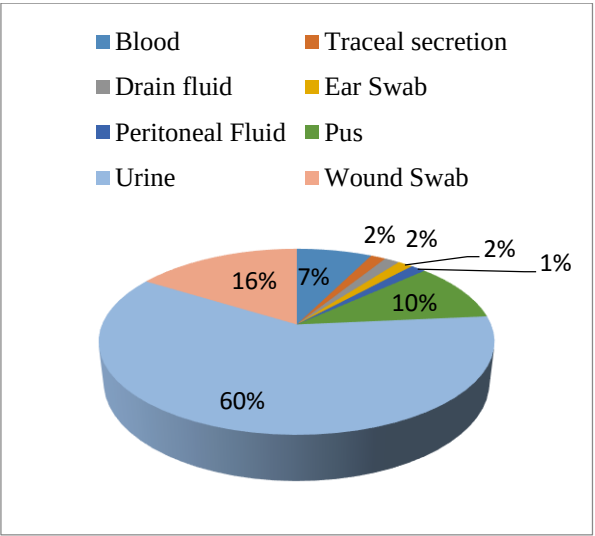


Figure 2. Distribution of sample types

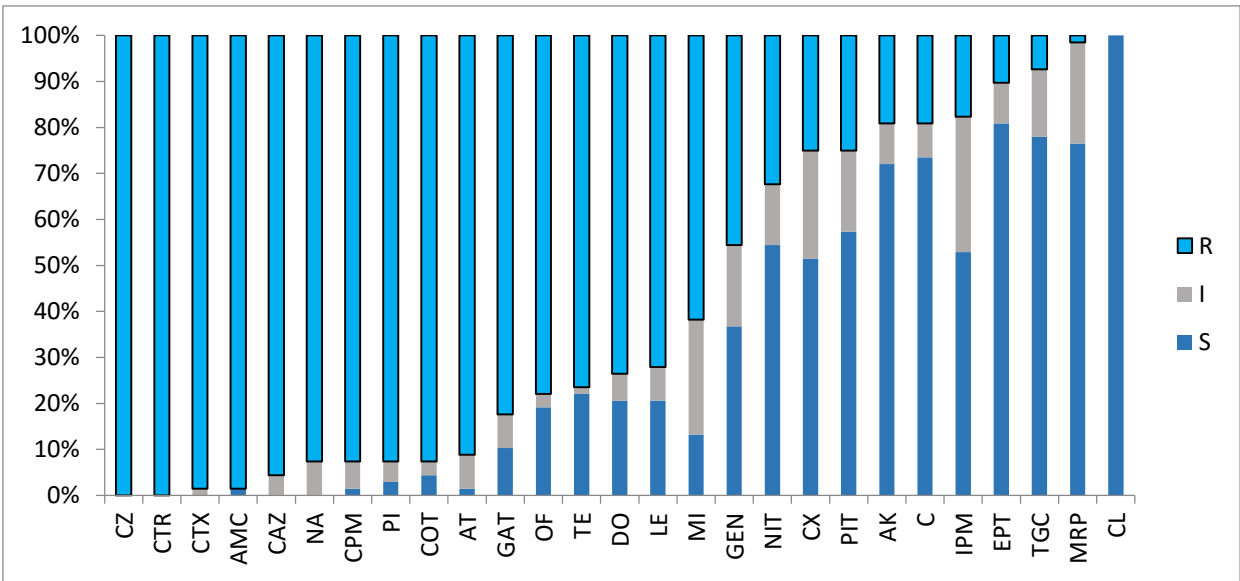


Figure 3. Antibiotic Sensitivity Testing: The susceptibility of the clinical isolates to different groups and classes of antibiotics was tested

Table 1. Overall distribution of ESBL genes

Genes	Isolates number
<i>bla</i> CTX-M ^a	95.38 (62)
<i>bla</i> TEM ^a	43.08 (28)
<i>bla</i> SHV ^a	15.38 (10)
<i>bla</i> SHV + <i>bla</i> CTX-M	3.08 (2)

<i>blaTEM</i> + <i>blaCTX-M</i>	26.15 (17)
<i>blaTEM</i> + <i>blaSHV</i> + <i>blaCTX-M</i>	12.31 (8)
Only <i>blaCTX-M</i> ^b	53.85 (35)
Only <i>blaTEM</i> ^b	4.62 (3)
None of the 3 genes	4.62 (3)

a - The presence of the indicated gene regardless of the presence or absence of the other genes

b - The word only means the presence of the indicated gene merely

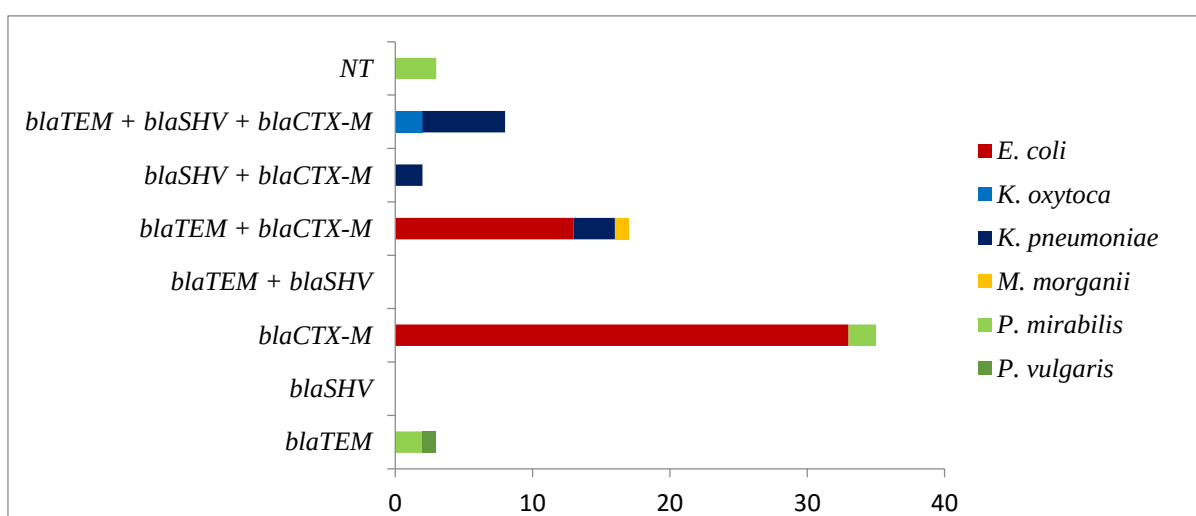


Figure 4. Distribution of ESBL genes amongst the isolates

Conclusions

1. All 68 isolates included in the study were confirmed to be ESBL producers.
2. High resistance was observed for various classes of drugs confirming MDR status of the isolates.
3. Most common isolates in the present study were *E.coli* harboring *blaCTX-M* gene.
4. The presence of genes in particular species highly correlated.
5. Antibiotic for treatment should be selected only after *In vitro* testing.

4. Molecular Diagnosis of *Mycobacterium tuberculosis* from AFB positive sputum samples by Multiplex PCR

Technical Team

1. Dr. Shashikant P Vaidya
2. Mr. Sankalp Kokare

Period

Jan 2016 - Apr 2016

Introduction

Tuberculosis (TB) is an infection with *Mycobacterium tuberculosis* (*M.tuberculosis*) which can occur in any organ of the body but is most well known in the lung. TB declined after the late 19th century but remained a major public health issue as it still is today. It is a major global health problem. In 2014, there were an estimated 9.6 million new TB cases and 1.5 million TB deaths. It is an infectious disease caused by the bacillus *M.tuberculosis*. TB is the only disease ever declared a global emergency by the WHO. Diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture. The challenge now is to combat the increasing TB cases. However a significant obstacle in controlling TB is the amount of time required to reach a diagnosis. Due to the slow growth rate of *M. tuberculosis*, the culture methods can take up to 6 weeks. This can cause significant delays in identifying TB cases, adjusting treatment regimens, and initiating epidemiological investigations. In view of these limitations, there is a need for more accurate and less time consuming tests.

Recently, attention has been drawn towards the development of molecular-based assays. Current advances in molecular biology have ushered in numerous exciting improvements in microbial diagnostics. For tuberculosis, this has resulted in more rapid and accurate diagnosis with impressive treatment outcomes. Assays that previously took days or even weeks to complete can now frequently be done with improved sensitivity and specificity in less than a day.

In the present study we shall perform a molecular method for the diagnosis of tuberculosis. The study shall be useful to identify the best possible methodology in the detection and diagnosis of tuberculosis

Objectives

The aim of this study is to diagnose *Mycobacterium tuberculosis* from smear positive sputum samples using Multiplex PCR. with the following objectives:

- To perform ZNCF staining
- To culture the sputum samples on L-J medium
- To perform PNB test
- To extract DNA from smear positive sputum samples
- To quantify DNA by Nanodrop
- To detect MTB Complex and *M.tuberculosis* from smear positive sputum samples by Multiplex PCR

Results and Conclusions

1. Table 1: AFB staining

No. of samples: 105

Sr No.	AFB status	Number of samples (%)
1	Positive	82
2	Negative	18

82% of the sputum samples were found to be positive for AFB by ZNCF Staining.

2. Table 2: Culturing on LJ Medium (TB results)

No. of samples: 105

Sr No.	Culture	Number of samples (%)
1	Positive	80
2	Negative	20

80% of the sputum samples were found to be Culture positive.

3. Table 3: Para-Nitro Benzoic acid Test: (Differentiation between MTB Complex and MOTT)

No. of samples: 84

Sr No.	Group	Number of samples (%)
1	MTB Complex	66.66
2	MOTT	33.33

66.66% of the all the sputum samples were from the Mycobacterium tuberculosis Complex whereas 33.33% were MOTT.

4. Table 8: Multiplex PCR (Differentiation between MTB Complex and MOTT)

No. of samples: 86

Sr No.	Status	Number of samples (%)
1	Mycobacterium tuberculosis Complex	66.27
Out of all MTB Complex positive samples (57 samples), 59.64% (34 samples) were <i>Mycobacterium tuberculosis</i>		

Out of 86 AFB positive samples, 57 samples were from the MTB Complex group of which 34 samples were found to be *M.tuberculosis*.

5. Table 5: Comparison of Conventional and Molecular method

	Conventional Method (LJ Culturing and PNB Test)	Molecular Method (Multiplex PCR)
TIME (Results) (Approx.)	28 - 42 Days	10 Hours
COST (Approx.)	Rs. 150 to 200/-	Rs. 800 to 1000/-
INFRA-STRUCTURE	Bio-safety	Bio-safety, Centrifuge, Conventional PCR, Gel Electrophoresis, Thermal Cycler
MAN-POWER	Skilled laboratory Personnel required	Skilled laboratory Personnel required

Laboratory diagnosis of TB still involves the smear examination and culture confirmation for TB. Conventional culture confirmation using LJ Medium has the disadvantage as the time required to observe the growth is high, since the TB bacilli takes around four weeks to grow. This contributes in delay of treatment, spread of the disease and leads to unnecessary treatment of non-specific pulmonary infections. Nevertheless, conventional method still remains the gold standard for culture of TB. It is useful for early confirmation of viable TB bacilli, observing response to treatment in TB. Significant advances have been made in the rapid and accurate diagnosis of TB. The use of molecular tests can reduce the need for invasive diagnostic procedures, which are both costly and pose an added risk to the patient. One of the means for diagnosis of TB is a molecular method, Multiplex PCR. An amplification assay based on the species-specific mtp40 genomic fragment, which is exclusively detected within the *M. tuberculosis* genome but not in other members of the MTB Complex group has been developed. Genetic markers, like IS6110 for species differentiation within the MTB Complex have been identified. A simple multiplex PCR based diagnostic method has been carried out to differentiate MTC members known to cause disease in humans and animals. PCR amplification with the mtp40 genomic fragment and IS6110 sequence provides a highly sensitive and specific technique for the detection of *M. tuberculosis* strains in clinical samples. It allows for the differentiation between *M. tuberculosis* and other related mycobacteria. This test is a reliable, rapid and easy to perform for the simultaneous detection of MTB Complex group and

M.tuberculosis strain in TB disease. With high sensitivity and high specificity obtained in this study with respect to detection of TB, we can conclude that this test strongly assists in adequate treatment of TB, long before the conventional LJ medium and PNB test results are available. Such molecular methods contributes in many ways; significant reduction in the time to diagnose TB, the earlier initiation of appropriate therapy and the potential to prevent transmission of drug-resistant strains constitutes the major advantages of such methods. These methods require skilled operators, good infrastructure and relatively high expenses. These tests serve as an early guidance of therapy, which can be followed by a phenotypic confirmation for all suspected TB patients.

5. Role of fibrotic cytokines in the disease activity and severity of systemic sclerosis (part of Ph.D project)

Technical Team

1. Dr. Shashikant P. Vaidya
2. Dr. Sunita D. Deshpande
3. Mr. Prasad V. Khadilkar
4. Ms. Manasvi Jain

Period

Jan 2016- Jun 2016

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by involvement of an autoimmune response, directed against tissues of the skin, lungs, heart and other organs. Presence of anti-topoisomerase I antibodies (anti-Scl70 abs) and anti-centromere antibodies are specifically associated with systemic sclerosis. An immune response involving excessive release of cytokines and subsequent activation of other cells like B cells, fibroblasts leads to the disease condition. Fibrosis is an important pathological event in systemic sclerosis.

Objectives

1. To collect blood samples of patients suffering from systemic sclerosis and healthy controls.
2. To evaluate presence and quantify the levels of IL-4 and TGF- β in the serum.
3. To check the gene expression pattern of the cytokines.
4. To statistically represent, compare and analyze the obtained data.

Results and Conclusion

20 clinically diagnosed systemic sclerosis patients (ACR/EULAR 2013 criteria) and 11 Age-Sex matched healthy controls have been enrolled since Jan-2016. Clinical Manifestations and demographic data has been recorded. Sample processing (Separation of serum) has been done and samples have been preserved. Autoantibody profiling has been performed using IFA and lineblot assay.

Table 2: Distribution of anti-nuclear antibodies and ANA specificities seen in enrolled systemic sclerosis patients

SR.	Title	Patient nos	ANA (IFA)		Anti-dsDNA (IFA)		AntiCentromere Abs (lineblot)		Anti-Scl-70 Abs (lineblot)	
			Pos	Neg	Pos	Neg	Pos		Pos	
1	Females	18	16	2	0	18	7		9	
2	Males	2	1	1	0	2	0		0	
3	Total	20	17	3	0	20	7		9	

- Test for ANA was performed using HEp2 cell lines provided in cavity slide by BIORAD, USA
- Test for anti-dsDNA antibodies was performed using *Crithidia lucidiae* cells provided in cavity slide by AESKU, GERMANY
- Test for anti-Centromere and anti-Scl-70 antibodies was performed using Line immunoassay provided in Blot strips by EUROIMMUN, Germany

6. Analysis of samples presented with pyrexia of unknown origin by Extended-Widal test

Technical Team

1. Mrs. Swati Sanp
2. Mrs. Vibhuti Thakur
3. Dr. Shital Vasave
4. Dr. Shashikant Vaidya

Period

Apr 2015 – Mar 2016

Introduction

The Extended-widal test comprises of analyzing serum antibodies for three infections namely:

1. Widal Test for Typhoid Fever
2. Weil-Felix Test for Rickettsial diseases
3. Brucellosis Test for Brucellosis

The Widal test is an old serological test for detecting IgM and IgG antibodies to the 'O' and 'H' antigens of Salmonella. In slide agglutination tests a known concentration of antigen and unknown concentration of antibodies in patient's serum are mixed, clumping occurs within a minute. Commercial antigens are available for detection of typhoid (O- and H-) and paratyphoid (AH- and BH-) antigens

The Weil-Felix test is an agglutination test for the rickettsial infection. Typhus group rickettsiae (*Rickettsia prowazekii*, *R.typhi*) reacts with *P. vulgaris* (OX19- antigen), Scrub typhus (*Orientia tsutsugamushi*) reacts with *P. mirabilis* (OXK-antigen). Spotted fever group rickettsiae (*R. rickettsii*, *R. africae*, *R. japonica* etc.) react with *P. vulgaris* (OX2-antigen) and (OX19- antigen), to varying degrees, depending on the species. The antigens necessary (OX2, OX19, and OXK) can be obtained commercially.

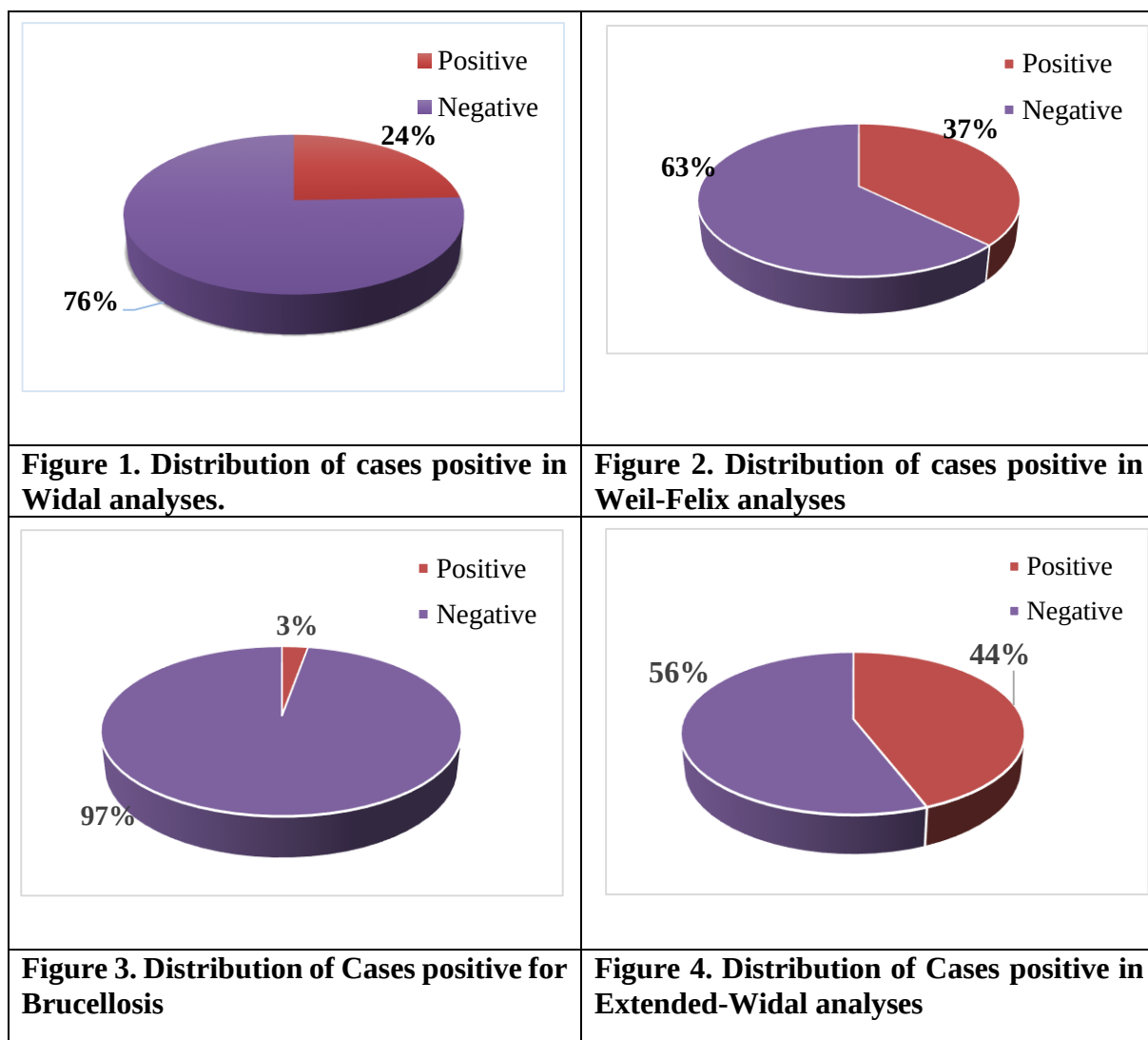
The Brucellosis Test for Brucellosis is an agglutination test for the Brucella infection. This infection caused by *Brucella melitensis* (Br-M) and/or *Brucella abortus* (Br-Ab).

Objectives

To analyse samples with pyrexia of unknown origin with the extended-Widal test of unknown origin and identify the presence of Typhus in the population

Results

The below graphs (figure 1 - 4) shows total number of widal tests performed between April 2015 and March 2016.



The department of Clinical Pathology received a total of 648 samples for the Widal test in the year 2015-16, out of which 366 were males and 282 were females. Of the total 648 samples tested, 24% (n=158) tested positive for Widal while 76% (n=490) were negative. Out of this 158 positive cases, 86 were male and 72 were female.

A total of 651 samples were received for the Weil-felix test in the year 2015-16, out of which 367 were males and 284 were females. Of the total 651 samples tested, 37% (n=243) tested positive for Weil-felix while 63% (n=408) were negative. Out of this 243 positive cases, 127 were males and 116 were females.

Department of Clinical Pathology received total 651 samples for the Brucellosis test in the year 2015-16, out of which 370 were males and 281 were females. Of the total 651 samples tested, 3% (n=18) tested positive for Brucellosis while 97% (n=633) were negative. Out of this 18 positive cases, 11 were males and 7 were females.

Department of Clinical Pathology received total 655 samples for the Extended Widal test in the year 2015-16, out of which 370 were males and 285 were females. Of the total 655 samples

tested, 44% (n=289) tested positive for Extended test while 56% (n=366) were negative. Out of this 289 positive cases, 155 were males and 134 were females.

Monthwise Distribution of cases

The following graph (figures 5-8) shows the month wise distribution of positive cases.

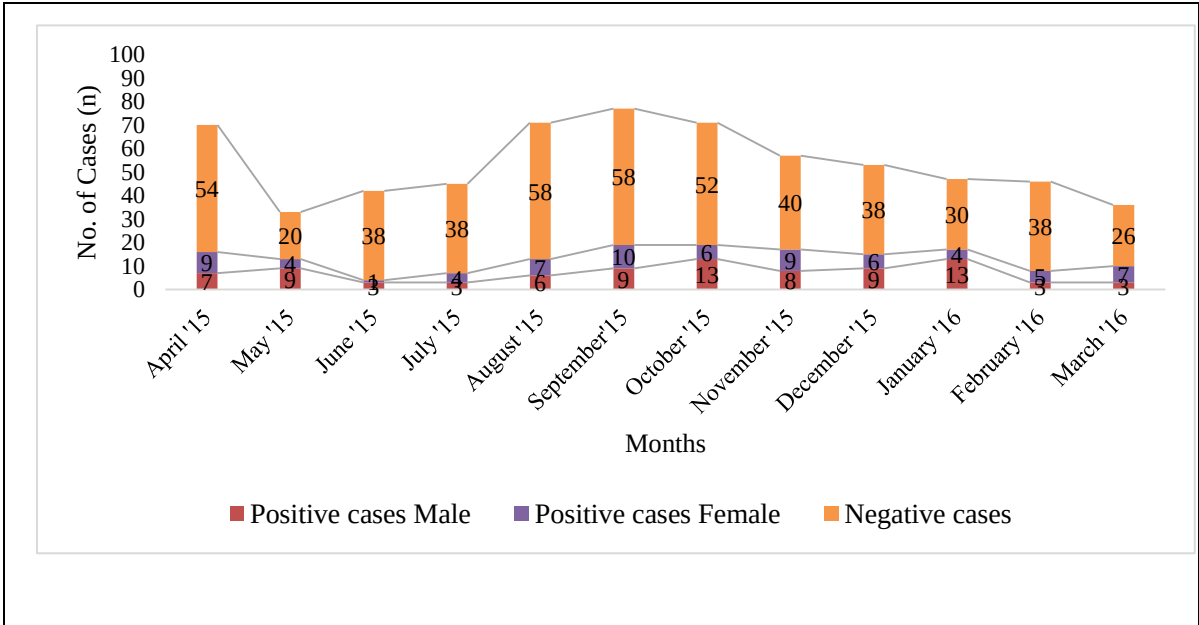


Figure 5. Month-wise distribution of total cases positive in Widal analyses

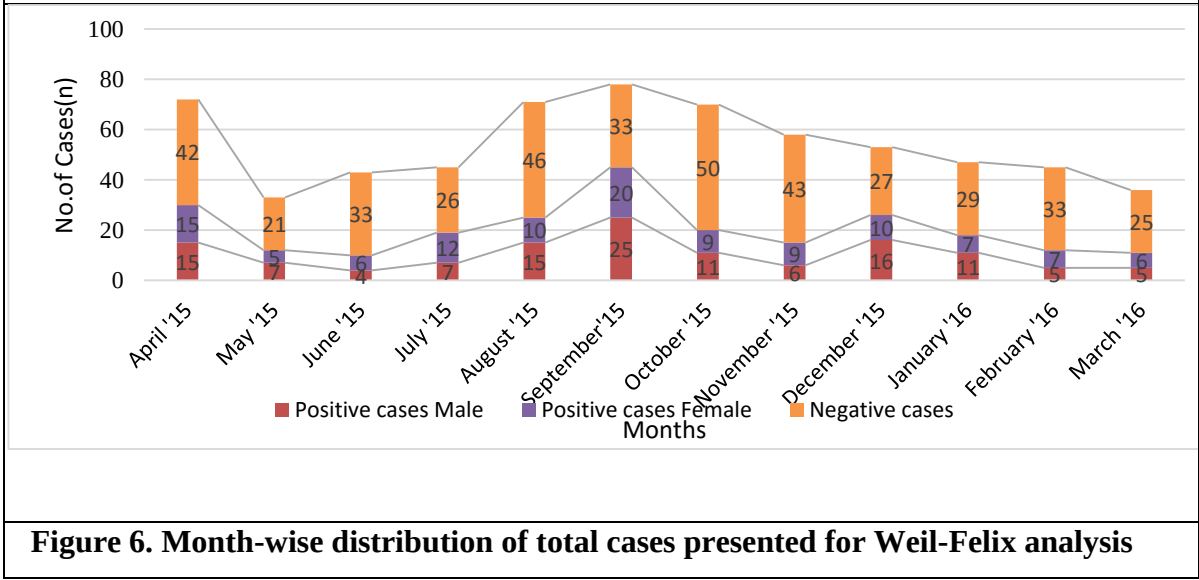


Figure 6. Month-wise distribution of total cases presented for Weil-Felix analysis

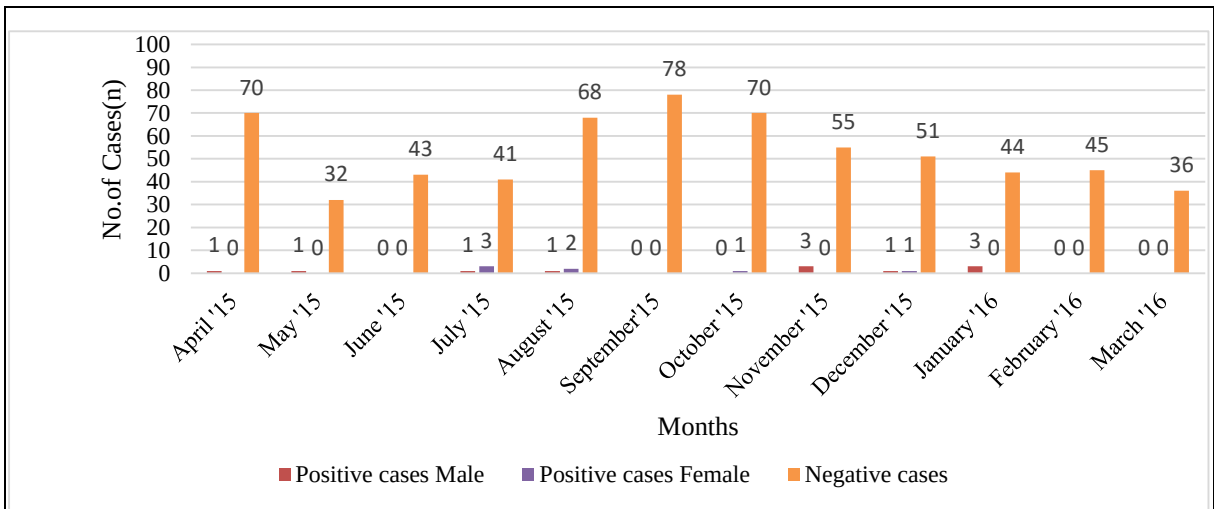


Figure 7. Month-Wise Distribution of Total Cases positive for Brucellosis

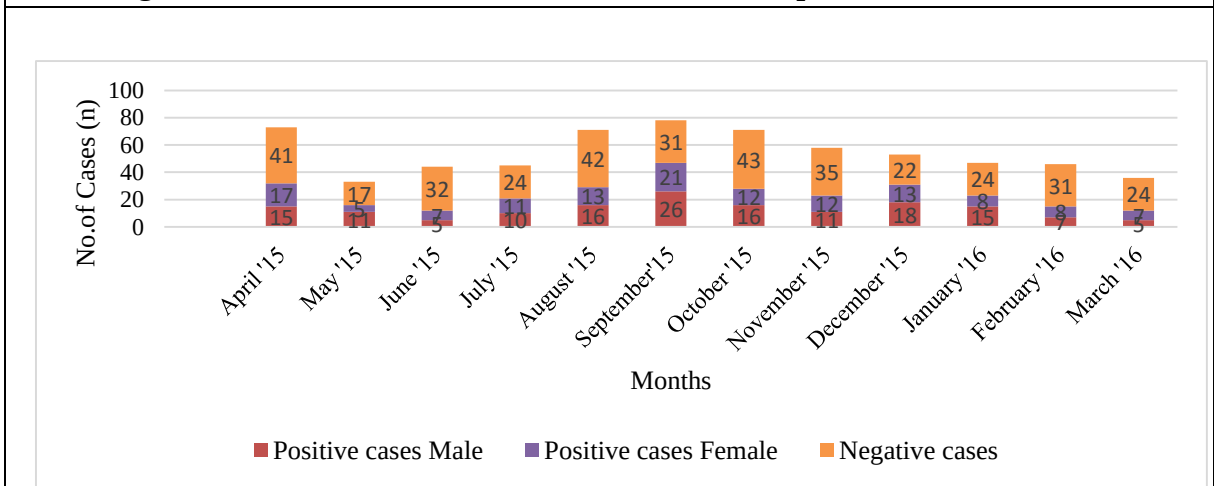


Figure 8. Month-wise distribution of total cases presented for extended-widal analyses

Percentage of positivity of cases

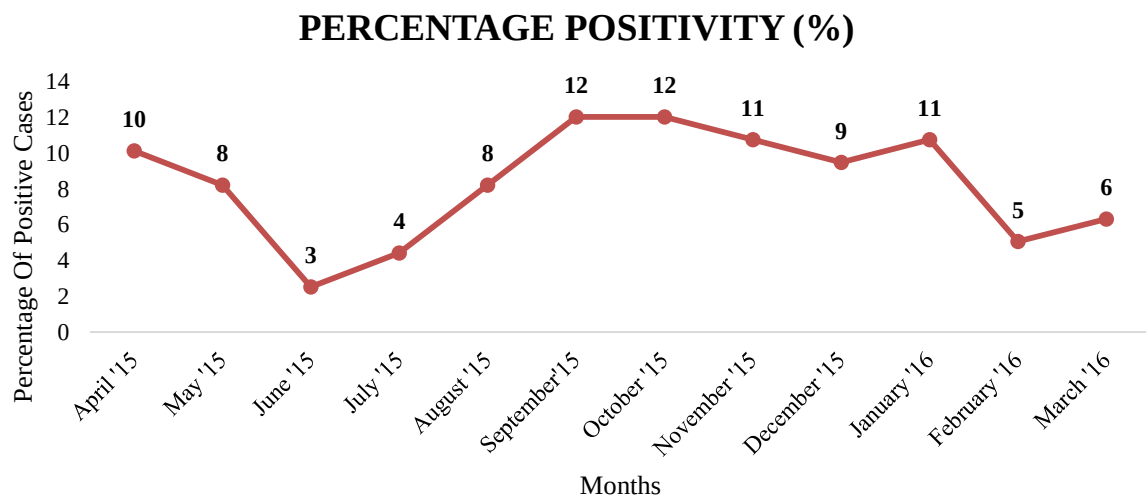


Figure 9. Month-Wise Distribution of Positive Cases in Percentage after Widal analysis

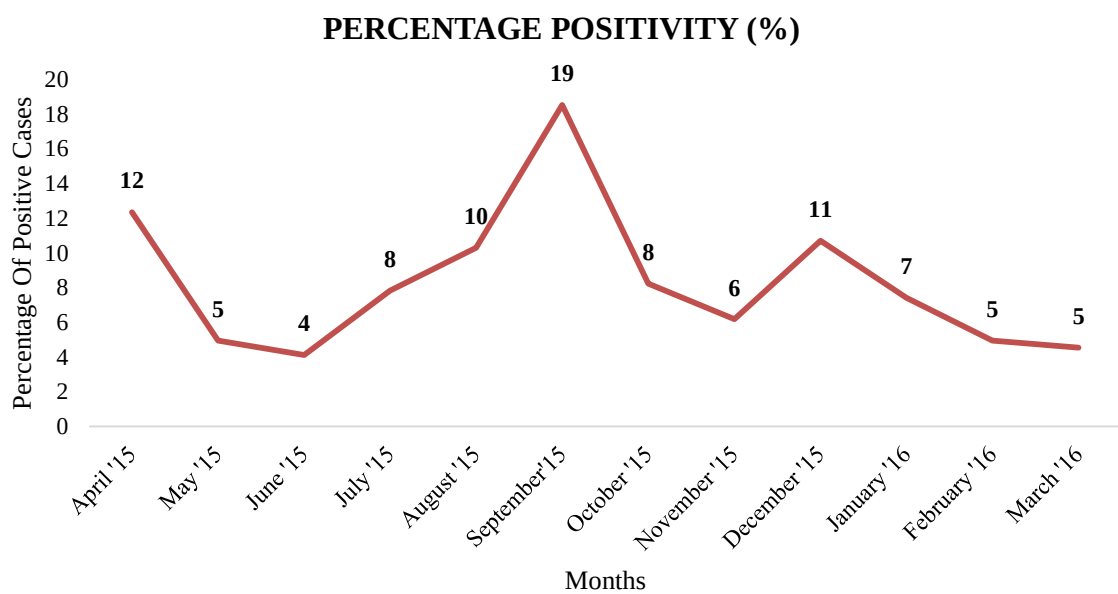


Figure 10. Month-Wise Distribution of Positive Cases in Percentage after Weil-Felix analysis

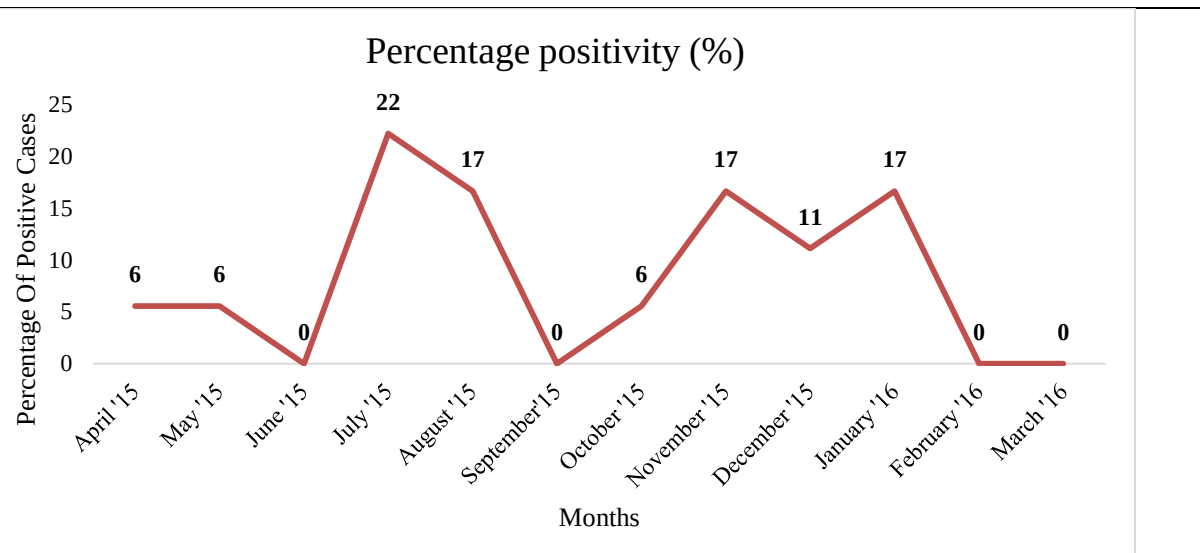


Figure 11. Month-Wise Distribution of Positive Cases in Percentage for Brucellosis

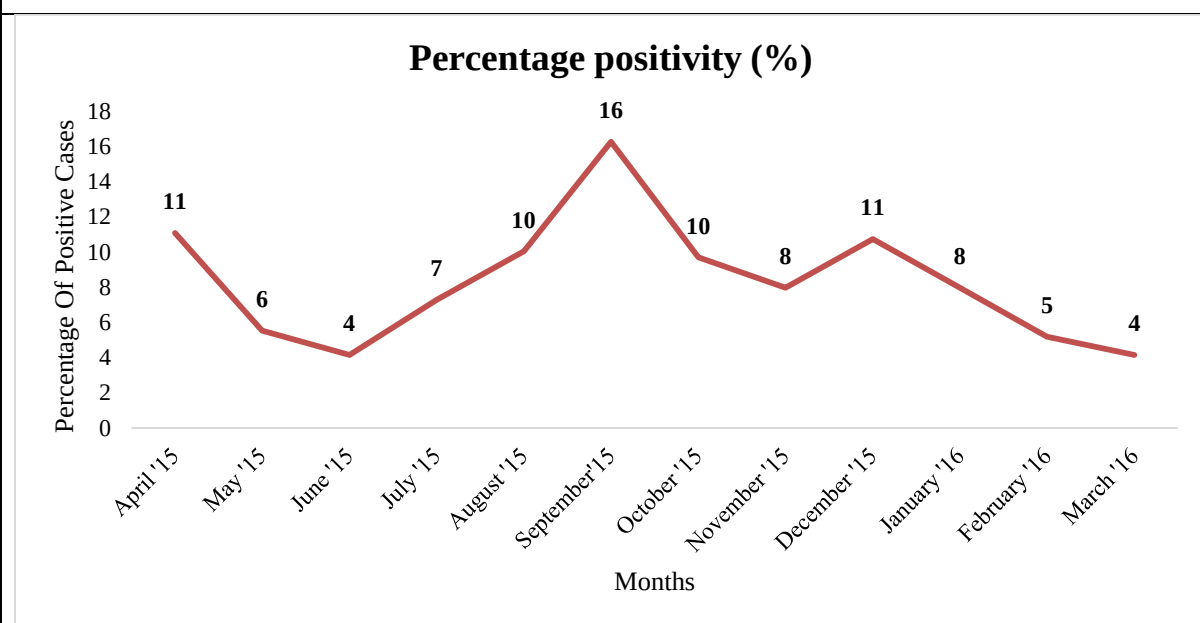


Figure 12. Month-Wise Distribution of Positive Cases in Percentage after Extended-Widal analysis

Age-wise and Gender-wise distribution

Table 1. Age-wise and Gender-wise distribution of positive cases after Widal analyses (p value= 0.163)

Age	Male %(n)	Female %(n)	Total %(n)
0-5	13 (20)	14(22)	27 (42)
5-10	32(51)	23(37)	56 (88)
10-15	9(15)	6(10)	16 (25)
above 15	0(0)	2(3)	2 (3)
Total	54(86)	46 (72)	100(158)

Table 2. Age-wise and Gender-wise distribution of positive cases after Weil-Felix analysis (p value= 0.313)

Age	Male %(n)	Female %(n)	Total %(n)
0-5	13 (32)	16(40)	30 (72)
5-10	27(66)	23(57)	51(123)
10-15	10(25)	6(15)	16(40)
above 15	2(4)	2(4)	3(8)
Total	52(127)	48(116)	100(243)

Table 3 Age-wise and Gender-wise distribution of positive cases for Brucellosis (p value= 0.487)

	Male %(n)	Female %(n)	Total %(n)
0-5	11 (2)	11(2)	22 (4)
5-10	33(6)	11(2)	44 (8)
10-15	11(2)	17(3)	28 (5)
above 15	6(1)	0(0)	6 (1)
Total	61(11)	39(7)	100 (18)

Table 4. Age-wise and Gender-wise distribution of positive cases in Extended-Widal analyses (p value= 0.247)

	Male %(n)	Female %(n)	Total %(n)
0-5	13(39)	16(45)	29(84)
5-10	22(65)	19(55)	42(120)
10-15	16(47)	10(29)	26(76)
above 15	1(4)	2(5)	3(9)
Total	54(155)	46(134)	100(289)

Conclusions

Widal analyses

A range of 10% to 39% of the samples received, test positive per month; while over 61% of the samples turn out to be negative. The proportion of male patients was observed to be higher than female patients throughout the year.

The data analysis revealed maximum number of samples belonging to age group 5-10, also number of male patients was observed to be higher as compared to number of samples of female patients. The other age groups 0-5, 10-15 and above 15 had comparatively lesser number of samples and no significant difference in was observed between male or female patients.

The maximum proportion of positive cases were observed in the months of September and October; while lowest number of positive cases were observed in June.

Weil-Felix analyses

A range of 23% to 58% of the samples received, test positive per month; while over 42% of the samples turn out to be negative. The proportion of male patients was observed to be higher than female patients throughout the year.

The data analysis revealed maximum number of samples belonging to age group 5-10, also number of male patients was observed to be higher as compared to number of samples of female patients. The other age groups 0-5, 10-15 and above 15 had comparatively lesser number of samples and no significant difference in was observed between male or female patients.

The maximum proportion of positive cases were observed in the months of September; while lowest number of positive cases were observed in June.

Brucellosis test

A range of 6% to 22% of the samples received, test positive per month; while over 78% of the samples turn out to be negative. The proportion of male patients was observed to be higher than female patients throughout the year.

The data analysis revealed maximum number of samples belonging to age group 5-10, also number of male patients was observed to be higher as compared to number of samples of female patients. The other age groups 0-5, 10-15 and above 15 had comparatively lesser number of samples and no significant difference in was observed between male or female patients.

The maximum proportion of positive cases were observed in the months of July; while lowest number of positive cases were observed in April and May.

Extended Widal

A range of 4% to 16% of the samples received, test positive per month; while over 84% of the samples turn out to be negative. The proportion of male patients was observed to be higher than female patients throughout the year.

The data analysis revealed maximum number of samples belonging to age group 5-10, also number of male patients was observed to be higher as compared to number of samples of female patients. The other age groups 0-5, 10-15 and above 15 had comparatively lesser number of samples and no significant difference in was observed between male or female patients.

The following graph shows the month wise distribution of positive cases. The maximum proportion of positive cases were observed in the months of September; while lowest number of positive cases were observed in March and June.

In summary, the above data gives us the warning about the number of fever cases in the community possibly due to typhoid or other febrile illness and supports to physician for the proper direction of the treatment.

7. Biosynthesis of Nanoparticles & its Potential Therapeutic Applications (Ph.D project)

Technical Team

1. Dr. (Mrs.) Sunita D. Deshpande
2. Dr. Shashikant P. Vaidya
3. Mr. Kapil Punjabi

Please see details under Nanoparticle research

Publications

1. Detection of Macrolide, Lincosamide and Streptogramin Resistance among Methicillin Resistant *Staphylococcus aureus* (MRSA). Subramanian A, Chitalia V, Vaidya S, Warke R, Chowdhary A, Deshmukh R, JKIMSU 2015, 4(3), 10 – 16. In Mumbai
2. Molecular Diagnosis of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates in and around Mumbai. Joshi S, Dahake R., Chitalia V, Vaidya S, Chowdhary A, JPAM 2015, 9(3):2263 – 2270.
3. Comparison of *Mycobacterium tuberculosis* DNA Extraction Methods. Joshi S, Dahake R., Chitalia V, Vaidya S, Chowdhary A. JPAM 2015, 9(2), 1129 – 1136.
4. Comparison of conventional method, Radiometric method and method using Oxidation-reduction dye for detection of Multidrug resistant Tuberculosis, Vaidya S, Sharma J, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, LIFE: International Journal of Health and Life-Sciences, 2015, 72-89.
5. Determination of efficacy of isoxyl, a mycolic acid inhibitor, in vitro against *Mycobacterium tuberculosis* strains, Vaidya S, Punjabi K, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, LIFE: International Journal of Health and Life-Sciences, 2015, 1-24.
6. Determination of in vitro inhibitory activity of mycolic acid inhibitors to clinical isolates of bacteria and *Mycobacterium tuberculosis*, Vaidya S, Punjabi K, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, LIFE: International Journal of Health and Life-Sciences, 2015, 37-54.
7. Relationship between RpoB mutations and Minimum inhibitory concentrations of Rifampicin in multi-drug resistant strains of *Mycobacterium tuberculosis*, Vaidya S, Maniar J, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, LIFE: International Journal of Health and Life-Sciences, 2015, 108-121.
8. To study incidence of multi drug resistant Tuberculosis, in Mumbai Vaidya S, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, , LIFE: International Journal of Health and Life-Sciences, 2015, 122-138.
9. Determining the efficacy of Isoxyl, a mycolic acid inhibitor, in vitro against *Mycobacteria* other than *Mycobacterium tuberculosis* (MOTT) strains, Vaidya S, Chitalia V, Mulye S, Koppikar G, Kulkarni M, Chowdhary A, LIFE: International Journal of Health and Life-Sciences, 2015, 90-107.
10. Prevalence of Candidiasis among children, in Mumbai Wandre S, Mukadam T, Sanap S, Vaidya S, Chowdhary A, , LIFE: International Journal of Health and Life-Sciences, 2015.

Department of Bacteriology

Head of Department's Overview

Department of Bacteriology is the oldest department of the Haffkine Institute. In 1896, plague broke out in Bombay in an epidemic form. Dr. Haffkine was invited by the Government to investigate the causes of the plague epidemic and to develop a prophylactic. He started his work in one of the rooms of the Petit Laboratory of Grant Medical College and this was the beginning of the Department of Bacteriology, with the main function of producing plague vaccine. In the year 1947, the Department of Bacteriology-then known as 'Vaccine Department'-supplied 23.5 million ml of plague vaccine, the highest in the history of the Institute. Department gradually undertook the production of Cholera & T.A.B. vaccines. The year 1963 saw the historic peak of supply of 23.5 million ml. of cholera vaccine.

Its approach has always been to tackle problems of public health, hence carrying out bacteriological analysis of water, food, milk, etc. was also been attached to this Department in the past.

Today, it is ISO 9001: 2008 Certified laboratory and good laboratory practices are followed while working in the department.

It provides testing facilities for carrying out Bacteriological examination of water samples from various industrial and household sources. Testing of water purifying systems includes UV-based, RO-based, and membrane filter-based water purifiers. Departmental testing activities also involve antimicrobial efficacy testing of sanitizers, disinfectants, antimicrobial coated/incorporated material as well as synthetic and herbal preparations.

Besides, department provides guidance and Research facilities to M.Sc./Ph.D. candidates and Short Term Research Interns (STRIP students).

Since its beginning, the department has been involved in research to tackle problems of immediate concern to public health. Hence, Department would continue its legacy of serving to mankind in the future.

Focus of the Department for Future

Department would like to focus on Clinical Bacteriology and would like to initiate following research work:

1. To study Antibiotic resistance of Enteric Bacteria.
2. To carry out Surveillance of Multi-drug resistant *Pseudomonas aeruginosa*.
3. To carry out Surveillance of Non-Typhoidal *Salmonella*.
4. To carry out Surveillance of Drug-resistant *Salmonella typhi*.

5. To study Extended-spectrum- β -lactamase (ESBL)-producing bacteria and their resistance pattern.

Department would also like to develop its infrastructure (especially instrumentation) to carry out above mentioned Research work.

Dr. Anuya Rege.

Research projects

1. Plasmid Diversity in *Pseudomonas aeruginosa* isolated From Different Clinical Sources.

Technical team

1. Mr. Rohan Dattatraya Dudhale
2. Dr. (Mrs.) Sunita D. Deshpande

Period

Jan 2013 - Dec 2016

Introduction

A plasmid is a genetic material that can replicate in a controlled way physically separate from the chromosome. Plasmids are extremely diverse & distributed universally in a range of hosts & ecological niches. The increasing use of antibiotics in human and veterinary medicine & agricultural production system has caused an increase in development of high levels of novel antibiotics resistance and the rapid global spread of antibiotic resistance gene. A consequence of the horizontal transfer of the genes (HGT) located on mobile genetic elements (MGE), especially on broad-host-range plasmid is the rising detection and level of resistance bacteria, particularly of MDR bacteria, both clinically and in natural environment. Therefore, detailed analysis of such plasmid genomes can help to understand bacterial evolution. The aims to study plasmid diversities in *Pseudomonas aeruginosa* leading to enhancement of antibiotic resistance and other virulence factors. The work therefore is significant, as it will provide a comprehensive analysis of genotypic and phenotypic factors responsible for enhanced virulence of *P. aeruginosa*.

Objectives

1. To isolate *Pseudomonas aeruginosa* from different sources and to assess Antibiotic Resistance patterns in the clinical isolates.
2. To investigate Heavy-Metal Resistance patterns and Hydrocarbon Degradation in the clinical isolates.
3. To isolate plasmid & to carry out sequencing of the isolated plasmids.
4. To perform sequence analysis of the plasmid sequences using bioinformatics tools and establish phylogenetic relationships if any.

Results

Pseudomonas aeruginosa bacterial isolates collection for the study:

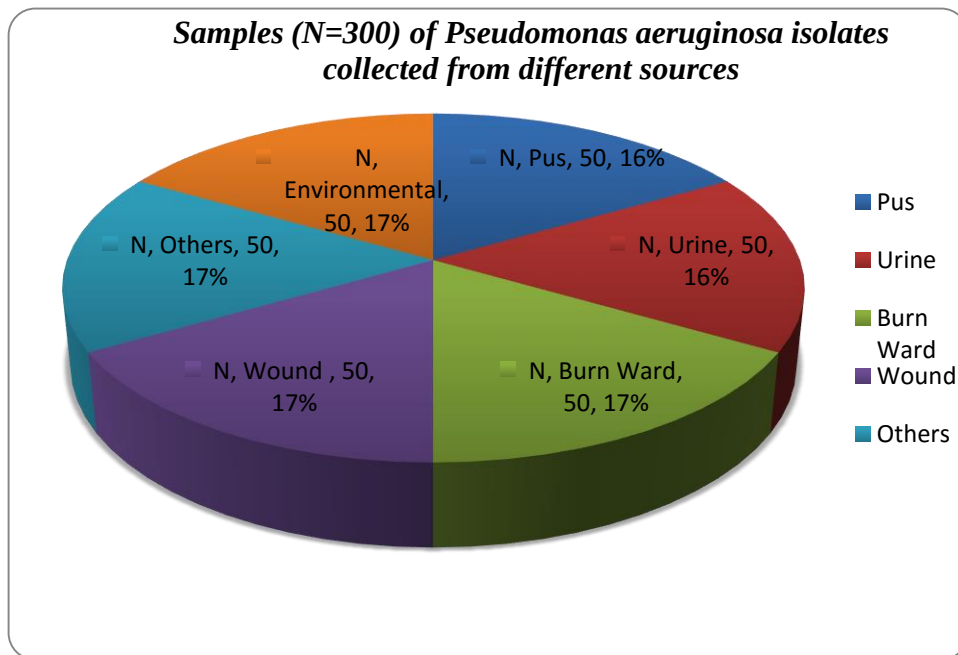


Fig: 1 - Pie-chart showing the sample collection

Antibiotic Resistance patterns in clinical isolates:

The antibiotic susceptibility of isolated *Pseudomonas aeruginosa* was performed on Muller-Hinton agar plates by Kirby-Bauer Disk Diffusion Method as described by the National Committee for Clinical Laboratory Standards. This method is accordance to Performance Standards for Antibiotic Disk Susceptibility Tests, CLSI (formally NCCLS).

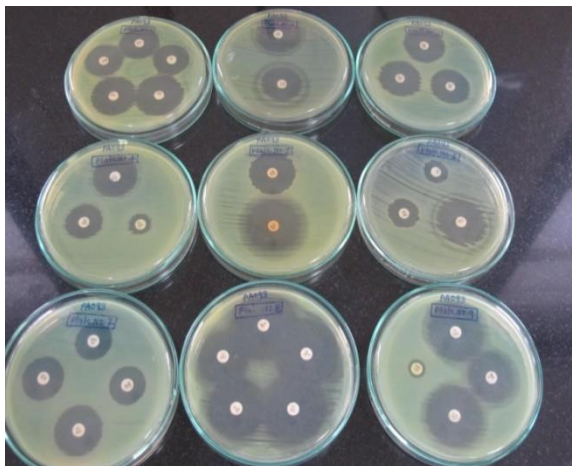


Fig: 2 - Images Showing Antibiotics susceptibility test results

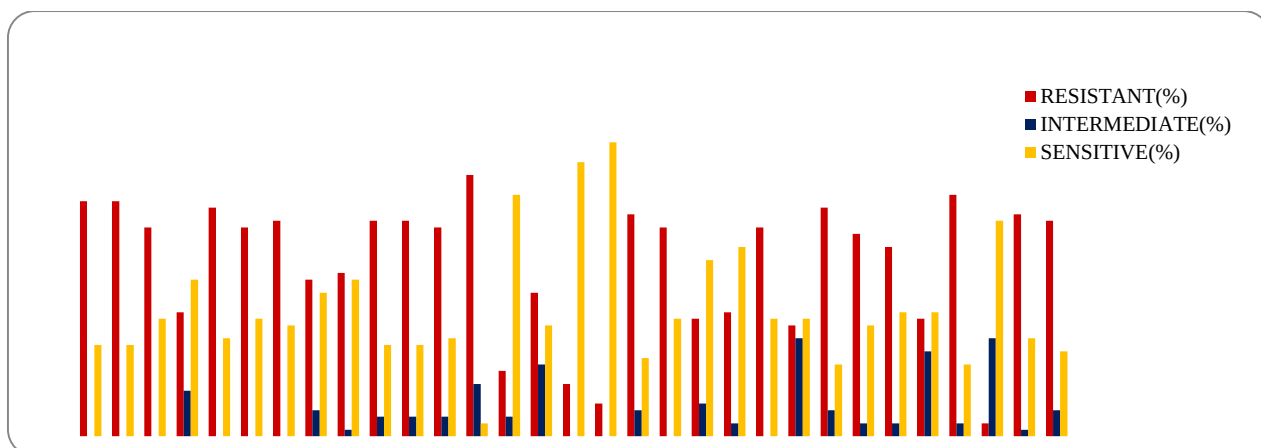


Fig: 3- Antibiogram for *Pseudomonas aeruginosa* isolated from burns patients

Where, MZ=Mezlocillin, TI=Ticarcillin, PI=Piperacillin, CB =Carbenicillin, AZ =Azlocillin, PIT=Piperacillin-tazobactam, TCC = Ticarcillin-calvulanic acid, CAZ =Ceftazidime, CPM= Cefepime, CTX =Cefotaxime, CTR= Ceftriaxone, CZX= Ceftizoxime, MX=Moxalactam, IPM =Imipenem,MRP=Meropenem,CL=Colistin, PB=Polymyxin-B, AT=Aztreonam, GEN= Gentamicin, AK= Amikacin, TOB= Tobramycin, NET= Netiliin, CIP= Ciprofloxacin, LOM= Lomefloxacin, OF= Ofloxacin, NX= Norfloxacin, GAT= Gatifloxacin, PRU= Prufloxacin, TGC= Tigecycline, SPX= Sparfloxacin, MO= Moxifloxacin.

***Pseudomonas aeruginosa* bacterial Biofilm Assay:**

Biofilm formation assay of clinical isolates in triplicate was performed as per the protocol explained by Toole *et al.* and standard strains of *Pseudomonas aeruginosa* ATCC 27853 and PAO1 was used for comparison.

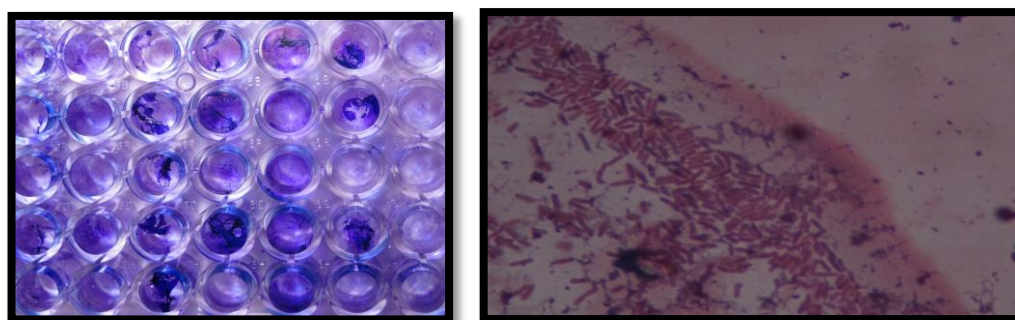
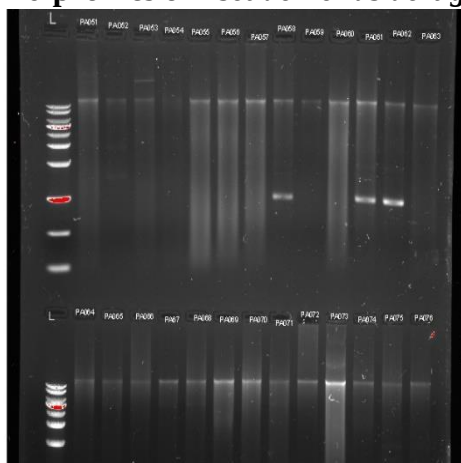


Fig 4:- Microtiter plate after staining with 0.1% crystal violet and microscopic image of Biofilm formed by *Pseudomonas aeruginosa* on cover slip

Plasmid Profiling of *Pseudomonas aeruginosa*:

The screening of plasmid has been carried out by using plasmid extraction methods of all isolates and further by routine Agarose gel electrophoresis for plasmid profiling.

Fig 5:- Plasmid profiles of *Pseudomonas aeruginosa* isolates



Outcome of the study

All the isolates are showing the Multi Drug Resistance (MDR) patterns as well as producing the biofilm formation which can result in the increasing drug resistance in such group of bacteria. *Pseudomonas aeruginosa* are mostly associated with hospital acquired infections which can play important role in the spreading drug resistance in other group of bacteria by various factors, such as producing the biofilm and sharing or uptake of plasmid from gene pool. The plasmid profiling showing the presence of plasmid in such bacteria is very important to carry/ transfer such genetic trait. In the current study, most of isolates were found to have high Kb plasmids that can lead to spreading the resistance into the bacteria very rapidly.

This study further focuses on molecular characterization of such plasmids which would predict type of genes they carry in clinical as well as in environmental isolates. We are looking forward to carry out the plasmid sequencing and their analysis to build the plasmid database which would help to overcome the resistance in environment and to understand the role of plasmid in bacterial resistance towards antibiotics.

Publications

1. Vishwe VS, Vaidya SP and Chowdhary AS: Optimization of Fermentation parameters for Enhanced production of Lipase from lipolytic *Pseudomonas* spp. Int Res J Biological Sci, 2015, 4(6): 16-21.
2. Vishwe VS, Gajbhiye SP, Vaidya SP and Chowdhary AS: Isolation and Characterization of Lipolytic *Pseudomonas* spp. from oil contaminated water samples. IOSR Journal of Biotechnology and Biochemistry, 2015, 1(6): 33-36.
3. Gajbhiye SP, Padmanabhan U, Kothari S, Patil A, Palep H and Chowdhary A: Immunostimulant activity of medical preparation Panchgavya. Int J Res Pharm Sci, 2014, 5(3): 1- (Please see section on Projects based on Ayurveda)

Department of Biochemistry

Head of Department's Overview

The Department of Biochemistry was established on the recommendations of the Advisory Board of the Indian Research Fund Association, in 1923. The Department of Biochemistry is actively involved in basic as well as applied research, in biochemical testing of clinical samples from human as well as animals and also in in-house and external training programmes.

Currently, various projects focused on diseases prevalent in India such as diabetes, cancer and neurological diseases are being pursued independently and in collaboration with clinicians and basic scientists. Understanding biochemical and molecular pathogenesis of the diseases would aid in better management of the diseases at different levels, i.e., diagnosis, therapy and prognosis. '*In vitro*' and '*in vivo*' investigations for understanding biological activities of herbs and their extracts, traditional medicinal formulations and synthetic compounds form an integral part of the departmental research activities. Pharmacological studies on traditional medicines will enable wider acceptance of traditional medicines and will also result in finding novel applications.

Biochemical analysis of clinical samples is a routinely offered testing service of the Department. One of the unique testing done in the Department is biochemical analysis of gall bladder stone and kidney stones. Another unique and novel testing service offered by the Department is chemical testing of honey. In view of the needs in the field of testing, the Department is focusing on development of rapid and economic tests and improvisation of the ongoing tests. Hence, in-house training of the modern techniques in molecular biology, biochemistry and cell biology is being given to the staff members.

NishigandhaNaik

Research projects

1 Studies to investigate role of steroid hormones in growth retardation & menstrual irregularities in EHPVO patients.

Technical Team

1. Dr.NishigandhaNaik
2. Dr.Shobna Bhatia
3. Dr.Akash Shukla
4. Dr.TaralaNandedkar

Period

Jun 2014 – Dec 2016

Introduction

Extrahepatic portal venous obstruction (EHPVO) is the commonest cause of portal hypertension and variceal bleeding in children. Diminished portal blood flow results in decreased insulin delivery to the liver and thereby decreased production of insulin-like growth factor-1 (IGF-I). Growth retardation due to growth hormone resistance is common in children with EHPVO. In young female patients, anaemia, irregular menstrual cycles and repeated abortions are observed. But molecular pathophysiology of growth retardation and recurrent abortions in the EHPVO females is not known. It is hypothesized that steroid hormones (E2 and P4), growth factors (IGF-1, VEGF) and interleukins (IL-6, IL-17 and LIF) might be the players in the pathophysiology of EHPVO.

Objectives

1. To study changes in oestradiol (E2) & progesterone (P4) in girls with EHPVO.
2. To compare steroid hormone levels in girls with normal cyclicity in EHPVO with normal controls.
3. To study relation of steroid hormone levels with expression of IGF-1, IL-6, IL-17, LIF and VEGF genes.

Results and Conclusions

Progesterone levels were higher in the EHPVO & non-EHPVO dyspepsia (NE) groups than the normal control (Figure 1)

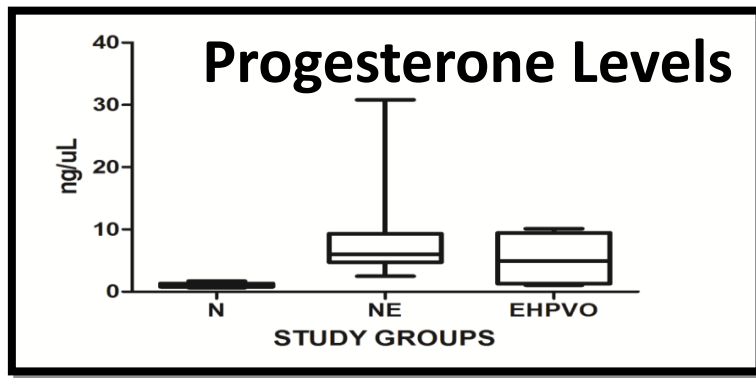


Figure 1: Progesterone levels in serum of all the three study groups.

Studies on expression of various genes are in progress.

2 Effect of fMLP on development and regeneration in zebrafish

Technical Team

1. Dr.NishigandhaNaik
2. Dr. Yasmin Khan
3. Dr.TaralaNandedkar

Period

Apr 2014 – Dec 2017

Introduction

N-formyl-Methionyl-Leucyl-Phenylalanine (f-Met-Leu-Phe or fMLP) is a classical chemo attractant that induces adherence, motility, degranulation and production of tissue-destructive oxygen-derived free radicals in phagocytic cells. N-formylated peptides are derived from bacterial secretion, protein degradation or from mitochondrial proteins upon tissue damage. fMLP receptors (FPR) are present on various human cell types, including stem cells. During development and regeneration of zebrafish (*Danio rerio*), cells show organized movement. However, precise role of FPR in zebrafish is not known. It is reported that fMLP induces osteogenesis and inhibits adipogenesis in zebrafish. Hence, it is hypothesized that FPR plays role in development and regeneration of zebrafish.

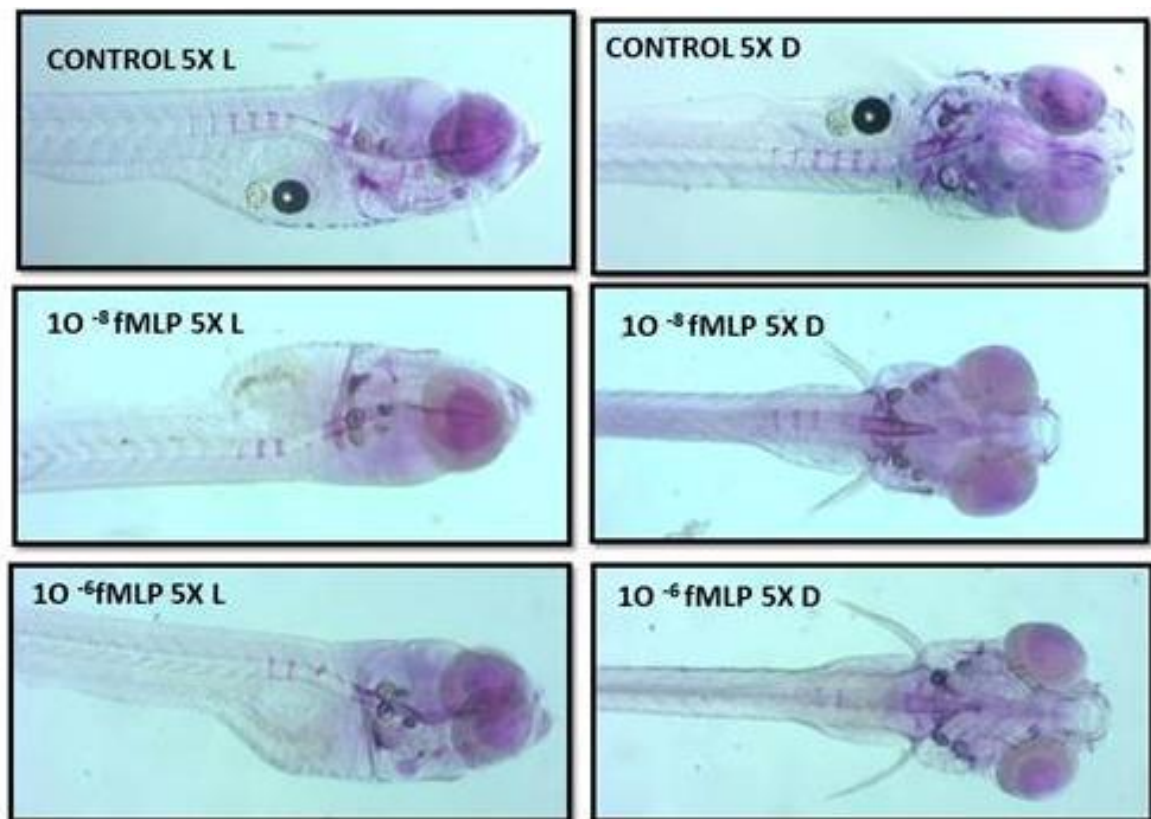
Objectives

1. To study role of FPR in early development of zebrafish.
2. To explore role of FPR in regeneration in zebrafish.

Results and Conclusions

In the preliminary studies, fMLP was found to be toxic at higher concentrations, but did not show any cardiotoxicity. It delayed hatching time, swim bladder development and bone development, but cartilage development was not affected (figure 2.1). On the other hand it appeared to enhance regeneration. Thus, preliminary studies indicate role of FPR in development as well as regeneration of zebrafish.

Figure 1



L –lateral view; D – dorsal view; fMLP concentration and magnification are indicated. Bone staining of the 6 days old embryos was done with Alizarin red. Control embryos showed more development, especially in the notochord region of the bones as compared to the fMLP treated embryos.

3 Analysis of kidney stones using conventional biochemical and FTIR techniques.

Technical Team

1. Dr. Nishigandha Naik
2. Dr. Anil Bobade
3. Dr. Ashish Asrondkar
4. Dr. Vrushali Patil

Period

Jan 2016 – Jan 2017

Introduction

The prevalence and incidence of nephrolithiasis is reported to be increasing across the world. Kidney stones constitute one of the commonest diseases in India and pain due to kidney stones is known as worse than that of labour pain. In India, approximately 5 - 7 million patients suffer from stone disease and at least 1/1000 of Indian population needs hospitalization due to kidney stone disease. One of the unique testing done in the Department is biochemical analysis of gall bladder stone and kidney stones. In comparison to the biochemical analysis, FTIR is a more sensitive technique for chemical analysis of kidney or gall stones. Development of FTIR techniques for analysis of kidney stones would enable more sensitive, precise, quick and economic analysis, which could be help in better treatment. Hence, development of FTIR based test for analysis of kidney and gall stones is initiated.

Objectives

To establish easy, rapid, sensitive and economic method for analysis of kidney stones and gall stones using FTIR.

Results and Conclusions

The project is recently initiated. Establishment of standard spectra using different standard chemical compounds such as ammonium oxalate, ammonium sulphate, calcium citrate is in progress.

5 Investigating the role of inositol pyro-phosphates in development of prostate cancer.

Technical Team

1. Dr. Usha Padmanabhan
2. Dr. Rosalind Marita

Period

Jul 2015 - Jun 2018

Introduction

Prostate cancer (PC) is the fifth leading cancer occurring in men above the age of 45. In India, it is the 10th cause of mortality among older men. Growth and differentiation of the prostate gland during development is dependent on the androgens - testosterone and dihydrotestosterone. Androgens play role in development of prostate by regulating Ip6 kinase (Ip6-K). Ip6-K is primarily involved in the synthesis of inositol phosphate - InsP_7 from InsP_6 . InsP_7 is degraded by the diphosphoinositol phosphate phosphatases (DIPPS) encoded by *NUDT* genes into DIPP. Over expression of Ip6-K and DIPP induces metastatic potential of prostate cells. Drug resistance and metastasis in PCa is attributed to the stem cells. Hedgehog (Hh) signalling pathway which is expressed in the stem cells is also involved in the development of prostate at the embryonic level and in malignant transformation of the prostate cells.

The purpose of this study is to investigate the role of InsP_7 through Ip6-K and/or DIPP in the malignant transformation of prostate cells, with particular emphasis on their role in modulation of Hedgehog signalling.

Objectives

To study -

1. Role of InsP_7 /Ip6-Ks/DIPP in PCa growth.
2. Effect of modulation of InsP_7 / Ip6-Ks/ DIPPS on growth of PCa in response to testosterone/ DHT.
3. Role of inositol pyrophosphates in prostate development.
4. Role of inositol pyrophosphates in Hh signalling.

Results and Conclusions

Effect of the Ip6-K inhibitor, TNP on growth / viability of prostate cancer cell line - PC3 has been evaluated. TNP induces a dose dependent cell death in PC3 cells (see Figure 1).

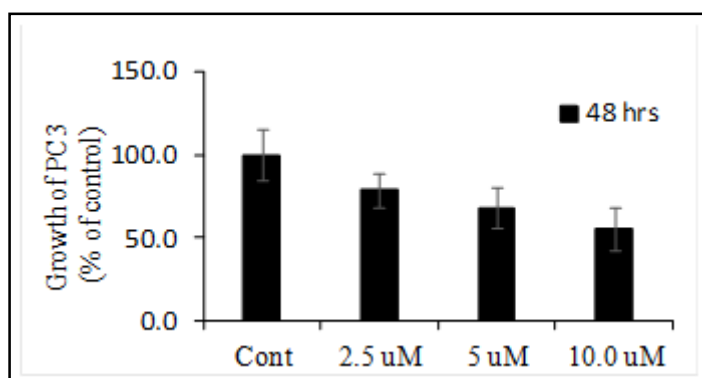


Figure 1. Ip6-K inhibitor - TNP causes cell death in PC3 cells: 0.7×10^6 cells/well were treated with various concentrations of TNP (as shown). At 48 hrs. the effect on cell growth was seen by using the Trypan blue exclusion assay.

6 Biochemical Parameters in HIV-TB co-infection. (Ph.D project)

Technical Team

1. Mr. ShreewardhanRajopadhye
2. Mr. Sandeepan Mukherjee
3. Dr. M H Ansari
4. Dr. R S Naanavare
5. Dr. LalitAnande
6. Dr. Vijay Karanjkar
7. Dr. SuchetaP.Dandekar
8. Dr. AbhayChowdhary

Period

2011 – Dec 2016

Introduction

As per the UNAIDS Global report and WHO, at the end of 2014 the total number of people living with HIV/AIDS was 38.3 million and 2.9 million people were infected with HIV and approximately 2.0 million deaths were due to AIDS . The magnitude as well as complexity of HIV-AIDS and the rapidity with which the syndrome has spread globally is one of the most important factors contributing to the necessity of its effective treatment.

TB is a leading cause of death among persons infected with HIV. About one-third of the AIDS patients are co-infected with TB. HIV can activate dormant TB in individuals. If left untreated, someone with active tuberculosis would infect about 10 to 15 people per year. Without proper treatment, 90% of people living with HIV will die within months of contracting TB. TB accounts for an estimated 13% of AIDS deaths worldwide.

Oxidative stress contributes significantly to the pathogenesis and prognosis of the both HIV& TB. One of the key host components in the prognosis of HIV disease is the binding of the transcription factor like NFκB to the LTR of infected HIV proviral DNA, thereby inducing transcription of the virus. TB induces the activation of NFκB cells. Hence, co-infection of HIV with TB may potentiate replication of the proviral DNA of HIV. Assessing role of such *activating factors* would help us in understanding the molecular mechanisms of HIV.

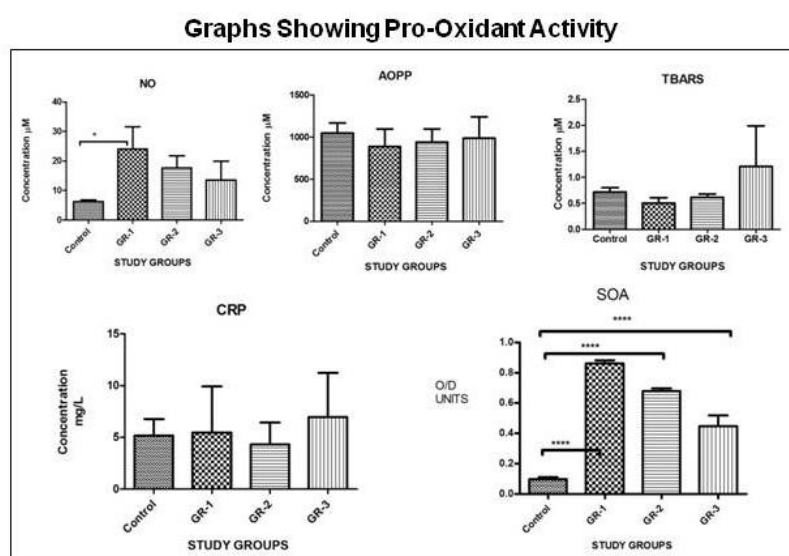
The present study mainly focuses on the role of oxidative stress, TNF α and NFκB in HIV/AIDS and TB which will help to understand their role and contribution in prognosis of HIV in HIV /TB co-infection. It will also help us to understand the molecular mechanism of the HIV disease and its progression.

Objectives

1. To assess oxidative stress markers in HIV, TB and HIV/TB co-infected patients.
2. To assess levels of proinflammatory cytokines in the study cohort.
3. To track activation of NFkB in the study cohort.
4. To correlate prognosis of HIV and TB with the above mentioned markers.

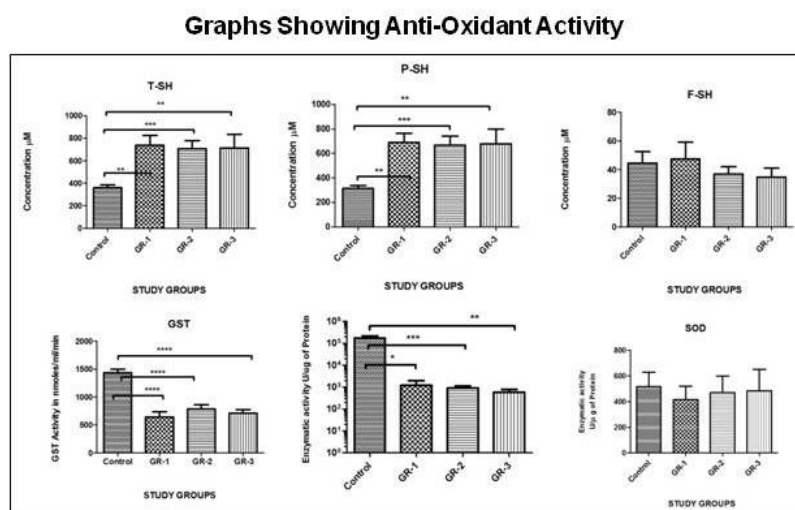
Results and Conclusions

1. Levels of pro-oxidants were significantly increased whereas antioxidant defence markers were significantly impaired in the HIV seropositive group (Figures 8.1 and 8.2).
2. The pro-oxidants exhibited a negative correlation when compared with CD4 counts whereas antioxidant markers showed a positive correlation.



Key: GR-1 CD4 <200, GR-2 CD4 200-499, GR-3 CD4 >500
 one-way annova. ordianry measures , *P<0.1, **P<0.01, ***P<0.0001

Figure 1 Levels of various pro-oxidant markers as indicated, in all the three study groups and control.



Key: GR-1 CD4 <200, GR-2 CD4 200-499, GR-3 CD4 >500
 one-way annova. ordianry measures , *P<0.1, **P<0.01, ***P<0.0001

Figure 2 Levels of various anti-oxidant markers as indicated, in all the three study groups and control.

These results are evidently indicative of oxidative stress in the HIV seropositive study population. Severely impaired antioxidant machinery leads to higher levels of pro-oxidants in the system. These in turn contribute negatively to the prognosis of HIV/AIDS.

9 Role of inflammation in Obesity and Type 2 diabetes. (Ph.D project)

Technical Team

1. Ms. GauriPatade
2. Dr. Vikrant Bhor
3. Dr. Satish Dharap
4. Dr. Priyadarshi Doctor
5. Dr. A. Rosalind Marita

Period

Oct 2013 – Dec 2016

Introduction

Type 2 diabetes has become the prime cause of morbidity and mortality in today's age. Almost 300 million people are affected worldwide. The increasing prevalence of type 2 diabetes in India is alarming and is attributed to the changing lifestyle. Increase in dietary calories combined with reduced physical activity levels result in metabolic disorders such as diabetes, obesity and cardiovascular disease. Smoking, stress, alcohol and genetic susceptibilities are other known risk factors. However, the exact pathogenesis of the disease is still not fully understood. Here, we propose to understand the role of inflammation, particularly contribution of the pro- and anti-inflammatory cytokines, in the pathogenesis of Type 2 diabetes and obesity. Hence, how pro-inflammatory M1 and anti-inflammatory M2 macrophages that reside in the adipose tissue, affect function of the adipose tissue and alter glucose homeostasis will be investigated.

Objectives

1. To assess the factors involved in the release of cytokines, using cell culture model of macrophages.
2. To evaluate the effect of cytokines in the development of insulin resistance, using cell models of insulin target tissues.
3. To assess the characteristic macrophages associated with the adipose tissue in human subjects with or without obesity or diabetes.
4. To characterize the defects in insulin signalling or glucose metabolism in the adipose tissue of these obese subjects.

Results and Conclusions

The factors responsible for the release of pro-inflammatory cytokines were assessed in RAW 264.7, a murine macrophage cell line. Fatty acids enhanced inflammatory signalling in

macrophages. Moreover, fatty acids with LPS resulted in higher expression of proinflammatory cytokines, as compared to the fatty acid or LPS alone (Figure 1).

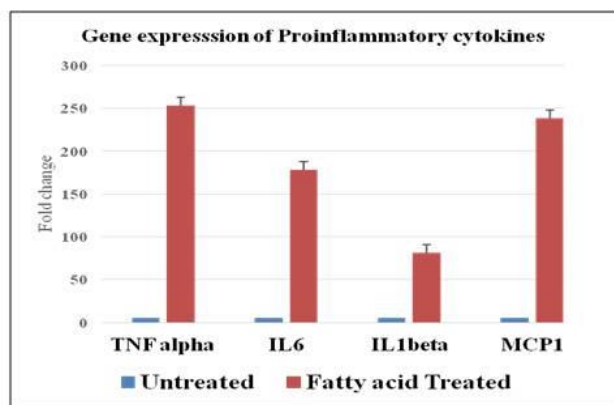


Figure 1 Fold changes in the gene expression of proinflammatory cytokines with respect to the house-keeping gene beta-actin, in RAW cells.

Further, we observed that on LPS stimulation the macrophages secrete TNF α and IL1 β , through the activation of Toll like receptors (Figure 2).

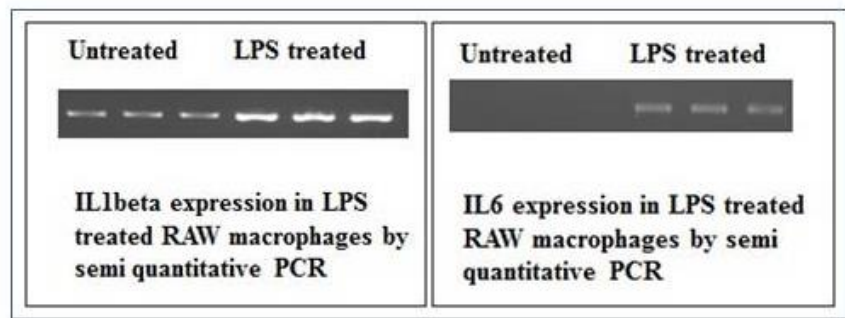


Figure 2 Effect of LPS on expression of IL1b and IL6 in RAW cellsas studied by conventional PCR. The PCR products were analyzed on 2% agarose gel. Representative gel pictures are shown.

We have analyzed 54 samples from human surgical adipose tissue for various parameters. Out of the 5 surgical samples analyzed for all the three cell surface markers, namely CD45, CD11c and CD206, one was diabetic. This one sample had 85% proinflammatory (CD11c⁺and CD206⁺) immune cells whereas the percentage of proinflammatory dual positive cells in control subjects were in the range of 15 – 50 % (n=4).

We have also looked at the mRNA expression of proinflammatory markers in the adipose tissue of these subjects. In the obese and diabetic subjects,an increase in the proinflammatory surface markers, expression of CD11c mRNA and size of adipocytes was found; indicating inflammation in the adipose tissue.Analysis of more samples for confirmation of these observations is in progress.

10 Serum Brain Derived Neurotrophic Factor and Gene Mutation in Autism Spectrum Disorders. (Ph.D project)

Technical Team

1. Nikhil Kasarpalkar
2. Dr. Sweta Kothari
3. Dr. Shilpa Kulkarni
4. Dr. Usha P. Dave
5. Dr. Abhay Chowdhary

Period

Aug 2014 - Jul 2016

Introduction

The Brain Derived Neurotrophic Factor (BDNF) belongs to the family of proteins, called neurotrophins that are responsible for the growth, development and maturation of neurons in the brain and plasticity in the nervous system. Involvement of BDNF is reported in many psychiatric disorders e.g. Alzheimer's Disease, Huntington's Disease, Bipolar Disorder. It is proposed to investigate the role of BDNF, if any, in the pathogenesis of Autism Spectrum Disorders.

Objectives

1. To study genotype of the BDNF gene in the autistic population in India for the Val66Met mutation (G196A polymorphism).
2. To investigate for correlation between the serum BDNF concentrations of children with ASD and further study genotype- phenotype correlation.

Results and Conclusions

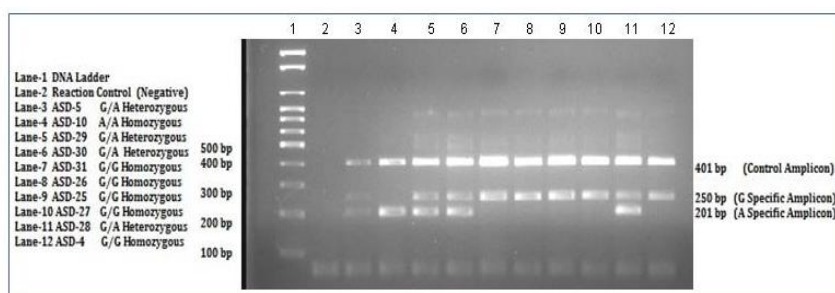


Figure 1A representative 2 % Agarose gel for the Val66Met mutation (G196A polymorphism)

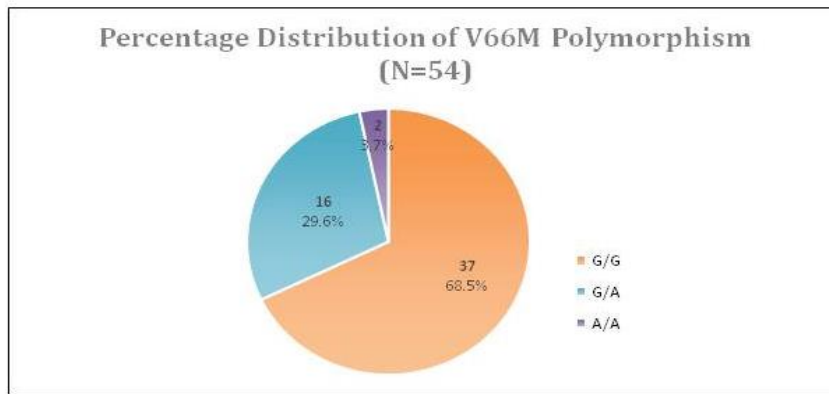


Figure 2 Distribution chart for the Val66Met mutation (G196A polymorphism)

A total of 54 case subjects have been collected and analyzed for the Val66Met polymorphism. Of these, 2 subjects were homozygous for the MET(AA) allele (Figure 1 and 2).

11 Genetics of Epilepsy: Mutational Diagnosis and Genetic Counseling.

Technical Team

1. Mr. Nikhil Kasarpalkar
2. Dr. VandanaKhumavat
3. Dr. UshaPadmanabhan
4. Dr. Usha Dave

Period

Jul 2014 – Jul 2016

Introduction

The World Health Organization (WHO) estimates that around 50 million people worldwide have epilepsy and that nearly 80% of the people with epilepsy are found in developing regions. Estimated number of epilepsy cases in India by the year 2015, is approximately 10-15 million. Hence, it is proposed to undertake mutation analysis study in familial epilepsy cases in Indian population.

Objectives

1. To screen and identify the families having more than one sibling with idiopathic epilepsy and/or family history of epilepsy/IGE or other known type of familial epilepsy proved by appropriate neurological examination & EEG pattern.
2. To conduct a mutational study on selected families (n = 10) for the epileptic conditions which are very prevalent in the Indian population - *SCN1A*, *PNKP* and *SYN1*.
3. To carry out genotype - phenotype correlation analysis in the diagnosed patients and characterize the nature and inheritance pattern of type of epilepsy & mutations, to generate genetic epidemiological data in Indian population with epilepsy.
4. To offer genetic counselling to the affected families and guidance for the treatment and its follow up.

Results and Conclusions

Genotyping of SCN1A in GEFS patients

PCR for exon 1 and exon 21 of the *SCN1A* gene were standardized and 80 samples from 10 families were analysed for the same (Figure 1). Sequencing of the PCR products is in progress.

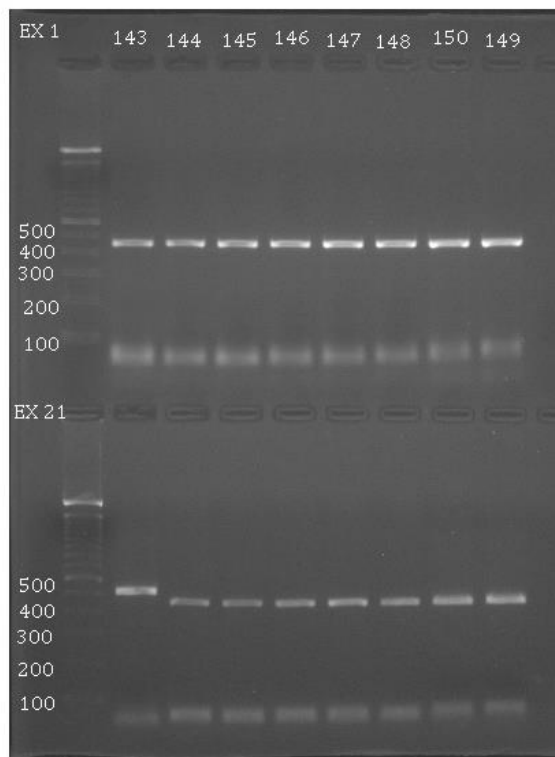


Figure 1 Representative gel showing PCR products for SCN1A - exon 1(441 bp) and exon 21 (519 bp). Numbers on the gels indicate patient codes.

12 Enhancement of rosmarinic acid, a neuroprotective, anti-viral compound by *in-vitro* tissue culture of *Menthapiperita*(L.)

Technical Team

1. Ms. Kshama Dhobale
2. Dr. Pratibha Chaturvedi
3. Dr. Abhay Chowdhary

Period

Feb 2015 – Jan 2019

Introduction

In vitro production of secondary metabolites in plant cell suspension cultures from various medicinal plants or bioreactors is the key step for their commercial production. Therapeutic compound, rosmarinic acid (RA) is a constituent of *Menthapiperita*(L.). The project aims at enhancement of rosmarinic acid (RA) in tissue culture of *Menthapiperita* (L.).

Objectives

1. Increase biomass production of *Menthapiperita* (L.), *in vitro*.
2. Biosynthesis, extraction, quantification and enhancement of antiviral bio-active principle, rosmarinic Acid in *Menthapiperita* tissue culture
3. Analysis of protein profile of treated sample *in vitro*.

Results and Conclusions

Various hormones such as dicamba, NAA, kinetin and zeatin were used alone or in combination to enhance biomass production of *Menthapiperita* (L.). Dicamba and NAA combination showed good colour and proliferation of callus, suggesting improved yield of RA. The developed technology in tissue culture might provide an affordable, economically feasible mean to bio-enhance production of RA, an antibacterial, antifungal and antiviral neuroprotective compound.

13 HIV induced reduction in hematopoiesis.

Technical Team

1. Dr. UshaPadmanabhan
2. Dr. AbhayChowdhary
3. Dr. RitwikDahake
4. Dr. Prasad S Koka

Period

Oct 2013 –Sept 2015

Introduction

In India, HIV+ population equates to over 2.1 million. In HIV+ individuals the CD4+ T / platelet cell number count gradually decreases over a period of 8-10 years. This leads to the development of severe cytopenias in patients, weakening the immune system and paving the way for opportunistic infections and AIDS. In healthy humans, any blood cell (including CD4+ T cells and platelets) depletion that may occur due to any infection, including HIV is counter balanced by circulating CD34+ stem cells. These cells undergo differentiation and maintain blood cell homeostasis. We hypothesized that HIV causes release of a factor/s either host or viral which inhibits CD34+ stem cell differentiation leading to cytopenias and increased susceptibility to AIDS. HIV modulated hematopoietic differentiation inhibition pathway could be a potential target for therapeutic intervention.

Objective

To identify viral and/or host factors which inhibit haematopoiesis of CD34+ stem cells.

Results and Conclusions

Recombinant HIV virus was produced *in vitro* by transfecting HEK293T cells with plasmids encoding the HIV strains pIndieC1 and pNL4-3 by using the CaCl₂ method. Recombinant viruses were used to infect CD4+ T cells, that were isolated from adult peripheral blood. Filtered culture medium from infected CD4+ T cellscontaining any host factors was used to assess its effect on differentiation of CD34+ stem cell isolated from cord blood (Figure 1).

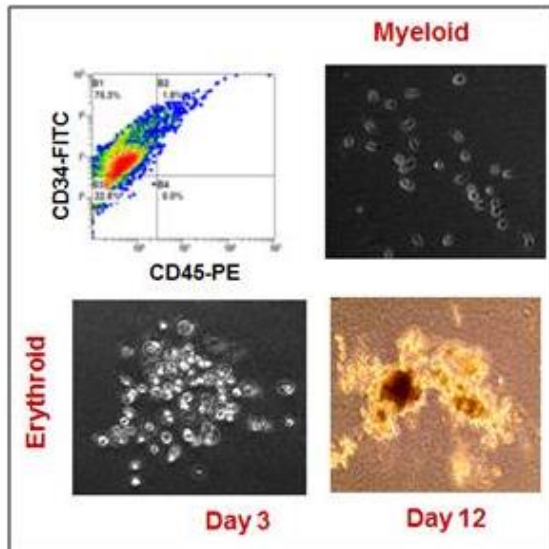


Figure 1 Isolation and differentiation of CD34+ stem cells: Top left panel shows the purity of CD34+ cells isolated as assessed by FACS. Top right panel shows myeloid differentiation of CD34+ stem cells on day 3. Bottom two panels show erythroid differentiation of CD34+ stem cells on day 3 and day 12, respectively.

Differentiation of CD34+ stem cells was inhibited by the filtered CD4+ T cell culture medium (Figure 2).

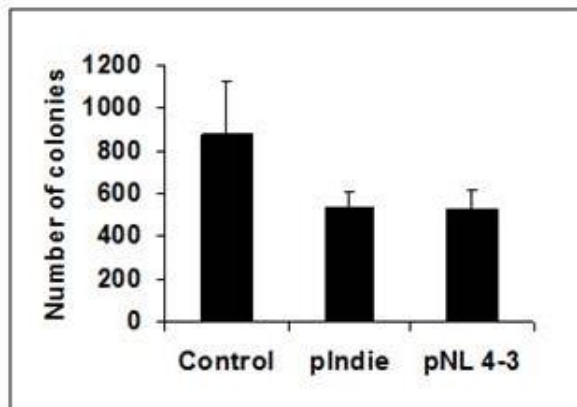


Figure 2
Filtred culture medium from infected CD4+ T cells containing the putative host factor inhibits CD34+ stem cell differentiation.

The host factor is identified as miRNA. Sequencing of the miRNA is in progress.

14 Kidney stones & their constituents

Technical Team

1. Dr. UshaPadmanabhan
2. Mr. PravinShirsat
3. Mr. Sameer Patil

Period

Jan 2014 – Jan 2018

Introduction

Renal calculi (or kidney stones) have affected mankind since history began and the disease appears in many parts of the world with variable intensity. High incidence areas are Scandinavian countries, Mediterranean-British isles, Central Europe, Northern Australia, parts of Malaysia, China, Pakistan and North-western India.

The origin of many stones is related to the amount of their constituents excreted into the urine. Urine is normally supersaturated with calcium oxalate and therefore, most urines are metastable, whether they are from normal or stone-forming individuals. Normal urine contains citrate that inhibits formation of calcium-containing crystals. Proteins such as calgranulin(an S-100 calcium binding protein), Tamm-Horsfall protein, glycosaminoglycans, uropontin, nephrocalcin, prothrombin, F1 peptide and bikunin present in urine also act as inhibitors to stone formation. However, when these substances fall below their normal proportions, the urine becomes supersaturated with one or more solutes which can precipitate out. A seed crystal may form by the process of nucleation. Other solutes within urine deposit onto the seed crystal which serves as the solid scaffold and heterogenous nucleation proceeds.

Objectives

1. To retrospectively analyze stone composition such and analyze if any correlation exists between stone formation and population demographics.
2. To explore better diagnostics for renal calculi by protein profiling and genetic analysis.

Results and Conclusions

250 kidney stone samples that were characterized biochemically during the period 2008-2014 were retrospectively analyzed. Majority of the samples presented to our centre were from male subjects. Children were more susceptible to the presence of sulphonamide and carbonate stones ($p = 0.003$ and 0.008 , respectively). Women were more prone to phosphate stones and moderate non-vegetarians were more prone to calcite stones. Taken together, the data suggested that all three factors age, gender and diet can influence composition of stones.

15 Evaluation of leptin levels in malnourished children from Nandurbar.

Technical Team

1. Dr. Usha Padmanabhan
2. Dr. Rosalind Marita
3. Dr. Mridula Phadke

Period

Apr 2015 – Mar 2016.

Introduction

Malnutrition is very prevalent in the State of Maharashtra and the Nandurbar district is the worst affected. The project to tackle malnutrition was a joint initiative of the RajmataJijau Mother Child Health & Nutrition Mission, Department of Women & Child Development, Govt of Maharashtra, UNICEF, TATA Trust and Public Health Department. The project basically involves giving nutraceuticals to children suffering from severe acute malnutrition (SAM) across 6 blocks of Nandurbar district and assessing their progress. In common parlance, leptin provokes hunger whereas grehlin (obestatin) provokes the feeling of satiation in healthy individuals. Role of the investigators was to estimate leptin levels in children with SAM.

Objective

To estimate leptin levels in malnourished children from Nandurbar.

Results and Conclusions

Leptin was estimated in 80 blood samples of children (ages ranged from 6 months – 4 yrs) from the Nandurbar district. In healthy Indian children leptin levels are 5.4 ± 1.7 ng/ ml¹. In the tested samples, leptin was in the range from 70 pg/ ml – 18.28 ng/ ml. Only 18 children (22.5 %) had leptin levels greater than 5 ng/ ml. Leptin levels in African children with SAM are reported to be in the range of 250 ± 320 pg/ ml². Our values are well within the ranges of those reported above, indicating that in majority cases, leptin could contribute to SAM.

References:

1. Dubey et al., IndianPediatr44(4): 257-62 (2007).
2. Bartz et al., J ClinEndocrinolMetab99(6): 2128–2137 (2014).

16 Studies to validate safety and to understand mechanism of Ayurvedic formulation RaupyaSuvarnaSootshekhar (RSS) and its therapeutic metallic ingredients used for neural therapy

Technical Team

1. Dr.VineetaDeshmukh
2. Dr.NishigandhaNaik
3. Dr. D. S. Joshi
4. Dr.Mansi Thakur
5. Ms.Smital Kulkarni

Please see details under Research Projects based on Ayurveda

17 Isolation, Characterization and Screening of potential anti-inflammatory drugs from Praval (Coral / *Coralliumrubrum*)

Technical Team

1. Dr. Usha Padmanabhan
2. Dr. Narendra Bhat
3. Dr. Vandana Kozarekar

Please see details under Research Projects based on Ayurveda

18 Characterization and assessment of anticancer activity of Indian honey. (Ph.D project)

Technical Team

1. Dr. Sucheta P. Dandekar
2. Ms. Amruta Naik
3. Dr. Nishigandha Naik

Please see the details of these projects in under Research Projects based on Ayurveda

Publications

1. Evaluation of the anti-angiogenic and antioxidant activity of Indian honeys in comparison with manuka honey from New Zealand, Naik A, Juwekar OD, Ansulkar P, Deshpande SD, and Chowdhary AS, Intl J PharmaSci Res 2015, 6(4), 1813-1818.
2. Correlation of biochemical constituents present in kidney stones and diet from an Indian population., Shirsat PL, Patil SS, Parkar SN, Rajopadhye SH, Bagal SB, Ansulkar PV, Naik A, Marita R, Chowdhary AS and Padmanabhan U, Intl J Res Pharm Sci, 2016, 6(1), 7 -11.
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5. Chemical and pharmacological study of the twigs of Thevetiaperuviana plant and its bio-activity profiles, Save SA, Padmanabhan U, Kothari ST, Lokhande RS, Chowdhary AS, Intl J Res Eng App Sci 2015, 5(6), 123 -138.
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7. Effect of Homeopathic preparation on Phenolics production in Tylophoraindica and Curcuma longa, Chaturvedi P, Mukherjee S and Chowdhary A, Adv Plant Sci, 2015, 28(1), 115-119.
8. Evaluation of anti-viral activity of essential oil of Trachyspermumammi against Japanese encephalitis virus, Roy S, Chaurvedi P, Chowdhary A, Pharmcog Res, 2015, 7, 263-267.
9. Anti-Influenza Potential of Alkaloidal Molecules of JatrophaCurcas Leaves, Chavan R, Chaturvedi P, Chowdhary A, Int J Pharm Sci Res, 2015, 6(11), 4705-4711.
10. Approaches for Antivirals Production in Tissue Culture of Medicinal Plant, Chaturvedi, P, Chowdhary, A, OMICS Publications US, 2015, ISBN No 978-1- 63278-052- 2.
11. Where do the hybrids go? Padmanabhan U, Curr Sci, 2016, 110(6), 957.
12. Preclinical model for identification of therapeutic targets for CML offers clues for handling imatinib resistance, Molli PR, Pradhan MB, Ingle AD, Naik NR*, Biomedicine & Pharmacotherapy, 2015, 73, 160-170.

Patents*

Granted

Naik NR* as an inventor in patents granted to the Piramal Enterprises Limited

Sr. No.	Patent No.	Country	Granted in
1	US 9,062,046 B2	USA	23 June- 15
2	JP 5750158 B	Japan	July-15
3	2013/01212	South Africa	July-15
4	607060	New Zealand	July-15

* The publication and patents granted are on the work done with the previous employer.

Chemotherapy Department

Head of Department's Overview

The Department of Chemotherapy is engaged in Training, Research & Testing activity.

Training:-

The department is affiliated to the University of Mumbai for M.Sc&Ph.D degrees for Organic Chemistry. During the year, three students were awarded the PhD degree and two students obtained M.Sc. degree. Currently in the Department, one student is registered for Ph D (Dr. BabasahebAmbedkarMarathwada University) and two students are registered for M.Sc degree for Organic Chemistry. The Department is going to present & published their research work in National & International Conferences. The department has started short term training programme for one month, 45 days, three months, six months (STRIP) for graduate & post-graduate students of M.Sc(Chemistry), B.Pharm, M.Pharm.

Research:-

Chemotherapy Department mainly focussed on the synthesis of heterocyclic compounds for their possible therapeutic values. Compounds were synthesized & characterized by using Analytical facility available in the Department. Chemotherapy department approaches synthesis of heterocyclic compounds by using green techniques (microwave irradiation).Additionally Department focused on research using in silico drug designing tool.

Testing:-

The department is involved in testing activities of HPLC (High Performance Liquid chromatography), GCMS (Gas chromatography Mass Spectrophotometer), and IR (Infrared Spectrophotometer) samples on commercial & academic purposes.

Future directions:-

To develop laboratory processes for synthesis of drug intermediates of commercial importance.

To train students and research scholars in newer methods of chemical synthesis.

To develop a well-equipped Chemistry Laboratory capable of undertaking sponsored Industrial Projects for Chemical Process Development.

To undertake Structure-Activity Relationship studies for New Drug Development in the area of Chemotherapy of Infectious Diseases.

The following staff members of the department were recognized guiding teachers at the University of Mumbai for the respective degrees and subjects mentioned below

Sr. No.	Name of the Guiding Teacher	Degree & subject for which recognised	Intake capacity	No students Registered
1	Dr. Ashish Asrondkar	M.Sc, Chemistry	8	1
2	Dr. VrushaliPatil	M.Sc, Chemistry	8	Nil

Dr. Ashish LawooAsrondkar, PhD, FISCA

Research projects

1. Synthesis & Biological evaluation of Benzothiazole derivatives.

Technical team

1. Dr. A.S. Bobade
2. Mrs. Priti Bendre

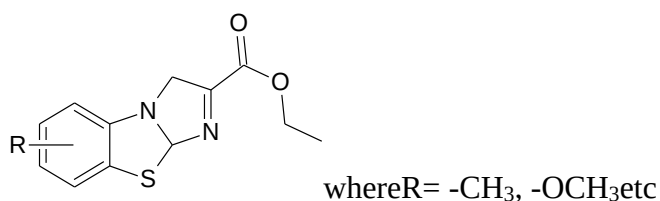
Period

Jul 2015- Jul 2018

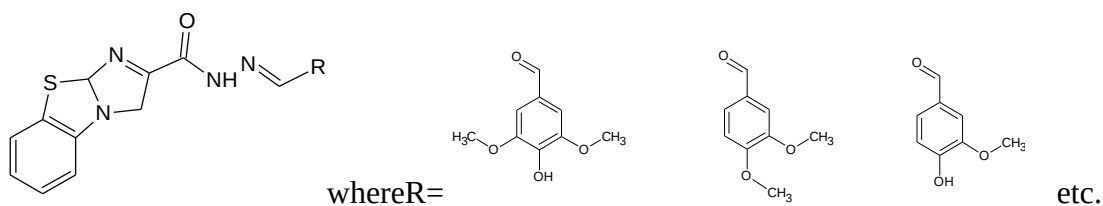
Introduction

In this project our aim to synthesized following heterocyclic compounds -

SERIES 1:- Synthesis of Ethyl substituted 3, 9a-dihydroimidazo [2, 1-*b*][1,3] benzothiazole - 2-carboxylate



SERIES 2:- Synthesis of *N'*-[(1*E*)-substituted]-3, 9a-dihydroimidazo[2,1- *b*] [1,3] benzothiazole -2-carbohydrazide



SERIES 3:- Synthesis of *N'*-(Substituted benzoyl)-3,9a-dihydroimidazo[2,1-*b*][1,3] benzothiazole-2-carbohydrazide

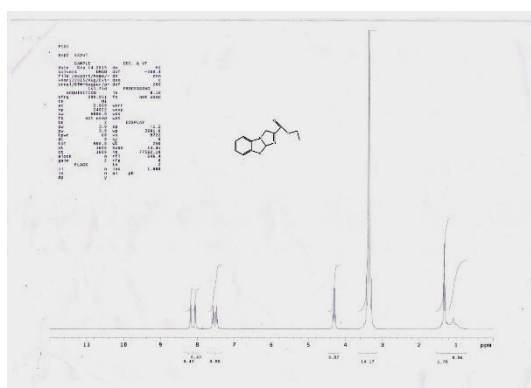


Objectives:

1. To establish the method of synthesis for the proposed compounds.
2. To synthesize the title compounds by appropriate methods.
3. To carry out the preliminary tests such as physical determination, solubility, TLC.
4. To confirm the structures of the synthesized compounds by Spectroscopic and chromatographic technique.
5. *In vitro* Screening of synthesized compounds for their biological activity.

Results and Conclusions.

Synthesis of Ethyl substituted 3,9a-dihydroimidazo [2, 1-*b*][1,3] benzothiazole-2-carboxylate completed following are the confirmatory results:



2. Synthesis & biological activity of thiadiazole & Benzothiazole derivatives (M.Sc project)

Technical team

1. Ashish Shinde
2. Dr. Ashish Asrondkar

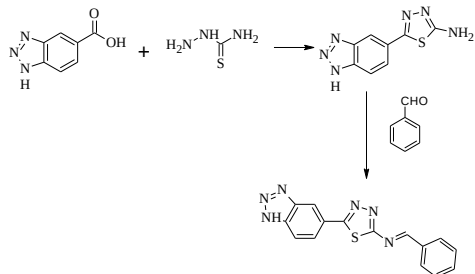
Period

Mar 2016 - Mar 2019

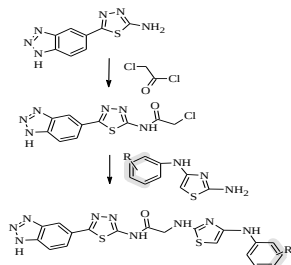
Introduction

The development in chemotherapy of synthetic drugs totally depends upon the biological results of chemotherapeutic agents which ultimately give the guidelines to the research workers in selection of agents for further studies and set the pattern of future drug evaluation. Taking this into consideration, the different heterocyclic derivatives were characterized and deduced their activity against microbes. Therefore in this project we prepared 4 different schemes for preparation of compounds.

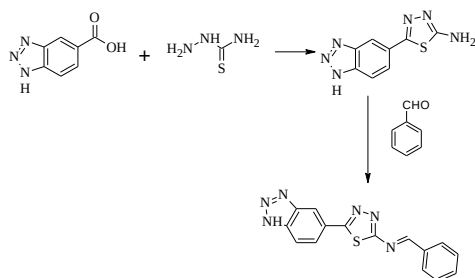
Scheme 1:-Synthesis of N- [2-(4-{[5-(1H-benzotriazol-5-yl)-1, 3,4-thiadiazol-2- yl] sulfamoyl} phenyl) ethyl]substituted phenylamide



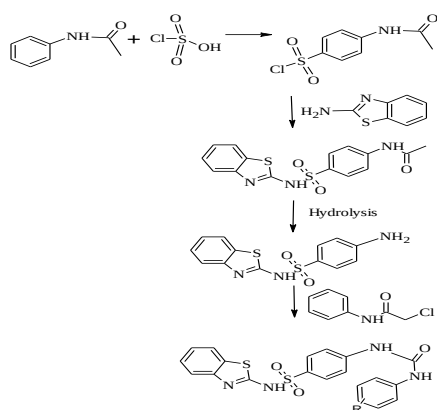
Scheme 2:-Synthesis of N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-2-(4-[substituted phenyl] amino)1,3thiazol-2-yl}amino)acetamide



Scheme 3:- Synthesis of (E)-N-[5-(1H-benzotriazol-5-yl)-1,3,4-thiadiazol-2-yl]-1-(Substituted phenyl) methanimine



Scheme 4:- Synthesis of N-(1 3-benzothiazol-2-yl)-3-methyl-4-[(substituted phenyl) amino] benzenesulfonamide



Objectives:

1. To establish the method of synthesis for the proposed compounds.
2. To synthesize the title compounds by appropriate methods.
3. To confirm the structures of the synthesized compounds by Spectroscopic and chromatographic technique.
4. *In vitro* Screening of synthesized compounds for their biological activity.

Results and Conclusions.

Registered on dated 29/04/2016 for M.Sc (by research) in the subject of Chemistry under Mumbai University.

3. Development of Synthetic methodology for various bioactive nitrogen, sulphur and oxygen heterocycles.

Technical team

1. Sanjeev Patil
2. Dr. A.S. Bobade

Period

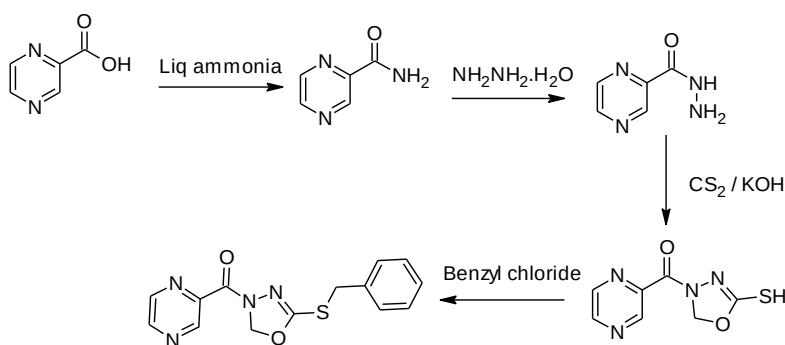
Jul 2013 - Jul 2018

Introduction

Pyrazinamide is a drug used to treat tuberculosis. The drug is largely bacteriostatic, but can be bacteriocidal on actively replicating tuberculosis bacteria. Due to the importance of pyrazine nucleus in drug discovery and our continuous search of new potent and safe anticancer agents the aim of this work is to present a series of fifty-one pyrazinehydrazone derivatives design by molecular hybridization, which was tested against biological evaluation.

Schematic representation of the project

The project is meant for synthesis, characterization and biological evaluation of pyrazine derivatives. Under this scheme in this project we prepared approx. 10-15. Compounds which will be used for further evaluation.

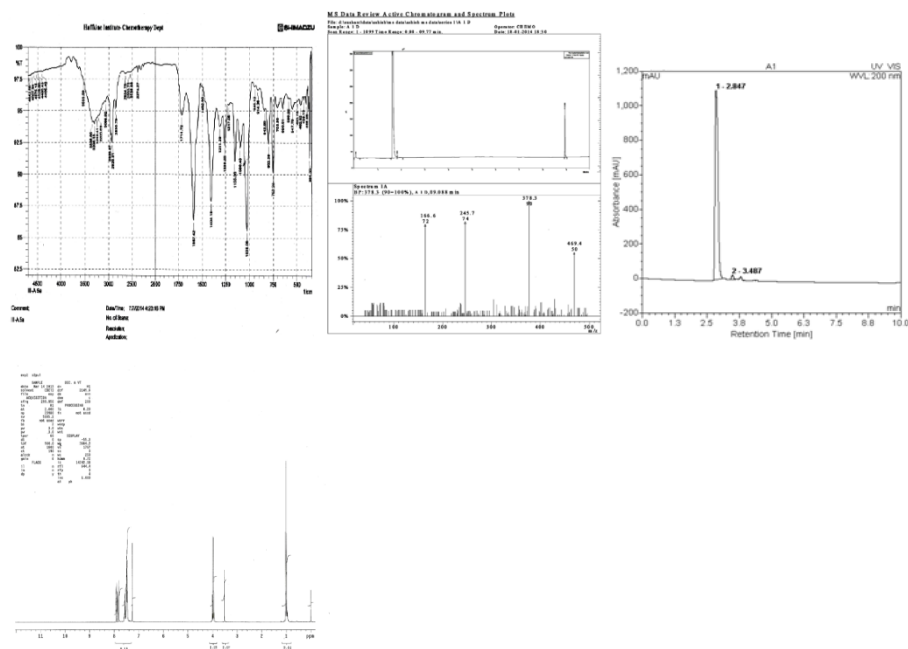


Objectives

1. To establish the method of synthesis for the proposed compounds.
2. To synthesize the title compounds by appropriate methods.
3. To carry out the preliminary tests such as physical constant determination, solubility, TLC.
4. To confirm the structures of the synthesized compounds by Spectroscopic and chromatographic technique.
5. To evaluate the proposed compounds for their biological activity

Results and Conclusions.

These are confirmatory results of some compounds



After validation of all compounds we will check all the compounds for their biological activity.

4. Analysis of kidney stones using conventional biochemical and FTIR techniques.

Technical Team

1. Dr. Nishigandha Naik
2. Dr. Anil Bobade
3. Dr. Ashish Asrondkar
4. Dr.VrushaliPatil

(Please see details under Research Projects, Department of Biochemistry)

5. Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations (completed)

1. Ramaiya M
2. Patil V N
3. Bobade AS
4. Ghag- Sawant M S
5. Narayakkadan A
6. Asrondkar A L

(Please see details in Projects based on Ayurveda)

Publications

1. Synthesis Characterization and analysis of some mercaptobenzothiazole Derivative, Asrondkar AL, Patil VN, Mishra N, Bobade AS, Chowdhary AS, World Journal of Pharmaceutical Research (WJPR), 2015,4(2),618-626 .
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5. Synthesis of Quinoline Analogues as Anti-microbial Agents, Desai GH, Asrondkar AL, Patil VN, Bobade AS, Chowdhary AS, IOSR Journal of Applied Chemistry, 8(5) Ver. II ; 2015,01-03.
6. Synthesis and Biological Evaluation of some Thiazolidinone-Quinoline Derivative, Desai GH, Asrondkar AL, Patil VN, Bobade AS, Chowdhary AS, World Journal of Pharmaceutical Research, 4(6); 2015, 1487-1494.
7. Synthesis and Characterization of Some Coumarin Derivatives, Chowdhary PS, Patil VN, Asrondkar AL, Bobade AS, Chowdhary AS, International Journal of Science & Research, 4(4);2015,2342-2344.
8. Tandem Synthesis of thia-oxadiazolophanes, Malghe YS, Thorat VV, Patil VN, Bobade AS, Chowdhary AS, Journal of Chemical And Pharmaceutical Research, 2015, 7(5), 729-734
9. Synthesis and Characterisation and Biological activities of new bis-1,3,4-oxadiazoles Malghe YS, Thorat VV, Patil VN, Bobade AS, Chowdhary AS, Journal of Chemical And Pharmaceutical Research, 2015, 7(6), 392-398
10. Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations, Patil VN, Ghag- Sawant MS, Narayakkadan A, Asrondkar AL, Bobade AS, Ramaiya M, International Journal Of Scientific and Research Publication, 2015, 5(12), 277-283.

Chemical Testing Unit

Head of Department's Overview

Prime function of the Testing unit is to perform analysis of samples submitted by State Excise Department of the Government of Maharashtra

The main aim and objective of the Chemical Testing Unit is to analyze the alcoholic beverages like Indian Made Foreign Liquors (I.M.F.L) Toddy, Country Liquors etc, also to analyze the Denaturant (such as Benzene, Acetone, Crotonaldehyde, Diethyl phthalate, Diethyl ether, Pyridine Base, Toluene, Diethyl amine) Bitterants (Denatonium Saccharide and Denatonium Benzoate) Colorant (Crystal Violet & Methylene Blue) those are added for preparing Ordinary Denatured Spirit (O.D.S) and Special Denatured Spirit (S.D.S) sent by State Excise Department of Government of Maharashtra.

Also to analyze the samples of Special Denatured Spirit (S.D.S), Ordinary Denatured Spirit (O.D.S), French Polish, Thinner etc. sent by State Excise Department of Government of Maharashtra.

Currently the department is conducting a training programme “Workshop on Testing of Alcoholic Beverages” since last 3 years and few research project are also been carried out in the department.

The department is planning to conduct more research project utilizing the sources available in the department. The department has started giving research Training to STRIP Students.

Ms. Suvarna Pachpore

Research projects

1. In-vitro evaluation of the dermato - protective potential of *Hibiscus rosasinesis* flower extract

Technical Team

1. Mrs. Amita Patil
2. Mrs. Suvarna Pachpore
3. Dr. Sweta T Kothari
4. Dr. Anil Bobade
5. Dr. Abhay Chowdhary

Please see details under Research Projects based on Ayurveda

2. Detection of chemical constituents presents in *Phyllanthus Emblica* fruit Juice.

Technical Team

1. Mr. Manoj K. Patil
2. Mr. Santosh B. Bagal
3. Dr. Anil Bobade

Please see details under Research Projects based on Ayurveda

3. Synthesis of Nanosilica from RHA for controlled drug release.

Technical Team

1. Mr. Manoj K. Patil (Principal Investigator)

Please see details under Nanoparticle research

Research at the Snake Farm

Venomous Animal Unit, Zoonosis Department

Head of Department's Overview

Venomous animal Unit is India's 1st premier unit working on snake and snake venom the building of snake farm was established in 1960 in the premises of Haffkine institute.

Historical Mile stones The history of venom/ anti venom research and procedure for venom extraction from live venomous snakes started way back before 1920 by honourable Dr. Dalal. In the year 1938, Department of Entomology was established under the leadership of Dr. M. Sharif, D.Sc., PhD, to work mainly of entomology with plague and its transmission.

Dr. P. J. Deoras, Assistant Director in 1960 had continued to work on plague and established Venomous animal for Public Health Importance. In 1970 the department was renamed as Department of Zoonosis under the guidance of Dr. M. V. Sant Deputy Director to concentrate more on communicable diseases of Zoonotic importance. Under department of Zoonosis two units were established namely Entomology unit and Venomous animal unit. Dr. D. M. Renapurkar in 1980's had taken the charge of the department of zoonosis as Assistant Director and flourished the activity of the department to study the snake repellency, study of anti snake and anti scorpion venom. Dr. N.G. Chanderkar, Assistant Director holds the charge and continued the same activity till 1999. After him Dr. B.D. Sathe, served the department as Assistant Director. Dr. L.S. Patil took the charge in 2006. Mr. K. R. Pawar, was posted as Head of the Venomous animal unit from 2009 to 2011. Dr Mira Ramaiya is heading the department of Zoonosis since 2012 till date. Dr. Mrunal Ghag- Sawant is posted as Incharge Venomous animal unit in 2012 and she continued the previous activities and prospers the department with newer Research, training and testing activities. **Present state of affairs** The venomous Animal Unit is actively engaged in Training, research and testing. Department is offering regular and short term training courses such as awareness training programmes on Snake and its importance for those who are working with the public health authorities, paramedical and medical institutes, colleges and schools at state and national levels. Department also undertake scientific workshop "Introduction to covert existence of snake" and "Snake bite death mitigation" which covers identification of venomous and non-venomous snakes, how to apply first aid measure post snakes bite and how snakes are important to maintain the ecological balance. Workshop on "Principle and practices of Laboratory animals" is regular hands on workshop encompass concepts of in vivo experiments and its applications in the field of research as well as ethical issues, welfare regulations. "Rodent Pest management" is 3 days field training to understand the Biology and Control of Rodent Vector for the betterment of Public Health diseases.

Department is involve in venom biology research right from its inception, various core areas of research includes screening of natural/ synthetic compounds to explore the repellent property against snake, Evaluation of naïve molecules from venom to explore its therapeutic potential, study of whole body profile of live venomous creatures, screening of natural/ synthetic compounds to explore the natural alterative to anti snake venom, Venomics of snake venom proteins.

Department conducts various testing activities of public importance such as the Anti rodent, Anti squirrel and Anti termite research and testing procedure for evaluating the efficacy of repellents coated in pipes, cables etc and testing of seized venom samples of Forest department. Apart from this department is also involved in lyophilization of venom as per WHO guideline and sale of venom for research, education and anti venom production.

Dr Mrunal Ghag-Sawant & Dr Mira Ramaiya
Incharge, Venomous Animal Unit & Head Department of Zoonosis

Research projects

1. Protective Armour Against Snake Bite

Technical Team

1. Dr. Mrunal Ghag – Sawant
2. Mr. M.F. Vohra,
3. Prof. Dr. Abhay Chowdhary

Period

Dec 2014 – Dec 2017

Introduction

Snake bite become common threat for public health problem in many tropical and subtropical countries. India has Big four deadly venomous snakes responsible for mortality. India has the highest number of deaths due to snake bites in the world with 35,000–50,000 people dying per year according to World Health Organization (WHO) direct estimates. In India, the farmers undergo snake bite and is one of the major health hazard for farmers. Snake bite is mostly, due to lack of protection during their farm work. There are two main reasons as No proper protection to the people during their farm work and Non-availability of anti-venoms collection for treatment. There is no proper cost effective protection material used in India for protection against the snake bites.

The rationale behind the concept is that we do not have scientifically tested protective armor available for farmer, paramilitary forces, adventures groups, hikers etc. in India. This project is aimed to develop snake bite protection armor for people which will enable proper protection and reduces the direct risk to snake bite and saves the human life.

We hypothesized that the textile under evaluation will provide the protection against snake bite.

Project schematic:

1. Preparation of Fabric and Characterization which includes Preparation of Fabric, designing, rubber coating, puncture resistance, tensile strength, stretch resistance etc.
2. Selection of snakes based on Species, Gender, Length of fangs and Length of snake
3. Clinical Observation of snakes which comprise Body weight, Locomotion, Mucus membranes, Visible signs /symptoms and Physical examination
4. Evaluation of Snake Bite
 - a. Assessment of snake bite parameters: Time taken for complete bite from start of experiment, Total Exposure time and Injury to the snake

b. Examination of protective textile : Total number of strikes and Visual assessment of bite area

5. Statistical Evaluation of the Results

Objectives of the project

1. To develop puncture resistant fabric with rubber coating.
2. To evaluate the efficacy of fabric with rubber coating against snake bite
3. To develop snake bites protective gloves, shoes for the farmers and footwear for agricultural workers also for paramilitary forces working in Jungles and Sports-persons, Hikers, adventure groups etc.

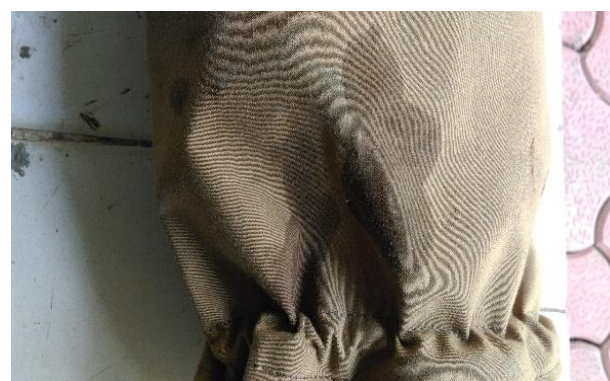
Results and Conclusions

Snakebite repellency record {Study No. : HITRT/ZNS_VAU/ (C)/RES-019/2014}

Sample no./ color	Snake species	Sex	Snake id	Snake length	Snake weight	Total no of strikes	Visual assessment of bite area	Time taken for completter bite from	Injury to snake	Total exposure time
X4-black	Cobra	Male	41	158 cm	1.313 kg	1	Complete bite	20 sec	No	37 sec
X4-black	R.Viper	Male	70	118 cm	0.844 kg	1	Complete bite	169 sec	Yes	174 sec
157016 yellow 1.68 mm	R.Viper	Male	79	130 cm	1.193 kg	5	No bite-only mark	239 sec	No	255 sec
157016 yellow 1.68 mm	Cobra	Male	64	167 cm	1.323 kg	4	No bite-only mark	245 sec	No	297 sec
Brown colour sample double coating 0.8mm	R.Viper	Female	85	135 cm	1.900 kg	1	Only mark	108 sec	No	200 sec
	R.Viper	Female	85	135 cm	1.900 kg	3	Only mark	188 sec	No	190 sec
	Cobra	Male	71	90 cm	0.480 kg	5	Only mark	162 sec	No	165 sec
	Cobra	Female	74	120 cm	0.998 kg	4	Only mark	150 sec	No	170 sec

Turtle skin (std.)	Cobra	Male	42	170 cm	1.482 kg	3	Complete bite	73 sec	No	120 sec
	Cobra	Male	71	90 cm	0.480 kg	8	Complete bite	90 sec	No	190 sec
	R. Viper	Female	76	115 cm	1.071 kg	6	Complete bite	10 sec	No	237 sec
	R.Viper	Male	79	130 cm	1.193 kg	5	Complete bite	28 sec	No	220 sec

Test Set Up:



Conclusion: Brown color sample double coating (0.8 mm) test sample is having good repellency compared to all other samples and standard marketed product (Turtle skin) however further investigation with more sample size is required.

2. Characterization of snake venom by using in vitro Techniques and in vivo Techniques

Technical Team

1. Dr. Mrunal Ghag – Sawant
2. Prof. Dr. Abhay Chowdhary

Period

Jun 2014 - Jun 2017

Introduction

Snakes are rich in diversity over the world. There are currently around 18 families of snakes in world and in between 2500 to 3000 species. In India Cobra, Krait, King Cobra, Russell's Vipers, Saw scaled viper and pit viper are the most popular venomous species of snakes. Snake Venom is highly valued because of their most usefulness in medicinal biology. The most important use of snake venom is to produce the snake anti venom. Considering to all importance of snake venom, it is necessary to evaluate the toxicity of protein components of snake venom. In Haffkine institute, the Snake venom is extracted (milked) from different types of venomous snakes. This extracted venom is stored in the form of lyophilized powder until its requirement for commercial production for anti snake venom or for any research organization who want to carry research on snake venom.

The rationale behind the concept is

1. Snake venom is a very costly substance, but unfortunately we don't have any data who states whether the efficacy of venom is increasing or decreasing in correlation with time of storage.
2. Haffkine institute are in the process of lyophilising the snake venom since Year 1960 till date.
3. In this experiment, we want to check the potency of various lyophilized venom in accordance to the time of storage.
4. It is hypothesised that, snake venom remain active for approximately 5 to 7 years from the date of its extraction from snake, so in this study we want to find out the potency of snake venom and also to determine its efficacy according to time.

We hypothesized that the potency of venom did not decline until 15 years from the date of extraction and lyophilization, we can make use of lyophilized venom for more than 15 year.

Project schematic

1. Selection of 4 years lyophilized old venom sample and fresh sample of snake venom
2. Protein profiling of venom using in vitro techniques

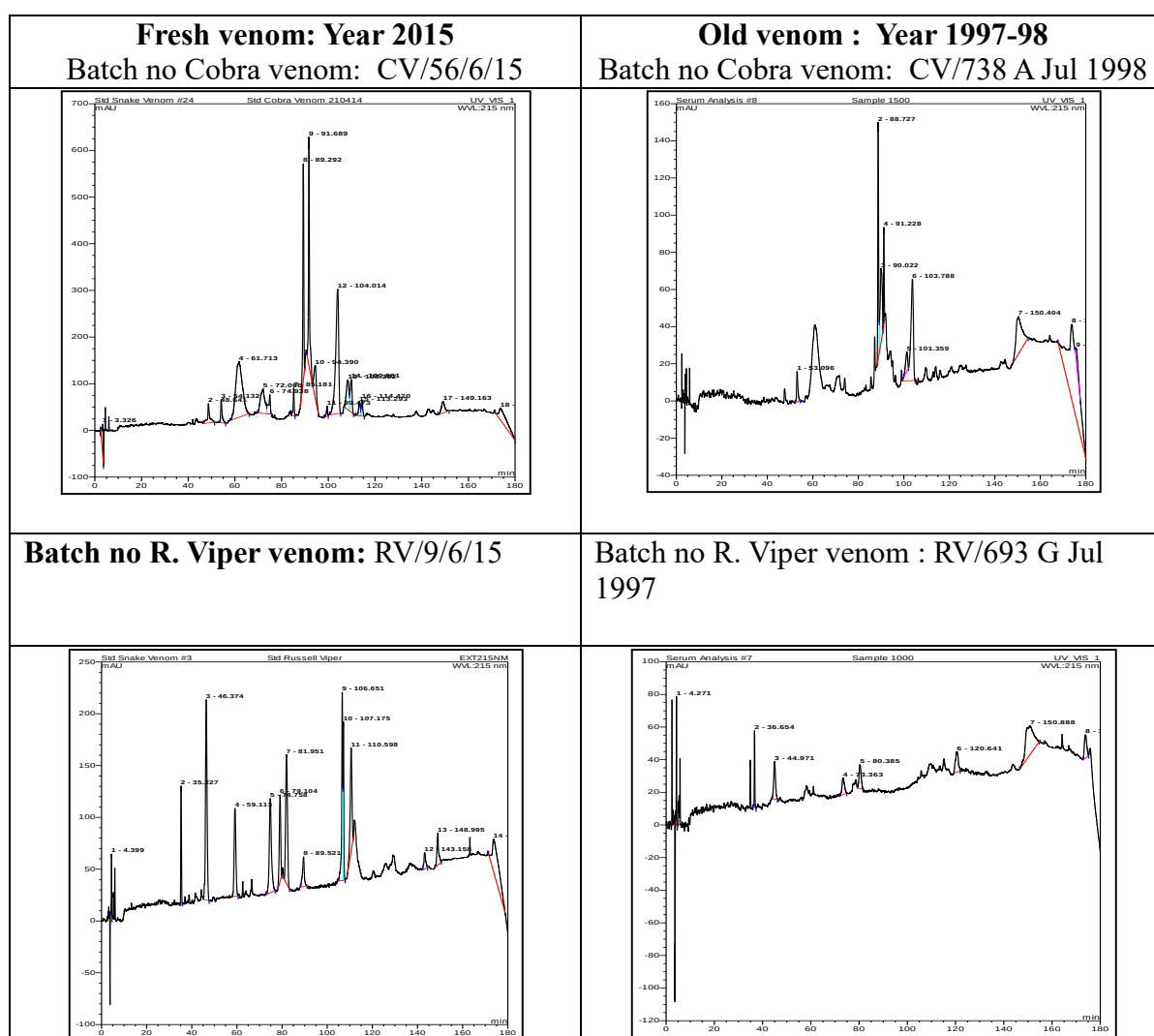
3. Physical parameters of snake venom
4. Screening of in vivo lethal toxicity of venom samples

Objectives of the project

1. To determine the Protein profiling of venom samples from different years.
2. To study the in vivo lethal toxicity of venom samples from different years.
3. To compare the potency of stored sample of venom with fresh venom sample

Results and Conclusions

HPLC analysis



LETHAL DOSE 50 (LD50)

Fresh venom: Year 2015

CV/56/6/15: 5.00 µg/ mouse

RV/9/6/15: 8.6958 µg/ mouse

Old venom: Year 1997-98

CV/738 A Jul 1998: In progress

RV/693 G Jul 1997: In progress

Conclusions: HPLC profile is completed and screening of other parameters is in progress.

3. An in vitro analysis of the effect of Common Cobra (*Naja naja*) venom on the Viability and Proliferation of Lung Cancer Cell Lines

Technical Team

1. Mr. Karan Gera
2. Dr. Mrunal Ghag – Sawant
3. Ms Nilam Sonavane

Period

Jan 2016 – May 2016

Introduction

Snake venom is a complex mixture of biologically active proteins and peptides which are similar in structure but not identical to those of a snake's prey. Some of these proteins and factors are toxic in nature with known effects including neurotoxicity, myotoxicity, nephrotoxicity, cardiotoxicity and anti-coagulation. On a cellular level, certain enzymes present in snake venom (e.g. Phospholipase A₂) are capable of producing inhibitory effects on cell growth, proliferation and viability leading to cytotoxicity depending on the concentration of envenomation. These compounds, while toxic to the human body can also be prospectively isolated to create therapeutic formulations for the treatment of several diseases and disorders. In fact, venoms of certain species of the *Elapidae* and *Viperidae* families have already been identified to have anti-bacterial, anti-inflammatory and anti-cancer activity.

The rationale behind the concept is that there are endless possibilities for the therapeutic uses of snake venom, apart from synthesizing anti-venoms. Many of the compounds isolated from snake venom such as 3FTx, Acetyl cholinesterase, L-amino oxidase, Cobra Venom Factor, and most notably the Phospholipase A₂ groups [IB and IIA] are known to cause neurotoxicity, depleted neuromuscular activity, tissue necrosis, apoptosis, cytolysis and aberrant lipase activity leading to tissue destruction respectively. Of course, all these effects may be controlled and targeted if operated between the Minimum Effective Concentration [MEC] and Minimum Toxic Concentration [MTC]. Snake venom is relatively easier to extract and the fractionation of its components can always be performed by various high-throughput forms of chromatography. This can be considered a suitable alternative to surgery in case tumours are no more localized. It is also more broadly applicable than hormone therapy and if combined with monoclonal antibodies at the drug formulation and development stage it can prospectively work as a good cytotoxic agent against cancer cells. It is absolutely vital for us to find as many effective solutions as possible for the treatment of lung cancers and if this project yields satisfactory results, this may be quite a viable one.

We hypothesized that Cobra venom has effect on cell viability and proliferation of cancer cell lines??

Project schematic:

The project is carried out in into following parts:

1. Standardization of the parameters of culture media, incubation intervals and temperature, buffer requirements, monolayer adherence of cells, passaging, etc. for all cell lines.
2. Synchronization of the cell cycles of all cell lines (using chemicals like Nocodazole, a known cell cycle arrest drug).
3. Standardization of the use of the MTT Tetrazolium Reduction Assay, Apoptosis and Cell Cycle analysis using Propidium Iodide (PI) Staining with Flow Cytometry.
4. Treatment of samples of each cell line with a concentration gradient of *Naja naja* lyophilized venom.
5. Analysis of Cell Viability by the Fluorimetric MTT Asssay.
6. Analysis of Cell Migration and Proliferation by the *in vitro* Scratch Assay.
7. Cell Cycle and Apoptosis analysis by PI staining and Flow Cytometry.
8. Analysis of the acquired assay data and assessing the LD₅₀ concentration of venom for each lung cancer cell line, also determining why one may be affected more than the other.

Objectives of the project

To test the effect of lyophilized Common Cobra (*Naja naja*) venom of various concentrations on Cell Viability, Cell Death by Apoptosis and Cell Migration using the following lung cancer cell lines:

NCI-H23 [Adenocarcinoma; non-small cell lung cancer]

NCI-H460 [large cell lung carcinoma]

NCI-H520 [squamous cell lung carcinoma]

Results and Conclusions

1. Standardised the laboratory set up for Animal tissue culture in Venomous animal unit.
2. Optimized the cell lines and freeze the stock for future use
3. Initiated scratch assay.

Research at Experimental Animal House

1. Validation of reproductive toxicity parameters in Wistar rat

Technical Team

1. Prof. Dr. Abhay Chowdhary, (M.D., D.M.V, D.H.A.) (Principal Investigator)
2. Dr. Mrunal Ghag – Sawant (M.V.Sc) (Co- Investigator)
3. Ms. Nilam Sonavane (B.Sc., D.M.L.T) (Project Coordinator)

Period

Dec 2013 - Jun 2017

Introduction

Reproductive toxicity includes the toxic effects of a substance on the reproductive ability of an organism and the development of its offspring.

Well established Guidelines for reproduction toxicity

The design of this study is based on the requirements of the following guidelines:

1. OECD Guideline for the Testing of Chemicals No. 414 “Prenatal Developmental Toxicity Study” in Rodents”; adopted: 22 Jan, 2001.
2. OECD Guideline for the Testing of Chemicals No. 415 “One-Generation Reproduction Toxicity Study” in Rodents”; adopted: 26 May, 1983.
4. Schedule Y, Drugs and Cosmetics Rule 1988 (8th amendment), Drug Control of India, Government of India, Drugs and Cosmetics (IInd amendment) Rules, effective on 20th Jan 2005.

The rationale behind the concept is to validate the male and female reproductive performance, such as gonadal function, oestrous cycle, mating behaviour, conception, parturition, lactation, weaning implantation, and development of pre implantation stages of the embryo in the females and permit detection of functional effects (e.g. on libido, epididymal sperm maturation) in males

We hypothesized that the test substance under evaluation will have the reproduction toxicity

Project schematic:

The design of this study is based on OECD Guideline for the Testing of Chemicals No. 414 and 415 wherein parameters evaluated was Animals Selection, Cohabitation, Vaginal smear examination, Maternal observation throughout gestation, C- section at 21 days of gestation, Gross Examination of fetus and dam, Fetal parameters, Maternal parameters and Statistical Evaluation of the Results

Objectives of the project

The Aim of this project is to validate the male and female reproductive performance, such as gonadal function, estrous cycle, mating behavior, conception, parturition, lactation, weaning

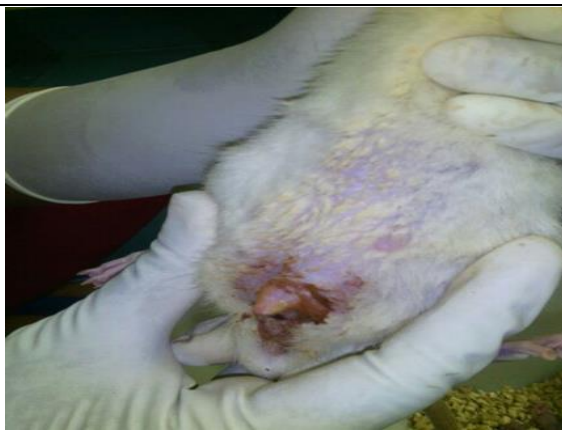
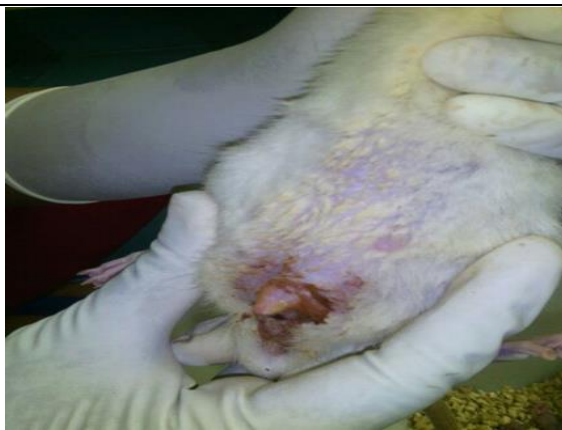
implantation, and development of pre implantation stages of the embryo in the females and permit detection of functional effects (e.g. on libido, epididymal sperm maturation) in males

The objective of this project is to

1. Screening of Reproductive toxicity
2. To validate the male and female reproductive performance

Results and Conclusions

Phase I: One generation Reproductive toxicity study

Figure 1: Vaginal cytology procedure 	Figure 1: Evaluation of sperm motility 
Figure 3: Profuse salivation post dosing of ethanol in Dam 	Figure 4: Wet perineum post dosing of ethanol in Dam 
Figure 5: Chromodacryorrhea post dosing of ethanol in Dam 	Figure 6: Foetal Mortality due to ethanol induce reproductive toxicity 



Conclusion:

Phase I: Based on this study, it can be concluded that, ethanol consumption causes reproductive cycle toxicity. After giving 6% ethanol, profuse salivation and entire litter mortality was observed in treatment group post-delivery. This is a clear indication of ethanol toxicity. Treatment animals also revealed gross changes such as enlargement of spleen and liver as compared to control animals. Ethanol has effect on the fertility potential of animals and there is also effect on the subsequent F1 generation. Further investigation is needed to explore the effects in the multiple generations with different toxic concentrations of the ethanol.

Figure 1: Fetal observation in Group no 1 (control)



Figure 3: Vaginal bleeding in Group no 2 (Ketoconazole)



Figure 5: External examination in fetus of Group no 2 (Ketoconazole)



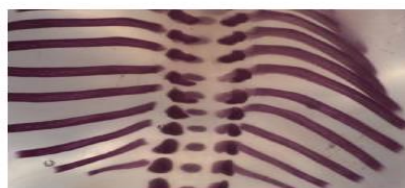
Figure 2: Fetal observation in Group no 2 (Ketoconazole)



Figure 4: Non gravid Uterus in Group no 2 (Ketoconazole)



Figure 6: Skeletal examination in fetus of Group no 1 (Control)



Phase II: Prenatal Developmental toxicity study

Conclusion

Phase II: Based on the results obtained in phase II it is stated that the test

substance used in these studies were Ketoconazole and Ca-poly sulphide a well-known developmental toxicant with immunomodulatory properties. Based on the data of foetal evaluation it reflects that pregnant animals treated with Ketoconazole had shown still birth and resorption of foetuses as shown in figures. The other evaluation as skeletal examination and visceral examination is still under progress.

2. Evaluation of Wound Healing Activity of Mesenchymal Stem Cells in Rats

Technical Team

1. Ms. Nilam Sonavane
2. Dr. Mrunal Ghag – Sawant
3. Dr Carolin Mathew
4. Prof. Dr. Abhay Chowdhary,

Period

Dec 2014- Dec 2016

Introduction

A wound is an injury to a part of the body, especially one in which a break is made in the skin. There are various types of wounds, including an excision wound, incised wound, lacerated wound, abrasion, contusion, ulcer, and burn wound. The process of wound healing involve a variety of biological responses, such as an acute inflammation, cellular proliferation and contraction of collagen lattice formed (Bodekar and hughes, 1998: Suresh Reddy *et al.*, 2002) wounds caused by experimental insults such as mechanical and chemical injuries may extend from epidermis deep into the muscles depending on the severity of damage. The healing process starts with clotting of blood and is ends with remodeling of the cellular layer of the skin.

The rationale behind the concept is that wound healing process is interfered due to inflammation and oxidative stress. Exploratory research on the wound healing compounds is one of the developing areas in the current biomedical sciences. Because stem cells have been shown to improve healing in several injury models, in present work it is hypothesized that mesenchymal stem cells (MSCs) will reduce injury progression and facilitates the wound closure in a excision wound model.

We hypothesized that the Mesenchymal stem cells will heal the excision wound better??

Project schematic:

This study was conducted to assess the wound healing activity of Mesenchymal cells in laboratory Wistar Rat. Healthy young adult wistar rats were obtained from Laboratory Animal house, Bharat serum and Vaccine Ltd, Wagle Estate, Thane. Mumbai and were allowed to acclimatize and housed to the standard experimental laboratory conditions for a period of nine days before exposure to the test substance. Animals were randomized and allocated in four group's viz. Normal Control (G1), Sham Control (G2), Vehical Control Cry solvent (G3), Media Treatment (G4), PBS Control (G5), Treatment with cryosolvent Low (G6), Treatment with cryosolvent Mid (G7), Treatment with cryosolvent High (G8), Treatment with PBS Low (G9), Treatment with PBS Mid (G10), Treatment with PBS High (G10). Each group was

comprised of six rats per group per dose. The wound site was prepared day prior to infliction of excision wound by clipping the fur on the dorsal thoracic region of the rat under injectable anesthesia by ketamine [100mg/kg, IM] and xylazine [16mg/kg,IM].

The rats were observed for mortality and morbidity twice daily. All visible signs and symptoms such as changes in the activity, behavioural pattern, change in skin and fur, eyes and mucous membranes, abnormal growth and other general changes were observed and recorded once daily till the end of experimental period. Body weights of rat were recorded before initiation of dosing and 4th day, 7th day, 14th day, 22nd day experimental period. Animals were provided with adequate quantity of feed sample every day. Measurement of wound closure is recorded on 1st day, 4th day, 8th day, 11th day, 18th day 22nd day of experimental period. Animals were humanely sacrificed at the end of experimental period. Gross pathological examination included an examination of the external surface of the body, all orifices, the cranial, thoracic and abdominal cavities and their contents were observed. Raw data was processed and analyzed between the controls and the treated groups using statistical software such as Graph Pad prism 6.0 Version.

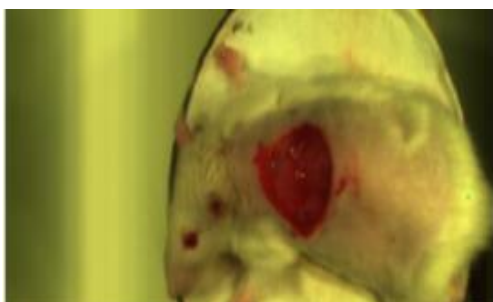
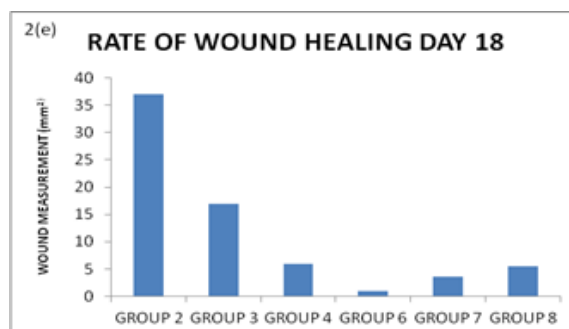
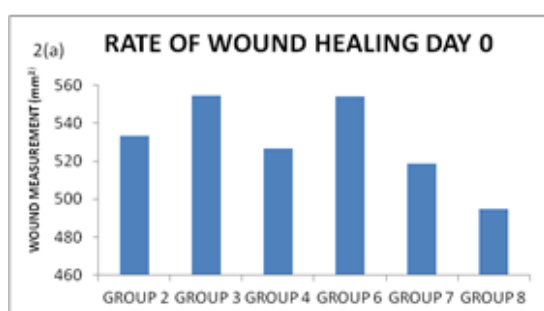
Objectives of the project

The Aim of this project is to Screen Mesenchymal stem cells to evaluate the wound healing potential.

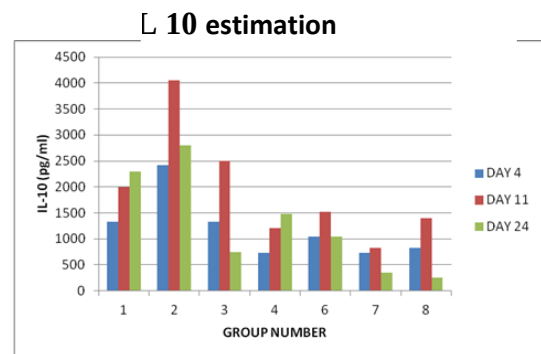
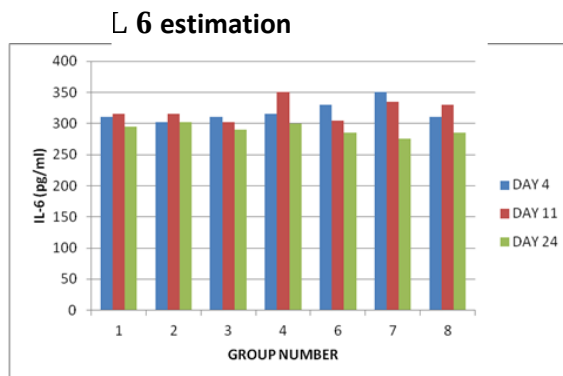
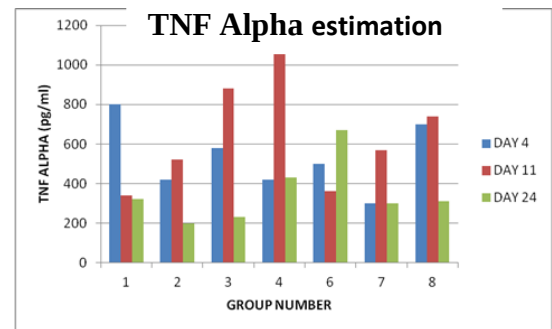
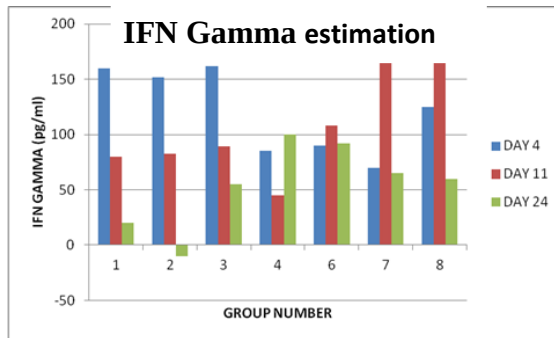
The objective of this project is to

1. To develop Animal model of Excision wound in Wistar rats.
2. To study wound healing potential of Mesenchymal stem cells.

Results and Conclusions



Rat no. 33 Group 6 (Cryo protectant mid dose group)



Conclusions: Based on the results in present study. It can be concluded that the cryosolvent groups had better wound healing potential compared to other groups.

3. DNA methylation and DNA Hydroxy methylation in Rat and Mouse & pre-implantation embryos

Technical Team

1. Dr Rahul Deshmukh
2. Dr. Mrunal Ghag – Sawant

Period

Dec 2013- Dec 2014

Introduction

DNA methylation and DNA hydroxymethylation plays important role in gene regulation. At least DNA methylation is known to be a reversible DNA modification where the DNA methylation when increased, gene expression is suppressed and when it is decreased, gene is expressed.

Mammalian embryonic development in vivo and in vitro is largely affected by these two chemical modifications of DNA. DNA methylation plays very important role during the embryonic development.

Although DNA methylation is studied extensively during the mammalian embryonic development, not much is known about the role of DNA hydroxymethylation during embryonic development.

Therefore, the purpose of this project will be to study the DNA hydroxymethylation during the mice and rat preimplantation embryonic development.

The rationale behind the concept is to study the DNA hydroxymethylation during the mice and rat preimplantation embryonic development.

We hypothesized that during preimplantation mouse and rat embryos DNA hydroxymethylation is developed in vivo??

Project schematic

1. Superovulation and in vivo Fertilization in mice and rats
2. In vivo embryo production
3. Uterus flushing and embryo Collection
4. Embryo and fibroblast cells Fixation for immunocytochemistry
5. Immunocytochemistry – DNA methylation/Hydroxy methylation

6. Fluorescence Microscopy
7. Image Analysis and Fluorescence Quantification
8. Statistical Analysis

Objectives of the project

1. To develop method for studying DNA methylation and hydroxymethylation by

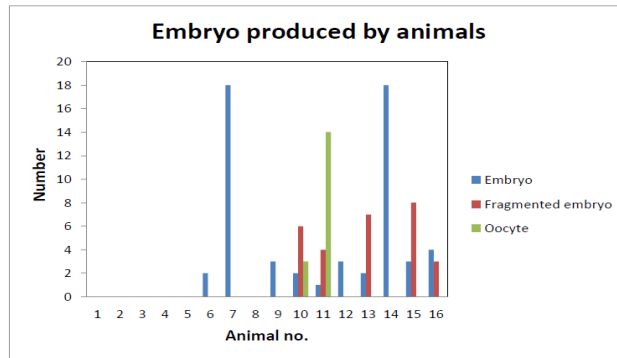


Figure 5.4: Graphical representation of all embryos produced by mice

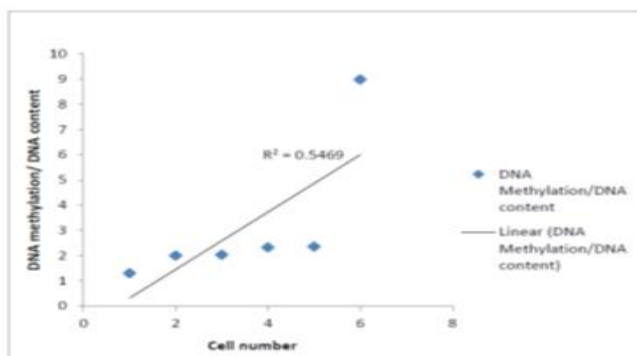


Figure 5.13: Normalised DNA Methylation pattern of each cell

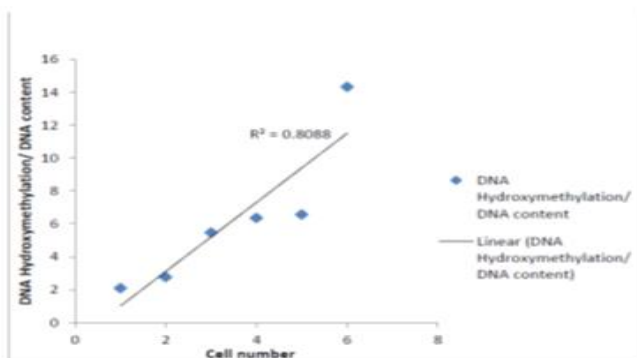


Figure 5.14: Normalised DNA Hydroxymethylation pattern of each cell

immunocytochemistry.

2. To study the DNA methylation and hydroxymethylation during mammalian preimplantation embryonic development in vivo.

3. To compare the DNA methylation levels with DNA hydroxymethylation during mammalian pre-implantation development.

Results and Conclusions

- Optimized the method of Embryo Production
- Optimized immunocytochemistry of cells and embryos
- Optimized method of quantification

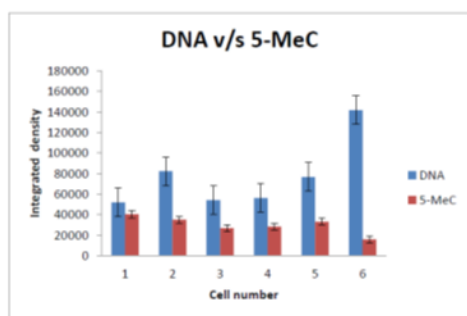


Figure 5.11: Graphical representation of integrated density of DNA and 5-MeC calculated using ImageJ

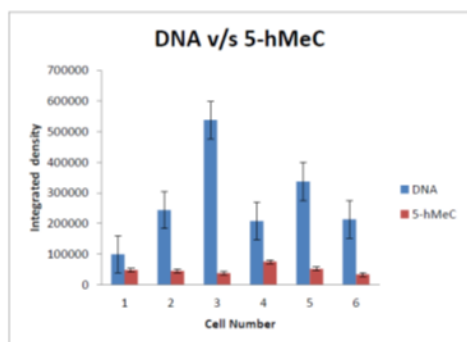


Figure 5.12: Graphical representation of integrated density of DNA and 5-hMeC calculated using ImageJ

Conclusions

This research project has provided with development of basic methodology to standardise the protocol of Embryo collection at different stages and Immunocytochemistry. Superovulation using concentration of 20 I.U. of FSH and hCG showed an increase in ovulation as compared to 10 I.U. concentration. However, at same time it has also resulted in fragmented embryos. Immunocytochemistry was standardized on NIH 3T3 cell and dilution of 1:200 for anti-5-MeC and 1:500 for 5-hMeC for primary antibody; 1:200 for both the secondary antibody was found to be optimum.

A future perspective of this research projected hints towards a comparative study between natural mated and superovulated embryo for evaluation of the quality of embryo. Standardisation of immunocytochemistry protocol with lower concentration of HCl i.e. 0.1 – 0.5 N may be taken into consideration for successfully staining while DNA denaturation step.

Publications

1. Evaluation of anti-osteoarthritic activity of *Vigna mungo* in papain induced osteoarthritis model. Patel D, Ghag-Sawant M and Kaur G, Indian Journal of Pharmacology. 2015; 47(1):59-64.
2. Experimental Evaluation of Antitumor Effect of Russell's Viper Venom on Breast Cancer Cell Line - Mda-Mb-231. Ahluwalia S, Ghag-Sawant M, Shah N, Chowdhary AS International Journal of Pharmacy and Pharmaceutical Research, 2015; 3 (3): 254-263.
3. Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations. Patil V, Ghag-Sawant M, Narayakkadan A, Asrondkar A, Bobade A and Ramaiya, M. International Journal of Scientific and Research Publications, 2015; 5, (12)
4. Study of neutralization of enzymatic activity of *Daboia russelii* venom by various plant extracts and their combinations using in vitro methods. Ghag-Sawant M, More TV, Samant LS and Chowdhary AS. International Journal of Pharmaceutical sciences and Research for Jun 2016 issue (In Press)

Research Projects Based on Ayurveda

Department of Virology

1. Mechanism Based Screening and Validation of Herbal Drugs Using Radiotracer Techniques

Technical team

1. Dr. Abhay Chowdhary
2. Dr. R. D. Lele
3. Dr. Tarala Nandedkar
4. Dr. H. S. Palep
5. Mr. Harish Chander
6. Mr. Sandeepan Mukherjee
7. Ms. Purna Charan
8. Ms. Pallavi Choudhary

Period

Dec 2012 -Nov 2016

Introduction

Based on experiential wisdom of over 5000 years, Ayurveda has described 50 categories of drugs with their clinical indications. Using current understanding of molecular biology, the anti-oxidant, anti-inflammatory and immunomodulatory actions of many Ayurvedic herbal drugs have been established. A classic example is Curcumin (active principle of haldi), which prevents NF- κ B activation via COX, LOX and induces apoptosis.

Ayurveda describes 12 “MedhyaRasayanas”—Memory enhancing drugs which need validation using the transgenic mouse model of Alzheimer’s disease (AD) which is now commercially available. These mice are normal at birth and within 4 months, they develop all the pathological changes in human AD, which can be studied by in vivo imaging, without killing the animals. We plan to study the potential of Ayurvedic herbal drugs for prevention as well as reversal of established diseases. The importance of this project can be appreciated by the fact that today there is no effective drug for AD yet. US\$15 billion are being spent by patients to buy drugs for AD. If our mission validates MedhyaRasayans, a world market of \$15 billion for this simple disease awaits us. The world market of herbal drugs is over \$100 billion of which Chinese share is 57%. India’s share is a mere 2%. Hence, the importance of supporting our project.

Objectives

- 1. To assess the bio-distribution of Tritium Labeled Extracts:**
 - a. To procure standard extracts of selected Ayurvedic MedhyaRasayanas
 - b. To assess acute oral toxicity of the extracts in Mice models

- c. To Label the extracts with Tritium
- d. To observe the bio-distribution of the labeled extracts in mice

2. To study the effects of medhyarasayanas on Alzheimer's Disease in Mice

- a. To procure and maintain Transgenic Alzheimer's Disease Mice model.
- b. To administer Selected MedhyaRasayanas to these mice
- c. To observe the animals for development of Neurofibrillary Tangles

Results and Conclusions

Limit Dose oral toxicity was performed and the extracts were found to be well tolerated and non toxic to the mice.

2. In-vitro evaluation of the dermato - protective potential of *Hibiscus rosasinesis* flower extract

Technical team

1. Mrs. AmitaPatil
2. Mrs. Suvarna Pachpore
3. Dr. Sweta T Kothari
4. Dr. Anil Bobade
5. Dr. Abhay Chowdhary

Period

Aug 2015 - Dec 2016

Introduction

Sunburn is a burn on the skin caused from exposure to ultraviolet (UV) radiation. Both UVA and UVB are responsible for photo-ageing (premature aging of the skin leading for wrinkles) and sunburn.

World Health Organization has been promoting research in identifying newer bioactive molecules from natural sources. It has been reported that the plants of Hibiscus genus have the potential to provide biologically active compounds that act as antioxidants that are able to deter the proliferation of malignant cells.

Vitamin C is an important part of a variety of bodily functions, ranging from bone formation to scar tissue repair. Vitamin C is a potent antioxidant drug that can be used topically in dermatology to treat and prevent changes associated with photo-ageing. Because it is unstable and difficult to deliver into the dermis in the optimum dosage, research is being directed to find stable compounds of Vitamin C and newer methods of delivery of Vitamin C into the dermis. Topical Vitamin C performs three different functions in skin metabolism: as an anti-oxidant, as an inhibitor of melanin and as a stimulator of collagen synthesis. As an antioxidant, Vitamin C prevents or suppresses the development of skin cancer caused by UVA and UVB exposure. As a melanin inhibitor, Vitamin C helps to lighten the skin. Additionally by stimulating collagen synthesis, Vitamin C accelerates skin firming and helps to prevent fine lines.

Anthocyanins have been hypothesized in the past to provide protection from DNA cleavage and elucidate anti-inflammatory response. They are also involved in decreasing capillary permeability and membrane strengthening. The potential of anthocyanins to interfere in carcinogenesis is also being studied. Scientific research has illustrated that anthocyanin may have a protective effect against UV-induced photo-aging of the skin.

We plan to evaluate the *in-vitro* dermato-protective activity of the flower extracts of *Hibiscus rosasinensis* on fibroblasts/dermal cells. The flower extracts will be studied for their effects on cell viability. The dermal cells/fibroblasts will be exposed to UV radiations and their collagen breakdown will be studied as an indicator of dermal damage.

Objectives

1. To assess the anti-oxidant property of *Hibiscus rosasinensis* flower extract
2. To assess the effects of hibiscus flower extracts on dermal cell viability
3. To assess the dermato-protective/anti-ageing activity of *Hibiscus rosasinensis* flower extract

Results

Ascorbic Acid was determined using Iodometric titration (end point colorless to blue), wherein the aqueous floral extract of *Hibiscus rosasinensis* gave a yield of 0.1 gm ascorbic acid per 5 gm of flowers (wet weight, Figure 1 & 2)



Figure 1: Colorimetric analysis of Vit C. From left to right Tube no. 1:10 mg; tube no. 2:1mg; tube no. 3:0.1 mg, tube no. 4:0.01 mg; tube no. 5 0.001mg.



Figure 2: Presence of Vit. C in Flower

Anthocyanin was determined using UV absorption at 516nm and 700nm, wherein the acidified aqueous floral extract of *Hibiscus rosasinensis* gave a yield of 0.55 gm ascorbic acid per 5 gm of flowers (wet weight, Figure 3)

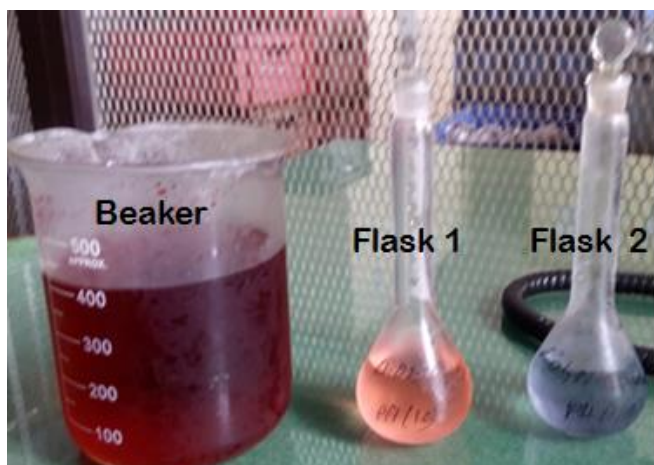


Figure 3: Beaker contains acidified aq. extract of *H. rosasinensis*, flask 1 is extract at pH 1.0 and flask 2 is extract at pH 4.5.

Conclusion: *Hibiscus rosasinensis* has a potential anti-oxidant activity, as seen by the simultaneous presence of Vitamin C and anthocyanin.

3. Assessment of Plant Proteins/Peptides as Potential Anti-Viral Agents.

Technical team

1. Mr. Sandeepan Mukherjee
2. Dr. Abhay Chowdhary

Period

Jun 2011 - Dec 2016

Introduction

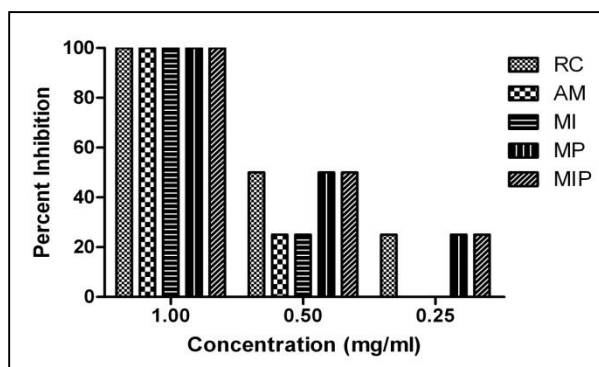
Anti-virals are the mainstay in treatment and management of Viral Diseases in Humans. However a large number of viral diseases have no known anti-virals. Also the fact, that emerging drug resistance makes the available anti-virals ineffective, calls for the development of novel anti-viral molecules. Plant peptides and secondary metabolites are known to have potent anti-viral activity. Although most such activities are directed against plant viruses, the mechanism of action remains common for most anti-virals. The present study investigated the potential antiviral activity in Plant secondary metabolites and protein/peptides against Influenza and Rabies Viruses.

Objectives

1. To collect Plant Material from field and its identification.
2. To prepare Protein Extracts and Methanolic extracts.
3. To carry out Phytochemical Analysis of extracts using HPTLC.
4. To Assess of Anti viral activity against Rabies Virus using RFFIT

Results and Conclusions

Methanol extracts and total protein extracts of Eranda (*Ricinus communis L.*), Mango (*Mangifera indica L.*) and Bael (*Aegle marmelos*) were assessed for their antiviral activities. The methanol extracts of all three plants exhibited promising anti Rabies Virus activity.



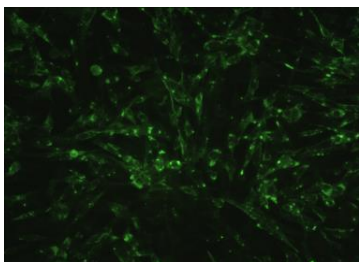


Figure 1: Detection of RABV-CVS infection using FAT. **A:** FAT analysis of Treated Cells; **B:** FAT analysis of Virus Control, stained using diluted FITC- tagged antibody specific against N antigen. **C:** %Inhibition of Rabies Virus in cell culture

Department of Clinical Pathology

1. Evaluation of Anti-Microbial Activity of Wild Apple and Gooseberry Fruit Juices

Technical Team

1. Mr. Kapil Punjabi
2. Dr. Shital Vasave
3. Dr. Shashikant P. Vaidya

Period

Sept 2015- Dec 2015

Introduction

The development of microbial resistance to currently available antibiotics due to its indiscriminate use has necessitated the search for new antimicrobial agents. With growing interest in eco-living lifestyle, increased attention is being paid to the natural antimicrobial agents.

The fruits and vegetable juices serve as a source of some essential nutrients and reportedly exhibit significant antimicrobial effect, the bioactivity being associated with mineral content and biologically active constituents. Hence these fruit juices with the property of bioavailability and the retention of certain minerals by polyphenolic compounds can be recommended for their use as alternative anti-infective agent in natural medicine for the treatment of infectious diseases.

Apples have high phenolic content that possess various biological functions such as Astringent, Antioxidant, Anticancer, Anti-inflammation and antibacterial activities.

Indian gooseberry (*Phyllanthus emblica linn*) or “Amla” fruit is reputed to contain the highest content of vitamin C compared with any other naturally occurring substances in nature. Active extract of *P. emblica* have been shown to possess several pharmacological properties including analgesic, anti-inflammatory, Antioxidant and chemo-protective activities.

Objectives

1. To determine the antimicrobial activity of the fruit juices qualitatively.
2. The quantitative assessment of antimicrobial activity by determining the Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentration.

Results and Conclusions

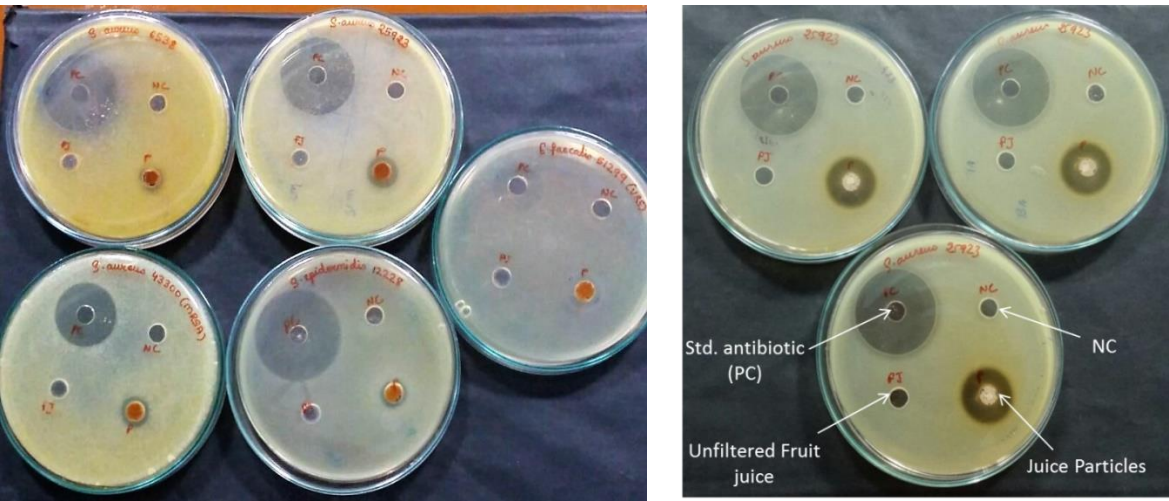


Figure 1. Screening of antimicrobial activity of Wild Apple and Gooseberry fruit juice

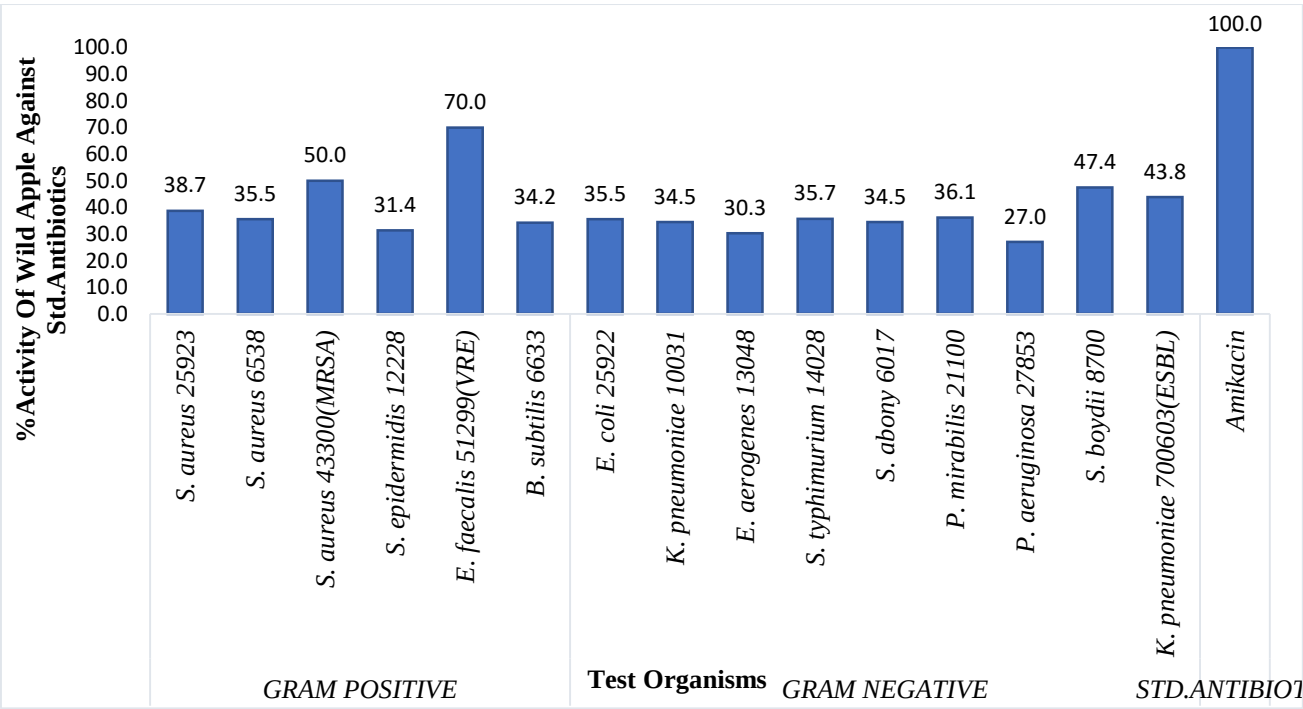


Figure 2. Percentage activity of Wild-Apple particles: The activity of the fruit juice particles against test organisms was determined using Amikacin as standard.

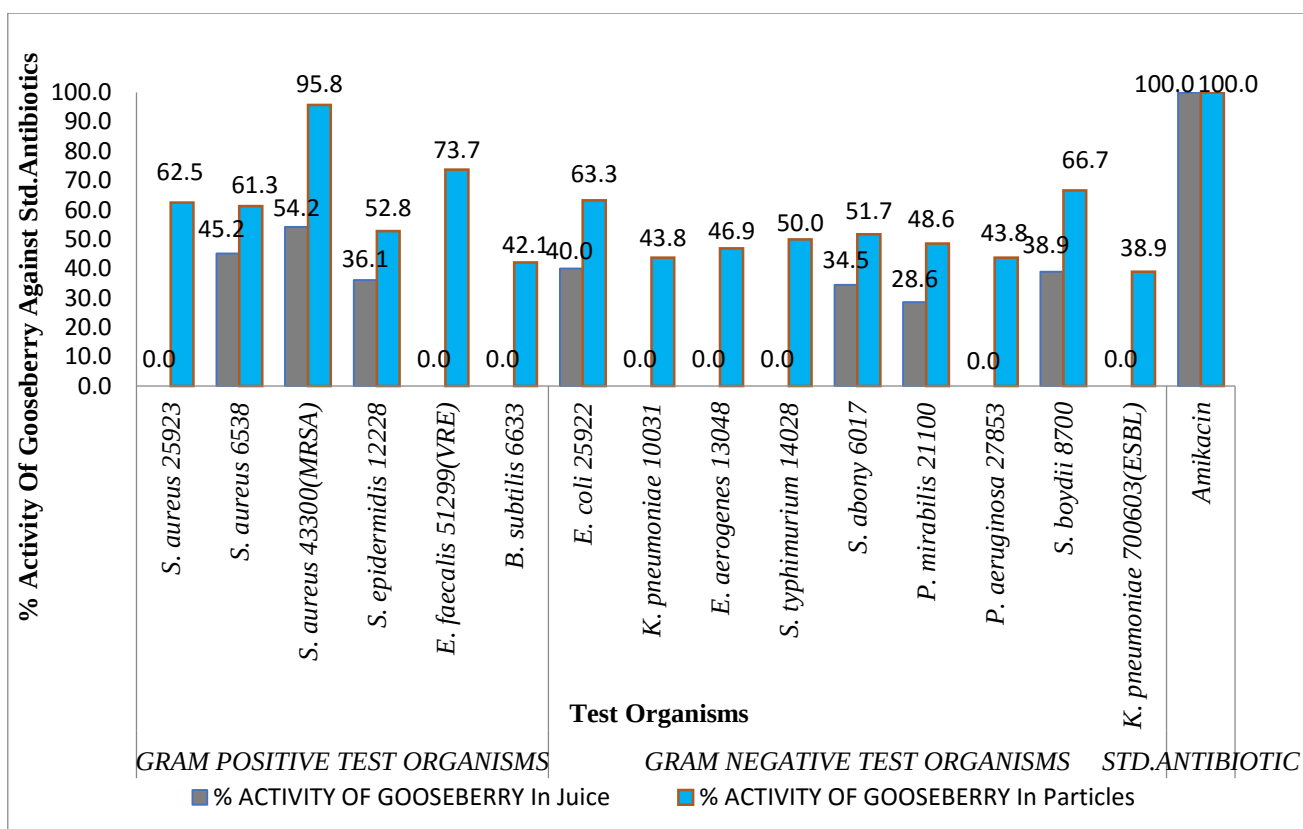


Figure 3. Percentage activity of Gooseberry Juice and particles: The activity of the fruit juice and particles against test organisms was determined using Amikacin as standard

Table 3. Minimum Bactericidal Concentration of Wild-Apple and Gooseberry Fruit Juice Particles against Gram Negative Organisms

Test Organism	Gooseberry particles				Wild apple particles				
	400	200	100	50	800	600	400	200	100
<i>E. coli</i> 25922	-	-	-	++	-	++	+++	+++	+++
<i>K. pneumoniae</i> 10031	-	-	-	++	-	-	+++	+++	+++
<i>K. pneumoniae</i> 700603	-	-	++	++	-	-	+++	+++	+++
<i>E. aerogenes</i> 13048	-	-	-	+++	+	++	+++	+++	+++
<i>S. typhimurium</i> 14028	-	-	+	+++	-	++	++	+++	+++
<i>S. abony</i> 6017	-	-	-	++	-	-	++	+++	+++
<i>P. mirabilis</i> 21100	-	-	+	++	-	-	-	-	++
<i>S. boydii</i> 8700	-	-	-	+	-	-		++	+++
<i>P. aeruginosa</i> 27853	-	-	-	+	-	-	++	+++	+++

All concentrations in mg/ml

Table 4. Minimum Bactericidal Concentration of Wild-Apple and Gooseberry Fruit Juice Particles against Gram Positive Organisms

Test Organism	Gooseberry particles				Wild apple particles			
	400	200	100	50	400	200	100	50
<i>S. aureus</i> 25923	-	-	+	++	-	+	+	++
<i>S. aureus</i> 6538	-	-	-	-	-	+	+	++
<i>S. aureus</i> 43300	-	-	+	++	-	++	++	++

<i>S. epidermidis</i> 12228	-	-	-	++	-	-	++	+++
<i>E. faecalis</i> 51299	-	+	++	++	-	+	++	++
<i>B. subtilis</i> 6633	-	-	-	-	-	-	-	-

All concentrations in mg/ml

Table 5. Minimum Bactericidal Concentration (MBC) of Wild Apple & Gooseberry Fruit Juices (Unfiltered Juices)

	Test Organism	Gooseberry fruit juice					Wild-Apple fruit juice				
		100 %	75 %	50 %	25 %	MIC range	100 %	75 %	50 %	25 %	MIC range
Gram positive	<i>S. aureus</i> 25923	-	-	-	-	< 25%	-	-	-	+	26-50%
	<i>S. aureus</i> 6538	-	-	-	-	< 25%	-	-	-	-	< 25%
	<i>S. aureus</i> 43300	-	-	-	+	26-50%	-	-	+	+	51-75%
	<i>S. epidermidis</i> 12228	-	-	-	-	< 25%	-	-	-	+	26-50%
	<i>E. faecalis</i> 51299	-	-	-	+	26-50%	-	-	+	+	51-75%
	<i>B. subtilis</i> 6633	-	-	-	-	< 25%	-	-	-	-	< 25%
Gram Negative	<i>E. coli</i> 25922	-	-	-	-	< 25%	-	-	++	++ +	51-75%
	<i>K. pneumoniae</i> 10031	-	-	-	-	< 25%	-	-	++	++ +	51-75%
	<i>K. pneumonia</i> 700603	-	-	-	-	< 25%	-	-	+	++ +	51-75%
	<i>E. aerogenes</i> 13048	-	++	++	++	76-100%	-	-	-	++ +	26-50%
	<i>S. typhimurium</i> 14028	-	-	-	++	26-50%	-	-	++	++ +	51-75%
	<i>S. abony</i> 6017	-	-	-	++	26-50%	-	+	++	++ +	76-100%
	<i>P. mirabilis</i> 21100	-	-	-	-	< 25%	-	-	-	-	< 25%
	<i>S. boydii</i> 8700	-	-	-	-	< 25%	-	-	-	++	26-50%
	<i>P. aeruginosa</i> 27853	-	-	-	++	26-50%	-	-	-	++ +	26-50%

Where +++ is Luxuriant Growth; ++ indicates Growth; + means Few colonies and – indicates No growth

Conclusions

1. Anti-bacterial activity observed against bacteria that are causative agents of various infections like Skin Enteric, Gastric, respiratory, urinary and septicemia treatment of which involves high dose of strong antibiotics.
2. As no activity was found against fungal pathogens.
3. Results indicate that the juices and its particles had higher activity against the drug resistant strains of bacteria.
4. With their therapeutic potential these juices can be a good source of supplemental therapy and preventive care with other health benefits as well.

2. Characterization and assessment of antimicrobial and antioxidant activity of Kombucha (M. Sc project)

Technical Team

1. Ms. Tehmeena Mukadam
2. Dr. (Mrs.) Sunita D. Deshpande
3. Dr. Shashikant P. Vaidya

Period

Dec 2012 - Aug 2016

Introduction

Kombucha a fermented tea beverage known worldwide by many, originated in China which later spread to Russia and rest of the world. Microbiologist earlier had classified Kombucha as lichen based on the fact that Kombucha is symbiotic association of fungi and AAB. However, typical lichen is a symbiosis of algae and fungi and required light to carry out photosynthesis, unlike Kombucha that grow in dark.

Kombucha culture appears as a white rubbery pancake and appearance changes during fermentation period. Initially it is creamy white colour but when brewed in black tea the culture darken due to presence of tannins in tea.

Mushroom culture is steeped in sugared tea for fermentation. During this process original mushroom floats in the tea and baby mushroom is produced on the surface of the original culture. This new mushroom can now be used as culture for further process of fermentation.

In various studies Kombucha is found to be a symbiotic association of bacteria such *Acetobacter xylinoides*, *A. pasteurianus*, *A.xylinum*, *A. aceti*, and *Bacterium gluconicum* and yeast like *Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailli*, *Brettanomyces bruttanomyces*, *B. bruxellensis*, *B.lambicus*, *B.custersii* and *pichia species*. The exact microbial composition also depends on the source of the inocula of the tea fermentation. Bacteria and fungus in Kombucha present powerful symbiosis which are able to inhibit the growth of potential contaminating bacteria.

Due to the variability of Kombucha, the bacteria and yeast present in given Kombucha from a certain source can vary at large. Due to this variability the fermentation of tea and its properties tend to differ.

Objectives

1. For Characterization of Kombucha

- A. To measure Sugar Content in Fermented tea.
- B. To measure Ethanol Content in Fermented tea

C. To determine organic acid in fermented tea

D. To analyse the poly phenols

E. To isolate and characterize microorganism

2. To determine Activity of Kombucha

A. To check antimicrobial activity of Kombucha

B. To check cell toxicity

C. To check antioxidant activity of Kombucha

Results and Conclusions

Characterization /identification of organism involve in fermentation

In this study twin cultures of the Kombucha were successfully identified as *Komagaeibacter saccharivorans* and yeast *Zygosacchomyces bailli*.

Estimation of sugar

Daily analysis of reducing sugars was done as the fermentation proceeds; it was found that concentration of reducing sugars in the fermented broth gradually increases.

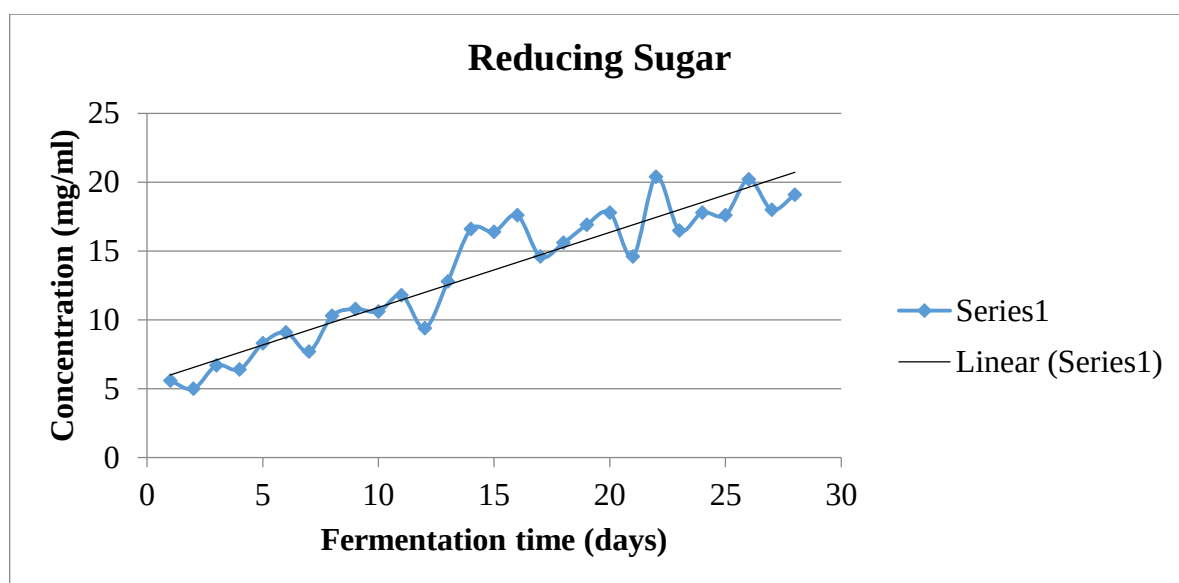


Figure 2. Estimation of Sugar content

Analysis of organic acid

Gluconic acid and acetic acid was identified and found to increase in concentration during fermented.

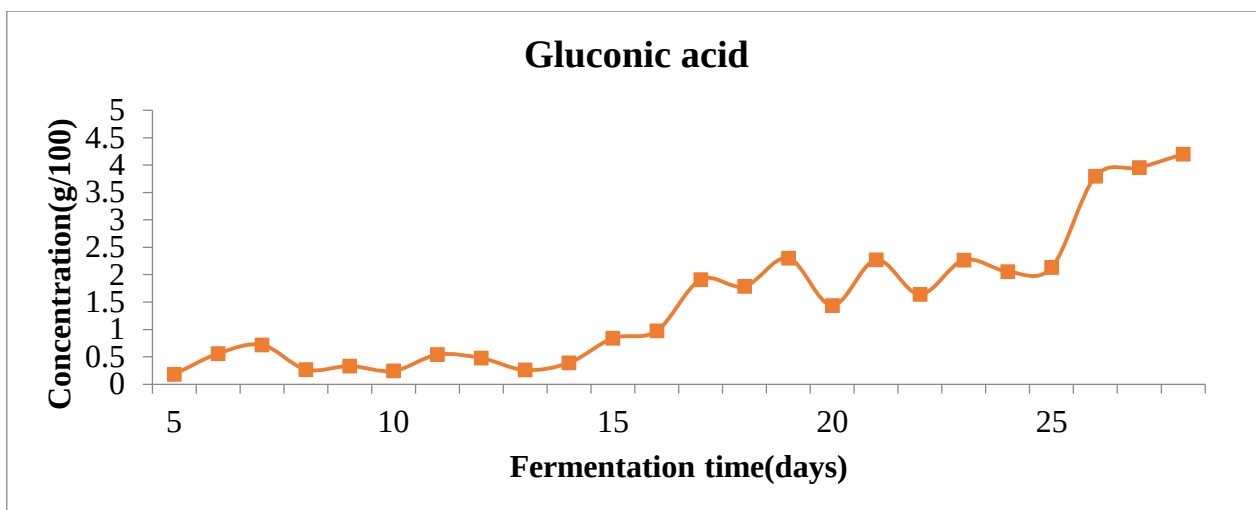


Figure 3. Analysis of organic acid

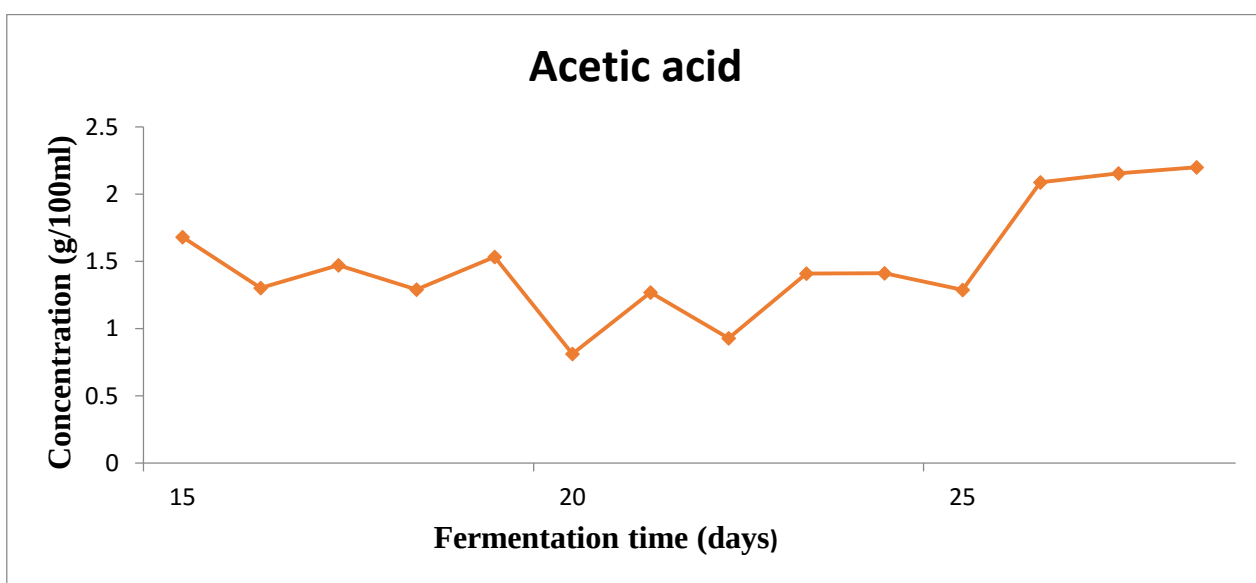


Figure 4. Analysis of Acetic acid content

Analysis of polyphenol

Catechin, Epicatechin galate, Epigalo-catechin, Epigalo catechin gallate and Theoflavin were identified in Kombucha tea.

Antioxidant Activity of Kombucha

The radical scavenging activity of Kombucha increases with increase in fermentation time. Maximum antioxidant activity was found at end of fermentation with about 80% DPPH Scavenging activity.

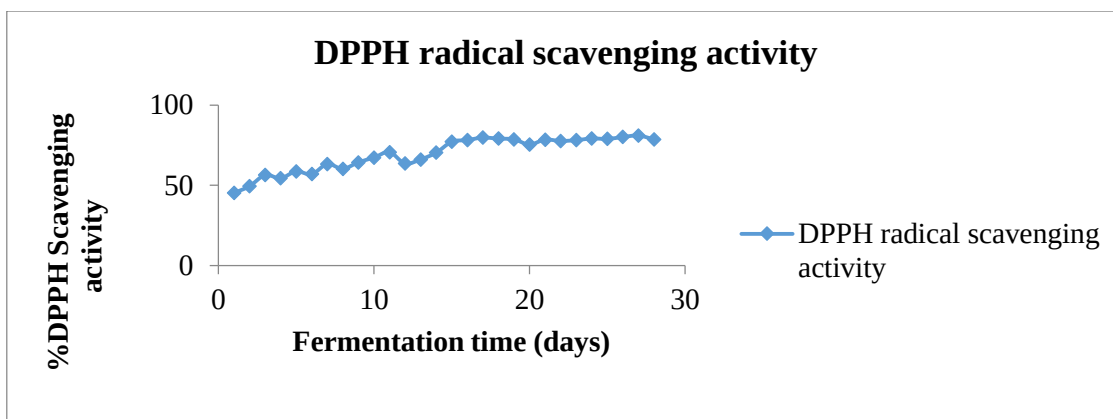


Figure 5. DPPH Scavenging activity

Antimicrobial activity of Kombucha

The antimicrobial activity increases with increase in fermentation time, maximum activity was found to be at day 28 of fermentation. Fermented Kombucha showed inhibitory effect against Standard ATCC strains of pathogens like *S.aureus*, *E.coli*, *K. pneumoniae*, *P. aeruginosa* except *Candida albicans*.

Determination of cell toxicity

Day wise effect of Kombucha on Vero cells were evaluated and observed that Kombucha was non- toxic even at 100% concentration.

Department of Biochemistry

1. Studies to validate safety and to understand mechanism of Ayurvedic formulation Raupya Suvarna Sootshekhar (RSS) and its therapeutic metallic ingredients used for neural therapy

Technical Team

1. Dr. Vineeta Deshmukh
2. Dr. Nishigandha Naik
3. Dr. D. S. Joshi
4. Dr. Mansi Thakur
5. Ms. Smital Kulkarni

Period

Jun 2014 – Dec 2018

Introduction

RaupyaSuvarnaSootshekhar (RSS) is an experience based herbo, mineral and metallic Ayurvedic formulation used in various diseases of nervous system. Its usage for treatment of diseases like memory loss, insanity, epilepsy, brain atrophy, brain tumours like astrocytoma, glioblastoma and retinoblastoma is found to be beneficial. It is observed that this formulation improves sensory and motor responses, memory and coordination in actions. It also minimizes symptoms related to nervous system like headache, diplopia, vertigo, slurred speech, insomnia and seizures to some extent. Concerns are often raised about toxicity due to heavy metals used in Ayurvedic formulations. Though, RSS is widely used in Ayurvedic clinical practices, its safety assessment and molecular mechanisms as per the modern medicine criteria are not known. Validating safety of the formulation will enable wider acceptance of RSS in the field. In depth understanding of the mechanism of action of the formulation will open new avenues for the targeted therapy, not only in the field of Ayurved, but in the modern medicine too. Hence, it is proposed to validate safety and to understand molecular mechanism of action of RSS and its therapeutic metallic components, using modern biology tools.

Objectives

1. Physical characterization of RSS and its therapeutic metallic ingredients.
2. Validation of safety of RSS and its therapeutic metallic ingredients using in vitro and in vivo models.
3. Understanding mechanism of action of RSS and its therapeutic metallic ingredients.

Results and Conclusions

Formulation of Copper (Cu) in the soluble form was prepared and was tested for Cu content by ICP-AES. Its toxicity was checked at various concentrations, using zebrafish embryos. The preliminary results showed that at high concentrations of Cu, there was delay in the development of the zebrafish in the first 48 hours post fertilization (hpf). On Cu treatment, significant increase in the motility was seen at 24 hpf while heart beats at 72 hpf, development of bones & cartilages as well as angiogenesis were unaffected. Thus, at lower concentrations, aqueous Cu did not show any adverse effects.

2 Isolation, Characterization and Screening of potential anti-inflammatory drugs from Praval (Coral / *Corallium rubrum*)

Technical Team

1. Dr. Usha Padmanabhan
2. Dr. Narendra Bhat
3. Dr. Vandana Kozarekar

Period

Feb 2016 - Jan 2019

Introduction

Inflammation is a complex immunovascular response to harmful stimuli, such as pathogens, damaged cells or chemical irritants that involves immune cells and blood vessels. Rheumatoid Arthritis (RA) is a common example of chronic inflammation and affects 1% of the population in India (Arthritis Foundation of India). Chronic inflammation continues unchecked leading to severe pain and swollen joints, eventually leading to cartilage decay and bone loss. There is no cure for RA.

The treatment of inflammatory conditions such as RA or uveitis involves medications such as steroids, Cox-2 inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), TNF alpha inhibitors, immuno-suppressants and occasionally, surgery. However, most of these medications have deleterious side effects. Hence, alternative therapeutics is needed.

The marine ecosystem offers a huge potential for the discovery of drugs. Modern biology has isolated anti-inflammatory molecules from a variety of coral (Praval) sub-species. Ayurveda has harnessed the active ingredients of several marine products for treatment of several chronic conditions such as inflammation. But a modern scientific approach is needed for acceptance of the traditional medicines into active clinical therapeutics of modern medicine.

Objectives

The approved objectives of the project are:

Procurement of Praval (Coral or *Corallium rubrum* and its varieties) from authorised dealer or licensed manufacturer having GMP certification and confirmation of the species by geographic origin and primary organoleptic analysis.

Preparation of formulations of Praval as per the standard guidelines proposed in the Ayurveda.

Chemical analysis of raw coral, intermediates formed during the ayurvedic formulation process and the final ayurvedic formulation, by using modern techniques such as HPTLC, HPLC and spectroscopic approaches.

Contribution to the scientific understanding of traditional medicinal knowledge in terms of modern medicine such a mode of action, target/s of the drug, etc..

Results and Conclusions

This project is recently sanctioned and procurement of coral is in progress.

3 Characterization and assessment of anticancer activity of Indian honey. (Ph.D project)

Technical Team

1. Ms. AmrutaNaik
2. Dr. Nishigandha Naik
3. Dr. Sucheta P. Dandekar

Period

Jul 2012- Dec 2016

Introduction

The traditional Indian medicines for the treatment of various diseases has received considerable attention and therefore, continuing search for new anticancer compounds in these is a realistic and promising strategy to deal with cancer. In ancient times, though traditional medicines were used for treating cancer, the mode of their anticancer action is not well explored in terms of modern science. As per Ayurvedic literature, Indian honey may act as inexpensive alternative to expensive therapeutic modalities for cancer either as separate entities or in combination. Understanding the anticancer action of honey, would enable us to enable use it more precisely and effectively. Medicinal properties of honey are gifted by the flora from which they have been collected and hence they vary from region to region. . Hence, the present study is proposed to assess the anti-cancer potential of four uni-floral Indian honeys and to elucidate its mechanism of action along with its anti-angiogenic potential.

Objectives

- To analyse phytochemical and biochemical constituents of different types of Indian Honey.
- To explore anticancer activity of Indian Honeys *in vitro*.
- To study the mechanism of anticancer activity.
- To evaluate anti-angiogenic potential of Indian Honeys.

Results and Conclusions

In-vitro cytotoxicity assay of 4 crude honey samples as well as their three organic solvent extracts was studied. All the four crude honey samples showed dose dependent cytotoxic activity in Cervical (HeLa) and Breast (MCF 7) cancer cell lines whereas any of the above-mentioned extracts of honey do not have anti-cancer activity *in vitro* (Figure 1).

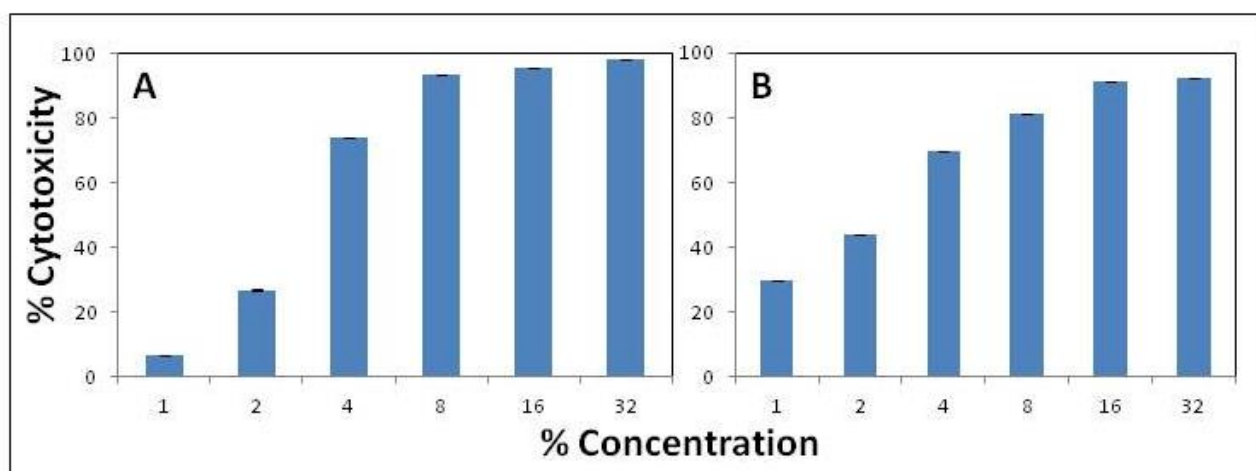


Figure 1.Representative data showing *in vitro* cytotoxicity of crude honey sample in (A) MCF-7 and (B) HeLA cells

Department of Chemotherapy

1 Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations (completed)

Technical team

1. Ramaiya M
2. Patil V N
3. Bobade AS
4. Ghag- Sawant M S
5. Narayakkadan A
6. Asrondkar A L

Period

Jan 2013 – Dec 2015

Introduction

The synthesis of benzothiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades

Objective

To evaluate the biological activity of Benzothiazole-ThioureaDerivatives, Plant Extracts and their Combinations

Results & Discussion

The structural formula of all derivatives was confirmed and characterized by elemental analysis and spectral studies. A methanol extract of Pongamiapinnata, Syzigiumcumini, Delonixregia, Adathodazeylanica was done by Soxhlet. The combinations of the plant extracts with the organic compounds were evaluated for antiinflammatory, anti-oxidant activity and checked for their cytotoxicity. Our findings indicate that the plant extract and organic compounds have a significant effect on biological evaluations.

Chemical Testing Unit

1. Detection of chemical constituents presents in *Phyllanthus Emblica* fruit Juice

Technical team

1. Mr. Manoj K. Patil
2. Mr. Santosh B. Bagal
3. Dr. Anil Bobade

Period

Sept 2015- ongoing

Introduction

Amla is a gift of nature to mankind. It is an indispensable part of the ayurvedic and unani system with amazing remedial qualities. In Sanskrit, it is called Amalaki or Dhartiphala. Amla is perhaps the single most often mentioned herb in "Charak Samhita", the Ayurvedic medicine literature. Amla is a wonder herb and one of the precious gifts of nature to humans. Amla is known as "Divya" and "Amrut" or Amrit Phala in Sanskrit, which literally means fruit of heaven or nectar fruit. The Sanskrit name, Amlaki, translates as the Sustainer or The Fruit where the Goddess of Prosperity Resides. In Hindu religious mythology the tree is worshipped as the Earth Mother as its fruit is considered to be so nourishing as to be the nurse of mankind

Emblica fruits are one of three "myrobalans," a term deriving from the Greek for acorn, which is a well-known astringent used in tanning. In fact, both emblic myrobalans and chebulic myrobalans (*Terminalia chebula*) are relied upon for their high content of tannins; chebulic myrobalans have long been used for tanning leather. The third fruit, beleric myrobalans, is a close relative of the chebulic myrobalans (*Terminalia belerica*) and this too is a tanning agent. These three fruits together make up the popular remedy "*Triphala*," a rejuvenating formula that is often applied to treating intestinal disorders (inflammation, infection, diarrhoea and constipation).

Objectives

Objective of the study is to detect the chemical constituent present in the amla (*PhyllanthusEmblica*) fruit juice which is used Ayurvedic medicine. Ayurvedic medicine has around a thousand herbs that are commonly used in this traditional system of medicine, but overall there may be more than 2,500 that are used across India in all forms of herbal medicine. Literature search we got the approx chemical constituent present in amla (*PhyllanthusEmblica*) fruit juice are

- 1) Ascorbic Acid, Gallic Acid, Chebulinic Acid, Mucic Acid, Chlogenic Acid, Ellagic Acid, Chebulagic Acid and Quecetin.

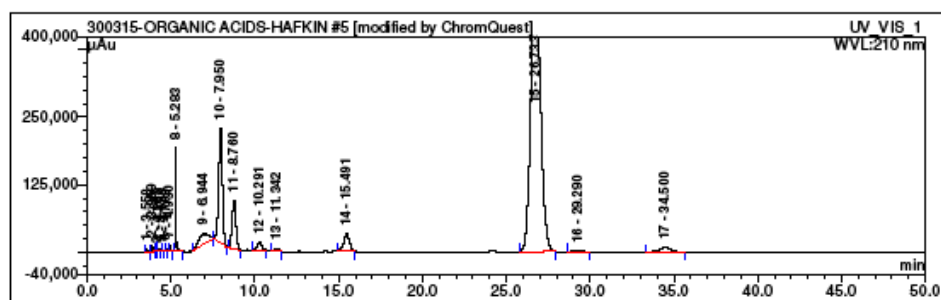
- Other ingredients like Alkaloids, Terpenes and Triglycidol glucose.

Results

Operator:ChromQuest Timebase:ACCELA-CCS-1 Sequence:300315-ORGANIC ACIDS-HAFKIN

Page 1-2
31.3.2015 3:16 PM

ThermoFisher Scientific ThermoFisher Scientific Demo Lab, Powai.			
Sample Name:	SAMPLE	Injection Volume:	20.0 µl
Vial Number:	C22	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	210 nm
Control Program:	ORGANIC ACIDS-HAFKIN	Bandwidth:	5
Quantif. Method:	ORGANIC ACIDS-HAFKIN	Run Time:	50.00 min
Recording Time:	30.3.2015 18:32	Sample Weight:	1.0000
Sample Comment:	ACCLAIM ORGANIC ACID:250 X 4 X 5UM;SR.NO:001908;LOT.NO:006-04-120		
		Sample Id:	DEMO LAB



No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Asymmetry	Plates(USP)	Plates(USPym)
	min		µAu	µAu*sec	%			
1	3.56	n.a.	6550.908	44906.026	0.16	1.74	9020	36080
2	3.97	n.a.	12099.624	95249.435	0.33	1.50	7231	28924
3	4.17	n.a.	791.907	2396.375	0.01	0.80	33421	133684
4	4.26	n.a.	3726.363	16944.952	0.06	1.41	22979	91912
5	4.53	n.a.	686.407	2678.943	0.01	0.79	40000	160000
6	4.70	n.a.	2069.817	9959.649	0.03	1.38	14395	57540
7	4.93	n.a.	9343.267	45227.399	0.16	1.06	22776	91104
8	5.29	n.a.	191225.182	426622.112	1.49	1.10	247262	989048

HAKFFINE INSTITUTE-ORGANIC ACID/Integration

Chromleon (c) Dionex 1996-2006
Version 6.80 SR13 Build 3967 (218758) (Demo-Installation)

11	8.76	n.a.	90524.202	1469503.276	5.09	0.99	6620	26480
12	10.29	n.a.	14998.182	291921.889	1.01	0.91	4025	16100
13	11.34	n.a.	4603.148	80602.144	0.28	0.85	2892	11568
14	15.49	n.a.	30539.683	739709.946	2.56	0.96	8609	34436
15	26.73	n.a.	527330.788	20946190.718	72.58	1.11	10750	43000
16	29.29	n.a.	2495.766	90795.016	0.31	0.92	4769	19076
17	34.50	n.a.	8472.801	412141.956	1.43	0.97	5326	21304

Figure 1: HPLC analysis of amla juice to identify the ingredients.

Conclusions

- Amla syrup shows Gallic acid, Ascorbic acid shows good response and Quercetin in very small amount.
- About the Mucic acid dissolving problem.
- About the Ellagic Acid dissolving problem.

Nano particle Research

Zoonosis Department

Comparative Assessment of immunomodulatory activities of three plant extracts and their synthesized nanoparticles (Ph.d. project)

Technical team

1. Mrs. Pallavi Kurkure Choudhary
2. Dr. Mira Ramaiya
3. Dr. Abhay Chowdhary

Period

Introduction

The basic function of immune system is to protect against foreign pathogens and infectious agents and follows a very tight rope. Even though the margin of error is very low, many immunodeficiency, autoimmunity and hypersensitivity diseases occur. A large number of diverse disease states threatening mankind, such as chronic and recurrent viral, bacterial, fungal, parasitic infections like human immunodeficiency virus (HIV) infection, allergies, autoimmune diseases, PID, and cancer, are believed to have resulted from altered functioning of the immune system. Therefore appropriate modulation of the immune system is must.

Currently available immunosuppressive and immunostimulating agents have major limitations. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathologies. Therefore, there is need to search for more effective and safer herbal agents exerting immunomodulatory activity. Immunomodulation using plant material can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defence mechanism has to be activated under the condition of impaired immune response.

Discovery of new molecules and manipulating those available naturally in nanosize could be appealing for their greater potential to improve health care. Hence present study aims to synthesize novel gold nanoparticles using plant extracts as reducing agents, characterized them and comparatively assess the immunomodulatory activity of plants and synthesized gold nanoparticles.

Objectives of the project

1. Screening of Plants and phytochemical analysis.
2. To establish novel protocols for synthesis of gold nanoparticles from selected plants.
3. Characterization of synthesized gold nanoparticles.
4. Toxicity study of plant extracts and synthesized nanoparticles.

5. To understand the immunomodulatory mechanisms of mentioned plants and their synthesized nanoparticles.

Results and Conclusions

1. Preliminary phytochemical analysis of methanolic leaves extracts reveals following data:

- *Alstonia scholaris* (AS) shows prominent presence of steroids, terpenoids, phenolic compounds, flavonoids, alkaloids. Tannins, glycosides and cardiac glycosides are moderately present.
- *Tridax procumbens* (TP) shows prominent presence of steroids, terpenoids, phenolic compounds, flavonoids, alkaloids, saponins. Tannins, glycosides and cardiac glycosides are moderately present.
- *Vitex negundo* (VN) shows prominent presence of steroids, terpenoids, flavonoids, alkaloids, saponins, tannins. Glycosides and cardiac glycosides are moderately present.

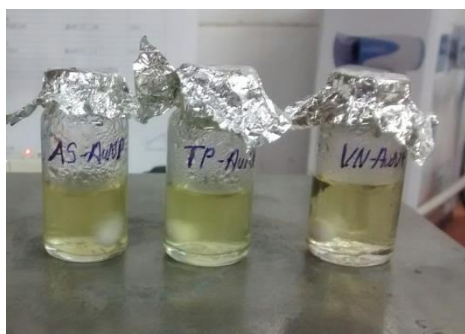
2. HPTLC Fingerprinting:

HPTLC fingerprinting of AS extracts confirms prominent peaks of the following active compounds.

Table 1: List of Active compounds identified

Phytochemicals	<i>Alstonia scholaris</i> (AS)	<i>Tridax procumbens</i> (TP)	<i>Vitex negundo</i> (VN)
Alkaloids	++	++	++
Flavonoids	+	+	++++
Glycosides	+	+	++++
Phenolic Compounds	++	+	++++
Phenols	+	+	+++
Saponins	++	+	++++
Sterols	++	++	+++
Tannins	+	+	+++
Triterpenoids	+	+	++

3. Standardization of gold nanoparticles synthesis was done using plant *Alstonia scholaris* (AS), *Tridax procumbens*(TP) and *Vitex negundo*(VN) with parameters as concentration of Chloroauric acid, PH, temperature, time etc.



Before Synthesis



After synthesis

Figure 1 Photographs of nano particle suspensions before and after synthesis

4. *In vivo* acute oral toxicity of all the three extracts was done using OECD guidelines and found to be non-toxic at limit dose of 2000mg/kg body weight.

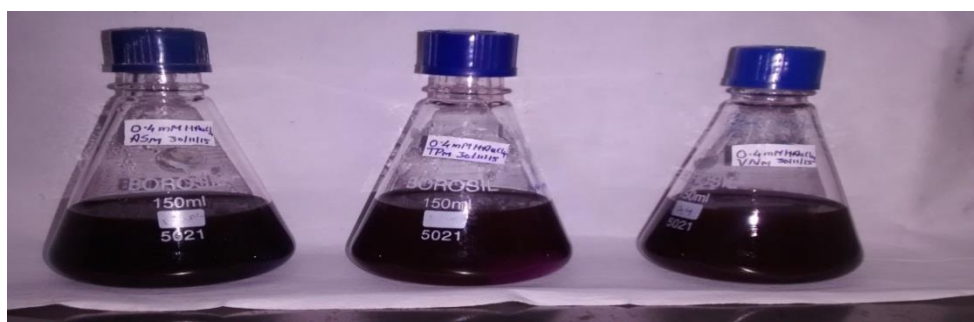


Figure 2: Photographs of nano particle suspensions before and after synthesis

Department of Clinical Pathology

Biosynthesis of Nanoparticles & its Potential Therapeutic Applications (Ph.D project)

Technical Team

1. Dr. (Mrs.) Sunita D. Deshpande
2. Dr. Shashikant P. Vaidya
3. Mr. Kapil Punjabi

Period

Mar 2013- Mar 2017

Introduction

Nanotechnology scale from 1 to 100nm & is the bridge between bulk materials & atomic structures. One nanometer is one billionth or 10^{-9} of a meter e.g. Diameter of DNA helix is 2nm. Nanoparticles accomplish extra properties compared to the bulk material. Nanoparticles are synthesized mainly by two approaches. Top down approach – Physical methods & Bottom up approach – Chemical & Biological methods. Biological methods are cost effective, non-toxic and comparatively easier. Various microorganisms, plants and DNA templates have been reported for nanoparticle synthesis. Nanoparticles are now widely applied in fields of technology, medicine, industries etc.

Objectives

1. Bacteria from soil and effluent of an electro plating industry will be screened for synthesis of nanoparticles.
2. Bacteria screened positive for synthesis of silver nanoparticles will be further analyzed for synthesis of other metal nanoparticles and identified by molecular characterization.
3. Evaluate the nanoparticles for its size, shape and conformity by electron microscopy and spectral studies.
4. Check the toxicity of nanoparticles (*in vitro*) to verify its usage for therapeutics
5. To determine antimicrobial properties of nanoparticles on Standard pathogenic strains & multi drug resistant pathogens.

Results and Conclusion

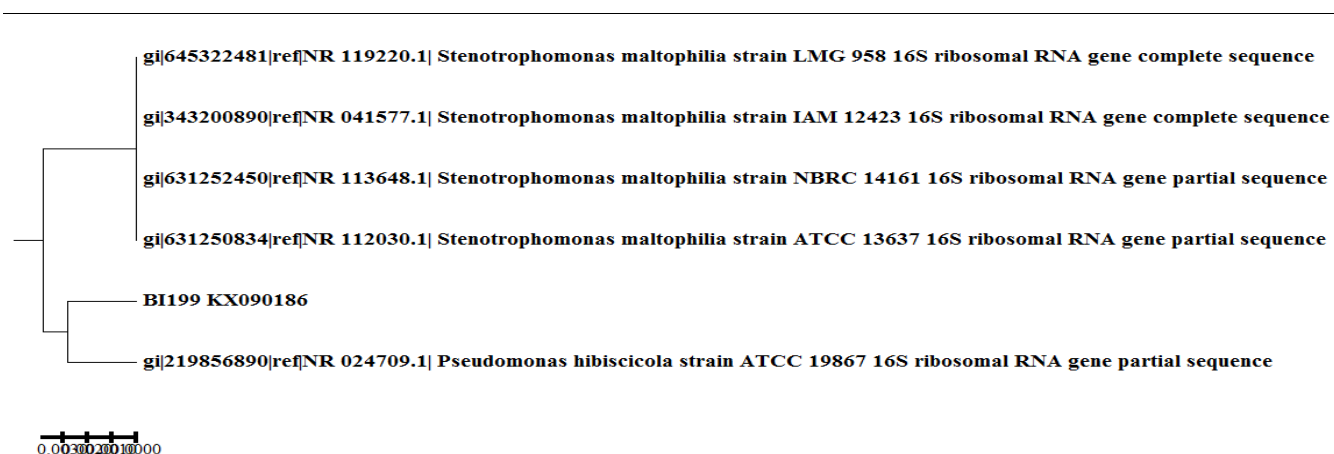


Figure 1: Dendrogram of potential isolates which synthesize nanoparticle

From total 20 isolates screened one was found to synthesize silver nanoparticles and it was found to be a closely related strain of *Pseudomonas hibiscicola* by molecular characterization (isolate identity no. BI199 Gene Accession No. KX090186)

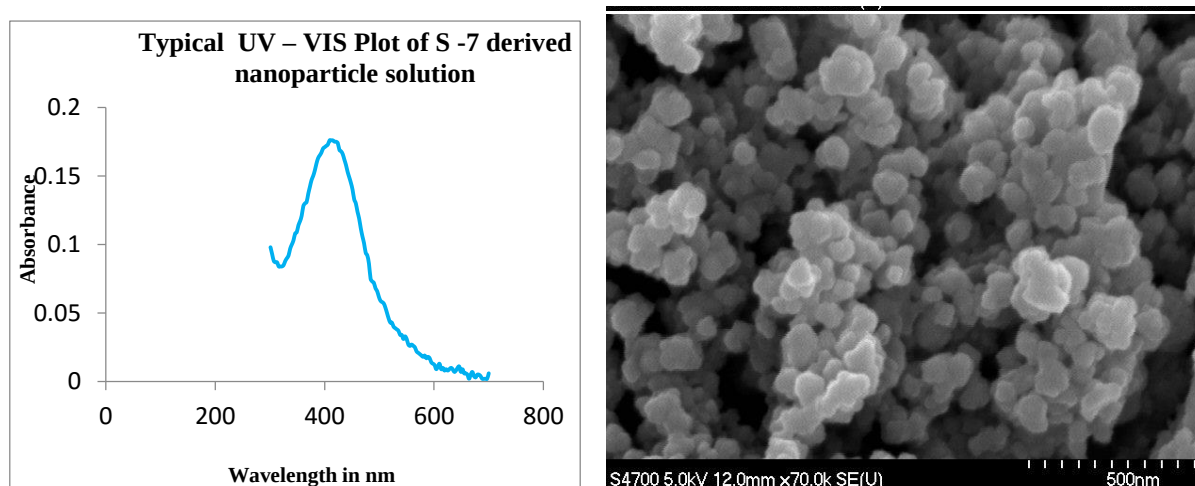


Figure 2: LHS: UV – VIS Plot of S -7 derived nanoparticle solution RHS: SEM micrograph of synthesized silver nanoparticles

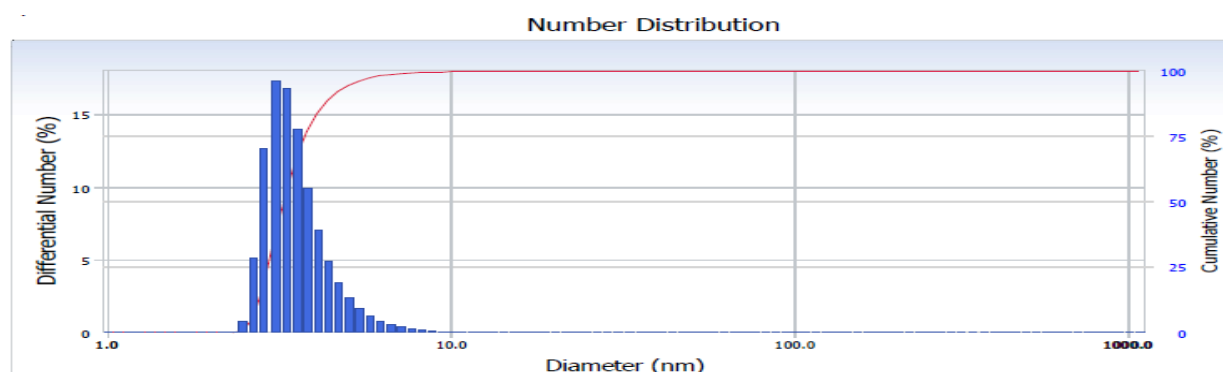


Figure 3: Nano Particle size determination using Direct Light scattering

Table 1: Analysis of particle size By DLS.

Particle Size	Dilute material	Lyophilized material
DLS Particle size analyzer	Z – Average size - 49.68 nm	Z – Average size - 58.18 nm

The UV-Vis absorption spectra obtained showed a characteristic peak at 420nm indicating presence of silver nanoparticles. The SEM micrograph shows presence of well dispersed nanoparticles of various shapes of about 50nm. The average size of the particles, size distribution, and polydispersity index (PDI) of the synthesized silver nanoparticles were determined by particle size analyzer. The average particle diameter was found to be 49.68 nm and Polydispersity index was 0.515. The average particle size and PDI revealed that the produced silver nanoparticles were monodispersed.

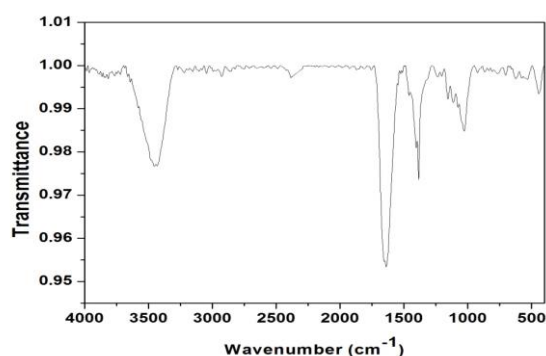
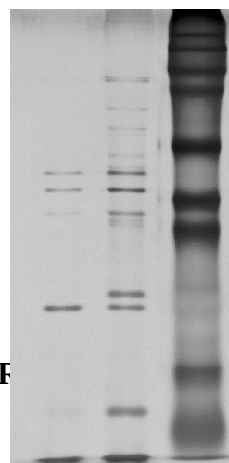


Figure 4 LHS: FTIR analysis showed Amide bonds F of Nanoparticle suspension. Rightmost lane is the marker.



FTIR analysis showed Amide bonds indicating presence of proteins in the test material. PAGE analysis confirmed presence of extracellular proteins and clear difference in band pattern between broth supernatant (control) and nanoparticle solution (test). Different molecular weight proteins involved in reduction of metal and capping of nanoparticles was observed.

Chemical Testing Unit

Synthesis of Nanosilica from RHA for controlled drug release.

Technical team

1. Mr. Manoj Patil
2. Jyoti Yadav

Period

April 2016- onwards

Introduction

India is a major producer of rice and finding ways to put the husk to use is imminent. The high silica content in the rice husk ash has attracted interest in discovering ways to use it commercially. Although silica occurs as a component of cells or cell walls in virtually all arial parts of the rice plant, it is most abundant in the husk. Owing to their small diameter, many technological applications, such as thermal insulators, composite fillers, etc., use ultrafine silica powders. Silica gel is a synthetic silica compound that can be extracted from rice husk ash by the sol-gel method. Silica is removed from residual ash in the form of sodium silicate, and in a later stage of the process, treatment with acid converts the silica into gel. This type of extraction can be done at ambient temperatures, making it an alternative to the thermal treatment. Previous studies on the preparation and characterization of extracted silica gel from rice husk ash have resulted in a simplified method for obtaining pure silica, and this process is called the sol-gel method.

Control release; Lack of specificity of a drug molecule will lead to high-dosage regimen and ultimately cause undesired interactions of a drug with healthy tissues or cells (Kwon et al., 2013). Recent developments in nanotechnology have shown that nanoparticles have a great potential as drug carriers Controlled release of therapeutics to the specific site over an extended period of time is an important determinant in delivery of biologically active agents. This local delivery will reduce potential side effects as well as maximise therapeutic effects by obtaining the optimal concentration of biologically active agents in local sites. The release of bioactive molecules from MSN is dependent on the size of pores, which can be controlled by the processing parameters as we have previously described. The controlled release of biomolecules is available through narrow mesoporous channels and nanoporous structure, which allows kinetics of drug release to be carried out with high precision.

Objectives

- 1) To synthesis silica from rice husk ash
- 2) Observe the control release of drug incorporated in nanosilica

3) Its biological activity

Results & Discussion FTIR SAMPLE 1

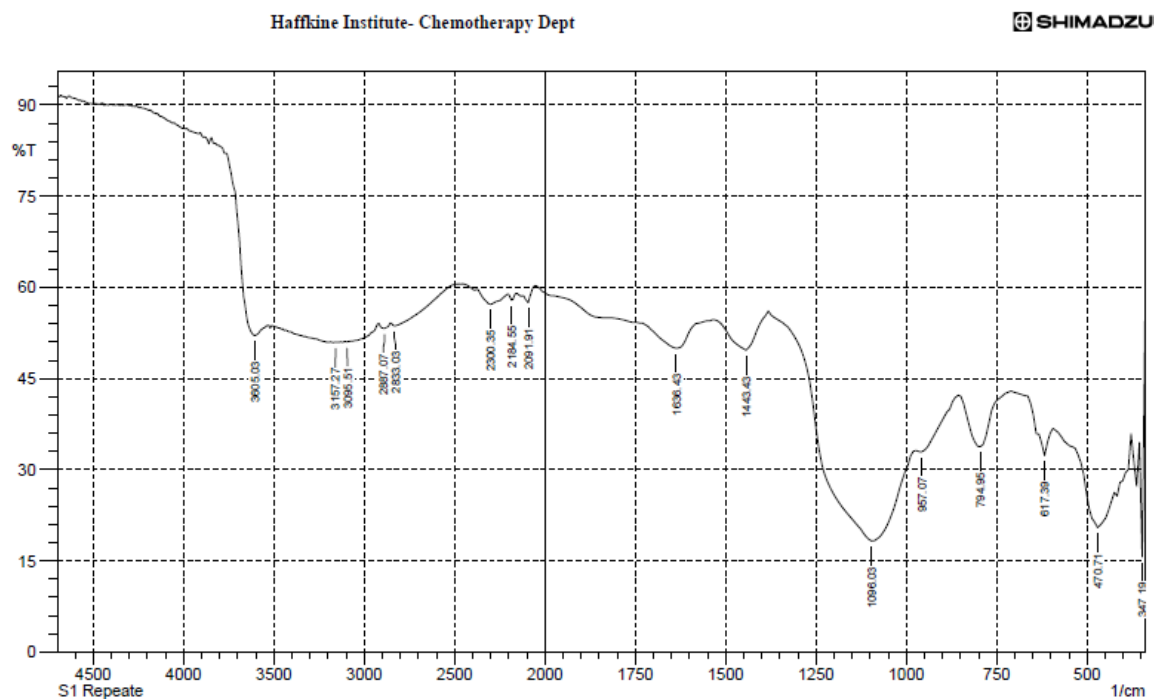


Figure 1: FTIR analysis of nanoparticles

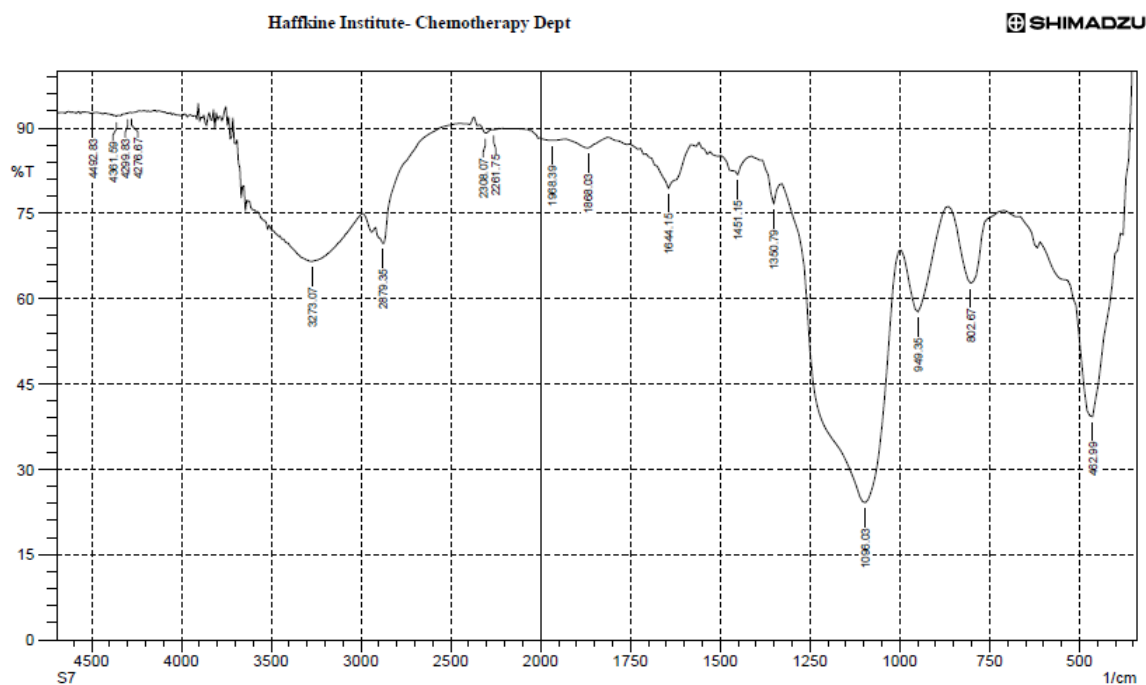


Figure 2: FTIR analysis of nanoparticles incorporated with Ascorbic acid

CONTROL RELEASE

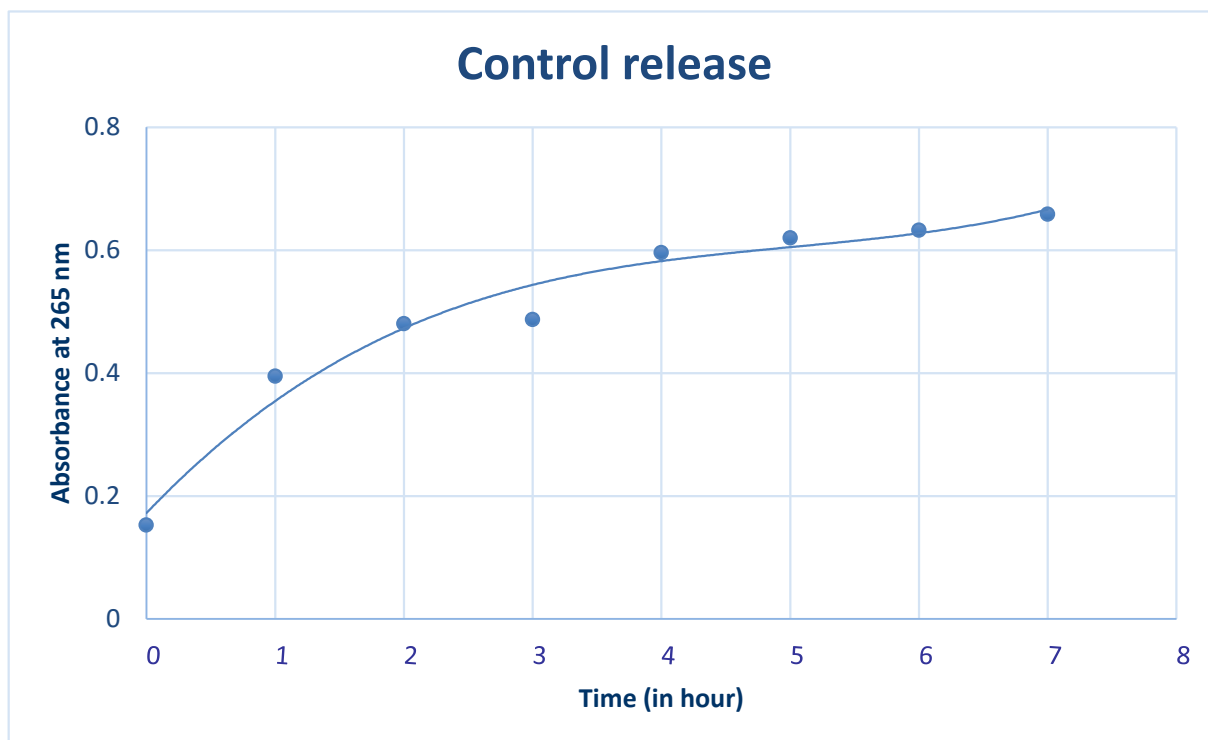


Figure 3: Controlled release of ascorbic acid as monitored by OD at 265 nm

From the above figures we have made the following observations

Figure 1 shows the peak of Si-O-Si at (1100cm^{-1}) which was synthesised from RHA.

Figure 2 shows the FTIR signal of silica nanoparticles incorporated with Ascorbic acid.

Figure 3 shows the graph of release of ascorbic acid incorporated in silica nanoparticle.in a controlled manner

The samples of nanosilica have been sent for further characterisation.

Publications

Some projects undertaken by various departments completed in previous years have resulted in publications during 2015-16. These projects may not have been adequately represented adequately in earlier reports. Abstracts of the publications have been included in this report.

Department of Virology

1. Novel evidence of microglial immune response in impairment of Dengue infection of CNS.

Bhatt R, Kothari S, Gohil D, D'Souza M, Chowdhary A.

Immunobiology, 2015, 220(10), 1170–1176.

Abstract

Objectives: Dengue, the most rampant zoonotic viral disease in tropics, contributes to 14% of acute febrile illness cases globally. Encephalitis in primary Dengue fever, with/without haemorrhage has been reported occasionally. Our study presents novel evidence for this rarity at the molecular level.

Methods: Murine microglia (BV2) were infected in-vitro with Dengue virus (DENV) serotypes (1-4) and their immune response was evaluated. Gene expressions of TNF- α , IL-10, IFN- γ , and IL1- β constituted the pro-inflammatory response, levels of MCP-1 and IL-6 represented the regulatory mechanism and changes in the levels of Occludin, MMP-2, MMP-9 and TIMP-1 encompassed the break-down of the blood-brain barrier (BBB). Cytokine response was studied using RT-PCR, with relative fold change assessed using $\Delta\Delta C_t$ method.

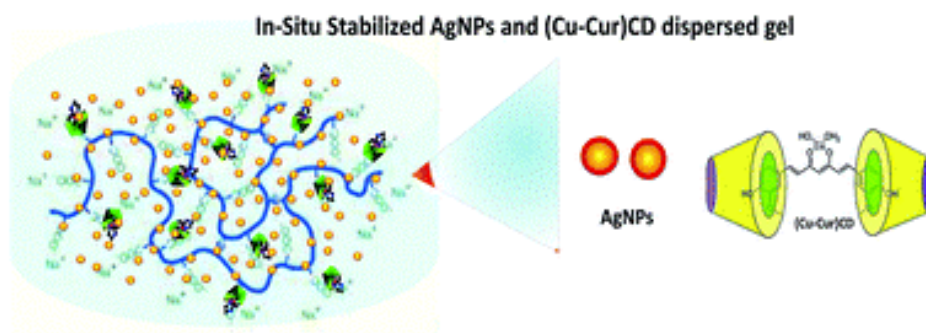
Results: We observed that DENV1 increased vascular permeability and trans-membrane transport, while DENV2 resulted in oxidative stress. DENV3 infection presented with impaired immune response and DENV4 manifested a chaotropic response of the BBB protein genes. However, no serotype was able to breakdown the BBB, thus validating the low prevalence of encephalitis in dengue. Our study is the first reported evidence of the microglial immune response resisting the entry of DENV into the CNS. It also supports the theory that primary Dengue infection results in the acute inflammation of the microglia, and the host immune response plays a critical role in development of encephalitis.

2. In-situ stabilized AgNPs and (Cu-Cur)CD dispersed gel. A topical contraceptive-anti-retroviral (ARV) microbicide.

Chauhan G, Kesarkar R, Kothari S, Rath G, Chowdhary A, Goyal A.

RSC Advances, 2015, 5, 83013-83028.

Abstract



Objectives: The increasing scenario of sexual HIV-1 transmission and unintended pregnancies demands modern approaches to concomitantly tackle these problems. Dually active strategies with “microbicidal” anti-human immunodeficiency virus (anti-HIV) as well as contraceptive properties constitute one such strategy. Nano-metal and metallo-herbal technology was explored to develop such an approach with minimal toxicity.

Methods: Synthesis of in situ stabilised silver nanoparticles (AgNPs) of 2–24 nm and a (copper-curcumin) β -cyclodextrin (Cu-Cur)CD inclusion complex was carried out. Cell viability and activity analysis revealed an acceptable dose of 500 $\mu\text{g ml}^{-1}$ of silver nanoparticles and 10 mg ml^{-1} of (Cu-Cur)CD respectively. AgNP dispersed (Cu-Cur)CD incorporated carbopol 974p gel was prepared and characterized for its pharmaceutical properties. AgNPs, (Cu-Cur)CD and the whole formulation was evaluated for in vitro anti-HIV, spermicidal and antifungal potential. **Results:** Results revealed that HIV-1 propagation and sperm motility was completely inhibited at 5 folds dilution levels. In vivo mating studies proved the contraceptive potential of the formulation. A pre-clinical toxicology study assures the acceptability of our formulation as an intravaginal product (which animal model used?). The novel nano-metallo-herbal strategy was found to possess a potential for topical contraceptive antiretroviral (ARV) microbicide action with an undeniably safe profile to be used as a vaginal product.

3. Oseltamivir Resistant Influenza A (H1N1) Virus Infection in Mumbai, India.

Gohil D, Kothari S, Shinde P, Chintakrindi A, Meharunkar R, Warke R, Kanyalkar M, Chowdhary A and Deshmukh R,

J Antivir Antiretrovir 2015, 7(4), 108-114.

Abstract

Objectives: Oseltamivir is an important antiviral agent approved for treatment of infections caused by Influenza virus. The emergence of an Oseltamivir resistant seasonal Influenza A (H1N1) virus in 2007 in Norway and pandemic Influenza A (H1N1) virus in 2009 [Influenza

A (H1N1) pdm 09] is of major concern. This study aimed for examining antiviral drug resistance of Influenza A viruses circulating during 2009 Influenza season in Mumbai.

Methods: Nasopharyngeal swabs positive for Influenza A virus were inoculated on Madin-Darby canine kidney cell line for virus isolation. Molecular analysis of neuraminidase gene and matrix gene was carried out to detect known mutations contributing to resistance. Resistance to neuraminidase was assayed using a commercially available chemiluminescence based NA-Star assay kit.

Results: Genotypically, a total of 47 isolates of Influenza A (H1N1) pdm 09 isolates were observed to harbour mutations known to confer resistance to Adamantane. However, no known mutations conferring resistance to neuraminidase (NA) inhibitors were detected. Resistance to Oseltamivir was observed in a single isolate of seasonal Influenza A (H1N1) isolate with an extremely high IC₅₀ value of 1261 nM. Conversely, the isolate was sensitive to Adamantane. Phylogenetic analysis revealed that NA gene of this Oseltamivir-resistant isolate from Mumbai was antigenically related to human A (H1N1) A/Brisbane/59/2007 vaccine strain.

Conclusions: Surveillance on drug susceptibility of circulating strains in Mumbai helped us to identify Oseltamivir resistance in seasonal Influenza virus. The study highlights the importance of continual surveillance on Influenza in India to ensure that antiviral prescribed by clinicians is effective when treating patients for Influenza infections.

4. A Novel Tetravalent Recombinant Envelope Domain III Vaccine Against Dengue: An In Silico Approach.

Kulkarni A, Shinde P, Kothari S, Warke R, Chowdhary A and Deshmukh R

Int J Pharm Sci Res. 2015, 6(6), pp 2441-2450.

Abstract

Objectives: The global rise in dengue cases is a major public health concern in terms of morbidity and mortality. The recent study reports 390 million dengue infections annually of which 96 million infections becomes clinically or subclinically severe. Therefore, development of an effective tetravalent vaccine against dengue is a top priority. Dengue envelope domain III is a surface exposed protein; involved in host cell binding and containing multiple, serotype-specific and subcomplex-specific neutralizing epitopes, thus becomes an ideal target for vaccine development. The rapid growth in bioinformatics or immunoinformatics area in terms of development of sophisticated tools assists researchers to predict immunodominant epitopes and study various characteristics of the predicted vaccine model. The combination of computer-aided or in silico methods and experimental methods are useful tools to address complex problems such as deciphering immune responses and vaccine design.

Methods: In the present study we aim to develop a recombinant tetravalent vaccine model using bioinformatics tools of our vaccine candidate containing envelope domain III of all four dengue serotypes (GenBank ID: KF 855114) and study its role and characteristics with its sequence and structure based features.

Results: In silico approach showed that our vaccine is stable, properly folded, antigenic and having multiple predicted B and T cell epitopes that are known to be immunogenic. Also the docking studies using a mouse monoclonal antibody (4E11), which neutralizes all four DENV serotypes, predicted a favourable and stable protein-protein interaction model. Further studies are underway to test its immunogenicity and efficacy in mice.

5. Seroprevalence of Measles, Mumps, and Rubella Antibodies in College Students in Mumbai, India.

Gohil D, Kothari S, Chaudhari A, Gunale B, Kulkarni P, Deshmukh R and Chowdhary
Viral Immunology, 2016, 29(3), 1-5.

Abstract

Objectives: Measles, Mumps, and Rubella (MMR) are vaccine preventable viral infections, which cause significant mortality and morbidity globally. Increased incidence rates of these infectious diseases are observed in young adults. Information on seroprevalence data on MMR in India is limited. The objective of this study was to determine the prevalence of IgG antibodies against MMR among young adults. This was a descriptive crosssectional study involving 192 healthy college students from Maharshi Dayanand College, Mumbai. The project was approved by the Institutional Ethics Committee of Haffkine Institute.

Methods: Between December 2012 and September 2013, blood samples were collected from individuals of age 18–23 years after obtaining written informed consent from them. The quantitative determination of IgG antibodies in serum specimens against MMR was determined using enzyme linked immunosorbent assay. Data on history of vaccination were also collected from participants. (Which ELISA kits were used?)

Results: Among 192 healthy college students (age 18–23 years), MMR seroprevalence was 91%, 97%, and 88%, respectively. The overall seropositivity of MMR was 79%. The highest level of seronegativity was seen with regards to rubella-specific antibodies in 12% of cases. About 96% of the participants did not know about their vaccination history while none of the participants knew about their history of MMR infections. Despite unknown vaccination status, a majority of college students in our study were found seropositive for all three infections, which indicate natural boosting. However, the proportion of seronegativity for measles and rubella was relatively higher. Especially since the study population belonged to reproductive

age group, there is a concern of congenital rubella syndrome in the offspring. Although a larger multicentric study is required to confirm the findings, the results indicate that a dose of measles–rubella (MR) vaccine should be offered to these college students.

6. Virological perspectives and characterization of seasonal influenza B viruses in Mumbai.

Gohil D, Kothari S, Correa A, Deshmukh R and Chowdhary A.

Current Science, 2016, 110(5), 823-827.

Abstract

Objectives: Acute respiratory infections caused by influenza viruses are major life-threatening infections affecting the global population. Information on influenza viruses in Mumbai region in India is limited. With an estimated population above one crore, it becomes imperative to identify the predominant circulating strains, seasonal evolution, epidemiology and impact of influenza virus infection on the public health of Mumbai. The present study is aimed to isolate and identify the prevalent influenza B virus strains circulating in the Mumbai region.

Methods: Virological analysis of 482 throat and nasal samples from patients presenting with suspected influenza-like illness was conducted from January to December 2012 at a research institute in Mumbai. Isolation of positive influenza B samples was performed on Madin Darby Canine Kidney (MDCK) cell line. The isolates were further characterized by Hemagglutination inhibition assay and one-step reverse transcriptase polymerase chain reaction.

Results: Among all the specimens tested, 71% were positive for influenza virus. The positivity for influenza B virus, influenza A (H1N1) pdm 09 and seasonal influenza A virus was 38%, 22% and 11% respectively. Of the 80 influenza B specimens cultured on MDCK, virus was isolated from 38 (48%) samples. Influenza B/Wisconsin/1/2010-like serotype belonging to the B/Yamagata/18/88 lineage was the predominant serotype identified. Influenza was a significant public health concern in the Mumbai region during the study period. Young children were the most affected age group. The study data generated will help understand the epidemiology of the disease in the Mumbai region as well as generate information for global influenza surveillance.

7. Postgenomics biomarkers for rabies-the next decade of proteomics.

Mehta SM, Banerjee SM, Chowdhary AS.

OMICS, 2015, 19(2), 67-79.

Abstract

Background: Rabies is one of the oldest diseases known to mankind. The pathogenic mechanisms by which rabies virus infection leads to development of neurological disease and

death are still poorly understood. Analysis of rabies-infected proteomes may help identify novel biomarkers for antemortem diagnosis of the disease and target molecules for therapeutic intervention. This article offers a literature synthesis and critique of the differentially expressed proteins that have been previously reported from various in vitro/in vivo model systems and naturally infected clinical specimens.

Conclusion: The emerging data collectively indicate that, in addition to the obvious alterations in proteins involved in synapse and neurotransmission, a majority of cytoskeletal proteins are relevant as well, providing evidence of neuronal degeneration. An interesting observation is that certain molecules, such as KPNA4, could be potential diagnostic markers for rabies. Importantly, proteomic studies with body fluids such as cerebrospinal fluid provide newer insights into antemortem diagnosis. In order to develop a complete integrative biology picture, it is essential to analyze the entire CNS (region-wise) and in particular, the brain. We suggest the use of laboratory animal models over cell culture systems using a combinatorial proteomics approach, as the former is a closer match to the actual host response. While most studies have focused on the terminal stages of the disease in mice, a time-series analysis could provide deeper insights for therapy. Postgenomics technologies such as proteomics warrant more extensive applications in rabies and similar diseases impacting public health around the world.

8. Unique Clinical and Imaging Findings in a First Ever Documented PCR Positive Rabies Survival Patient: A Case Report.

Netravathi M, Udani V, Mani R, Gadad V, Ashwini M, Bhat M, Mehta S, Chowdhary A, Pal P, Madhusudana S.

J Clin Virol. 2015, 70, 83-88.

Abstract

Background: Rabies is fatal encephalitis which is a major public health problem in Asian and African countries. Till date, only 12 cases have been reported who have survived after rabies.

Case Report: In this communication we report a patient who is unique as the first documented long term rabies survivor with PCR positivity even after 4½ years of illness. Child sustained dog bite following which he received adequate prophylaxis. Within two weeks, child developed encephalopathy requiring evaluation. Child continues to have persistent myoclonic jerks, seizures, is dependent on all activities with severe neurological deficits. Nested reverse transcriptase PCR (RT-PCR) specific for rabies nucleoprotein gene in CSF and nuchal skin biopsy were positive for rabies viral RNA. The nuchal skin biopsy was also positive for rabies nucleoprotein antigen by fluorescent antibody test (FAT). We describe the clinical evolution and sequential MRI brain changes in this child.

Conclusions: Despite the uniformly dismal prognosis of human rabies, these unusual reports of survival of rabies patients may provide an impetus to explore newer therapeutic strategies for this otherwise fatal disease.

9. Oxidative Stress in HIV/AIDS Patients in Mumbai, India.

Rajopadhye S, Mukherjee S, Dandekar S, Chowdhary A.

J Virol. Curr. Res. 2015; 1(1): 555553.

Abstract

Background: Viral and host factors are responsible for oxidative stress in HIV disease, which in turn activates the replication of HIV provirus by various pathways. Oxidizing stress is a pathologic phenomenon resulting from imbalance between the system producing active oxygen species and those defending the organism. The present study was aimed to assess oxidative stress markers in HIV patients.

Methods: The study included 65 HIV sero-positive patients attending OPD in a tertiary care hospital in Mumbai. 30 age and sex matched healthy volunteers served as controls. Patients were categorized on the basis of their absolute CD4 counts into 3 groups - Group-1 (<200 CD4 cells/mm³), Group-2 (200–499 CD4 cells/mm³), and Group-3 (>500 CD4 cells/mm³). Pro-oxidant markers like the Thiobarbituric Acid Reactive Species (TBARS), Nitric Oxide, and Advanced Oxidation Protein Product (AOPP) were studied from serum, while generation of Superoxide Anion in the PBMC's was assessed. Antioxidant parameters including enzymes like Catalase (CAT); Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST) and protein thiols were also assessed.

Results: Levels of pro-oxidants were significantly increased whereas antioxidant defense markers were significantly impaired in the HIV seropositive group. The pro-oxidants exhibited a negative correlation when compared with CD4 counts where as antioxidant markers showed a positive correlation.

Conclusion: Severely impaired antioxidant machinery leads to higher levels of pro-oxidants in the system. These in turn contribute negatively to the prognosis of HIV/AIDS. Very few comprehensive assessments have been made correlating oxidative stress and HIV in Indian population. This study may therefore generate important leads in surrogate prognostic markers for while assessing simple biochemical parameters. Also, the role of anti-oxidants may be investigated in the management of HIV/AIDS in Indian population.

10 Evaluation of antiviral activity of essential oil of *Trachyspermum ammi* against Japanese encephalitis virus.

Soumen Roy, Pratibha Chaturvedi, Abhay Chowdhary.

Abstract

Background: Japanese encephalitis is a leading form of viral encephalitis, prevalent mostly in South Eastern Asia caused by Japanese encephalitis virus (JEV). It is transmitted by the mosquitoes of the *Culex* sp. The disease affects children and results in 50% result in permanent neuropsychiatric disorder. There arises a need to develop a safe, affordable, and potent anti-viral agent against JEV. This study aimed to assess the antiviral activity of ajwain (*Trachyspermum ammi*: Umbellifereae) essential oil against JEV.

Methods: Ajwain oil was extracted by distillation method and in vitro cytotoxicity assay was performed in vero cell line by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay method. JEV titer was determined by plaque assay and in vitro antiviral activity of ajwain oil was quantified by the plaque reduction neutralization test (PRNT).

Results: Cytotoxic concentration of the oil was found to be 1 mg/ml by MTT assay. The titer of the virus pool was found to be 50×10^7 PFU/ml. we observed 80% and 40% virus inhibition in 0.5mg/ml of ajwain oil by PRNT method in preexposure treatment and postexposure treatment (antiviral activity), respectively.

Conclusion: Our data indicate ajwain oil has potential in vitro antiviral activity against JEV. Further, the active biomolecule will be purified and evaluated for anti-JEV activity and also to scale up for in vivo trial to evaluate the efficacy of ajwain oil in future.

11. In silico pharmacokinetics analysis and ADMET of phytochemicals of *Datura metel* Linn. and *Cynodon dactylon* Linn.

Soumen Roy, Lalit R. Samant and Abhay Chowdhary.

J. Chem. Pharm. Research. 2015; 7(11): 385-388

Abstract

Background: Rabies causes acute encephalitis and it still remains an important public health problem in the world. Although rabies associated fatalities may be prevented with timely immunoprophylaxis, but till date a therapeutic molecule has remained elusive. Plant secondary metabolites have tremendous potential to be antiviral agents. *Cynodon doctylon* and *Datura metel* are been used in ayurveda for treatment of various ailments. Thus these two medicinal plants may therefore be explored for potential antiviral activity against Rabies virus by the in-silico model approach.

Conclusion: ADME properties have shown promising results for all the selected phytochemicals. Whereas in silico toxicity study has shown more care should be taken while adjusting dose for atropine and scopolamine which are also good candidate for GPCR receptors.

Atropine, scopolamine and phytol has shown potential to become various inhibitors after subtle modifications.

12. Exogenous interferon prolongs survival of rabies infected mice.

Mehta S, Roy S, Mukherjee S, Yadav N, Patel N, Chowdhary A.

Virus Dis, 2015, 26(3), 163-169.

Abstract

Background: Rabies is an acute viral infection that causes encephalomyelitis in almost all warm blooded animals and is invariably fatal once the clinical signs appear. The present study was carried out to assess the effect of recombinant human interferon alpha (rhIFN α -2A) treatment on the survival of rabies infected mice and its correlation with cytokines expression.

Methods: The gene expression of TNF- α and IL-6 was measured by SYBR Green Real Time PCR for two groups-"Pre-exposure" (mice were inoculated with rhIFN α -2A prior to rabies infection) and "Post-exposure" (mice were inoculated with rhIFN α -2A post rabies virus infection).

Conclusion: Delayed mortality was observed in interferon treated infected groups. In addition, statistically significant decrease ($P < 0.0001$) in the expression of TNF- α and IL-6 was observed, both in the pre-exposure and post-exposure groups. These findings indicate that modulation of cytokine secretion using exogenous biologicals such as rhIFN may offer novel therapeutic approaches to treat diseases such as rabies.

13. Rabies Virus Severely Influences Host Protein during Terminal Stages of Infection.

Mehta S, Chowdhary A.

Int J Curr Microbiol App Sci, 2015, 4(10), 129-137.

Abstract

Background: Rabies, one of the most ancient diseases that cause encephalitis in all mammals is completely preventable provided appropriate post-exposure prophylaxis is initiated on time. Yet, large numbers of rabies deaths are reported each year and currently no effective treatment is available for clinical rabies.

Methods: In the present study 2-3 week old mice were intracerebrally inoculated with street virus strain to study the effect of rabies virus infection on host protein expression. Brain tissues were harvested at periodic time intervals and analyzed for rabies antigen detection and difference in host protein profiles.

Results: N protein antigen remained undetected 48 h pi (post infection), but was found to be positive for rabies N gene by PCR. With progress in infection and development of clinical manifestations, viral antigen concentration was found to be inversely proportional to the total

protein content in the brain tissues. By the terminal stages, a 40% reduction in protein content was observed and was thought to be a result of inhibition of host translational mechanisms.

Conclusion: Studying virus-host protein interactions on a time-point analysis can provide a deeper insight into the disease pathogenesis and help find druggable targets for timely therapeutic intervention or diagnostic biomarkers.

14. Molecular characterization of nucleoprotein gene of rabies virus from Maharashtra, India.

Mehta S, Charan P, Dahake R, Mukherjee S, Chowdhary A.

J Postgrad Med, 2016; Vol.62(2): 105-108.

Abstract

Background: Rabies poses a serious public health concern in developing countries such as India.

Objective: The study focuses on molecular diagnosis of street rabies virus (RABV) from human clinical specimens received from Maharashtra, India.

Methods: Nucleoprotein gene from eight (of total 20 suspected samples) rabies cases that tested positive for rabies antigen using reverse transcriptase-polymerase chain reaction (RT-PCR) were sequenced.

Results: Sequence analysis using basic local alignment search tool (BLAST) and multiple sequence alignment (MSA) and phylogenetic analysis showed similarity to previously reported sequences from India and those of Arctic lineages.

Conclusion: The circulating RABV strains in Maharashtra, India show genetic relatedness to RABV strains reported from Indo-Arctic lineages and India-South and Japan.

15. Pathway Analysis of proteomics profiles in Rabies infection: Towards future biomarkers? Mehta S, Sreenivasamurthy S, Banerjee S, Mukherjee S, Prasad K, Chowdhary A. OMICS. 2016; 20(2): 97-109.

Abstract

Background: Rabies is a zoonotic viral disease that invariably leads to fatal encephalitis, which can be prevented provided post-exposure prophylaxis is initiated timely. Ante-mortem diagnostic tests are inconclusive, and rabies is nontreatable once the clinical signs appear. A large number of host factors are responsible for the altered neuronal functions observed in rabies; however their precise role remains uninvestigated.

Results: We therefore used two-dimensional electrophoresis and mass spectrometry analysis to identify differentially expressed host proteins in an experimental murine model of rabies. We identified 143 proteins corresponding to 45 differentially expressed spots ($p < 0.05$) in neuronal

tissues of Swiss albino mice in response to infection with neurovirulent rabies strains. Time series analyses revealed that a majority of the alterations occur at 4 to 6 days post infection, in particular affecting the host's cytoskeletal architecture. Extensive pathway analysis and protein interaction studies using the bioinformatic tools such as Ingenuity Pathway Analysis and STRING revealed novel pathways and molecules (e.g., protein ubiquitination) unexplored hitherto.

Conclusion: Further activation/inhibition studies of these pathway molecular leads would be relevant to identify novel biomarkers and mechanism-based therapeutics for rabies, a disease that continues to severely impact global health.

16. Evaluation of in vitro cytotoxic and antioxidant activity of *Datura metel* Linn. and *Cynodon dactylon* Linn. Extracts.

Roy A, Pawar S, Chowdhary AS.

Pharmacognosy Res. 2016; Vol.8 (2): 123-127.

Abstract

Objective: To evaluate in vitro cytotoxicity and antioxidant activity of *Datura metel* L. and *Cynodon dactylon* L. extracts.

Methods: The extraction of plants parts (*datura* seed and fruit pulp) and areal parts of *durva* was carried out using soxhlet and cold extraction method using solvents namely methanol and distilled water. The total phenolic content (TPC) and total flavonoid content (TFC) was determined by established methods. The in vitro cytotoxicity assay was performed in vero cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. In vitro antioxidant activity of the extract was performed by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method.

Results: We found that the highest amount of TPC and TFC in methanolic extracts of seed (268.6 µg of gallic acid equivalence/mg of dry plant material) and fruit pulp (8.84 µg of quercetin equivalence/mg dry plant material) of *D. metel*, respectively prepared by Soxhlet method. The methanolic extract of *C. dactylon* prepared using soxhlation has shown potent free radical scavenging activity with 50% inhibitory concentration (IC₅₀) value of 100 µg/ml. The IC₅₀ of a methanolic cold extract of *datura* fruit was found to be 3 mg/ml against VERO cell line.

Conclusion: We observed that plant parts of *C. dactylon* and *D. metel* have a high antioxidant activity. Further research is needed to explore the therapeutic potential of these plant extracts.

Summary: In the present study we observed a positive correlation was between the phenolic and flavanoid content of the *Datura metel* and *Cynodon doctylon* (durva) extracts with the free radical scavenging activities. Both were found to have a high antioxidant activity.

Department of Zoonosis

1. Antimalarial activity of Argemone Mexicana: an in vivo and in vitro study.

Bapna S, Choudhary K. P, Ramaiya M, Chowdhary A.

World journal of Pharmaceutical Research: 2015, 4(11), 1653-1663.

Abstract

The present study reports the evaluation of anti-plasmodial effect of crude alkaloidal extract and its berberine fraction isolated from leaves of *Argemone mexicana* L. (family, Papaveraceae) which is commonly used in traditional medicine in India. Berberine demonstrated promising in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7 (IC₅₀ value 34.18 µg/mL) as compared to crude alkaloidal extract (IC₅₀ value 73.84 µg/mL). The toxicity of the extracts were monitored with Brine shrimp lethality assay and showed no toxicity in both the extract tested. The results of in vivo activity against *Plasmodium berghei* in mice, demonstrated more promising results with crude alkaloidal extract as compared to berberine. When both the extracts were co-administered there was tremendous increase in survival time and parasite inhibition as compared to control in *Plasmodium berghei* infected mice. The successful combination of crude and purified fractions may help in translating our research on *Argemone mexicana* into practical use for people at risk of contracting malaria.

2. Larvicide and growth retarding activity of alkaloidal extract of two plants towards *Aedes aegypti* (Dipteria: culicidae)

Bapna S, Shinde. R. Satvekar T, Ramaiya M.

World journal of Pharmaceutical Research: 2015, 5(1), 998-1004.

Abstract

The crude alkaloidal extract from leaves of *Ricinus communis* L. (family: Euphorbiaceae) and *Argemone Mexicana* L. (family: Papaveraceae) were evaluated for larvicidal and growth inhibitory activity against *Aedes aegypti* mosquito larvae. The extracts of both the plants demonstrated promising larvicidal activity and prolonged larval and pupal period with an overall increase in the development period of mosquito life cycle. *A. Mexicana* affected moulting of larva into pupa and reduced adult emergence as compared to *R. Communis* extract. Growth index was considerably reduced with extract concentrations (500 ppm and 1000 ppm) as compared to control signifying its anti-juvenile activity. The results thus indicates that both larvicidal and growth inhibiting potential of alkaloidal extracts from leaves could be used as promising mosquito control strategy particularly in view of continuing problem with resistance to synthetic insecticides and environmental pollution.

Sent for publication

1. Control of dengue vector *Aedes aegypti* using house hold disinfectants

Bapna S, Satvekar T, Ramaiya M

Indian Journal of Medical Research May 2016.

Abstract

The mosquito *Aedes aegypti* is responsible for transmitting dengue virus. These mosquitoes are associated with the living spaces of humans. They generally spend their entire lives in and around the houses where their eggs hatched. Accessing indoor larval habitats of *Ae. Aegypti* to apply larvicides is a major limitation in many urban contexts. Keeping this in mind the present study was aims to evaluate the potential of different household disinfectants to check *Aedes aegypti* mosquito larvae in domestic environment. Susceptibility of late third stage larvae of *Aedes aegypti* was evaluated to each of five selected household disinfectants (Mopz TM, Lizol, Dettol, Savlon, Sterillium® hand Sanitizer) using WHO standard protocol.

Out of five disinfectants only mopzTM and Lizol demonstrated promising larvicidal activity with 100% mortality below 0.04 % concentration followed by Dettol the same activity at 0.08% concentration. Savlon and hand sanitizer Sterillium®, were not found effective against larvae. The promising larvicidal activity obtained with Mopz TM, Lizol and Dettol, which are easily available in market and very convenient to use makes them important measures for *Aedes* control. Further research is needed to develop a handy household larvicidal agent for better control of *Ae. aegypti* mosquitoes in domestic environment.

2. A novel environmental friendly mosquito control strategy without chemical use.

Bapna S.

International Journal of Mosquito Research (Accepted) May 2016.

Abstract

The emergence and rapid spread of environmental pollution and insecticide resistance undermine the current mosquito control strategies. There is a global consent that new tactics are needed to suppress mosquito densities especially in areas of high insecticide resistance. The present opinion introduces a completely novel idea to attract mosquitoes to lay eggs in man-made breeding sites where they will be monitored at regular interval and developing stages will be destroyed before adult emergence. This may prove a safest mosquito control measure and will have a huge impact on our strategies for mosquito management without causing environmental pollution.

3. Surveillance of JE virus infection in *Culex* mosquito larvae in and around Mumbai

Kulkarni S, Sheikh A, Sheikh R, Sinde R, Mahale P, Bapna S, Ramaiya M, Chowdhary AS.

Journal of Arthropod-borne Diseases May 2016.

Abstract

The incidences of vector-borne diseases are increasing alarmingly due to many factors including uncontrolled urban developments that support breeding of vector mosquitoes. Japanese Encephalitis (JE) which is an important mosquito-borne viral disease and one of the leading causes of viral encephalitis and neurological infections in Asia and Culex mosquitoes are the primary vector. Japanese encephalitis virus (JEV) is maintained in an enzootic cycle between mosquitoes and amplifying vertebrate hosts, mainly pigs and wading birds. An entomo-epidemiological survey was undertaken to evaluate the present scenario of entomological indices and presence of JEV in mosquito larvae in and around Mumbai area to assess potential global health risks. This study describes surveillance of JEV in Culex mosquito larvae collected from different areas in and around Mumbai using JEV nested reverse transcription polymerase chain reaction (RT PCR)

It is observed that all the entomological parameters were above the critical level but none of the larvae screened had JEV infection. The present study was undertaken to evaluate the entomo-epidemiological risk of Culex mosquito borne diseases (VBD) in and around Mumbai region. Such molecular epidemiological surveillance helps in predicting the outbreaks of disease and mosquito population from the study areas.

Department of Clinical Pathology

1 Detection of Macrolide, Lincosamide and Streptogramin Resistance among Methicillin Resistant *Staphylococcus aureus* (MRSA) in Mumbai.

Subramanian A, Chitalia V, Vaidya SP, Warke RV, Chowdhary AS, Deshmukh RA.

JKIMSU 2015, 4(3), 10 – 16.

Abstract

Background:

The increase in incidence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and its extraordinary potential to develop antimicrobial resistance has highlighted the need for better agents to treat such infections. This has led to a renewed interest in use of new drugs for treatment with clindamycin and quinupristin-dalfopristin being the preferred choice for treatment. Aim & Objectives: This study was undertaken to detect the prevalence of Macrolide Lincosamide-Streptogramin (MLS) resistance among clinical isolates of MRSA

Material and Methods: Two hundred and thirty clinical isolates of *S. aureus* were subjected to routine antibiotic susceptibility testing including cefoxitin, erythromycin and quinupristindalfopristin. Inducible resistance to clindamycin was tested by 'D' test as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of all *S. aureus* isolates, 93.91% were identified as MRSA. In the disc diffusion testing, 81.5% of isolates showed erythromycin resistance. Among these, the prevalence of constitutive (cMLS), inducible (iMLS) and MS-phenotype were 35.80%, 31.82% and 32.39% respectively by the D-test method. 77.8% of isolates were resistant to quinupristin-dalfopristin and the Minimum Inhibitory Concentration (MIC) ranged from 4–32 µg/ml. 89.20% of isolates were resistant to both quinupristin-dalfopristin and erythromycin of which 35.03%, 35.67% and 29.30% belonged to iMLS, cMLS and MS phenotype respectively.

Conclusion: The emergence of quinupristin dalfopristin resistance and MLS phenotypes brings about the need for the simple and reliable D-test in routine diagnosis and further susceptibility testing for proper antimicrobial therapy.

2. Molecular Diagnosis of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates in and around Mumbai

Joshi S, Dahake R, Chitalia V, Vaidya SP, Chowdhary AS.

JPAM 2015, 9(3), 2263 – 2270.

Abstract

Mycobacterium tuberculosis (MTB), resistant to both isoniazid (INH) and rifampicin (RIF) and leading to multidrug-resistant tuberculosis (MDR-TB) can be successfully identified by an 8-

week long phenotypic method. However, there is a need for faster diagnostic techniques. In the present study, we evaluated the use of PCR followed by sequencing as a rapid diagnostic tool and determined the incidence of *rpoB* and *katG* resistance-associated mutations in *Mtb* isolates in and around Mumbai. Fifty-Five sputum samples from clinically suspected tuberculosis patients were decontaminated and screened for MTB using Ziehl Neelsen (ZN) staining and Lowenstein–Jensen (LJ) culturing. Extracted DNA from positive samples was subjected to conventional PCR for *rpoB* and *katG* genes. In addition, Drug Susceptibility Testing (DST) of isolates was performed by the standard proportional method. PCR products of selected samples of either type i.e., resistant and susceptible by both techniques were sent for sequencing. Fifteen samples (27.27%) were found to be resistant to both drugs phenotypically by DST and by PCR for *rpoB* and *katG* genes. Sequencing analysis revealed point mutations Ser 531 Leu for *rpoB* gene and Ser 315 Thr for *katG* gene. The results obtained by direct PCR followed by sequencing method were in accordance with phenotypic method (DST) with high sensitivity and specificity for both genes. The results suggest that the use of molecular techniques can be more suitable for rapid diagnosis of multi-drug resistance in clinical isolates of MDR-TB.

Key words: - Multi drug Resistant tuberculosis (MDR TB) Polymerase chain reaction (PCR) Sequencing, *rpoB* & *katG* genes

3. Comparison of *Mycobacterium tuberculosis* DNA Extraction Methods.

Joshi S, Dahake R, Chitalia V, Vaidya SP, Chowdhary AS.

JPAM 2015, 9(2), 1129 – 1136.

Abstract

Extraction of DNA from sputum samples of patients with tuberculosis is often the most time consuming step in the entire diagnostic procedure that increases the overall time required to process a clinical specimen. Hence there is a need to develop a quicker but efficient and cost effective method. The present study was designed to optimize the time required for DNA extraction without compromising the final yield, purity and therefore the end result for diagnosis. Thirty five samples were collected from clinically suspected tuberculosis patients and processed using standard decontamination method. DNA from these samples were extracted using three techniques-Qiagen QIAmp DNA mini kit, Hi media *Mycobacterium tuberculosis* DNA extraction kit and an In-house method (SDS, TE buffer and triton X). All the methods were assessed for yield and purity using nanodrop followed by conventional Nested PCR to amplify the IS6110 region. The optimum incubation period in lysis buffer for all three methods was found to be three hours, with no statistically significant change occurring in the yield and purity. The total time required for extraction was maximum for M/s. Qiagen i.e.,

~6 h and minimum for in house method ~3.45h. In-house method was found to be the relatively inexpensive. The in-house method of extraction was found to be the quickest and most cost effective. However, the time consuming and laborious preparatory procedures may increase the chances of manual error, especially in laboratories with high sample load.

4. Comparison of conventional method, Radiometric method and method using Oxidation-reduction dye for detection of Multidrug resistant Tuberculosis,

Vaidya SP, Sharma J, Mulye S, Koppikar G, Kulkarni M, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, 2015, 72-89

Abstract

Today Tuberculosis (TB) has become the most important communicable disease in the world. The emergence of multidrug resistant (MDR) TB has become the main threat to TB treatment and control programs. Rapid detection of TB is critical for the effective treatment of patients. Recently, a method using the Oxidation - Reduction dye has been proposed for drug susceptibility testing of *Mycobacterium tuberculosis* (M.tuberculosis). In the present study, we have compared Drug Susceptibility Testing (DST) by conventional method, Radiometric method like BACTEC460TB (B460TB) System and Oxidation-Reduction Assay such as Microplate Alamar Blue Assay (MABA) for detection of MDR TB. Total 60 clinical isolates of M.tuberculosis were included in the study. The results obtained by MABA for DST of M.tuberculosis were compared with those obtained by B460TB system and Conventional method. DST results obtained by MABA showed good agreement with results obtained by B460TB system and Conventional method for Isoniazid and Rifampicin. In our study specificity and sensitivity for susceptible and resistant strain was found to be 98% and 100% for Rifampicin respectively and 100% and 95% for Isoniazid respectively for MABA. MABA appears to be a reliable method for the rapid and simultaneous detection of MDR-TB and DST of M. tuberculosis. It is simple and inexpensive method for DST of M.Tuberculosis with minimal biohazard risk.

5. Determination of efficacy of isoxyl, a mycolic acid inhibitor, in vitro against *Mycobacterium tuberculosis* strains

Vaidya SP, Punjabi K, Mulye S, Koppikar G, Kulkarni M, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, 2015, 1-24

Abstract

The enzymes involved in the biosynthetic pathways of the critical components including mycolic acids offer attractive and selective targets for the developments of novel anti-mycobacterial agents. Isoxyl (ISO), a mycolic acid inhibitor, is an old drug, which was used for

the treatment of tuberculosis was evaluated. Determination of Minimum Inhibitory Concentration (MIC) pattern of clinical isolates of Mycobacterium tuberculosis (M. tuberculosis) to mycolic acid inhibitors namely ISO, Isoniazid (INH) and Ethionamide (ETH) by agar and broth dilution Method was done. Also the Minimum bactericidal concentrations were evaluated. Total 40 MDR and 20 susceptible strains of M tuberculosis were tested. The result of the MIC studies showed that ISO is capable of inhibiting the growth of M. tuberculosis in a range of 1-20µg/ml. Inhibitory activity of ISO was higher than activity of ETH in solid media. Amongst three anti tuberculosis drugs, INH showed highest bactericidal activity against M. tuberculosis strains followed by ETH. While ISO exhibited lowest bactericidal activity. Amongst, three drugs tested, ISO shows highest MBC/MIC ratio with lowest bactericidal activity. ISO showed significantly lower bactericidal activity against MDR strains than susceptible strains of M. tuberculosis. MBC/ MIC ratio of ISO was similar to MDR and susceptible strains of M. tuberculosis. Overall study implies that ISO may be suitable for the treatment of Tuberculosis, particularly multi-drug resistant kind. (I could not understand this abstract.)

6. Determination of in vitro inhibitory activity of mycolic acid inhibitors to clinical isolates of bacteria and mycobacterium tuberculosis

Vaidya S, Punjabi K, Mulye S, Koppikar G, Kulkarni M, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, 2015, 37-54

Abstract

Today tuberculosis (TB) has become the most important communicable diseases and a leading cause of death in the world. The emergence of drug resistance has become the main threat to TB treatment and control programs. The presence of mycolic acids in cell wall gives Mycobacterium tuberculosis (M. tuberculosis) many characteristics that defy medical treatment, increased resistance to chemical damage and dehydration, and prevent the effective activity of hydrophobic antibiotics. In addition, the mycolic acids allow the bacterium to grow readily inside [macrophages](#), effectively hiding it from the host's immune system. In this study, in vitro inhibitory activity of mycolic acid inhibitors namely Triclosan (TCN), Thiolactomycin (TLM) and Isoxyl (ISO) was determined against 87 bacterial clinical isolates and 20 clinical isolates of M.tuberculosis by agar dilution method. Significant difference was observed in the inhibitory activity of TCN, TLM and ISO to all bacterial strains. Clinical isolates of Pseudomonas species were found to be more resistant to TCN than other bacterial strains. While clinical isolates of Staphylococcus aureus and Klebsiella species were found to be most susceptible to

TCN. Inhibitory activity of ISO against clinical isolates of *M. tuberculosis* in both, susceptible and MDR strains were seen. For susceptible strains, the MIC of ISO ranged from 0.3 - 2.5 mcg/ml and for MDR strains it was found to be in the range of 1.2 – 20 mcg/ml. TCN was found to be a better mycolic acid inhibitor compound in inhibiting test organisms amongst three compounds. ISO was found to be least active against bacterial isolates, but was effective against susceptible as well as MDR strains of *M. tuberculosis*.

7. Relationship between RpoB mutations and Minimum inhibitory concentrations of Rifampicin in multi-drug resistant strains of *Mycobacterium tuberculosis*

Vaidya SP, Maniar J, Mulye S, Koppikar G, Kulkarni M, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, 2015, pp. 108-121

Abstract

Tuberculosis is an infectious disease caused by obligate intracellular bacteria *Mycobacterium tuberculosis*, which has created a health problem worldwide. Incidence of Multi-drug resistance tuberculosis creates the scenario dangerous, which is resistant to both the first line drugs namely Rifampicin and isoniazid. Mutation in *rpoB* and *inhA* confers resistance to rifampicin and isoniazid respectively. In the present study we studied the relationship between *rpoB* mutations and minimum inhibitory concentrations of Rifampicin in Multi drug resistant (MDR) strains of *Mycobacterium tuberculosis*. Total 20 MDR strains were selected for the study. The mutations in the *rpoB* gene were identified by sequencing the PCR products. The minimum inhibitory concentrations of Rifampicin, was determined by Absolute Concentration method. Mutations conferring resistance to Rifampicin in *Mycobacterium tuberculosis* occurs in the defined region of 81 base pair of *rpoB* gene. The most frequent mutation found in Rifampicin resistant strain is at position 532, 526 and 516. Mutation occurring at this position results in high level of Rifampicin resistance. Mutation at 511, 518 and 522 is associated with low level of Rifampicin resistance.

8. To study incidence of multi drug resistant Tuberculosis in Mumbai,

Vaidya SP, Shreyasi Mulye S, Geeta Koppikar G, Mohan Kulkarni M, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, 2015, 122-138

Abstract

Isoniazid and Rifampicin, the two most potent anti-tuberculosis drugs are rendered ineffective in Multidrug-resistant Tuberculosis (MDR-TB). India, China and Russia contribute to more than 62% of MDR-TB globally. In India, endemic areas like Mumbai are “hotspots” for the dissemination of MDR-TB. The aim of the study was to investigate the trends in incidence of MDR-TB in cases of pulmonary tuberculosis in Mumbai. Total hundred and two clinical

isolates of *M. tuberculosis* along with Standard strain of *M. tuberculosis* H37Rv was tested in the study. Drug susceptibility testing of these strains were carried by Resistance Ratio method to anti-tuberculosis drugs namely Isoniazid (INH), Streptomycin (SM) and Ethambutol (EMB) and by absolute concentration method for Rifampicin (RF). In our study highest resistance (46 %) was observed to INH followed by RF (42.16 %), SM (29.41 %) and EMB (25.49 %). While, resistance to Pyrazinamide (PZ) was least (7.8%). MDR TB cases were found to be 41.18%. There was significant difference between resistance pattern of INH and EMB, INH and SM, PZ and EMB, PZ and SM, EMB and RF, PZ and RF. (chi square with Yates correction = 8.5, $p < 0.01$). Detection of MDR TB strain would not only eliminate non-essential use of antibiotics, but would also help in the selection of most effective drug regimen and guide therapy in chronic cases.

9. Determining the efficacy of Isoxyl, a mycolic acid inhibitor, *in vitro* against Mycobacteria other than *Mycobacterium tuberculosis* (MOTT) strains

Vaidya SP, Shreyasi Mulye S, Geeta Koppikar G, Mohan Kulkarni M, Chowdhary AS
LIFE: International Journal of Health and Life-Sciences, 2015, 90-107.

Abstract

Mycobacteria other than *Mycobacterium tuberculosis* (MOTT) cause infections more commonly in the presence of predisposing factors and underlying diseases. They are also notably resistant to commonly used anti tuberculosis drugs. Total 11 clinical isolates MOTT were included in the study. Drug susceptibility testing of these isolates was performed by Resistant Ratio method. Minimum inhibitory concentration (MIC) pattern of these isolates of MOTT to mycelia acid synthesis inhibitors namely, Isoxyl (ISO) and Isoniazid (INH) were determined by agar dilution and broth dilution method. Minimum bactericidal concentration (MBC) pattern of these isolates to ISO and INH were also determined. Out of 11 MOTT isolates, 3 isolates were characterized as *Mycobacterium scrofulaceum*, 3 isolates as *Mycobacterium fortuitum*, 2 isolates as *Mycobacterium flavescens*, 1 isolates as *Mycobacterium terrae* and 2 isolates as *Mycobacterium kansasii* depending upon the results of biochemical tests. The MBC range of INH was found to be 0.025 to 6.4 µg/ml and of ISO was found to be 0.6 to 20 µg/ml. Bactericidal activity of ISO was 7.25 times lower than the activity of INH. It is well known that most MOTT species are more resistant to chemotherapeutic agents other than tubercle bacilli. The inhibitory activity of ISO was more to MOTT strains than *Mycobacterium tuberculosis* strains. There was low bactericidal activity of ISO to MOTT strains, but better than for *Mycobacterium tuberculosis* strains.

10. Prevalence of Candidiasis among children in Mumbai

Wandre S, Mukadam T, Sanap S, Vaidya SP, Chowdhary AS

LIFE: International Journal of Health and Life-Sciences, ISSN 2454- 5872 Special Issue, 2015, 25-36

Abstract

This study aims to determine the prevalence of infection of *Candida* species and its resistance to anti-fungal agents amongst children in Mumbai. Total 169 clinical isolates of *Candida* species from various clinical specimens namely blood, urine, stool; Cerebral spinal fluid, liver abscess, Endotracheal secretion and ventric tip were included in the study. They were screened and identified by staining, culture and microscopy, followed by isolation on specialized chromogenic agar. Antifungal susceptibility tests of these isolates were carried out against Amphotericin B (50mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Ketoconazole (30mcg), Miconazole (30mcg), and Fluconazole (10mcg) and were reported based on CLSI guidelines (2008). The prevalence of *Candida* infections was found to be greater in males (69%) as compared to female (31%) children. In the age group of 0-5 years, the occurrence of *Candida* infection was substantially higher as compared to the others age group. *Candida albicans* was found to be the major causative agent of fungal infections. The susceptibility of isolates to antifungal agents was found to differ in male and female patients. Overall, highest resistance was observed to Fluconazole (27.1%) while, lowest resistance to Amphotericin B (28.7%). Males exhibited highest resistance to Fluconazole (27.1%) while, females showed highest resistance to Amphotericin B (28.7%). Higher resistance was found to the azole group of antifungal agents. Hence, Amphotericin B can be the choice of treatment of *Candida* infection in children followed by Miconazole.

Department of Bacteriology

1. Optimization of Fermentation parameters for Enhanced production of Lipase from lipolytic *Pseudomonas* spp.

Vishwe VS, Vaidya SP and Chowdhary AS

Int Res J Biological Sci, 2015, 4(6), 16-21.

Abstract

Lipases (Triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes which catalyze the enzymatic hydrolysis of triacylglycerol into diacylglycerol and fatty acids. Lipases are one of the most important enzymes that are used in various industries like Detergent, Food, Leather, Textiles, Pharmaceuticals, etc. Though various organisms used for the production and purification of lipase for industrial applications, *Pseudomonas* spp. remains considerably less explored. The present study thus explores the ability of *Pseudomonas* spp. isolated from oil contaminated soil samples for production of extracellular lipase enzyme. For the present study, 29 oil contaminated soil samples were collected from different regions of Pune, Solapur and Mumbai. Total 50 lipolytic *Pseudomonas* spp. were isolated by using Tributyrin agar medium containing Rhodamine B. Isolate showing maximum lipase activity (SP45) was then selected for further optimization studies. During optimization of fermentation parameters, SP45 showed optimum lipase activity of 50.4967 U/ml when Glucose was used as a carbon source, while lipase activity of 61.6567 U/ml was observed when Yeast extract was used as a nitrogen source. On optimizing the pH and incubation temperature of the fermentation medium, SP45 showed maximum lipase activity of 66.2567 U/ml in fermentation medium of pH 7 at 37°C.

2. Isolation and Characterization of Lipolytic *Pseudomonas* spp. from oil contaminated water samples.

Vishwe VS, Gajbhiye SP, Vaidya SP and Chowdhary AS

IOSR Journal of Biotechnology and Biochemistry, 2015, 1(6), 33-36.

Abstract

Oil contaminated water samples collected from different areas in Pune were screened for the selective isolation of lipolytic *Pseudomonas* spp. Screening medium containing Tributyrin (1% v/v) and Rhodamine B was used for the specific isolation of lipolytic *Pseudomonas* spp. from oil contaminated water samples. Lipolytic bacteria showing zone of clearance around the colonies were selected for the present study. Isolated bacteria were identified upto genus level with the help of morphological and biochemical testing by following Bergey's manual. Spectrophotometric Lipase assay showed that out of 49 lipolytic *Pseudomonas* spp., WP23 exhibits the maximum Lipase activity of about 45.4733 U/ml.

3. Immunostimulant activity of medical preparation Panchgavya.

Gajbhiye SP, Padmanabhan U, Kothari S, Patil A, Palep H and Chowdhary A

Int J Res Pharm Sci, 2014, 5(3): 1-5.

Abstract

Panchagavya finds mention in the Shushrutasamhita for its wide variety of applications in the treatment of mania, epilepsy, apart from being suggested for treatment of hepatic diseases such as jaundice and hepatitis. It is a blend of five products derived from the cow namely cow dung, urine, milk and ghee (clarified butter). These are mixed in proper ratio and then allowed to ferment. In this study we prepared Panchagavya (PG) as per the guidelines suggested both by traditional texts and that used by other authors. Mice were fed with this preparation of PG and significant weight gain was observed compared to controls. Further the animals fed with PG displayed increments in both humoral immune response, as assessed by haemagglutination assay and cell mediated immunity as assessed by the neutrophil adhesion test. Our study therefore highlights the effect of PG on diet and in immune-stimulation, both aspects important for combating diseases during re-convalescence, thus validating the traditional use of PG in treatment of hepatic diseases.

Department of Biochemistry

1. Evaluation of the anti-angiogenic and antioxidant activity of Indian honeys in comparison with manuka honey from New Zealand.

Naik A, Juwekar OD, Ansulkar P, Deshpande SD, and Chowdhary AS

Intl J Pharma Sci Res 2015, 6(4), 1813-1818.

Abstract

Honey possesses a wide range of medicinal properties due to presence of phytochemicals including flavonoids and phenolic acids. Free radicals and process of angiogenesis are one of the major contributors to cancer development and the use of phyto-constituents as reactive oxygen species scavengers and as an anti-angiogenic moiety has clinical potential and may be having less negative side effects than the existing drugs. This study has explored the phyto-constituents as well as anti-angiogenic and anti-oxidant activity of Indian honey (Kashmir honey and Dhanwantari honey) in comparison with Manuka honey. The total phenolic content was measured by folin ciocalteau method and antioxidant activity was estimated by DPPH scavenging assay. Anti-angiogenic activity of honey samples were evaluated by chorioallantoic membrane (CAM) assay. Drabkin's assay was performed in order to check the haemoglobin content of the chorioallantoic membrane sections. Presence of flavonoids and tannins were observed in all honey samples. Higher total phenolic content was found in kashmir honey (6662.67 mg GAE /100 gm honey) than manuka honey (6078.67 mg GAE /100 gm honey) followed by dhanwantari honey (2286 mg GAE /100 gm honey). Kashmir honey ($IC_{50}= 5.8+_{-}3.03$ mg/ml) showed most potent anti-oxidant activity followed by manuka honey ($IC_{50}= 9.1+_{-}3.00$ mg/ml) and dhanwantari honey ($IC_{50}=12.3+_{-}2.75$ mg/ml). All three honey samples showed a dose dependent anti- angiogenic potential in chick embryo model which was then confirmed by dose dependent decrease of haemoglobin observed in drabkin's assay. Thus, we conclude that, among these three honey samples, Kashmir honey is more potent anti-angiogenic and antioxidant.

2. Correlation of biochemical constituents present in kidney stones and diet from an Indian population.

Shirsat PL, Patil SS, Parkar SN, Rajopadhye SH, Bagal SB, Ansulkar PV, Naik A, Marita R, Chowdhary AS and Padmanabhan U

Intl J Res Pharm Sci, 2016, 6(1), 7 -11.

Abstract

Renal calculi (or kidney stones) post-surgical intervention from the various hospitals in the Mumbai region were analyzed for the presence biochemical components such as calcium,

magnesium, oxalate, phosphate, sulphonamide, cholesterol and carbonate at our department. In this study, we retrospectively analyzed 250 kidney stone samples which were characterized biochemically during the period 2008-2014 to evaluate the predominant component and to establish any correlation which may exist between the biochemical components in these samples and the diet of these patients. The population was statistically analyzed using the Z-proportion test for age, gender and diet; diet was segregated into a moderately non-vegetarian (MNV) and non-vegetarian (NV) and presence of the each of the above components. Majority of the samples presented to our centre were from male subjects. Children were more susceptible to the presence of sulphonamidine and carbonate stones. Women more prone to phosphate stones and moderate non-vegetarians were more prone to calcite stones. Taken together, the data suggest that all three factors age, gender and diet can influence composition of stones.

3. Oxidative Stress in HIV/AIDS Patients in Mumbai, India

Rajopadhye SH, Mukherjee SR, Dandekar SP, Chowdhary AS,

J Virol Curr Res, 2015, 1(1), 555553.

Abstract

Background: Viral and host factors are responsible for oxidative stress in HIV disease, which in turn activates the replication of HIV provirus by various pathways. Oxidizing stress is a pathologic phenomenon resulting from imbalance between the system producing active oxygen species and those defending the organism. The present study was aimed to assess oxidative stress markers in HIV patients.

Methods: The study included 65 HIV sero-positive patients attending OPD in a tertiary care hospital in Mumbai. 30 age and sex matched healthy volunteers served as controls. Patients were categorized on the basis of their absolute CD4 counts into 3 groups - Group-1 (<200 CD4 cells/mm³), Group-2 (200–499 CD4 cells/mm³), and Group-3 (>500 CD4 cells/mm³). Pro-oxidant markers like the Thiobarbituric Acid Reactive Species (TBARS), Nitric Oxide, Advanced Oxidation Protein Product (AOPP) were studied from serum, while generation of Superoxide Anion in the PBMC's was assessed. Antioxidant parameters including enzymes like Catalase (CAT); Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST) and protein thiols were also assessed.

Results: Levels of pro-oxidants were significantly increased whereas antioxidant defense markers were significantly impaired in the HIV seropositive group. The pro-oxidants exhibited a negative correlation when compared with CD4 counts whereas antioxidant markers showed a positive correlation.

Conclusion: Severely impaired antioxidant machinery leads to higher levels of pro-oxidants in the system. These in turn contribute negatively to the prognosis of HIV/AIDS. Very few comprehensive assessments have been made correlating oxidative stress and HIV in Indian population. This study may therefore generate important leads in surrogate prognostic markers for while assessing simple biochemical parameters. Also, the role of anti-oxidants may be investigated in the management of HIV/AIDS in Indian population.

4. Immunostimulant activity of a medical preparation panchagavya,

Gajbhiye SP, Padmanabhan U, Kothari S, Patil A, Palep H, Chowdhary A

Intl J Res Pharm Sci. (2015); 5(3), 1 – 5.

Abstract

Panchagavya finds mention in the Shushruta samhita for its wide variety of applications in the treatment of mania, epilepsy, apart from being suggested for treatment of hepatic diseases such as jaundice and hepatitis. It is a blend of five products derived from the cow namely cow dung, urine, milk and ghee (clarified butter). These are mixed in proper ratio and then allowed to ferment. In this study we prepared Panchagavya (PG) as per the guidelines suggested both by traditional texts and that used by other authors. Mice were fed with this preparation of PG and significant weight gain was observed compared to controls. Further the animals fed with PG displayed increments in both humoral immune response, as assessed by haemagglutination assay and cell mediated immunity as assessed by the neutrophil adhesion test. Our study therefore highlights the effect of PG on diet and in immunestimulation, both aspects important for combating diseases during re-convalescence, thus validating the traditional use of PG in treatment of hepatic diseases.

5. Chemical and pharmacological study of the twigs of Thevetia peruviana plant and its bio-activity profiles,

Save SA, Padmanabhan U, Kothari ST, Lokhande RS, Chowdhary AS

Intl J Res Eng App Sci 2015, 5(6), 123 -138.

Abstract

Thevetia peruviana is a native evergreen tropical shrub of Mexico and Central America primarily cultivated in India, and is a close relative to Nerium oleander. The seeds, flowers, leaves, barks, and roots of the plant have been extensively studied for their biochemical activity. This study was aimed to confirm the medicinal value in the twigs of Thevetia peruviana plant. It subsequently performed chemical and pharmacological experiments to determine the bioactivity in it by extracting, isolating, and characterizing bioactive compounds present therein.

Seventeen bio-active compounds were isolated using column chromatography technique that comprised of an eleven solvent system of increasing polarities used for its extraction procedure. Purified compounds were further characterized analytically and subsequently analyzed for various biological assays on its hydro alcoholic extract, that demonstrated a broad spectrum of bioactivity such as a four folds increase in B-cells proliferation in immunomodulation experiment, strong cytotoxicity observed in pancreas, lung, prostate, breast and colon cancer cells and ten percent moderate anti-inflammatory activity. However, this extract did not demonstrate any anti-microbial activity.

This study confirms that the twigs of *Thevetia peruviana* can be considered as a candidate for bioprospection and drug development for diseases such as anaemia, cancer, arthritis, and microbial allergies

6. Vaccines against HIV

Padmanabhan U, Chowdhary AS, Dahake R, Koka SP

J Vir Curr Res, 2015, 1(1), 1 - 5.

Abstract

The human immunodeficiency virus-1 (HIV-1) infects helper CD4(+) T cells, and causes CD4(+) T-cell depletion and immunodeficiency. Although significant progress has been made in antiretroviral therapy in the past 30 years, an effective vaccine and a functional cure awaits discovery. This review summarizes the molecular basis of HIV infection and the various hurdles for vaccine development and in this perspective analyzes the status of vaccines against HIV.

7. Effect of Homeopathic preparation on Phenolics production in *Tylophora indica* and *Curcuma longa*

Chaturvedi P, Mukherjee S and Chowdhary AS

Adv Plant Sci, 2015, 28(1), 115-119.

Abstract

Emerging trends of exploiting plant cell culture for the production of high value phyto-pharmaceuticals of *Tylophora indica* (Asclepeadaceae) and *Curcuma longa* (Zingiberaceae) were used. The present study deals with the enhancement in the production of their active compounds Kaempferol and Curcumin respectively. In this study different concentrations of Jasminoic acid (5, 10, 15, 20 μ M) and diluted homeopathic medicine Calendula 30 C (1:20) were used to increase the production. Zenk Production media was used as basal media in all the instances. Directly plant material (leaves of *Tylophora indica* and rhizome of *Curcuma longa*) were used as explants instead of callus, which reduced the time as well expenditure. The maximum content of both the compounds (50.98% Kaempferol; control 5.27% and Curcumin

48.112% control 9.66%) were significantly increased in Zenk media supplemented with 15 μ M of Jasminoic acid in which explants were dipped for 1min duration in aqua-diluted Calendula 30 c(1:20). This study was a new kind of study, where homeopathic medicine was used to enhance the secondary metabolites production in tissue culture. There are many reports which reveal the use of Jasminoic acid as phytoalexin enhancer which in turn helps in defence mechanism of plant and eventually bio-enhancement in the secondary metabolites production obtained but we did not find any report regarding use of Homeopathic medicine for enhancement of natural product *in vitro*. The study showing the synergistic effect of Jasminoic acid and Calendula in production of phenols *in vitro*.

8. Evaluation of anti-viral activity of essential oil of *Trachyspermum ammi* against Japanese encephalitis virus

Roy S, Chaurvedi P, Chowdhary AS

Pharmacog Res, 2015, 7, 263- 267.

Abstract

Japanese encephalitis is a leading form of viral encephalitis, prevalent mostly in South Eastern Asia caused by Japanese encephalitis virus (JEV). It is transmitted by the mosquitoes of the *Culex* sp. The disease affects children and results in 50% result in permanent neuropsychiatric disorder. There arises a need to develop a safe, affordable, and potent anti-viral agent against JEV. This study aimed to assess the antiviral activity of ajwain (*Trachyspermum ammi*: Umbelliferae) essential oil against JEV. Ajwain oil was extracted by distillation method and *in vitro* cytotoxicity assay was performed in vero cell line by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay method. JEV titer was determined by plaque assay and *in vitro* antiviral activity of ajwain oil was quantified by the plaque reduction neutralization test (PRNT). Cytotoxic concentration of the oil was found to be 1 mg/ml by MTT assay. The titer of the virus pool was found to be 50×10^7 PFU/ml. we observed 80% and 40% virus inhibition in 0.5mg/ml of ajwain oil by PRNT method in preexposure treatment and postexposure treatment (antiviral activity), respectively. Our data indicate ajwain oil has potential *in vitro* antiviral activity against JEV. Further, the active biomolecule will be purified and evaluated for anti-JEV activity and also to scale up for *in vivo* trial to evaluate the efficacy of ajwain oil in future.

9. Anti-Influenza Potential of Alkaloidal Molecules of *Jatropha Curcas* Leaves

Chavan R, Chaturvedi P, Chowdhary AS

Int J Pharm Sci Res, 2015, 6(11), 4705-4711.

Abstract

The Influenza viruses are major etiologic agents of human respiratory infections, and inflict sizable health and economic burden. The present study reports the *in-vitro* antiviral effect of isolated alkaloidal compound of *Jatropha curcas* leaves against Influenza virus by Hemagglutination (HA) reduction assay in two different layouts of simultaneous and post-treatment assay. The alkaloidal compound was used for anti-influenza activity in the non-cytotoxic range. The separated compound (R_f 0.5) was eluted, crystallised and identified by using NMR and FTIR analysis and co-TLC with standard compound of Tetra Methyl Pyrazine(TMP). The Thin Layer Chromatographic analysis showed that ethyl acetate: dichloromethane (1:1) was the best solvent for alkaloidal separation. The identified Tetra-Methyl Pyrazine showed 100% reduction in HA in the post-treatment assays at the concentration of 10mg/ml and 5mg/ml. In simultaneous assay, HA was reduced to 71% and 42% at concentration of 10mg/ml and 5mg/ml respectively. These results suggest that TMP has strong anti-Influenza virus activity that can inhibit viral attachment and/or viral replication and may be used as viral prophylaxis.

10. Approaches for Antivirals Production in Tissue Culture of Medicinal Plant,

Chaturvedi, P, Chowdhary, AS. OMICS Publications US, 2015, ISBN No 978-1- 63278-052- 2.

Abstract

Lack of effective therapeutics for the most of viral diseases, emergence of antiviral drug resistance, and high cost of some antiviral therapies necessitate finding new effective antiviral compounds. Additionally, the existing antiviral therapies are not always well-tolerated or quite effective and satisfactory. Hence, the increasing requirement for antiviral substances will be more highlighted. Plants as a rich source of phytochemicals with different biological activities including antiviral activities are in interest of scientists. Tissue culture has given an alternative mean for production of medicinally important secondary metabolites in controlled condition. This gives a uniformity in production of these compounds and also save green cover which ultimately serves as a tool for ecosystem balance.

There is growing interest worldwide in the utilization of plant materials and phytochemicals to support sustainable and environmentally friendly lifestyle. The chemicals extracted from plants are expected to have low toxicity and high degree of bio degradation. Therefore, plant extracted phyto-chemicals have been used in nutraceuticals, pharmaceuticals, herbal medicines, spices, insect repellents, cosmetics, perfumes and many other beneficial secondary metabolites. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new

sources of economic phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies . Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action.

11. Where do the hybrids go? Padmanabhan U, Curr Sci, 2016, 110(6), 957.

No Abstract, correspondence

12. Preclinical model for identification of therapeutic targets for CML offers clues for handling imatinib resistance.

Molli PR, Pradhan MB, Ingle AD, Naik NR*,

Biomedicine & Pharmacotherapy, 2015, 73, 160-170.

Abstract

Success of imatinib in chronic myeloid leukemia (CML) therapy has undoubtedly proved utility of signalling molecules as therapeutic targets. However, development of imatinib resistance and progression to blastic crisis are the current challenges in clinics. To develop therapeutic alternatives for CML, understanding of signalling events downstream of bcr-abl might be helpful. Current CML cell lines do not give comprehensive picture of signalling events involved in pathogenesis of CML. Hence, there is a major unmet need for a better preclinical model for CML. Here, we report on development of RIN9815/bcr-abl, a novel cell line model that mimics signalling events in CML PMNL. Studies on crucial signalling molecules i.e., ras, rac, rhoA and actin in this cell line identified rhoA as the key regulator involved in CML cell function as well as proliferation of both, imatinib sensitive and resistant cells. Hence, RIN9815/bcr-abl could serve as the unique preclinical model in understanding pathogenesis of CML and in drug development.

Patents*

Granted Naik NR* as an inventor in patents granted to the Piramal Enterprises Limited

Sr. No.	Patent No.	Country	Granted in
1	US 9,062,046 B2	USA	23 June-15
2	JP 5750158 B	Japan	July-15
3	2013/01212	South Africa	July-15
4	607060	New Zealand	July-15

* The publication and patents granted are on the work done with the previous employer.

Chemotherapy

1. Synthesis Characterization and analysis of some mercapto benzothiazole derivative

Asrondkar AL, Patil VN, Mishra N, Bobade AS, Chowdhary AS

World Journal of Pharmaceutical Research (WJPR), 2015, 4(2), 618-626 .

Abstract

In present study we synthesized some 2-amino mercapto benzothiazole and Acid Chloride derivatives and screen synthesized compounds for their anti-microbial activity. 2-amino mercapto benzothiazole on reacting with ethyl chloro acetate forms ester derivative of benzothiazole which on further treatment of hydrazine hydrate formed hydrazino derivatives of mercaptobenzothiazole, It was then treated with carbon disulphide to form oxadiazole derivative of mercapto benzothiazole and finally it was condensed with different substituted Acid chloride to form final compound. Synthesized compounds have been confirmed on the basis of spectral studies by using FT-IR, NMR, Mass Spectrophotometer. All the compounds were screened for their *in vitro* anti-inflammatory, *in vitro* anti oxidant and *in vitro* antibacterial activity against *E. coli*, *S. typhi*, *P. aeruginosa*, *Kleb pneumoniae*, *Vibrio chlorae*. Antifungal strain *Candida albicans* and *Aspergillus niger* using tube dilution method shows promising activity.

2. Characterization, Synthesis and analysis of some benzothiazole Derivative

Patil VN, Asrondkar AL, Mishra N, Bobade AS, Chowdhary AS

World Journal of Pharmaceutical Research, (WJPR), 2015,4(2),842-850.

Abstract

Substituted 1, 3-benzothiazole derivatives are an important class of heterocyclic compounds. The benzothiazole nucleus containing compounds in this work is been treated with hydrazine hydrate to form hydrazone derivative then it is treated with acetophenone further cyclisation is done by Vilsmeier-Haack reaction using DMF/POCl₃ finally the intermediate is condensed by using substituted thiourea to form the desired products. All the synthesized compounds was confirmed by spectral analysis such as FT-IR, NMR Mass Spectrophotometer for structure confirmation and synthesized compounds were screened for various *in-vitro* biological activities such as anti-oxidant, anti-inflammatory, anti-microbial activity It was found that the synthesized compounds showed enhancing biological activity.

3. Synthesis Characterization and analysis of some Coumarin Derivative.

Mishra N, Patil VN, Asrondkar AL, Bobade AS, Chowdhary AS

***World Journal of Pharmaceutical Research (WJPR)*, 2015,4(2),627-636 .**

Abstract

A series of some coumarin derivative have been synthesized by the interaction of 4-hydroxy coumarin with chloroethylacetate to form ester derivative of coumarin. Further treatment with hydrazine hydrate leads to formation of hydrazone derivative which was further treated with substituted benzaldehyde to form schiff base and finally on treatment with thioglycolic acid in presence of alcohol forms thiadiazole derivative of coumarin. The identities of these synthesized compounds have been established on the basis of chemical transformation and FT-IR, ¹H NMR and Mass spectral studies. In the present study synthesized compounds were studied for their *in vitro* anti inflammatory activity, anti-oxidant and anti microbial activity. all compounds studied shows satisfactory results.

4. Comparative Study of Synthesis of ethyl (quinolin-8-yloxy)acetate by conventional, Microwave & Ultrasound Irradiation Techniques

Desai GH, Asrondkar AL, Patil VN, Bobade AS, Chowdhary AS

***Der Pharma Chemica*, 2015, 7(5):263-266.**

Abstract

Microwave and Ultrasound assisted organic synthesis has emerged as a new “lead” in organic synthesis. The technique offers simple, clean, fast, efficient, and economic for the synthesis of a large number of organic molecules. In the recent year microwave assisted organic reaction has emerged as new tool in organic synthesis. Important advantage of this technology include highly accelerated rate of the reaction, Reduction in reaction time with an improvement in the yield and quality of the product. A series of various ethyl (quinolin-8-yloxy)acetate derivatives were synthesized by the reaction of chloroethylacetate with substituted 8-hydroxy quinoline under conventional, Ultrasound irradiation and microwave irradiation conditions, purified by recrystallisation and the structure of all the compounds have been confirmed by IR, NMR and Mass spectral data.

5. Synthesis of Quinoline Analogues as Anti-microbial Agents

Desai GH, Asrondkar AL, Patil VN, Bobade AS, Chowdhary AS

***IOSR Journal of Applied Chemistry*, 8(5) Ver. II ; 2015,01-03.**

Abstract

Various substituted Quinoline containing different functional group have been synthesized by conventional method. The quinoline derivative is synthesized by using 8-hydroxy quinoline) was first treated with ethyl chloroacetate to form ester intermediate which was subsequently

treated with Hydrazine Hydrate result into formation of Hydrazone derivative then this intermediate was made to react with substituted benzoic acid. The final structures have been established on the basis of their chemical analysis and spectral data. Final compounds were further evaluated for in-vitro anti-microbial activity using standards.

6. Synthesis and Biological Evaluation of some Thiazolidinone-Quinoline Derivative

Desai GH, Asrondkar AL, Patil VN, Bobade AS, Chowdhary AS

***World Journal of Pharmaceutical Research*, 4(6); 2015, 1487-1494.**

Abstract

N'-[2-(quinolin-8-yloxy) acetyl] benzohydrazide (2) obtained from 8-hydroxy quinoline (1) was converted to the corresponding 2-(quinolin-8-yloxy) acetohydrazide (3) by treatment with hydrazine hydrate. The basic treatment of (3) and substituted benzaldehyde yielded the corresponding schiff base derivatives (4a-e). The synthesis of N-(4-oxo-2- substituted phenyl-1, 3-thiazolidin-3-yl)-2-(quinolin-8-yloxy) acetamide (5a-e) was performed from the reaction of (4) with thioglycolic acid in basic media. The newly synthesized compounds were characterized by elemental analyses, IR, ¹H-NMR, and mass spectra. The antimicrobial activity study revealed that newly synthesized compounds dose not showed moderate activity against a variety of microorganisms (*E. coli*, *S. aureus*, *C. albicans*).

7. Synthesis and Characterization of Some Coumarin Derivatives

Chowdhary PS, Patil VN, Asrondkar AL , Bobade AS, Chowdhary AS

***International Journal of Science & Research*, 4(4);2015,2342-2344.**

Abstract

A series of coumarin derivatives were synthesized. The present study reveals the synthesis of coumarin derivative in which 4- hydroxycoumarin was reacted with chloroethylacetate in presence of K₂CO₃ to form an intermediate which was further treated with hydrazine hydrate to form hydrazino derivative of coumarin and finally the intermediate is condensed by substituted isothiocyanate derivatives to form final compound, the synthesized compounds were then Characterized by using FT-IR, ¹H-NMR and Mass spectrophotometer, All the compounds were screened for their anti-microbial activity. It was found that the derivatives showed moderate activity as compared to standard.

8. Tandem Synthesis of thia-oxadiazolophanes

Malghe YS, Thorat VV, Patil VN, Bobade AS, Chowdhary AS

***Journal of Chemical And Pharmaceutical Research*, 2015, 7(5), 729-734.**

Abstract

Present work reports the synthesis of series of thia-oxadiazolophanes using Tandem method. Reaction of dihydrazide (oxalic acid dihydrazide, malonic acid dihydrazide, succinic acid dihydrazide, glutaric acid dihydrazide, pimelic acid dihydrazide, suberic acid dihydrazide, azelaic acid dihydrazide and sebacic acid dihydrazide) with KOH and CS₂ in alcohol resulted in the formation of K-salt of bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] alkane with evolution of H₂S gas. After complete removal of H₂S gas, 1,2-dibromoethane was added and the mixture was further refluxed. Structures of newly synthesized compounds were established using FTIR, ¹H NMR and elemental analysis. All the compounds are screened for their anti-inflammatory activities. IC₅₀ values reveal that the newly synthesized compounds exhibit significant anti-inflammatory activities. Representative samples were studied for cytotoxicity. Results of cytotoxicity study reveal that the compounds exhibit poor cytotoxicity.

9. Synthesis and Characterisation and Biological activities of new bis-1,3,4-oxadiazoles

Malghe YS, Thorat VV, Bobade AS, Chowdhary AS,

Journal of Chemical And Pharmaceutical Research, 2015, 7(6),392-398.

Abstract

In present work, a series of 1,n-bis[5-(3-methoxy-4-hydroxy-5-nitrophenyl)-1,3,4-oxadiazol-2-yl] alkanes/benzene have been synthesized. Condensation of different dihydrazides with 5-nitrovanillin gave corresponding dihydrazones. Dihydrazones on oxidative cyclization using chloramine-T yielded the corresponding bis-1,3,4-oxadiazole derivatives. Structures of newly synthesized compounds were established using FTIR, ¹H NMR and elemental analysis. All compounds were screened for their anti-inflammatory activities. IC₅₀ values reveal that newly synthesized compounds exhibit better anti-inflammatory activities. Representative samples were studied for cytotoxicity. Results of cytotoxicity reveal that compounds exhibit moderate cytotoxicity.

10. Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations

Patil VN, Ghag- Sawant M, Narayakkadan A, Asrondkar AL, Bobade AS, Ramaiya M

International Journal Of Scientific and Research Publication, 2015, 5(12), 277-283.

Abstract

The synthesis of benzothiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades. The structural formula of all derivatives was confirmed and characterized by elemental analysis and spectral studies. A methanol extract of *Pongamia pinnata*, *Syzigium cumini*, *Delonix regia*, *Adathoda zeylanica*

was done by Soxhlet. The combinations of the plant extracts with the organic compounds were evaluated for antiinflammatory, anti-oxidant activity and checked for their cytotoxicity. Our findings indicate that the plant extract and organic compounds have a significant effect on biological evaluations.

Department of Toxicology

1. Preclinical model for identification of therapeutic targets for CML offers clues for handling imatinib resistance

Molli PR, Pradhan MB, Ingle AD, Naik NR

Biomedicine & Pharmacotherapy 2015, 73, 160-170

Abstract

Success of imatinib in chronic myeloid leukemia (CML) therapy has undoubtedly proved utility of signalling molecules as therapeutic targets. However, development of imatinib resistance and progression to blastic crisis are the current challenges in clinics. To develop therapeutic alternatives for CML, understanding of signalling events downstream of bcr-abl might be helpful. Current CML cell lines do not give comprehensive picture of signalling events involved in pathogenesis of CML. Hence, there is a major unmet need for a better preclinical model for CML. Here, we report on development of RIN9815/bcr-abl, a novel cell line model that mimics signalling events in CML PMNL. Studies on crucial signalling molecules i.e., ras, rac, rhoA and actin in this cell line identified rhoA as the key regulator involved in CML cell function as well as proliferation of both, imatinib sensitive and resistant cells. Hence, RIN9815/bcr-abl could serve as the unique preclinical model in understanding pathogenesis of CML and in drug development.

Patents

Granted Inventor in 4 patents granted to the Piramal Enterprises Limited

Filed Diagnostic probes made of aptamers and methods making the same.*

Applicant & Inventor: Nishigandha Naik No. 1485/MUM/2015*

Please note that all these publications are from the work done at the previous employer.

**Snake Farm & Experimental Animal House, Venomous Animal Unit,
Department of Zoonosis**

1. Evaluation of anti-osteoarthritic activity of *Vigna mungo* in papain induced osteoarthritis model.

Patel D, Ghag-Sawant M and Kaur G

Indian Journal of Pharmacology. 2015, 47(1), 59-64.

Abstract

Aim: This study was carried out to evaluate the effect of *Vigna mungo* hydroalcoholic extract (VMHA) by papain induced osteoarthritis (OA) in the rat model.

Materials and Methods: OA was induced by intra-articular injection of papain (4% w/v) along with cysteine (0.03 M) on day 1, 4 and 7 in rats and VMHA was administered orally in three doses (100, 200 and 400 mg/kg) after last papain injection. The anti-osteoarthritic activity was evaluated by measuring knee joint diameter, grip strength, locomotion activity and hanging time. Histopathological analysis and acute toxicity study were also performed.

Results: VMHA improved inflammatory condition with all the doses, but significant ($P < 0.05$) attenuation of inflammation was present only with 400 mg/kg dose. The grip strength, locomotion activity and hanging time were also significantly ($P < 0.05$) improved at dose level of 100 mg/kg however other two doses (200 mg/kg and 400 mg/kg) were not found to be effective. VMHA did not show any mortality or any toxic clinical signs after oral administration of 2 g/kg dose.

Conclusion: VMHA improved arthritic condition by significantly reducing pain and inflammation.

2. Experimental Evaluation of Antitumor Effect of Russell's Viper Venom on Breast Cancer Cell Line - Mda-Mb-231.

Ahluwalia S, Ghag Sawant M, Shah N, Chowdhary AS.

International Journal of Pharmacy and Pharmaceutical Research, 2015, 3(3), 254-263.

Abstract

Objective: The treatment of breast cancer needs to be changed from chemotherapy to biotherapy using biologicals, which have minimum or no adverse effects. dr-CT-1, a heat-stable protein present in *Daboia russelli* venom, has shown antineoplastic activity against HepG2. Some PLA2s isolated from Viperidae venoms are capable of inducing antitumoral activity. The objective of this study was to see the effect of Russell's Viper venom on inhibition of human breast cancer cells in vitro.

Methods: Lyophilised snake venom was used to check its effect on breast cancer cell line MDA-MB-231. MTT assay was performed to calculate cytotoxicity and study the antiproliferative activity of the venom. Antioxidant potential was determined using TBARS assay. Immunocytochemistry was performed to understand the effect of venom on migration of cells. The protein fractions obtained using SDS-PAGE were referenced to identify the component of the venom which might act as the anticancer agent.

Results: CC50 value obtained using MTT assay was found to be 0.82 µg/ml. 1 µg/ml of venom also showed effect against proliferation of the cells. With increasing concentration of venom, % TBARS also increased. 10 µg/ml of venom helped in inhibiting the tubulin fibers of the cells, thereby affecting the migration. 5 different protein bands were separated using SDS-PAGE.

Conclusion: The results suggest that the snake venom might contain dr-CT-1 as well as PLA2 proteins which have shown their effect on proliferation and migration of tumour cells. Thus, the use of Russell's viper venom may have antitumor effect against breast cancer.

3. Biological Evaluation of Benzothiazole-Thiourea Derivatives, Plant Extracts and their Combinations.

Patil VN, Ghag-Sawant M, Narayakkadan A, Asrondkar A, Bobade A and Ramaiya M. International Journal of Scientific and Research Publications, 2015, 5, (12)

Abstract

The synthesis of benzothiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades. The structural formula of all derivatives was confirmed and characterized by elemental analysis and spectral studies. A methanol extract of *Pongamia pinnata*, *Syzigium cumini*, *Delonix regia*, *Adathoda zeylanica* was done by Soxhlet. The combinations of the plant extracts with the organic compounds were evaluated for anti-inflammatory, anti-oxidant activity and checked for their cytotoxicity. Our findings indicate that the plant extract and organic compounds have a significant effect on biological evaluations.

4. Study of neutralization of enzymatic activity of *Daboia russellii* venom by various plant extracts and their combinations using in vitro methods.

Ghag-Sawant M, T. V. More TV, Samant LS and Chowdhary AS.

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Abstract

Snakebite is an important cause of morbidity and mortality and is one of the major health problems in India. Russell's viper (*Daboia russellii*) appears to be the commonest cause of fatal

snakebite in many Asian countries and especially in the Indian subcontinent. Intravenous administration of anti venom serum neutralizes the systemic actions, but it is of little effect on the reversal of local symptoms and often induces adverse reactions, a context that drives the search for complementary treatments for snakebite accidents. Plant extracts with a range of antiophidian activities constitute an excellent alternative. The Methanolic extracts of seeds of *Pongamia pinnata*, fruits of *Piper longum* and *Sapindus laurifolius* and leaves of *Adhatoda zeylanica* were tested for the snake venom neutralizing capacity using *in vitro* methods. The venom of *Daboia russelii* (Russell's viper) was used to evaluate the anti-venom activity of these extracts individually and in combinations. The parameters assessed were the neutralization of procoagulant activity, proteolytic activity and phospholipase A2 activity. All the extracts studied exhibited significant activity in neutralizing the venom effects and among the four extracts tested *Pongamia pinnata* showed superior activity followed by *Piper longum*. The present study suggests the effectiveness of the above extracts in the treatment of snake bite and also supports the use of herbal extracts by tribal and Vaidya's.

Training Services

Training Department

Head of Department's Overview

Haffkine Institute has emerged as a multi-disciplinary Institute engaged in training, research and testing of various aspects of infectious diseases. The Institute serves as a premier teaching institution in the field of biomedical sciences.

The University of Mumbai has affiliated Haffkine Institute as training Centre for the M.Sc. and Ph.D. degree courses in Applied Biology, Microbiology, Biochemistry, Biotechnology and Organic Chemistry. Maharashtra University of Health Sciences (MUHS), Nashik has also affiliated Haffkine Institute as a training center for the Ph.D. degree courses in Medical Microbiology. Research on subjects such as Bacteriology, Biochemistry, Biotechnology, Zoonosis, Applied Biology, Virology, Microbiology, Chemotherapy and toxicology are conducted through these degree courses. The students enrolled for M.Sc. By research degree course are awarded Junior Research fellowship of Rs. 2000/- and students enrolled for Ph.D. program are awarded Senior Research fellowship of Rs. 3000/- as a stipend.

Training Department has initiated PG Diploma in Clinical Research Management and Regulatory Affairs course (PGD-CRM & RA) as a weekend program in 2009. This course provides the platform for training and knowledge acquisition in Clinical Research Management and Regulatory Affairs to health care and allied professionals, and interested science graduates to enhance their career prospects in the field of Clinical Research both in India and abroad. So far 9 batches of this course have been successfully completed.

Haffkine Institute also gives a research platform through Short Term Research Internship Program (STRIP) to the students who are pursuing their master degrees under the recognized universities. The research internship Program offers short term (4 – 6 months) research opportunities primarily to students who require completing dissertations for the partial fulfilment of their B. Tech, M. Tech, B.E or M. Sc. degrees. Selected candidates get to work on projects encompassing various multi-disciplinary areas of Biological and Chemical Sciences.

Training department is also engaged in coordination and implementation of Annual Biotechnology Training Program (ABT) which is combination of lectures & demonstration of various topics in Microbiology, Molecular Biology, Zoology and Chemistry. This course is implemented with active participation of all institutional departments.

Training department also organizes visit to Haffkine Institute's Museum for school and college students registered under various Boards/ Universities, Medical & Nursing students of various

Hospitals, Foreign tourists, and general public of the society and staff of the Haffkine Institute /HBPCL.

The detail information of the various training programs and activities are given.

Dr. Anuya Rege

Programs conducted by Training Department

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1	Annual Biotechnology Training Program (ABT)	13 th Apr 2015 To 08 th May 2015	19	Students of B.Sc. / B. Tech. / M.Sc. / M. Tech. (Microbiology / Biotechnology / Zoology / Botany / Life Science) registered under any recognized University.
2	Short Term Research Internship Training Program	Nov.2015 to Apr 2016	04	Final year students of M.Sc., B. Pharm, M. Pharm, B.E., B. Tech., M. Tech. / M. Tech. Integrated registered under any recognized university.
		Jan.2016 to Jun 2016	15	
3	PG- Diploma In Clinical Research Management and Regulatory Affairs	Nov.2014 To Apr 2015 Convocated in : May 2015	16	B. Pharm, M. Pharm, B. Sc, M. Sc, MBBS, BDS, MDS, MD/MS Graduate with Science /Medical background
4	Workshop on Testing of Alcoholic Beverages	23 rd Apr 2015 To 24 th Apr 2015	11	Students from any Science stream.
		8 th May 2015 To 9 th May 2015	5	
		12 th May 2015 to 13 th May 2015	9	
5	Workshop on Analytical Training for Instrumentation	Apr 2015 to Mar 2016	155	Pharm, Pharm., M.Sc. & B.Sc. (Chemistry, Analytical Chemistry, Microbiology, Biochemistry, Biotechnology.)
6	Hands on Training in Techniques in Virology and Immunology	Jun 2015 to Jul 2015	32	UG/PG Students of Life Sciences/Biotechnology

7	Bio Safety - Training	25 th Nov. 2015	83	Staff & Students of Haffkine Institute
8	Workshop on Principal and Practices of Laboratory Animal Care	9 th Apr 2015 to 10 th Apr 2015	11	Students who are studying/ have passed their B.Sc. /B. Tech. /M.Sc. /M. Tech degrees in Microbiology/ Biochemistry/ Biotechnology/ Zoology/ Botany/ Life Sciences
		7 th May 2015 to 8 th May 2015	14	
9	Introduction to Covert Existence of Snakes	16 th Jul 2015 to 17 th Jul 2015	10	Std. XII or Higher Education.
10	Training on Rodent Paste Management	17 th Jun 2015 To 19 th Jun 2015	70	Pest control officers, Sanitary inspectors, scientist, Professionals and student working in this field to learn and understand the biology of commensal and wild rodent pests
11	A Seminar on role of snake Friends in snake bite death mitigation	29 th Sep 2015	46	Snake friends/ NGOs/ Forest department
12	Mission to control on mosquito menace	10 th Apr 2015	36	Under graduate students from any branch of science
		6 th Jul 2015	43	
		28 th Jul 2015	31	
13	Haffkine Institute's Museum Visitors	1 st Apr 2015 To 31 st Mar 2016	2476	School, College students registered under various Boards/Universities, Medical and Nursing students of various hospitals, Foreign tourists, Haffkine Staff and general public.etc

Training Programs provided by other Departments

Department of Virology

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1	Short Term Research Internship Projects	Nov 2015 to Jun 2016	4	Final Year in the Degree of Life Sciences/Biotechnology
2	Hands on Training in Techniques in Virology	Jun 2015 to July 2015	32	UG/PG Students of Life Sciences/Biotechnology
3	Bio Safety	1 Day Training (25.11.2014)	83	Staff & Students of Haffkine Institute

The Short Term Internship provided to the students in the Department of Virology has allowed them the chance of working with equipments like RealTime Thermal Cyclers, Biosafety Cabinets, Multimode microplate reader, Gel Documentation System, etc. They also got an exposure in how to handle cell lines and rabies virus.

Hands on Training in Techniques in Virology has been well received by the students since its inception in 2014, the factors attributing to this are the application oriented lectures, their exposure to virology techniques and most importantly the attribute that the students can perform the experiments 'hands-on'. This is significant in terms that it is one of the few application based courses available in infectious Virology in Mumbai region. By virtue of this training, the Department contributed revenue of Rs. 2,08,000/- (Rupees Two lakhs eight thousand only). Depending upon the resources that might be available to the Department of Virology, we plan to initiate similar courses in Applied Biology as well.

Training in Biosafety was a mandatory exercise with Haffkine Institute, where we received an overwhelming response from both scientific (students and technical personnel) as well as non-scientific cadre. Its significance lies in the implementing of the 1989 Guidelines of use of hazardous materials, laid down by the Ministry of Environment and Forests, Government of India. Today, this training has sensitized the cadre at the Institute towards appropriate handling and discard of biohazardous material.

Zoonosis Department

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1.	Mission to Control Mosquito Menace (MCMM)	10/04/15 (One day workshop)	36	Under graduate students from any branch of science
		06/07/15 (One day workshop)	43	
		28/07/15 (One day workshop)	31	

Analysis & Significance

The significant contribution of this training programme is to create public awareness among college students. We have feedback of last 1 year .Approximately 85-90% students gave excellent remarks to our workshop and they were quite satisfied with overall workshop programme. This programme helps in creating public health awareness among participants and making them vigilant for vector control. We have chosen student force for our workshop because students can easily grasp the subject & spread information among friends and family members.

Department of Bacteriology

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised
1	STRIP (Short Term Research Internship Program)	Jan 16 - Jun 16	03	B. Pharm, B.Sc, B. Tech, M. Pharm M.Sc, M. Tech

Analysis and Significance

Short Term Research Internship Projects (STRIP) students get an opportunity and hands-on experience of working on a Research Project. The department hosted three students: Ms. Shraddha Rane, Ms. Pooja Bhosale and Mr. Tanmay Shirsat. The feed back given by them are as follows.

Some of the comments received from students were

“We were able to perform all techniques without any interference. Unavailability of machines required for Molecular Biology.”

“Proper guidance was provided. It would be much better if some of the equipment’s are provided.”

“The daily usage instruments are well maintained though there is shortage of some basic equipment’s (like test tubes). The department can be improved by including molecular facilities.”

Department of Chemotherapy

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1.	Analytical Training for Instrumentation Course (3 Days)	Apr 15-Mar 2016	155 Students (13 Batches)	B.Pharm, M.Pharm, M.Sc. & B.Sc (Chemistry, Analytical Chemistry, Biotech, Microbiology, Biochemistry)
2.	15 Days Training	Apr 15-Mar 2016	11 Students	B.Sc, B.Pharm,
3.	1 Month Training	Apr 15-Mar 2016	09 Students	B.Sc, B.Pharm, M.Sc,
4.	45 Days Training	Apr 15-Mar 2016	13 Students	M.Sc (analytical chemistry)
5.	3 Month Training	Apr 15-Mar 2016	16 Students	M.Sc, M.Pharm
6.	6 Months Training (STRIP)	Apr 15-Mar 2016	02 Students	M.Pharm, B-tech

Analysis & Significance

Data of Analytical Training for Instrumentation Course for last 5 yrs

Sr. No.	Year	No. Of Batches Taken	Total no. Of students
1	2011	03	59
2	2012	05	75
3	2013	03	41
4	2014	06	65
5	2015	13	155

Data of Short term Training programme for last 5 yrs

Sr. No.	Year	15 Days	45 Days	1Month	3 Month	6 Month	Total no. of students
1	2011	-	-	4	-	-	4
2	2012	-	-	20	-	-	20

3	2013	-	-	-	2	5	7
4	2014	-	-	7	-	-	7
5	2015	11	13	9	16	2	51

Feedback

From feedback form filled by students, Ratings are as follows

Excellent-30, Very Good- 70, Good-27, Adequat-1.

Following were some of the suggestion received from the students

- Increase Course duration (ATI course for 15/30 Days)
- Add more Analytical Techniques

Significance

This course is an overview of Analytical techniques. Students will learn facts about major classes of instruments used in analysis. This Training provides basic information about handling & scope of application of Chromatographic & Spectroscopic Instruments.

Chemical Testing Unit

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1	Workshop on Testing of alcoholic beverages	Batch I – 23 to 24 Apr 2015	11	Students from any Science stream
		Batch II – 12 to 13 May 2015	09	
		Batch III – 08 to 09 May 2015	05	

Analysis and Significance

Significance and Response received from students is that students have given overall rating as “Excellent” training programme. They seem to be satisfied about arrangement, schedule and content of above said training programme. They find this training as very helpful from future point of view.

**Snake Farm & Experimental Animal House, Venomous Animal unit, Zoonosis
Department**

Sr. No.	Name of Training Program conducted by Department	Period over which conducted	Number of students	Min. qualification of students eligible for the course as advertised.
1	Workshop on “Principle and Practices of Laboratory Animal Care”	Batch I 09 th to 10 th Apr 2015	11	Students who are studying/ have passed their B.Sc. /B. Tech. /M.Sc. /M. Tech degrees in Microbiology/ Biochemistry/ Biotechnology/ Zoology/ Botany/ Life Sciences
		Batch II 07 th to 08 th May/2015	14	
2	Workshop on “Introduction to Covert Existence of Snake”	16 th to 17 th Jul 2015	10	12 th or Higher education
3	Training on “Rodent Pest Management”	17 th to 19 th Jun 2015	70	Pest control officers, Sanitary inspectors, scientist, Professionals and student working in this field to learn and understand the biology of commensal and wild rodent pests
4	Seminar on “Role of Snake Friends In Snake Bite Death Mitigation in State of Maharashtra”	29 th Sept 2015	46	Snake friends/ NGOs/ Forest department
5	Awareness training on “Identification of Venomous and non-venomous snakes and first aid measures post bite”	Regular	1471	Sanitary Inspector, Nursing, Agriculture, Veterinary, Medical & para medical Students

Feedback & Analysis

Workshop on “Principle and Practices of Laboratory Animal Care”: Feedback data

Sr. No.	Date	Total Student	Good	Very Good	Excellent
1	09 & 10/04/2011	15	09	04	02
2	04 & 05/06/2011	10	06	02	02
3	23 & 24/04/2012	12	04	07	01
4	14 & 15/07/2012	09	00	00	09
5	04 & 05/05/2013	14	04	04	06
6	15 & 16/06/2013	06	02	03	01
7	12 & 13/04/2014	14	04	05	05

8	03 & 04/05/2014	12	07	05	00
9	14 & 15/06/2014	06	00	02	04
10	09 & 10/04/2015	11	01	06	04
11	07 & 08/05/2015	14	02	08	04

Significance

This hands-on workshop encompass concepts of principle and practices of laboratory animal care in invivo experiments and its wide applications in the field of research as well as ethical issues, welfare regulations of animal experiment.

Workshop on “Introduction to Covert Existence of Snake: Feedback data

Sr. No.	Date	Total Student	Good	Very Good	Excellent
1	27 & 28/12/2011	04	00	02	02
2	11 & 12/05/2013	11	01	06	04
3	09 & 10/10/2014	04	01	02	01
4	16 & 17 / 07/ 2015	10	00	06	04

Significance

This workshop has wide application in the field of research. By knowing the first aid measures and Awareness regarding secret life of snakes one will have better understanding and insight of serpentology.

Training on “Rodent Pest Management”: Feedback data

Sr. No.	Date	Total Student	Good	Very Good	Excellent
1	17 th to 19 th Jun 2015	70	8	30	24

Significance

This training facilitates 3 days field work to understand the Biology and Control of Rodent Vector for the betterment of Public Health diseases. This program is designed to benefit the environmental health professionals such as pest control officers, Insecticide officers, Sanitary inspectors, scientist, Professionals and student working in this felid to learn and understand the biology of commensal and wild rodent pests in order to more effectively and efficiently address the growing number of vector-borne diseases and improve the control measures in their jurisdiction and communities.

Seminar on “Role of Snake Friends in Snake Bite Death Mitigation in State of Maharashtra”: Feedback data

Sr. No.	Date	Total Student	Good	Very Good	Excellent
1	29.09.2015	45	13	11	21

Significance

This seminar encompass the insights of Snake bite and fatality arising out of it is a major concern in our state and role of snake friends towards snake bite death mitigation efforts in our rural areas of Maharashtra.

Testing Services

Testing services provided by the various departments

Department of Virology

Sr. No.	Name of testing service
1	Detection of pdm H1N1 Influenza A virus
2	Detection of Rabies
3	In-vitro evaluation of anti-viral efficacy of new chemical/natural compounds/entities
4	In-vitro evaluation of virucidal efficacy of chemicals/disinfectants/hygiene products
5	Analytical testing of physiological parameters from human serum/plasma samples from Clinical Research

H1N1 Influenza A virus testing is a part of the Laboratory Surveillance for Influenza Like Illness, a project undertaken by the NCDC-IDSP on an National Level (for details please refer to the research section)

Last year, we received 08 samples (01 positive detected) for detection of rabies virus gene/antigen from anté-mortem and post-mortem cases. The anté-mortem matrices consisted of CSF, corneal smear, and nuchal biopsy, while brain samples were received for the post-mortem cases. This service, typically for the post-mortem cases caters to the police stations as a part of their report during death occurring after exposure or bite of the canine animals. To our knowledge, our Department is one of the very few government labs offering this test across the country.

In-vitro virucidal/anti-viral activity evaluation is a specialised contract research service offered by the Department of Virology. Here, both qualitative and quantitative evaluations are provided under the prevailing European Standard (EN 14476). This service is offered to various pharma companies, start-ups and FMCG manufacturers. In the last year, an amount of around Rs. 20,00,000/- (Rupees Twenty lacs only) was generated due to these Departmental services. Department of Virology has started providing analytical testing services for Clinical Research Organizations/Units from 2014, wherein till date, two projects have been sanctioned to our Department. While one study on cytokine response after administration of herbal formulations has been successfully completed last year, one study on the Irritable Bowel Syndrome is currently underway. Again, our department is one of the very few government laboratories offering such value added services.

Zoonosis Department

Sr. No.	Name of testing service
Mosquito	
1	Bio-efficacy study of test compound against <i>Culex quinquefasciatus</i> , <i>Aedes aegypti</i> and <i>Anopheles stephensi</i> mosquitoes
2	Testing of impregnated net against mosquitoes species.
3	Bio-efficacy of liquid vaporiser repellent sprays and mosquito coil against mosquitoes species.
4	Testing of mosquitoes repellent cream, fabric/garment staining property and moisturizing property
5	Bioefficacy of herbal tablet against mosquitoes species.
6	Bio- efficacy study of mosquito larvicidal oil
Cockroaches	
7	Bioefficacy, Persistency and Water resistance study of test compound against <i>Periplaneta americana</i> and <i>Blattella germanica</i> cockroaches.
8	Bioefficacy testing of fumigator and gel against cockroach species
9	Bioefficacy and surface persistency of mopping liquid against cockroach species.
House Fly	
10	Bioefficacy and surface persistency test of aerosol and oil spray against insects (mosquitoes, housefly and cockroach)
11	Bioefficacy testing of insect's repellent floor cleaner against house fly
Other Insects	
12	Bioefficacy testing of test compound (bait /granules) against common red ants
13	Bioefficacy testing of insect's repellent floor cleaner against common red ants
14	Bioefficacy of plant extract against termite
15	Bioefficacy of test compound against Silver fish
16	Bioefficacy of test compound against Louse

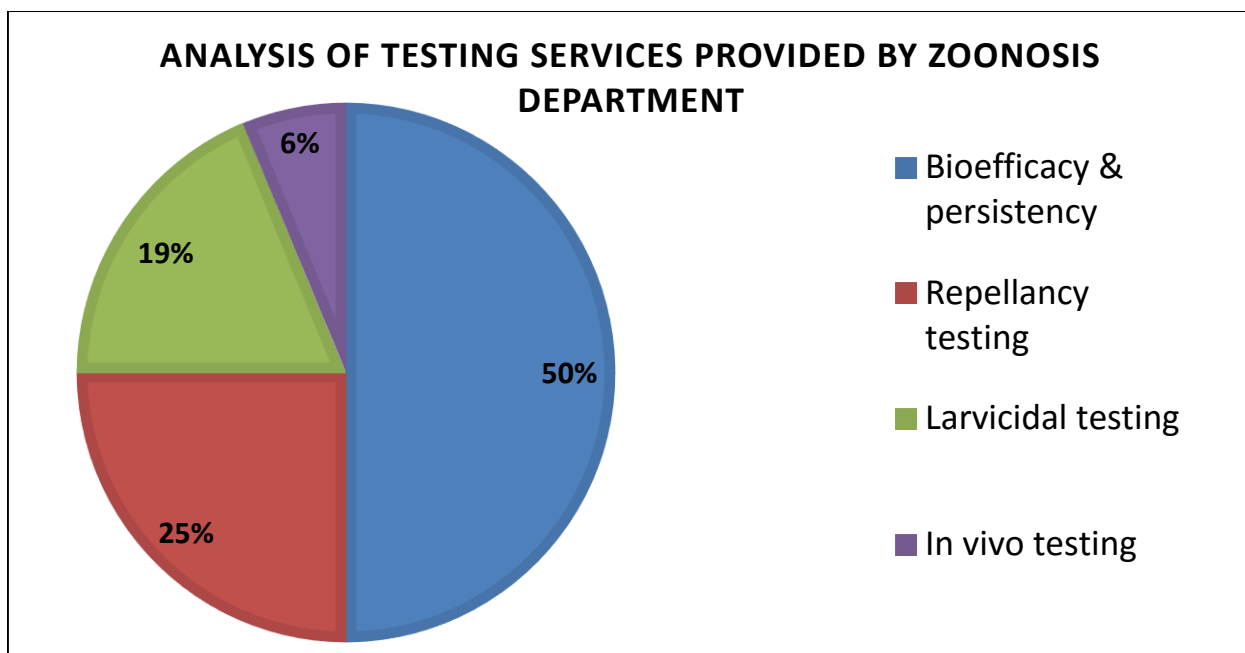
Significance

WHO has declared the mosquito as a public health menace number one; they can cause disease, distress and discomfort. In India malaria, dengue, chikungunya, zika virus and other arbovirus infections are transmitted by *Aedes*, *Culex* and *Anopheles* mosquito. We have made it our priority to help in control of mosquitoes and mosquito borne diseases.

All the testing activities in the department are as per WHO guidelines and HITRT protocols. The testing services help to evaluate test compounds for their efficiency as efficient insecticides, to minimize the menace cause due to these insects, but at the same time

We are aware of several anti-insect products which are currently in the market after successful testing at our department.

The analysis of the testing services provided by the department in 2015-16 are as follows



Some of our major customers are Godrej Consumer Pvt. Ltd., Dabur India Ltd., Syngenta India Ltd. Sumitomo Chemical Pvt. Ltd., Tagros Chemical India Ltd., Hicare services Pvt. Ltd., MCGM etc.

Department of Clinical Pathology

Sr. No	Name of testing service		Number of Tests
1	Hematology	CBC	82
		ESR	14
		MP	26
		Urine (R/M)	38
2	Serology	Extended Widal Test	657
3	Microbiology	OT Swabs	1936
		Aerobic Culture and AST	09
4	Mycology	Fungal Culture & Sensitivity	07
5	Rheumatology Testing	Anti-cardiolipin antibodies	05
		Anti-beta 2 Glycoprotein antibodies	07
	Total		2781

Department of Clinical pathology of Haffkine Institute has well established testing laboratory where department carries out testing like Haematology, Serology, Bacteriology and Mycology. Department receives blood samples for **Extended Widal Test** from various government hospitals and also from some private dispensaries / practitioners and most of the sample received are of children.

This testing consist of following tests,

1. **Widal Test** for Typhoid Fever
2. **Weil-Felix Test** for Rickettsial diseases
3. **Brucellosis Test** for Brucellosis

This tests can be done by following methods.

- Slide Screen Method
- Slide Semi-Quantitative Method
- Quantitative Method

Department of Clinical Pathology carries out rapid **slide agglutination method** for the detection from the patient's serum.

It is a simple, rapid and convenient screening test based upon the agglutination reaction between antibodies of patient's serum and antigens. Agglutination is a positive test result indicating presence of anti- antibodies in the patient serum. No agglutination is a negative result.

Widal test

The Widal test is an old serological test for detecting IgM and IgG antibodies to the 'O' and 'H' antigens of Salmonella. In slide agglutination tests a known concentration of antigen and unknown concentration of antibodies in patient's serum are mixed, clumping occurs within a

minute. Commercial antigens are available for detection of typhoid (O- and H-) and paratyphoid (AH- and BH-) antigens

Department of Clinical Pathology received a total of 648 samples for the Widal test in the year 2015-16.

Weil-Felix Test

This is an agglutination test for the rickettsial infection. Typhus group rickettsiae (*Rickettsia prowazekii*, *R. typhi*) reacts with *P. vulgaris* (OX19- antigen), Scrub typhus (*Orientia tsutsugamushi*) reacts with *P. mirabilis* (OXK-antigen). Spotted fever group rickettsiae (*R. rickettsii*, *R. africae*, *R. japonica* etc.) react with *P. vulgaris* (OX2-antigen) and (OX19-antigen), to varying degrees, depending on the species.

The antigens necessary (OX2, OX19, and OXK) can be obtained commercially.

Department of Clinical Pathology received total 651 samples for the Weil-felix test in the year 2015-16.

Brucellosis test

This is an agglutination test for the Brucella infection. This infection caused by *Brucella melitensis* (Br-M) and/or *Brucella abortus* (Br-Ab).

Department of Clinical Pathology received total 651 samples for the Brucellosis test in the year 2015-16,

Extended Widal Test

Extended Widal test comprises of analyzing serum antibodies for three infections namely:

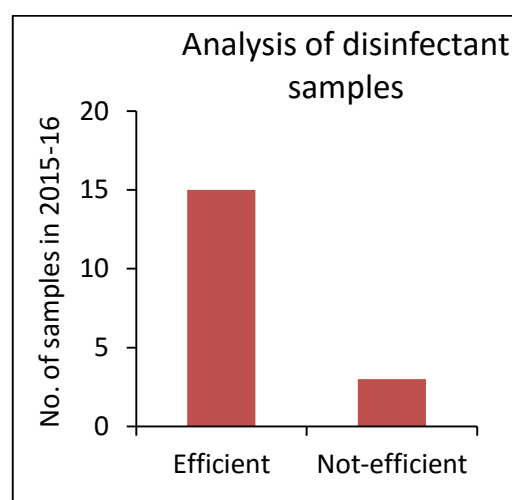
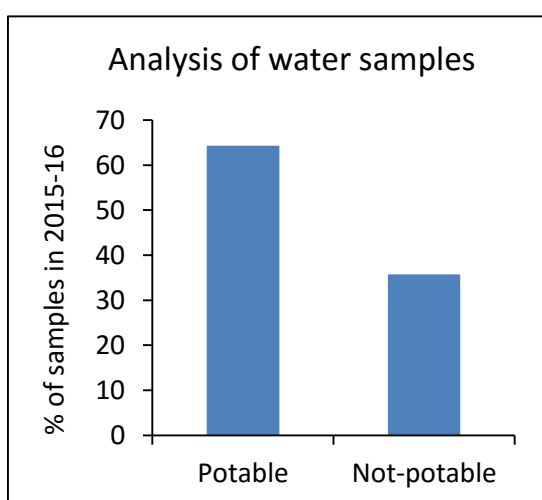
4. Widal Test for Typhoid Fever
5. Weil-Felix Test for Rickettsial diseases
6. Brucellosis Test for Brucellosis

Department of Clinical Pathology received total 655 samples for the Extended Widal test in the year 2015-16.

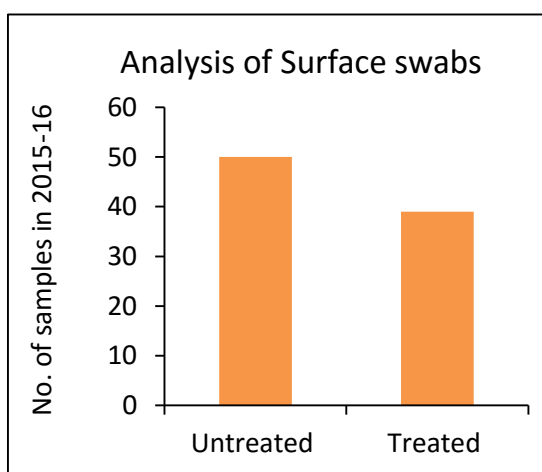
Department of Bacteriology

Sr. No.	Name of testing service and No. of Samples Tested
1	Water Sample testing (42)*
2	Water Purifier testing (03)
3	Disinfectant Testing (18)**
4	Testing of Surface Swab Samples for the efficacy of Antimicrobial coating (89)#
5	Antimicrobial testing of antimicrobial coated Material (02)
6	Chlorine Dioxide Estimation (01)
7	Antimicrobial testing of synthetic drug (01)
8	Testing of Swab samples for presence of <i>Clostridium</i> spp. (10)

Analysis and Significance:



Out of 42 Water samples subjected to testing, 27 found to be Potable and fit for Human consumption. Out of 18 disinfectant samples tested, 15 samples (83.33%) inactivated test microbial species.



Out of 89 Surface Swab Samples tested for efficacy of Antimicrobial coating, 50 were uncoated surface swab samples, whereas, 39 were coated/treated surface swab samples. These 39 coated/treated surface swab samples revealed no microbial growth, hence found to be effective.

Department of Biochemistry

Sr. No.	Name of testing service
1	Blood Biochemistry – Glucose, Bilirubin, SGOT, SGPT, Cholesterol, Triglycerides, BUN, Creatinine
2	Kidney & gall stone testing

Significance

Biochemical analysis of blood is helpful in the diagnosis per se or in confirming the diagnosis of various anomalies that are identified clinically. It is also helpful in monitoring progress of the disease.

The prevalence and incidence of nephrolithiasis is reported to be increasing across the world. Kidney stones constitute one of the commonest diseases in India. However, chemical analysis of kidney and gall stone is done at handful of the place in India. Having this facility available in Mumbai enables physicians to guide patients in avoiding recurrence of the incidence.

Department of Chemotherapy

Sr. No.	Name of testing service
1	Snake venom analysis by HPLC (06 samples)(Jan 2015-Dec.2015)
2	Antioxidant Activity (52 samples)(Year 2015-16)

In the last 5 years, a total 22 samples are tested for Snake venom analysis.

Snake venom analysis:

Significance: Snake venom analysis helps Forest Department to investigate the confirmation of venom samples from various sources.

Observation: With the help of HPLC, we analyses samples whether they are snake venom or not.

Antioxidant Activity : The role of Antioxidant testing are to detect possible drug intermediate that can produce free radical to inhibit oxidation leading to chain reactions that may damage cells.

Department of Toxicology

Sr. No.	Name of testing service
1	Acute oral toxicity testing of FERMSEPT® -S (J) in mice.
2	Acute dermal irritation/ corrosion test of Silicon Fluid in rabbits.
3	Acute oral toxicity test of Chlorine Dioxide in rats.
4	Acute oral toxicity - LD50 determination of CRISTOL OB – 77 in rats.

In the field of drug discovery as well as new chemical entities, toxicity testing is required to assess safety of the test substance before their usage. ‘In vitro’ toxicity testing could serve as a guideline for evaluating safety of the compound for any usage, but for the ultimate application in the field ‘in vivo’ toxicity testing is mandatory worldwide. In the Department of Toxicology, testing is done by following guidelines that are accepted worldwide e.g. OECD, FDA. Hence, the ‘in vivo’ toxicity testing services provided by the Department of Toxicology are useful in grading substances on the basis of their toxicity and thus, getting approval from the regulatory bodies for utilization of the products as well as marketing.

Snake Farm & Experimental Animal house, Venomous Animal Unit, Zoonosis

Department

Sr. No.	Name of testing service
1	Evaluation of Anti Termite activity of test compound
2	Evaluation of Anti Rodent activity of test compound
3	Evaluation of Anti squirrel activity of test compound
4	Identification of Unknown Venom Sample

Significance

Rodents/ squirrel causes significant damage to underground cables, pipelines and other polymeric materials. The polymeric material composition and texture is such that it attracts rodents/squirrel to them. Moreover in order to keep their continuously growing incisors in shape rodents keep gnawing. This can cause significant damage to various applications such as wires and cables, plastic pipelines and so on.

Similarly Termites are the prominently destructive pests which pose a potential threat to almost every structure. In addition to the threat that they pose to building constructions and the timber industry termites, especially subterranean termites cause a lot of destruction to underground laid wires and cables which may include telecom cables, signalling cables, fiber optic cables, power supply cables and many flexible, light weight cables. It is estimated that the amount of damage caused by termites in India is few million rupees per year.

Thus, in order to safeguard these cables against rodent/termite/squirrel attack, it is often proposed that cables be incorporated with the suitable rodent repellents or rodenticides in the outer sheathing. The effectiveness of these doping compounds may depend again on the percentage of doping as also the life span of these compounds i.e. the half-life of these compounds. Thus it is essential to evaluate these compounds in terms of their bio-efficacy before they are nominated for large scale use in these critical applications. This test method has been developed taking into account the evaluation of these compounds once incorporated in fiber optic samples (which may be PVC, PE and so on based) under both field as also laboratory testing conditions at venomous animal unit, Haffkine institute. This test method can be extended to testing samples of pipes, ducts as also other polymeric materials. Hence this testing is aiding to evaluate the better o test compound to minimize the losses/menace cause due to these notorious pest to society and overall economy of the country.

Regarding venom testing, it is observed that Venom is snaking its way from India to overseas destinations and slithering back as a value added product to be mixed with narcotic drugs.

Recently it came into light that these illegal trade is trying to bypassing enforcement agencies in India. Wildlife crime detection organisation/ forest anti-poaching units and police department all over India is seizing these venom samples and sending to Haffkine institute for confirmation of the seized sample as venom or any other sample. Hence this testing is supporting all these government authorities to identify the unknown venom samples in order to reduce the crime against Indian forest act.

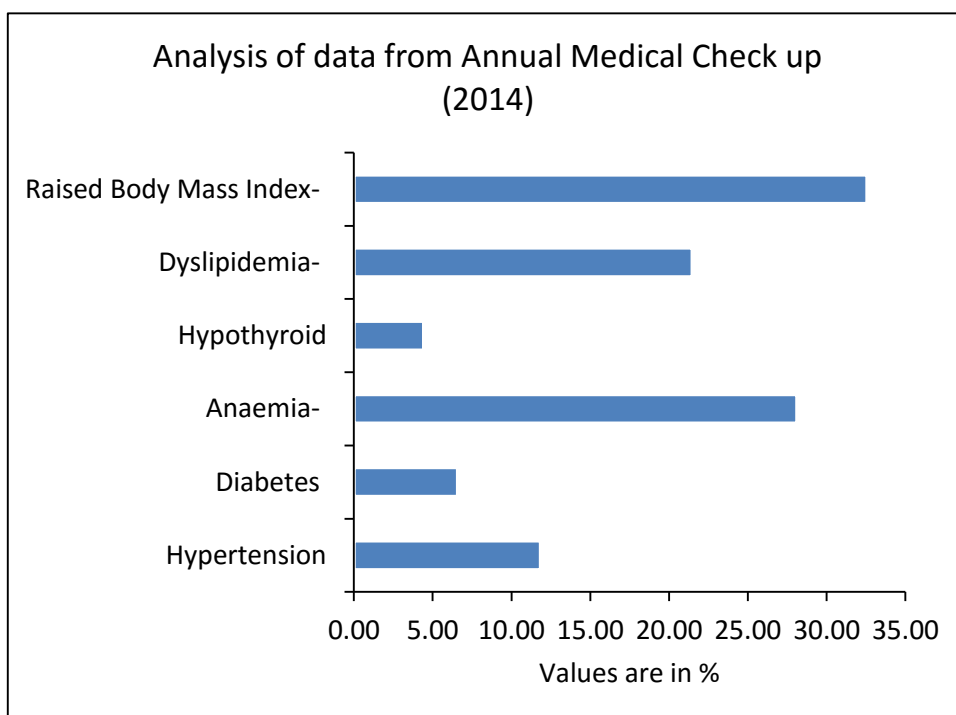
Chemical Testing Department

Department is primarily involved in testing of alcoholic beverage samples received from State Excise department of Maharashtra. The number of samples received are as follows:

Sr. No.	Nature of the Sample Tested & the Test Performed	Total Number of Sample Tested
1	TDL,ODS,SDS	4468
2	CL-I	9069
3	CL-III	6089
4	Toddy	3371
5	TL(Beer, IMFL)	345
6	TCL	39
7	TFS	4
8	TAC	0
9	CL-II	2
10	Miscellaneous Sample	0
11	Revenue Archieved By Giving Duplicate Report to the Party	5200
12	Total Revenue in Rs.	1,34,48,900

Dispensary, Department of Clinical Pathology

Sr. No.	Name of testing service
1.	OPD Consultation and treatment provided for employees, their family members and students of HITRT. Monthly medications are provided from dispensary for chronic illness like HTN, diabetes, Anaemia etc.
2.	Pre and Post exposure Vaccinations provided to H.I.T.R.T. employees and students as follows:
	a) Pre exposure prophylaxis for Hepatitis-B
	b) Pre exposure prophylaxis for Rabies
	c) Pre and Post exposure prophylaxis for Tetanus Toxoid
	d) Pre exposure prophylaxis Influenza Vaccine
3.	Antenatal care provided
4.	For Hypertensive patients -Blood Pressure monitoring, diet counselling is done, medications and investigations advised.
5.	For diabetic patients- blood sugar monitoring, clinical parameters (checked periodically), diet counselling and treatment for the same is provided.
6.	Dressing of Wounds.
7.	OPD Consultation was provided for employees of Haffkine Biopharmaceuticals Corporation Ltd.(May-2015)
8.	Clinical assistance provided for Various Department Research Project A] Participated in project of Virology Department “Seroprevalance of MMR antibodies in Adolescence “at Parel in Dec-2012.
	B] Clinical data provided for Project of Biochemistry Department on “Analysis of Angiotensin Converting Enzyme (ACE) polymorphism in samples of worksite Population.”(2013)
	C] Clinical Data provided for Project of Biochemistry Department on “Identity potential susceptibility markers of type-2 diabetes in a sample of Indian Population.”(2013)
	D] Medical Examination of Volunteers in Project of Virology Department “Comparison of Post Licensure Safety Surveillance of Bivalent and Quadrivalent HPV Vaccine in Healthy Women’s.”(2014)
	E] Medical Examination of volunteers for the Research Project “Bio-efficacy evaluation of N.N.Diethyl Benzamine based gel and wipe formulations” of Zoonosis department.(2015-2016)
9.	Annual Medical check-up was done for last 4 years on HITRT Employees & students



Analysis of Annual Medical Check-up of 2014]:

a) Hypertension- 11.85%

b) Diabetes- 6.6%

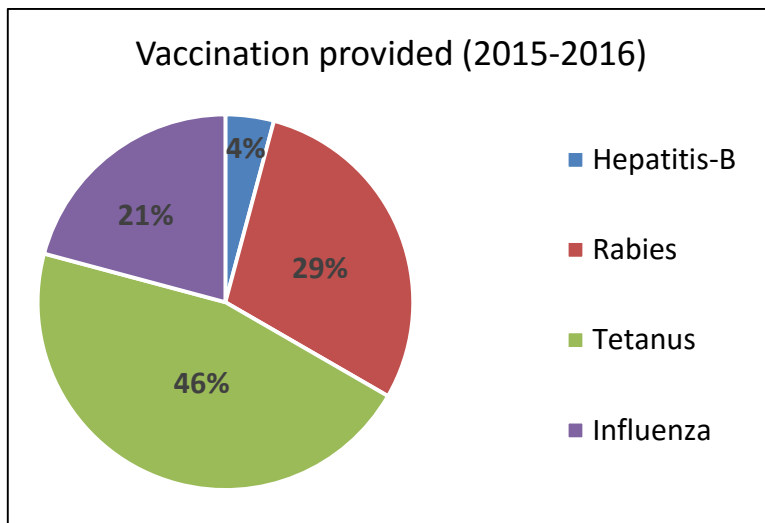
c) Anaemia- 28.14%

d) Hypothyroid- 4.44%

e) Dyslipidemia- 21.48%

f) Raised Body Mass Index- 32.59%

Analysis of Pre and Post exposure Vaccinations provided to HITRT. employees and students (2015-2016) is as follows:



Pre exposure prophylaxis for Hepatitis-B: 3 doses for 6 patients.

Rabies -3 doses for 21 patients.

Tetanus Toxoid for 33 patients.

Influenza Vaccine- 1 dose for 15 patients.

Antenatal care provided was provided for 6 patients. In

addition to all the above services provided, the Dispensary also provides OPD Consultation and treatment provided for employees, their family members and students (1231) of HITRT.

Other Scientific Activities

Training programs attended by Departmental staff

Sr. No.	Name of staff	Designation	Department	Name of course attended	Period of training
1.	Dr. Shashikant P. Vaidya	Assistant Director & Head	Department of Clinical Pathology	Mentoring Skills	22 - 24 Jun 16
				Direct Training Skills	7 -11 Dec 15
				Ethics in Research	Feb 16
2.	Dr.Mira Ramaiya	Assistant Director & Head	Zoonosis Department	Rodent Pest Management	7 – 8 May 15
3.	Dr. Sweta T Kothari	Sr. Scientific Officer & In-Charge, Dept. of Virology	Department of Virology	Foundation Training under the State Training Policy	9 Mar 15 - 22 May 15
				Biorisk Management Capacity Building Workshop (CDC-NCDC)	5 May 15 - 9 May 15
				Current Regulatory Requirements for Members of Institutional Ethics Committees	17 Feb 16 - 18 Feb 16
				17th Indo-US Cytometry Symposium	12 Mar 16
4.	Dr. Sandesha Pashte	Medical Officer	Dispensary	Foundation Training course under State training policy conducted at Yashada, Pune.	Phase 1: 9 - 29 Mar 15. Phase 2: 30 Mar 15 – 14 Apr 15. Phase 3: 20 - 22 May 15
5.	Dr. Shital U. Mane.	Medical officer	Dispensary	Foundation Training course under State training policy conducted at Yashada Pune.	Phase 1:13 Apr - 3 May 15. Phase 2: 4 - 22 May 15.
6.	Dr. AnuyaRege	Sr. Scientific Officer & In-Charge, Dept. of Bacteriology	Department of Bacteriology	UK-India Workshop on “Design for Infection Prevention & Control” at HindujaHospital, Mumbai.	15 Mar 16

7.	Dr. Usha Padmanabhan	Senior Scientific Officer	Department of Biochemistry	Animal Handling workshop	7- 8 May 15
8.	Dr Mrunal Ghag-Sawant	Scientific Officer	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Mandatory Training for CPCSEA Nominee, NIAW, Haryana	18 – 22 May 15
				One day CME training “ Preclinical Imaging and Drug Discovery”	10 Aug 15
				Current regulatory requirements for members of Institutional ethics committee	17 - 18 Feb 16
9.	Mrs. Jayashree A. More	Scientific Officer – CL II	Department of Bacteriology	Workshop on “Principle and Practices of Laboratory Animal Care” at Haffkine Institute.	7 - 8 May 15
10.			Department of Bacteriology	UK-India Workshop on “Design for Infection Prevention & Control” at Hinduja Hospital, Mumbai.	15 Mar 16
11.	Mrs. Trupti Satvekar	Scientific officer – CL II	Zoonosis Department	Rodent Pest Management	17 - 19 Jun 15
12.				Principle & Practices of laboratory animal care (PPLAC)	7 – 8 May 15
13.	Mrs. Amita S. Patil	Scientific Officer – CL II	Department of Bacteriology	Workshop on “Principle and Practices of Laboratory Animal Care” at Haffkine Institute.	7- 8 May 15
14.	Mrs. Swati U. Mubarakar	Scientific Officer -CL II	Department of Toxicology	PPLAC	7 - 8 May 15
				Rodent Pest Management	17 - 19 Jun 15
				Animal Tissue Culture – In-house Training	18 - 28 Sept 15
15.	Mr. Pradeep Jadhav	Senior Technical Assistant	Zoonosis Department	Principle & Practices of laboratory animal care (PPLAC)	7 – 8 May 15
				Rodent Pest Management	7 – 8 May 15
16.	Mrs. Dipti Kolte	Senior Technical Assistant	Zoonosis Department	Rodent Pest Management	7 – 8 May 15
17.	Ms Nilam Sonavane	Senior Technical Assistant	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Principle and practices of Laboratory animal care	7 - 8 May 15
				Rodent Pest Management	17 - 19 Jun 15

18.	Mr Sanjay Mane	Senior Technical Assistant	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Principle and practices of Laboratory animal care	7 - 8 May 15
19.	Mr Sanjay Mane	Senior Technical Assistant	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Rodent Pest Management	17 - 19 Jun 15
20.	Mr Ashirwad Raorane	Snake Man	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Principle and practices of Laboratory animal care	7 - 8 May 15
				Rodent Pest Management	17 - 19 Jun 15
21.	Mr Rohan Nimbalkar	Snake Man	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Principle and practices of Laboratory animal care	7 - 8 May 15
				Snake bite death Mitigation	29 Sept 15
22.	Mr Vagesh Walhekar	Snake Man	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	Principle and practices of Laboratory animal care	7 - 8 May 15
23.	Mr Baburao Kotare	Mali	Snake Farm & Experimental Animal House, Venomous	Principle and practices of Laboratory animal care	7 - 8 May 15
				Introduction to covert existence of snakes	16 - 17 Jul 15
				Snake bite death Mitigation	29 Sept 15

			Animal Unit, Department of Zoonosis		
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Conferences/Seminar/Colloquium attended by departmental staff

Sr. No.	Name of staff	Designation	Department	Name of Conference attended	Period of the conference	Type of presentation (oral / poster)
1.	Dr. Shashikant Vaidya	Assistant Director & Head	Department of Clinical Pathology	Good Clinical Practices & Current Regulatory Requirements of Institutional Ethics Committee	16 – 18 Feb 16	Nil
2.	Dr. Nishigandha Naik	Assistant Director & Head	Department of Biochemistry	Institutional Scientific Review Committee (SRC) of the NIRRH, Mumbai	NA	Invitee
				Indo-US Workshop on Traditional Medicine, organized by the Ministry of AYUSH	3 & 4 Mar 16	Invited participant
				Insights into Biological Research, organized by the Wilson College, Mumbai.	26 Feb 16	Invited speaker
				Consultative Meet to Prioritize AYUSH Research Activities in the field of Cancer for International Collaboration, organized by the Indian Association for the Study of Traditional Asian Medicine (IASTAM)	21 & 22 Feb 16	Invited participant and speaker
				Workshop on ‘Confocal Microscopy’ organized by the Venture Center, Pune	2 - 3 Feb 16	Invited Anchor faculty

				Time and Again: Challenging Science – Science Academies’ Lecture Workshop.	8 - 9 Jan 16	Invited participant
				Department of Molecular Medicine & Biotechnology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGI), Lucknow	30 Nov 15	Invitee
				Association of Radiation Oncologists of India - Annual Meeting 15’ (AROICON-15)	27 - 29 Nov 15	Invited speaker
				Science Academies’ Refresher Course for University teachers on ‘Developmental Biology’	16 – 28 Nov 15	Course Coordinator and invited speaker
				Meeting of the Society of Biological Chemists, India - Mumbai Chapter 15	22 Aug 15	Invited participant and Jury
				Seminar and Awareness Workshop for students on Integrative Oncology, organized by the Integrated Cancer Treatment & Research Center, Wagholi.	2 Aug 15	Invited speaker
3.	Dr. Mira Ramaiya	Assistant Director & Head	Zoonosis Department	International conference on ‘Promotion of Animal Research Welfare & harmonization of Laboratory animal Science”at ACTREC	15-16 Oct 15	-
				UK-India Workshop on Design for Infection Prevention & Control at Hinduja Hospital	15 Mar 16	-
				Science Honours programme called “JIGYAASA”at K.C.College.	5 Apr 16	-

4.	Dr. Shital U. Mane.	Medical officer	Dispensary	Foundation Training course under State training policy conducted in collaboration with Yashada Pune, & H.I.T.R.T.	20 – 22 May 15	Oral presentation:
				Monsoon related Diseases conducted by I.M.A.	12 Jul 15	Attended
				Multispecialty conference by Indian Medical Association.	9 Aug 15	Attended
				Multispecialty oncology conference by Indian Medical Association.	13 Aug 15	Attended
				World Heart Day conference by Indian Medical Association.	27 Aug 15	Attended
				Hypertension Learning academy conference by Indian Medical Association	28 Feb 16	Attended
				Multispecialty and cosmetology conference by Indian Medical Association.	20 Mar 16	Attended
				International Tuberculosis Day conference by Indian Medical Association.	25 Mar 16	Attended
5.	Dr. Sandesha Pashte	Medical officer	Dispensary	Foundation Training course under State training policy conducted in collaboration with Yashada Pune, & H.I.T.R.T.	20 – 22 May 15	Oral presentation: “Medical Bill Reimbursement Procedure”
				Prevention of Sexual Harassment at Workplace	15 Jan 16	Attended
				Intellectual Property Management	Apr 15	Attended

				Conference conducted by Indian Medical Association on Respiratory Updates	Apr 15	Attended
				Conference conducted at Fortis Hospital on Heart Surgeries and Dialysis	Apr 15	Attended
				Conference at Fortis Hospital on Cardiac updates	Jul 15	Attended
				Conference conducted by Indian Medical Association on Common Psychological problems in children's	Jul 15	Attended
				Conference at Fortis Hospital on Liver transplant	Jul 15	Attended
				Conference conducted by Indian Medical Association on Lymphoma overview	Oct 15	Attended
				Conference conducted by Indian Medical Association on Radiotherapy in Oncology	Aug 15	Attended
				Conference conducted by Indian Medical Association on Skin Cosmetic and laser therapies	Jan 16	Attended
				Conference conducted by Indian Medical Association on Renal Basics	Jan 16	Attended
				Conference at Fortis Hospital on Arrhythmias, Diabetes and Febrile Seizures	Feb 16	Attended
6.	Dr. Shital Vasave	Senior Scientific Officer	Department of Clinical Pathology	UK-India Workshop on Design for Infection Prevention & Control at P. D. Hinduja Hospital, Mahim	15 Mar 16	Nil

				17 th Indo- US Cytometry Symposium at NIRRH	12 Mar 16	Nil
7.	Dr. AnuyaRege	Senior Scientific Officer & In-Charge, Dept. of Bacteriology	Department of Bacteriology	Colloquium “Four New Approaches for Validation of Ayurvedic Herbal Drugs” by Dr. R. D. Lele.	8 Mar 16 at UM-DAE Centre for Excellence in Basic Sciences, Mumbai.	NA
8.	Dr. Usha Padmanabhan	Senior Scientific Officer	Department of Biochemistry	24 hour Stem Cell Event	3 Dec 15	Invited Oral (e-presentation, video conferencing)
				Healthcare: Challenges & Potential Challenges	13 Feb 16	Invited Speaker
9.	Dr. Mrunal Ghag-Sawant	Scientific Officer	Snake Farm & Experimental Animal House, Venomous Animal Unit, Department of Zoonosis	International conference on “Promotion of Animal Research, Welfare and harmonization of Laboratory animal science” at ACTREC, Kharghar	15 – 16 Oct 15	Poster
10.	Dr. Ashish Asrondkar	Scientific Officer & in-charge Chemotherapy	Department of Chemotherapy	National conference on chemical sciences: Emerging Scenario & Challenges (NCS 2016)	29-30 Jan 2016	poster
11.	Dr. Vrushali Patil	Scientific Officer	Department of Chemotherapy	National conference on chemical sciences: Emerging Scenario & Challenges (NCS 2016)	29-30 Jan 2016	poster
12.	Dr. Saroj Bapna	Scientific officer	Zoonosis Department	UK-India Workshop on Design for Infection Prevention & Control at Hinduja Hospital	15 Mar 16	-

				Science Honours programme called “JIGYAASA”at K.C.College.	5 Apr 16	Oral presentation
13.	Mrs. Jayashree A. More	Scientific Officer – CL II	Department of Bacteriology	“National Seminar on recent advances in Research and development in medicinal & aromatic plants-A country Scenario.”	27 & 28 Nov 15 at Jabalpur	Oral
14.	Mrs. Trupti Satvekar	Scientific officer- CL II	Zoonosis Department	Science Honours programme called “JIGYAASA”at K.C.College.	5 Apr 16	-
				Attended the Lecture on Zika virus at KEM Hospital	18 Feb.16	-
15.	Mrs. Vibhuti Thakur	Scientific Officer- CL II	Department of Clinical Pathology	17 th Indo- US Cytometry Symposiumat NIRRH	12 Mar 16	Nil
16.	Mrs. Swati Sanap	Senior Technical Assistant	Department of Clinical Pathology	UK-India Workshop on Design for Infection Prevention & Controlat P. D. Hinduja Hospital, Mahim	15 Mar 16	Nil
17.	Mrs. Dipti Kolte	Sr.tech.assistant	Zoonosis Department	Attended the Lecture on Zika virus at KEM Hospital	18 Feb.16	-
18.	Mrs. Priti Bendre	JRF	Department of Chemotherapy	National conference on chemical sciences: Emerging Scenario & Challenges (NCS 2016	29-30 Jan 2016	Poster

Lectures given by Departmental staff

Sr. No.	Name of staff	Designation	Department	Title of Lecture & Program	Date of the Lecture
1.	Dr. Nishigandha Naik	Assistant Director & Head	Dept. of Toxicology and Dept. of Biochemistry	Laser Confocal Microscopy and Applications, M. Sc. course in Biotechnology	4 th Sept 15
2.	Dr. Sandesha Pashte	Medical Officer	Dispensary	Information on Service book Program: Maharashtra Civil Service Day	20-4-15
3.	Dr. Sandesha Pashte	Medical Officer	Dispensary	Management of Snake Bite Program : Importance of covert existence of snakes	17-7-15
4.	Dr. Shital U Vasave	Senior Scientific Officer	Department of Clinical Pathology	MCS(Conduct) Rules” 1979 On the occasion of Civil Service Day	20 th Apr 15
5.	Dr. Saroj Bapna	Scientific Officer	Zoonosis Department	Malaria and Mosquito and mosquito borne diseases ,MCMM(Mission to Control Mosquito Menace workshop)	06/07/15 28/07/15
6.	Mrs. Trupti Satvekar	Scientific Officer	Zoonosis Department	Collection & Identification of Mosquitoes, MCMM (Mission to Control Mosquito Menace workshop)	10/04/15 06/07/15 28/07/15
7.	Mrs Swati U. Mubarakar	Scientific officer CL- II	Department of Toxicology	PPLAC - Demonstration and Hands-on training	9, 10 Apr 15
				Same as above	7, 8 May 15

**Other Activity at Snake Farm & Experimental Animal House, Venomous Animal Unit,
Department of Zoonosis**

Department is actively engaged in lyophilization of Snake and scorpion venom and sale of venom to research / anti venom production organizations.

Snake and scorpion	Opening stock (gm) Apr 15	Closing stock (gm) Mar 16
Cobra	142.256	153.021
R. Viper	21.088	30.059
Krait	0.047	0.148
Echis	14.891	14.756
Red scorpion	10.276	10.406
Bamboo Pit viper	0.025	0.025

Department provides certificates for Identification of different types of Venomous snakes. Total number of identification report issued during the period 1st Apr 15 to 31st 16 are as follows:

Sr. No.	Period of work	Total number of snakes received for identification	Total number of reports issued
1	Apr 15 - Mar 16	67	67

Supporting Departments

Dispensary, Department of Clinical Pathology,

Head of Department's Overview

The Dispensary of Haffkine Institute provides Outpatient Department facility, monthly medication for chronic illness for staff and their family members and students. It provides free consultation and treatment to PWD staff and their contract workers. It provides Pre and Post exposure prophylaxis vaccination for Tetanus toxoid, Rabies, Hepatitis-B and Influenza Vaccine to employees and students. It conducts Pre employment medical check-up for new recruits of HITRT. The Dispensary complies with I.S.O .9001:2008 norms.

Following are the significant contributions of Dispensary in this year. (2015-2016)

The Medical officers provided clinical assistance by conducting Medical Examination of volunteers as per inclusion criteria for the Research Project “Bio-efficacy evaluation of N.N.Diethyl Benzamine based gel and wipe formulations” of Zoonosis department.(2015-2016)

1. Haffkine Institute employees were vaccinated for:
 - a. Pre exposure prophylaxis for Hepatitis-B.
 - b. Pre exposure prophylaxis for Rabies.
 - c. Pre and Post exposure prophylaxis for Tetanus Toxoid.
 - d. Pre exposure prophylaxis for Influenza Vaccine.
2. OPD facilities were provided for employees and students of HITRT.
3. Antenatal care was provided to the HITRT employees.
4. OPD facilities were provided for Haffkine Biopharmaceuticals Corporation Ltd. staff in May 2015
5. Medical Officers did Medical Examination of volunteers for the Research Project “Bio-efficacy evaluation of N.N.Diethyl Benzamine based gel and wipe formulations” of Zoonosis department.
6. Dr.Sandesha Pashte Medical Officer, made presentation on “Medical Bill Reimbursement” in foundation course assignment in May 2015 as part of State Training Policy.
7. Dr.Sandesha Pashte Medical Officer, delivered lecture on “Information on Service Book” on occasion of Maharashtra Civil Service Day on 20.4.15.
8. Dr.Sandesha Pashte Medical Officer, delivered Guest Lecture on “Management of Snake Bite” during workshop “Introduction to covert existence of snakes” organised by Venomous Animal Unit on 17.7.15.

9. Dr.Shital Mane presented project on “Knowledge Attitude Practices Regarding Customer Satisfaction process amongst H.I.T.R.T.employees” successfully for foundation course assignment in May 2015 as a part of State Training Policy
10. Dr.Sandesha Pashte, Medical Officer, attended lecture on “Intellectual Property Management”at Haffkine Institute in Apr 2015.
11. Dr.Sandesha Pashte, Medical Officer, attended lecture on “Prevention of Sexual Harassment at Workplace” at Savitribai Phule Gender Resource Center, Mumbai on 15.01.16.
12. Dr.Sandesha Pashte, Medical Officer, Dr. Shital Mane, Medical Officer, attended various multi-specialty Medical CME Organised by Indian Medical Association & General Practitioner’s Association.
13. Dr Sandesha Pashte, Medical Officer, Dr. Shital Mane, Medical Officer completed Foundation Training Course of 42 days duration under State Training Policy at YASHADA, Pune.
14. Dr Sandesha Pashte was appointed as chairperson of Women’s Committee for Prevention of Sexual Harassment at work place, member of Write off Committee, and member of Executive Committee (till Jan 2016).
15. Dr. Shital Mane was selected as Management Representative for I.S.O.9001:2008, and worked for organising internal audits, management review meetings, surveillance audits by external auditors as per I.S.O. norms

Future Directions

- 1) To set up speciality clinics [e.g. Fever clinic, diabetes, Hypertension clinic] operating periodically as there are increasing number of patients requiring speciality consultation.
- 2) To provide comprehensive Primary Health Care facility by incorporating other allied branches of medicine like Ayurveda, Yoga, and Homeopathy.

Dr. Shashikant P.Vaidya

Library

Librarian's overview

The aim and objective of the Haffkine Institute Library is to collect, organize, search and retrieve information in the areas of prime activities of the institute i.e. Biomedical and allied sciences with a special reference to the communicable diseases. The library subscribes to specialized national and international journals from various renowned publishers and scientific societies.

This year the library continued to provide online scientific search and retrieval facilities to the staff, researchers and students. During the period total 566 users made use of the online search and retrieval facility offered by the library. Users mainly consisted of Scientific Staff, Research Fellows and the students enrolled for the various programmes of the Institute. The library also provided reference service by Telephone, Email, Post, Courier, etc. to the distant users who could not come to the library.

The library made available different research and scientific publications from other libraries to the scientists and students of the Haffkine Institute for 3 times on the basis of Inter Library Cooperation. Similarly, 7 publication available in our library were made available to other libraries in the current year.

The library regularly imparted induction and orientation training to outside visitors and new students of the institute on the library usage and how to conduct the literature search effectively.

Library Collection and Users Statistics

YEAR	2015-2016
Total Titles	10575
Total Bound Volumes of Journals	27161
Total No. of Theses	484
Outside Visitors visiting the library	908
Staff and Students of the Institute visiting the library	2225

Highlights

Theses, Annual Reports and other documents were scanned using in house digital repository software and made available to the users.

Smt. Sarita Wadekar

Funding

Intramural Funding

Department	Sr. No.	Title of Project	Investigators	Starting date – Ending date	Total projected budget	Total amount spent so far for the project	Total amount spent in year 15 16
Department of Virology	1	Molecular characterization of the circulating 15 16 strains of H1N1 viruses for genomic mutations conferring resistance - neuraminidase inhibitors and adamantanes and virulence	Dr. Sweta Kothari Dr. Abhay Chowdhary Srg. Cdr. (Dr.). Kavita Anand Ms. Devanshi Gohil	Oct 15 – Oct 17	3,00,000	Nil	Nil
	2	Detection of TORCH infection in children and it correlation with birth defects.	Dr. Sweta Kothari Dr. Usha Dave Dr. Vandana Kumavat Dr. Abhay Chowdhary Ms. Payal Panchal	Aug 15 - Dec 17	6,00,000	4,20,000	2,00,000
	3	- Study siRNA Associated Suppression of Rabies Virus Infection and Assess its Therapeutic Potential	Ms. Purna Charan Dr. Abhay Chowdhary	Jun 11- Dec 16	5,00,000	3,40,000	Nil
	4	To study siRNA associated suppression of Rabies Virus infection in vitro	Dr. Abhay Chowdhary Mr. Sandeepan Mukherjee Ms. Purna Charan Ms. Pracheta Thorat	Jan 16 - Jun 16	25,000	NIL	NIL

	5	Assessment of in vitro Immuno modulatory activity of Anti inflammatory agents	Mr. Sandeepan Mukherjee Ms. Perna Charan Mr. Siddhant Gawli	Nov 15 - Apr 16	25,000	12,500	12,500
	6	Interplay of Gaso transmitters in in vitro infection of Rabies Virus	Mr. Sandeepan Mukherjee Ms. Perna Charan Mr. Rohit Beher	Jan 16 - Jun 16	25,000	10,000	10,000
	7	Interplay of Gaso transmitters in in vitro infection of Japanese Encephalitis Virus	Mr. Sandeepan Mukherjee Ms. Perna Charan Ms. Amruta Anvekar	Nov 15 - Apr 16	25,000	12,500	12,500
	8	In vitro evaluation of the dermato protective potential of <i>Hibiscus rosasinensis</i> flower extract	Dr. Abhay Chowdhary Dr. Anil Bobade Dr. Sweta T Kothari Mrs. Suvarna Pachpore Ms. Amita Patil	Aug 15 - Dec 16	5,00,000	10,000	10,000
	9	Assessment of Plant Proteins/Peptides as Potential Anti Viral Agents.	Mr. Sandeepan Mukherjee Dr. Abhay Chowdhary	Jun 11- Dec 16	5,00,000	3,00,000	Nil
				Total	25,00,000	11,05,000	2,45,000
Zoonosis Department	1	Assessment of Dendritic Cell Vaccines as a Potential Immunotherapeutic Agent in Malaria	Dr. Ranjana Deshmukh, Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Rahul Gosavi	Oct. 10- Nov 15	5,00,000	4,90,000	95,000

	2	Interplay of Trace Elements in the pathophysiology of Murine Model of Malaria.	Ms. Mansi Malik Dr. Mira Ramaiya Dr. Abhay Chowdhary	Oct. 12- Dec 17	5,00,000	4,90,000	95,000
	3	To Assess the Contribution of Sweat in Parasite Induced Attractiveness to Insect Host in Malaria Patients	Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Chinmayanand Oroskar	Dec 11 - Aug 17	5,00,000	11,000	Nil
	4	In vitro production of Plasmodium falciparum gametocytes and screening of malaria transmission blocking drugs	Ms.Trupti Satvekar Dr. Saroj Bapna Dr. Mira Ramaiya	Dec 15- Apr 17	75,000	2800/-	72,200/-
	5	Evaluation of herbal, biological and common household against mosquito larvae.	Ms.Trupti Satvekar Dr. Saroj Bapna Dr. Mira Ramaiya	Jan 16- Aug 16	Nil	Nil	Nil
	6	Assessment of BCG as an Adjuvant in Preparation of Japanese Encephalitis Vaccine	Dr. Abhay Chowdhary; Dr. Mira Ramaiya Mr. Sahil Kulkarni	Oct 10- Oct 16	5,00,000	4,89,000	Nil
	7	Comparative assessment of immunomodulatory activities of three plant extracts and their synthesized nanoparticles	Ms.Pallavi Kurkure Choudhary Dr. Mira Ramaiya Dr. Abhay Chowdhary	Oct 12- Dec. 17	5,00,000	75,755	47,600
				Total	25,75,000	15,55,755	2,37,600

Department of Clinical Pathology	1	Comparative Analysis of Different Molecular Methods for Diagnosis of Multi Drug Resistant Tuberculosis	Dr. Shashikant Vaidya Mr. Jaishankar Sharma	Dec 12 - April 16	2,50,000	2,22,189	nil
	2	The study of differential expression of matrix metalloproteinase genes in macrophage infected with <i>Mycobacterium tuberculosis</i>	Dr. Shashikant Vaidya Mr. Jinesh Maniar	Mar 14 - Dec 16	2,50,000	1,17,826	54,543
	3	Immunobiology in understanding the pathophysiology of Active Scleroderma patients from Western India	Dr. Sunita D. Deshpande Dr. Vandana Pradhan Mr. Prasad Khadilkar	Jun 14 - Dec 18	5,00,000	20,000	9,000
	4	Biosynthesis of Nanoparticles & its Potential Therapeutic Applications	Dr. Sunita D. Deshpande Mr. Kapil Punjabi	Mar 13 - Mar 17	3,50,000	1,27,067	28,234
	5	Characterization and assessment of antimicrobial and antioxidant activity of Kombucha	Dr. Sunita D. Deshpande Ms. Tehmeena Mukadam	Dec 12 - Aug 16	2,50,000	24,134	5,152
	6	Evaluation of AntiMicrobial Activity of Wild Apple and Gooseberry Fruit Juices	Dr. Shashikant P. Vaidya Dr. Shital Vasave Mr. Kapil Punjabi	Sept 15 - Dec 15	1,00,000	60,000	60,000

	7	Comparative Analysis of Different Molecular Methods for Diagnosis of Multi Drug Resistant Tuberculosis	Dr. Shashikant Vaidya Mr. Jaishankar Sharma	Dec 12 - April 16	2,50,000	2,22,189	nil
	8	The study of differential expression of matrix metalloproteinase genes in macrophage infected with <i>Mycobacterium tuberculosis</i>	Dr. Shashikant Vaidya Mr. Jinesh Maniar	Mar 14 - Dec 16	2,50,000	1,17,826	54,543
	9	Immunobiology in understanding the pathophysiology of Active Scleroderma patients from Western India	Dr. Sunita D. Deshpande Dr. Vandana Pradhan Mr. Prasad Khadilkar	Jun 14 - Dec 18	5,00,000	20,000	9,000
	10	Characterization and assessment of antimicrobial and antioxidant activity of Kombucha	Dr. Sunita D. Deshpande Ms. Tehmeena Mukadam	Dec 12 - Aug 16	2,50,000	24,134	5,152
	11	Evaluation of AntiMicrobial Activity of Wild Apple and Gooseberry Fruit Juices	Dr. Shashikant P. Vaidya Dr. Shital Vasave Mr. Kapil Punjabi	Sept 15 - Dec 15	1,00,000	60,000	60,000
	12	Biosynthesis of Nanoparticles & its Potential Therapeutic Applications	Dr. Sunita D. Deshpande Mr. Kapil Punjabi	Mar 13 - Mar 17	3,50,000	1,27,067	28,234
				Total	34,00,000	11,42,432	3,13,858

Department of Bacteriology	1	Plasmid Diversity in <i>Pseudomonas aeruginosa</i> from different clinical sources	Mr. Rohan Dudhale	01 Jan 13 - 31 Dec 16	5,00,000	1,30,000	80,000
				Total	5,00,000	1,30,000	80,000
Department of Biochemistry	1	Studies to investigate role of steroid hormones in growth retardation & menstrual irregularities in EHPVO patients.	Dr. Nishigandha Naik, Dr. Shobna Bhatia, Dr. Akash Shukla, Dr. TaralaNandedkar	Jun 14 – Dec 16	3,00,000	1,00,000	Nil.
	2	Effect of fMLP on development and regeneration in zebrafish.	Dr.NishigandhaNaik, Dr. Yasmin Khan, Dr.TaralaNandedkar.	Apr 14 – Dec 17	1,50,000	30,000	Nil.
	3	Analysis of kidney stones using conventional biochemical and FTIR techniques.	Dr.NishigandhaNaik, Dr. Anil Bobade, Dr. Ashish Asrondkar, Dr.VrushaliPatil.	Jan 16 – Jan 17	6,00,000	Nil	Nil
	4	Biochemical Parameters in HIV-TB infection.	Dr. Sucheta Dandekar, Dr. Abhay Chowdhary, Dr. M H Ansari, Dr. R S Naanavare, Dr. LalitAnande, Dr. Vijay Karanjkar, Mr. Sandeepan Mukherjee, Mr. Shreewardhan Rajopadhye.	11 – Dec 16	3,50,000	3,50,000	10,000

	5	Role of inflammation in Obesity and Type 2 diabetes	Dr. A. Rosalind Marita, Dr. Vikrant Bhor, Dr. Satish Dharap, Dr. Priyadarshi Doc-r, Ms. Gauri Patade	Oct 13 – Dec 16	3,50,000	2,00,000	50000
	6	Kidney stones & their constituents	Dr. Usha Padmanabhan, Mr. Pravin Shirsat, Mr. Sameer Patil	Jan 14 – Jan 18	1,00,000	Nil	Nil
	7	Evaluation of leptin and adipokine levels in malnourished children from Nandurbar	Dr. Mridula Phadke, Dr. Rosalind Marita, Dr. Usha Padmanabhan	Jan 15 – Jan 16	72,000	72,000	72,000
	8	Studies to validate safety and to understand mechanism of Ayurvedic formulation Raupya Suvana Sootshekhar (RSS) and its therapeutic metallic ingredients used for neural therapy.	Dr.Vineeta Deshmukh, Dr.Nishigandham,Naik, Dr. D. S. Joshi, Dr.Mansi Thakur.	Jun 14 – Dec 18	70,00,000	30,000	Nil
	9	Characterization and assessment of anticancer activity of Indian honey.	Dr.Sucheta Dandekar Ms.Amruta Naik Dr.Nishigandha Naik.	Jul 12 - Dec 16	3,50,000	92,564	37,564
	10	Enhancement of rosmarinic acid, a neuroprotective, anti-viral compound by <i>in-vitro</i> tissue culture of <i>Mentha piperita</i> (L.).	Dr. Pratibha Chaturvedi, Dr. Abhay Chowdhary, Ms. Kshama Dhobale	February 2015 – January 2019	3,50,000/-	50,000/-	50,000/-

	11	Effect of Homeopathic preparations on production of polyphenols in <i>Curcuma longa</i> and <i>Tylophoraindica</i> tissue culture and elucidation of its potential molecular mechanism	Dr. Abhay Chowdhary, Dr. Rajesh Shah, Dr. Pratibha Chaturvedi, Ms. Geetanjali Talele, Mr. Sandeepan Mukherjee, Ms. Kshama Dhobale	Sept 15 – May 16	2,65,000	1,20,000	1,20,000
				Total	95,37,000	9,94,564	2,89,564
Department of Chemotherapy	1.	Synthesis and biological evaluation of benzothiazole derivatives.	Dr. A.S. Bobade Mrs. Priti Bendre	Jul 15 - Jul 18	3,00,000	1,85,000	1,85,000
	2	Synthesis & biological activity of thiadiazole & Benzothiazole derivatives.	Dr. Ashish Asrondkar Mr. Ashish Shinde	Mar. 16 - Mar 19	3,00,000	47,000	47,000
	3	Development of Synthetic methodology for various bioactive nitrogen, sulphur and oxygen heterocycles.	Dr A.S. Bobade Sanjeev Patil	Jul 13 - Jul 18	5,00,000	1,00,000	Nil
	4	Analysis of Kidney stone using conventional Biochemical & FT-IR techniques	Please see details above (Department of Biochemistry)				
				Total	11,00,000	3,32,000	2,32,000

Department of Toxicology	1	Development of MTT assay for cyto-toxicity testing.	Dr. Nishigandha Naik, Mrs. Swati Mumbarkar Mrs. Jayshree Kale	Jan 16 – Dec 16	1,00,000	61,851.81	54551.81
				Total	1,00,000	61851.81	54551.81
Chemical Testing Unit	1	In-Vitro evolution of the dermato protective potential of <i>Hibiscus rosasinesis</i> flower extract.	Please see details above (Department of Virology)				
	2	Synthesis of Nanosilica from RHA for controlled drug release.	Mr. Manoj Patil	Jan 16-Jul16	5,000	2,000	2,000
				Total	5000	2000	2000
Snake Farm (Venomous Animal Unit), Zoonosis Department	1	Protective Armour Against Snake Bite	Dr. Abhay Chowdhary Dr. Mrunal Ghag – Sawant	Dec 14 - Dec 17	1,00,000	30,000	20,000
			Mr. M.F. Vohra, Chairman & MD, Zenith Industrial Rubber Products Pvt. Ltd		20,00,000	9,50,000	200,000
	2	Characterization of snake venom by using in vitro Techniques and in vivo techniques	Dr. Abhay Chowdhary, Dr. Mrunal Ghag–Sawant	Jun 14 – Jun 17	3,50,000	58,188	57,688
	3	An in vitro analysis of the effect of Common Cobra (Najanaja) venom on the Viability and Proliferation of Lung Cancer Cell Lines	Dr. Mrunal Ghag–Sawant Ms Nilam Sonavane Mr. Karan Gera	Jan 16 – May 16	30,000	28,741	28,741

	4	The study on neutralization of enzymatic activity of Russell viper venom by <i>Aris-lochiaindica</i> , <i>Achyranthesaspera</i> and <i>Vitexnegundo</i> plant extracts	Dr. Mrunal Ghag–Sawant Ms Nilam Sonavane Mr. Abhijit Pole	Jan 16 – May 16	30,000	15,400	15,400
				Total	25,10,000	10,66,929	3,06,429
Experimental Animal House (Venomous Animal Unit), Zoonosis Department	1	Validation of reproductive toxicity parameters in wistar rat	Dr. Abhay Chowdhary Dr. Mrunal Ghag–Sawant Ms. Nilam Sonavane	Dec 13 - Jun 17	1,50,000	72,000	Nil (Halt due - insufficient staff)
	2	Evaluation of Wound Healing Activity of Mesenchymal Stem Cells in Rats	Prof. Dr. Abhay Chowdhary Dr. Mrunal Ghag–Sawant	Dec 14 - Dec 16	2,00,000	170,000	170,000
			Dr. Carolin Mathew	----- Live phase completed Dec 15	6,00,000	501,800	501,800

	3	DNA methylation and DNA hydroxy methylation in Rat and Mouse pre implantation embryos	Dr. Rahul Deshmukh Dr. Mrunal Ghag–Sawant	Dec 13 - Dec 14 — Phase I (Mouse study) completed Dec 14 Phase II (Rat study) not initiated due to insufficient staff	4,00,000	2,00,000	NIL (Halt due - insufficient staff)
				Total	13,50,000	9,43,800	6,71,800

ExtraMural Funding

Department	Sr. No.	Title of Project	Investigators	Funding agency	Starting date – Ending date	Total projected budget	Total amount spent so far for the project	Total amount spent in year 15 16
Department of Virology	1	Laboratory surveillance of Influenza like illness and severe acute respiratory illness (ILI-SARI) in India	Dr. Abhay Chowdhary Dr. Sweta T Kothari Mr. Pavankumar Todkar Ms. Devanshi Gohil	NCDC, MoHFW-GoI	Apr-15-Mar 16	9,40,000	8,81,303	8,81,303
	2	Structure Aided Drug Design, Synthesis and Evaluation of Newer Neuraminidase Inhibitors in Treatment of Pandemic Influenza	Dr. Meena Kanyalkar Dr. Abhay Chowdhary Dr. Sweta T Kothari	ICMR	Jan 15-Dec18	4,99,340 (Haffkine Component)	1,77,000	1,77,000
	3	Studies on neuronal proteomics, virus-host interaction and immune-pathological alternations in rabies encephalitis in murine model	Dr. Abhay Chowdhary Dr. S. Madhusudhana (NIMHANS) Dr. Keshava Prasad (IOB) Mr. Sandeepan Mukherjee Ms. Purna Charan, Mr. Paras Mahale	DBT	Mar-13-Mar-15	45,90,000 (Haffkine Component)	39,67,048	NIL
	4	Mechanism based screening and validation of herbal drugs using radiotracer techniques	Dr. Abhay Chowdhary Dr. R. D. Lele Dr. H.S. Palep Dr. Tarla Nandedkar Mr. Sandeepan Mukherjee Ms. Purna Charan, Ms. Pallavi Choudhary	DST	Dec 12-Nov 16	3,54,80,800	3,07,34,141	1,83,434
					Total	4,15,10,140	3,57,59,492	1,24,1737

Zoonosis Department	1	Assessment of Dendritic Cell Vaccines as a Potential Immunotherapeutic Agent in Malaria	Dr. Ranjana. Deshmukh Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Rahul Gosavi	ICMR- SRF Fellow ship	Jun 12 - Nov 15	Stipend 1042800 Cont 60000 Total 1102800	Stipend 916000 Cont 43200 Total 959200	Stipend 436000 Cont 13200 Total 449200
	2	To Assess the Contribution of Sweat in Parasite Induced Attractiveness to Insect Host in Malaria Patients	Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Chinmayanand Oroskar	ICMR- SRF Fellow ship	Sep 14 – Aug 17	Stipend: 1297400 Cont 60000 Total 1357400	Stipend 373400 Total 373400	Nil
	3	Interplay of Trace Elements in the pathophysiology of Murine Model of Malaria.	Dr. Abhay Chowdhary Dr. Mira Ramaiya Ms. Mansi Malik	ICMR- SRF Fellow ship	May 14 – May 17	Stipend 1245400 Conti 60000 Total 1305400	Stipend 530316 Conti 20000 Total 550316	Nil
	4	Isolation & Identification of the novel bacterium having mosquito larve-cidal activity	Dr. Avinash Kale Dr. Abhay Chowdhary Dr. Mira. Ramaiya	DAE	Jan14 - Jan 17	Nil	Nil	Nil
	5	Assessment of BCG as an Adjuvant in	Dr. Abhay Chowdhary	ICMR- SRF Fellow ship	Oct 13 - Sept 16	Stipend 1154400	Stipend 7,17,600	Stipend 2,18,400

		Preparation of Japanese Encephalitis Vaccine	Dr. Mira Ramaiya Mr. Sahil Kulkarni			Cont 60000 Total 12,14,400	Cont 30000 Total 7,47,600	Cont 10,000 Total 2,28,400
					Total	37,65,600	18,82,916	4,49,200
Department of Biochemistry	1	Serum Brain Derived Neurotrophic Factor (BDNF) and Gene Mutation in Autism Spectrum Disorders (ASDs)	Dr. Usha P. Dave Dr. Abhay Chowdhary Dr. Sweta Kothari Dr. Shilpa Kulkarni.	DBT	Aug 14 - Jul 16	26,26,000	20,16,367	17,71,067
	2	Genetics of Epilepsy: Mutational Diagnosis and Genetic Counseling.	Dr. Usha Dave \$, Dr. Vandana Khumavat Mr. Nikhil Kasarpalkar	Moastumoto Inst. of Life Sc. – India\$	14 – Dec 16	Not Applicable	2,00,000	Not Applicable
	3	Investigating the role of inositol pyrophosphates in development of prostate cancer.	Dr. Usha Padmanabhan Dr. Rosalind Marita	DBT	Jul 15 - Jun 18	24,97,000	3,62,542	3,62,542
	4	HIV induced reduction in hematopoiesis.	Dr. Prasad Koka, Dr. Usha Padmanabhan, Dr. Abhay Chowdhary, Dr. Ritwik Dahake	Ramlingswami fellowship to Dr. Koka, DBT	Oct 13 - Sept 15	10,00,000	10,00,000	Nil
	5	Isolation, characterization and screening of potential anti-inflammatory	Dr. Usha Padmanabhan, Dr. Narendra Bhat, Dr. Vandana Kozarekar	MoES/ CDRI	Feb 16 - Jan 19	45,00,000	Nil	Nil

		drugs from Praval (Coral / <i>Corallium rubrum</i>)						
					Total	1,06,23,000	35,78,909	21,33,609
§ Funding was received by the PI – Dr. Usha Dave at her organization.								
Chemical Testing Unit	1	Detection of chemical constituents presents in <i>PhyllanthusEmblica</i> fruit Juice.	Dr. Anil Bobade Mr. Manoj K. Patil Mr. Santosh Bagal	Exotic Juices Ltd Manipur	Jan 15- Jun-16	90,000	30,000	30,000
					Total	90,000	30,000	30,000

Department	Sr. No.	Title of Project	Investigators	Funding agency	Starting date – Ending date	Total projected budget	Total amount spent so far for the project	Total amount spent in year 15 16
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	2	Structure Aided Drug Design, Synthesis and Evaluation of Newer Neuraminidase Inhibitors in Treatment of Pandemic Influenza	Dr. Meena Kanyalkar Dr. Abhay Chowdhary Dr. Sweta T Kothari	ICMR	Jan 15-Dec18	4,99,340 (Haffkine Component)	1,77,000	1,77,000
	3	Studies on neuronal proteomics, virus-host interaction and immune-pathological alternations in rabies encephalitis in murine model	Dr. Abhay Chowdhary Dr. S. Madhusudhana (NIMHANS) Dr. Keshava Prasad (IOB) Mr. Sandeepan Mukherjee Ms. Purna Charan, Mr. Paras Mahale	DBT	Mar-13-Mar-15	45,90,000 (Haffkine Component)	39,67,048	NIL
	4	Mechanism based screening and validation of herbal drugs using radiotracer techniques	Dr. Abhay Chowdhary Dr. R. D. Lele Dr. H.S. Palep Dr. Tarla Nandedkar Mr. Sandeepan Mukherjee Ms. Purna Charan, Ms. Pallavi Choudhary	DST	Dec 12-Nov 16	3,54,80,800	3,07,34,141	1,83,434
					Total	4,15,10,140	3,57,59,492	1,24,1737

Zoonosis Department	1	Assessment of Dendritic Cell Vaccines as a Potential Immunotherapeutic Agent in Malaria	Dr. Ranjana. Deshmukh Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Rahul Gosavi	ICMR- SRF Fellow ship	Jun 12 - Nov 15	Stipend 1042800 Cont 60000 Total 1102800	Stipend 916000 Cont 43200 Total 959200	Stipend 436000 Cont 13200 Total 449200
	2	To Assess the Contribution of Sweat in Parasite Induced Attractiveness to Insect Host in Malaria Patients	Dr. Abhay Chowdhary Dr. Mira Ramaiya Mr. Chinmayanand Oroskar	ICMR- SRF Fellow ship	Sep 14 – Aug 17	Stipend: 1297400 Cont 60000 Total 1357400	Stipend 373400 Total 373400	Nil
	3	Interplay of Trace Elements in the pathophysiology of Murine Model of Malaria.	Dr. Abhay Chowdhary Dr. Mira Ramaiya Ms. Mansi Malik	ICMR- SRF Fellow ship	May 14 – May 17	Stipend 1245400 Conti 60000 Total 1305400	Stipend 530316 Conti 20000 Total 550316	Nil
	4	Isolation & Identification of the novel bacterium having mosquito larve-cidal activity	Dr. Avinash Kale Dr. Abhay Chowdhary Dr. Mira. Ramaiya	DAE	Jan14 - Jan 17	Nil	Nil	Nil
	5	Assessment of BCG as an Adjuvant in	Dr. Abhay Chowdhary	ICMR- SRF Fellow ship	Oct 13 - Sept 16	Stipend 1154400	Stipend 7,17,600	Stipend 2,18,400

		Preparation of Japanese Encephalitis Vaccine	Dr. Mira Ramaiya Mr. Sahil Kulkarni			Cont 60000 Total 12,14,400	Cont 30000 Total 7,47,600	Cont 10,000 Total 2,28,400
					Total	37,65,600	18,82,916	4,49,200
Department of Biochemistry	1	Serum Brain Derived Neurotrophic Factor (BDNF) and Gene Mutation in Autism Spectrum Disorders (ASDs)	Dr. Usha P. Dave Dr. Abhay Chowdhary Dr. Sweta Kothari Dr. Shilpa Kulkarni.	DBT	Aug 14 - Jul 16	26,26,000	20,16,367	17,71,067
	2	Genetics of Epilepsy: Mutational Diagnosis and Genetic Counseling.	Dr. Usha Dave \$, Dr. Vandana Khumavat Mr. Nikhil Kasarpalkar	Moastumoto Inst. of Life Sc. – India\$	14 – Dec 16	Not Applicable	2,00,000	Not Applicable
	3	Investigating the role of inositol pyrophosphates in development of prostate cancer.	Dr. Usha Padmanabhan Dr. Rosalind Marita	DBT	Jul 15 - Jun 18	24,97,000	3,62,542	3,62,542
	4	HIV induced reduction in hematopoiesis.	Dr. Prasad Koka, Dr. Usha Padmanabhan, Dr. Abhay Chowdhary, Dr. Ritwik Dahake	Ramlingswami fellowship to Dr. Koka, DBT	Oct 13 Sept 15	10,00,000	10,00,000	Nil
	5	Isolation, characterization and screening of potential anti-inflammatory	Dr. Usha Padmanabhan, Dr. Narendra Bhat, Dr. Vandana Kozarekar	MoES/ CDRI	Feb 16 - Jan 19	45,00,000	Nil	Nil

		drugs from Praval (Coral / <i>Corallium rubrum</i>)						
					Total	1,06,23,000	35,78,909	21,33,609
§ Funding was received by the PI – Dr. Usha Dave at her organization.								
Chemical Testing Unit	1	Detection of chemical constituents presents in <i>PhyllanthusEmblica</i> fruit Juice.	Dr. Anil Bobade Mr. Manoj K. Patil Mr. Santosh Bagal	Exotic Juices Ltd Manipur	Jan 15- Jun-16	90,000	30,000	30,000
					Total	90,000	30,000	30,000

**Funding generated by the different departments through Testing Services
(Apr 15 - Mar 16)**

Department of Virology

Sr. No..	Name of testing service	Total Revenue Generated
1	Detection of pdm H1N1 2009 Influenza A virus (Samples tested:1586)	Free of cost
2	Detection of Rabies by FAT/PCR (Samples tested: 07)	9,000
3	In-vitro evaluation of anti-viral efficacy of new chemical/natural compounds/entities	8,44,470
4	In-vitro evaluation of virucidal efficacy of chemicals/disinfectants/hygiene products	16,15,652
5	Analytical testing of physiological parameters from human serum/plasma samples from Clinical Research	2,00,375
	Total	26,69,497

*1: pH1N1 testing is offered free of cost as it is a part of the National Project of Laboratory Surveillance of Influenza like Illness in Mumbai region.

*2: Detection of rabies by FAT/PCR is provided free of cost to medilegal post-mortem samples received from police stations across the country, while it is provided at a 50% rebate to the patients referred by government/civil/municipal hospitals, who are below the poverty line.

Zoonosis Department

Sr. No..	Name of testing service	Company name	Revenue generated
		Plant Protection Consultant	370296
1	Insecticide testing against flying and crawling insects.	MCGM	21660
		Apar Industries	43436

Department of Clinical Pathology

Sr. No.	Name of testing service		Total revenue generated
1	Hematology	CBC	3,000
		ESR	1,500
		MP	1,500
		Urine (R/M)	1,000
2	Serology	Extended Widal Test	3,44,925
3	Microbiology	OT Swabs	2,53,250
		Aerobic Culture and AST	3,100
4	Mycology	Fungal Culture & Sensitivity	1,700
5	Rheumatology Testing	Anti-cardiolipin antibodies	5,000
		Anti-beta 2 Glycoprotein antibodies	7,000
	Total		6,21,975

Department of Biochemistry

Sr. No..	Name of testing service	Total Revenue Generated
1	Blood Biochemistry – Glucose, Bilirubin, SGOT, SGPT, Cholesterol, Triglycerides, BUN, Creatinine	2,464
2	Kidney & gall stone testing	28,500
	Total	30,964

Department of Toxicology

Sr. No..	Name of testing service	Total Revenue Generated
1	Acute oral toxicity testing of FERMSEPT® -S (J) in mice.	27,800.00
2	Acute dermal irritation/ corrosion test of Silicon Fluid in rabbits.	46,900.00
3	Acute oral toxicity test of Chlorine Dioxide in rats.	64,000.00
4	Acute oral toxicity - LD50 determination of CRISTOL OB – 77 in rats.	52,250.00
	Total	190,950.00

Department of Chemotherapy

Sr. No..	Name of testing service	Total Revenue Generated
1	Snake venom analysis by HPLC (06 samples)(Jan 15-Dec.15)	3,000
2	Antioxidant Activity (52 samples)(Year 15-16)	11,890
Total		14,890

Chemical Testing Department

Serial No.	Nature of the Sample Tested & the Test Performed	Total Number of Sample Tested	Total Revenue Generated
1	TDL, ODS, SDS	5,416	81,24,000
2	CL-I	11,091	33,27,300
3	CL-III	7,271	21,81,300
4	Toddy	4,187	25,12,200
5	TL(Beer, IMFL)	3,45,413	1,65,200
6	TCL	3,952	46,800
7	TFS	07	4,200
8	CL-II	02	600
9	Miscellaneous Samples	04	21,500
Total		28,443	1,63,83,100

Snake Farm and Experimental Animal House (Venomous Animal Unit) Zoonosis Department

Sr. No..	Name of testing service	No of test completed	Total Revenue generated
1	Evaluation of Anti Rodent activity of test compound	1	2,20,880
2	Evaluation of Anti squirrel activity of test compound	1	2,48,240
3	Identification of Unknown Venom Sample	4	14,722
Total			4,83,842

Other Activity from Snake Farm and Experimental Animal House (Venomous Animal Unit) Zoonosis Department

Department is actively engaged in lyophilization of Snake and scorpion venom and sale of venom to research / anti venom production organizations.

Snake and scorpion	Opening stock (gm) April 15	Closing stock (gm) March 16	Total Revenue Generated (Rs.)
Cobra	142.256	153.021	8,67,000
R. Viper	21.088	30.059	2,54,000
Krait	0.047	0.148	NIL
Echis	14.891	14.756	NIL
Red scorpion	10.276	10.406	18,800
Bamboo Pit viper	0.025	0.025	NIL
Total			11,39,800

Staff & Students

Administration

Sr. No.	Name of staff/student	Designation
1.	Smt. Seema Vyas IAS	Director
2.	Shri. Dinesh B. Malekar	Chief Administrative Officer
3.	Smt. Hemangee N. Khanolkar	Statistician
4.	Smt. Supriya P. Gulekar	Stenographer
5.	Shri. Anant M. Kandalgaonkar	Senior Clerk
6.	Shri. Mahendra S. Bhurkunde	Senior Clerk
7.	Shri. Hemant S. Shinde	Clerk
8.	Smt. Sundar G. Ingale	Clerk
9.	Shri. Sandeep I. Ingawale	Clerk
10.	Smt. Sonali J. Kale.	Clerk
11.	Smt. Ruchira R. Chavan	Telephone Operator
12.	Shri. Navnath M. Ghode	Driver
13.	Shri. Mandar Y. Shinde	Driver
14.	Shri. Shishupal G. Kamble	Laboratory Attendant
15.	Smt. Seema B. Borate	Laboratory Attendant
16.	Shri. Hemant S. Salvi	Peon
17.	Kum. Swapnali S. Kadam	Peon
18.	Shri. Suresh D. Madgude	Peon
19.	Shri. Paresh S. Ghosalkar	Servant
20.	Shri. Rohidas M. Kahandole	Servant
21.	Smt. Mayadevi B. Walmiki	Sweeper
22.	Shri. Sunilkumar L. Gorla	Sweeper
23.	Shri. Nanjibhai M. Baria	Sweeper
24.	Shri. Santosh R. Gurav	Sweeper
25.	Smt. Trupti R. Ghadi	Sweeper
26.	Shri. Anil W. Bukane	Sweeper
27.	Shri. Swapnil S. Kasale	Sweeper
28.	Shri. Amol Gaikwad	Sweeper

Accounts

Sr. No.	Name of staff/student	Designation
1.	Smt. Vidya D. Sankhe	Accounts Officer
2.	Smt. Priti S. Palekar	Assistant Accounts Officer
3.	Kum. Vaishali V. More	Senior Clerk
4.	Shri. Prasad R. Manjrekar	Clerk
5.	Smt. Pramila K. Tambe	Clerk
6.	Shri. Sachin U. Somshetti	Peon
7.	Shri. Mahmud Fakir Kasim	Laboratory Attendant
8.	Shri. Rohan Mhatre	Laboratory Attendant

Stores

Sr. No.	Name of staff/student	Designation
1.	Smt. Darshana D. Bandarkar	Senior Clerk
2.	Shri. Santosh M. Mungekar	Clerk

3.	Smt.Vimal B. Ahire	Hawaladar
4.	Shri.Raju A. Mavlankar	Laboratory Attendant
5.	Shri.Madhukar S.Shengal	Laboratory Attendant

Training

Sr. No.	Name of staff/student	Designation
1.	Dr. Anuya Rege	Senior Scientific Officer
2.	Shri.Nilesh Gaikwad	Medical Social Worker

Library

Sr. No.	Name of staff/student	Designation
1.	Smt.Sarita Wadekar	Librarian
2.	Shri.Tushar B.Borse	Asst. Librarian
3.	Shri.Shashank G. Sarang	Laboratory Assistant
4.	Shri.Sunil G. Bawaskar	Laboratory Attendant
5.	Shri.Abhishek V. Manjrekar	Laboratory Attendant

Department of Bacteriology

Sr. No.	Name of staff/student	Designation
1.	Mr. Shrikant P. Gajbhiye	Head and Addl. Assistant Director
2.	Dr. Anuya Rege	Senior Scientific Officer (Department In charge)
3.	Mrs. Jayashree A. More	Scientific Officer (CI-II)
4.	Mrs. Amita S. Patil	Scientific Officer (CI-II)
5.	Mr. Chetan R. Zope	Senior Technical Assistant
6.	Mr. Jitendra S. Dalvi	Lab-Assistant
7.	Mr. Ravindra T. Bire	Lab-Assistant
8.	Mr. Bhagyawan A. Pawar	Lab-Attendent
9.	Mr. Sandip V. Kegede	Lab-Attendent
10.	Mr. Rohan Dudhale	Senior Research Fellow

Department of Biochemistry

Sr. No.	Name of staff/student	Designation
1.	Dr. Nishigandha Naik	Head & Assistant Director
2.	Dr. Usha Padmanabhan	Senior Scientific Officer
3.	Kum. Amruta A. Naik	Scientific Officer
4.	Smt. Sanika N. Parkar	Senior Technical Assistant
5.	Shri. Sameer S. Patil	Senior Technical Assistant
6.	Shri. Praveen L. Shirsat	Laboratory Assistant
7.	Shri.Samrat B.More	Laboratory Attendant

Chemical Testing Department

Sr. No.	Name of Staff / Student	Designation
1.	Mrs. Suvarna Pachpore	Scientific Officer CL – I (Department In charge)
2.	Mr. Manoj Patil	Scientific Officer CL - II

3.	Mr. Santosh Bagal	Scientific Officer CL - II
4.	Mrs. Shweta sawant	Sr. Tech. Asst.
5.	Mrs. Prachi Ghadage	Sr. Tech. Asst.
6.	Mrs. Deepali Bange	Sr. Tech. Asst.
7.	Mr. Subhash Kulal	Sr. Tech. Asst.
8.	Mr. Sandeep Ingawale	Clerk
9.	Mr. Praveen Thakur	Lab. Asst.
10.	Mr. Ravindra Katkar	Lab. Asst.
11.	Mr. Dilip S. Pawar	Lab. Asst.
12.	Mrs. Bhargavi sawant	Lab. Asst.
13.	Mr. Anil Rawale	Lab. Attendant
14.	Mr. Sandesh Kadam	Lab. Attendant
15.	Mr. Nikhil Bhendkar	Lab. Attendant
16.	Mrs. Pramila Ransinge	Peon
17.	Mr. Swapnil Kasle	Sweeper

Department of Chemotherapy

Sr. No.	Name of Staff / Student	Designation
1.	Dr. Ashish L Asrondkar, Ph.D, FISCA	Scientific Officer, (Department In charge)
2.	Dr. Vrushali N Patil, Ph.D, FISCA	Scientific Officer
3.	Mr. Sushant R Kaple, M.Sc	Scientific Officer
4.	Mr. Nishant N Bharati	Senior Technical Assistant
5.	Mr. Pramod D Andhere	Lab. Assistant
6.	Mr. Rohan A Patil	Lab. Attendant
7.	Mr. Maruti V Chile	Lab. Attendant
8.	Mr. Sanjeev Patil	SRF
9.	Mrs. Priti Bendre	JRF
10.	Mr. Ashish V Shinde	JRF

Dispensary, Department of Clinical Pathology

Sr. No.	Name of Staff / Student	Designation
1.	Dr. Shashikant P.Vaidya	Head & Assistant Director
2.	Dr.(Mrs).Sandesha N. Pashte	Medical Officer
3.	Dr.(Mrs).Shital U. Mane	Medical Officer
4.	Mrs.Rupali K. Ghugare	Nurse
5.	Mr.Shashikant Kasle	Attendant

Department of Clinical Pathology

Sr. No.	Name of Staff / Student	Designation
1.	Dr. Shashikant P. Vaidya	Head & Assistant Director
2.	Dr. Shital U. Vasave	Senior Scientific Officer
3.	Smt. Vibhuti A. Thakur	Scientific Officer
4.	Mrs. Swati Sanap	Sr. Technical Assistant
5.	Mr. Subhash Sawant	Lab. Assistant
6.	Mr. Shashikant Kasale	Lab. Assistant
7.	Mr. Avinash More	Lab. Attendant
8.	Mr. .Arunagiri Subramanian	Senior Research Fellow

9.	Mrs. Suvarna Joshi	Senior Research Fellow
10.	Mr. Kapil Punjabi	Senior Research Fellow
11.	Mr. Prasad Khadilkar	Senior Research Fellow
12.	Mr. Jaishankar Sharma	Junior Research Fellow
13.	Ms. Tehmeena Mukadam	Junior Research Fellow
14.	Mr. Jinesh Maniar	Junior Research Fellow
15.	Mr. Anuruddha Barua	STRIP Student
16.	Ms. Manasvi Jain	STRIP Student
17.	Mr. Sankalp Kokare	STRIP Student
18.	Mr. Darshil Maru	STRIP Student

Department of Toxicology

Sr. No.	Name of Staff	Designation
1.	Dr. Nishigandha Naik	Head & Assistant Director
2.	Mrs. Swati Mubarakar	Scientific officer (CI-2)
3.	Mrs Jayshree Kale	Laboratory Assistant (till – 31 st Dec 2015)
4.	Mr. Mahesh Gaonkar	Laboratory Attendant

Department of Virology

Sr. No.	Name of Staff	Designation
1.	Dr. Shweta T. Kothari	Senior Scientific Officer
2.	Shri. Sandeepan R. Mukherjee	Scientific Officer
3.	Shri. Pavankumar T. Todkar	Scientific Officer
4.	Smt. Meenal S. Mulkutkar	Senior Technical Assistant
5.	Smt. Manjula V.Jadhav	Senior Technical Assistant
6.	Shri. Prakash S. Gawade	Laboratory Assistant
7.	Smt. Monika M. Bhurkunde	Laboratory Assistant
8.	Smt. Rajeshree V. Patil	Laboratory Attendant
9.	Shri. Santosh V.Gujar	Laboratory Attendant
10.	Shri. Amol A. Rane	Laboratory Attendant

Venomous Animal Unit, Zoonosis Department

Sr. No.	Name of Staff / Student	Designation
1.	Dr Mira Ramaiya.	Assistant Director
2.	Dr. Mrunal Ghag-Sawant	Scientific Officer
3.	Mr. Sanjay Mane	Senior Technical Assistant
4.	Ms. Nilam Sonavane	Senior Technical Assistant
5.	Mr. Ashirwad Raorane	Snake Man
6.	Mr. Rohan Nimbalkar	Snake Man
7.	Mr. Vagesh Walhekar	Ass. Snake man
8.	Mr. Vikrant Ghadi	Animal Attendant
9.	Mr. Pankaj Javir	Laboratory Attendant

Non-technical auxiliary personnel of Institute appointed under Venomous Animal Unit, Department of Zoonosis

Sr. No.	Name of Staff / Student	Designation
1.	Mr. Sanjay Surve	Mukadam

2.	Miss. Vandana Mane	Mali
3.	Mr. Prakash Kalsulkar	Mali
4.	Mr. Raju Gaikwad	Mali
5.	Mr. Laxmikant Avale	Mali
6.	Mr. Baburao Khotare	Mali
7.	Mr. Anil Somshetty	Mali cum watchman
8.	Mr. Shrikant Mohite	Mali cum Shetrapal

Zoonosis Department

Sr. No.	Name of Staff / Student	Designation
1.	Dr. Ramaiya Mira L	Assistant Director
2.	Dr. Saroj Bapna	Scientific Officer- CL II
3.	Mrs. Trupti A.Satvekar	Scientific Officer- CL II
4.	Mr. Pradeep Jadhav	Sr. Tech. Assistant
5.	Mrs. Dipti Kolte	Sr. Tech. Assistant
6.	Mrs. Jyostna Naik	Lab. Assistant
7.	Mr. Kailas Aaglave	Insect Collector
8.	Mr. Siddhesh Thool	Lab. Attendant
9.	Mrs. Yugantika Mokashi	Lab. Attendant
10.	Mr. Sunil Khaire	Lab. Attendant
11.	Mr. Sahil Kulkarni	Senior Research Fellow
12.	Mr. Rahul Gosavi	Senior Research Fellow
13.	Mr. Chinmayanand Oroskar	Senior Research Fellow
14.	Miss. Mansi Malik	Senior Research Fellow
15.	Mrs. Pallavi Kurkure	Senior Research Fellow

Editor and Compiler's Note

I am thankful to Mrs. Vyas, Director-in-charge of the Institute for the opportunity to interact with the scientists and research scholars of all the departments for free and frank discussion on ongoing research activities and future plans after a gap of almost 15 years. It was heartening to see the thirst for new knowledge, spirit of dedication and the efforts to excel in research even under adverse conditions. This is obvious from the diverse research projects reported by all the departments in this Annual Report. On the downside, one could see that most of the posts of Assistant Directors and senior scientists have remained vacant for a long time and the laboratories have suffered structural damages in the aging building. While congratulating the scientific staff for their perseverance, I very much wish that substantial improvements happen very quickly.

Dr. J.M. Deshpande

Technical Consultant to the Director

Capturing the Institute's wide range of projects and activities during the past year has been a very interesting experience and an education in itself for me. I believe that every member of this Institution, like myself, feels quite honored to be part of the Institute and will strive to achieve more goals the next year. I would like to thank my colleagues and co-workers for their co-operation, which has helped me to present an accurate picture of the various activities in their respective departments. I would also like to thank Dr. J.M. Deshpande, for his constructive suggestions and valuable inputs during preparation of the report. Finally, I would like to thank Mrs. Seema Vyas for giving me the opportunity to compile this report and be part of the Institute's continually evolving history.

Dr. Usha Padmanabhan

Senior Scientific Officer

Biochemistry department

