



97th Indian Science Congress

January 3-7, 2010 Thiruvananthapuram

PROCEEDINGS

SECTION OF

New Biology

**(Including Biochemistry, Biophysics and Molecular
Biology & Biotechnology)**

President

Prof. P.K. Seth

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PROCEEDINGS

I

Presidential Address

President

Prof. P.K. Seth

New Biology and Environmental Health

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**Keywords: Biomarkers, Proteomics, Genomics,
Environmental Health**

Association of a number of environmental chemicals with ailments like respiratory and cardiovascular disorders, neurological disturbances; reproductive and immune dysfunctions, developmental defects and cancer has been observed in recent years. It has been observed in studies undertaken in India and elsewhere that certain individual showed differential response to the same level of given toxicant. We have observed that miners of Balaghat and Ukawa manganese mines near Nagpur, India show a differential degree of illness on exposure to the manganese dust. Similarly, arsenic exposure has been found to have differential effect in studies undertaken in Kolkatta by Indian Institute of Chemical Biology (IICB) under a CSIR multi-centric project on toxicogenomics. Certain individuals in spite of having detectable amount of arsenic did not show any lesions or clinical symptoms of arsenic poisoning while others with the same level of arsenic in their blood showed well marked skin lesions and enzyme changes. Further study showed that this difference was due to the differences in the enzymes involved in the methylation of the arsenic. The process of disease development whether due to an organic cause or induced by an environmental chemical is quite similar and, therefore, identification of impact of chemical exposure at early stages, prediction of the outcome of exposure and identification of the population sensitive to chemicals or diseases are challenging tasks but key to prevention of health ailments. We need a scientific understanding of the biological basis for susceptible populations and human health research priorities require focus to improve scientific foundation of risk assessment. It requires a scientific study that how genomes respond to the environmental stresses / toxicants and how the chemical interact with the genome. Gene expression pattern may provide a signature profile of specific toxicant or mechanism. Hence, identification of biomarkers,

which could help in identification of diseases and persons susceptible to diseases much before an irreversible damage is done, would be of unestimatable value. The current approaches to detect or monitor the diseases are based on animal data and mathematical modeling. However, these do not take into account the inter-individual variability at the genomic (e.g. SNPs) and proteomic levels. As we know that people differ significantly in their response to xenobiotics including drugs and toxic substances. We need an integrated approach employing bioanalytic, bioinformatic and their applications to understand changes at genomic, transcriptomic and proteomic levels. This approach would help to understand physiology and pathophysiology of the disease and such basic science understanding will bring benefit to the patient. The post genome era has led to an increased use of genomics in disease identification and monitoring.

Microarray is now being used not only for identifying the genes involved in disease but also for identification of biomarkers of exposure and effect. The conventional biomarkers of exposure are DNA adducts, protein adducts, albumin, hemoglobin and WBC's in blood tissues or urine. Application of proteomics and genomics and now metabolomics help in having markers of not only exposure but effect, which could also serve as markers for prediction of disease. Using such markers, it is possible to provide early indicators for timely intervention and prevention, target sensitive populations and redefine sensitive population to the level of individual. An ideal biomarker should be able to identify early and reversible biochemical events in target cells or tissues, predictive of later structural or functional damage, distinguish reversible from progressive damage and measurable in peripheral accessible media. SNPs are being used for identifying the population susceptible to certain chemicals as well as in deciphering the mechanism of diseases such as cancer. Cytochrome P450s in lymphocytes have recently gained importance as biomarkers for risk assessment and are being seen as a breakthrough in the area of molecular epidemiology. Lymphocytes have obvious advantages for use in development of non-invasive assays to screen population and applicability of these cell types as indicative of toxicant exposure. They have been shown to express several members of P450 gene family, whose protein products are involved in the metabolism of environmental carcinogens. Our studies provided evidence for the expression of CYP1A1 and 1A2 isoenzymes in freshly isolated rat blood lymphocytes. These were also found to express arylhydrocarbon receptor (Ahr) and aryl hydrocarbon nuclear transporter (Arnt), the transcription factors involved in the induction of CYP1A isoenzymes. Further we observed similarities in the catalytic activity of CYP1A1 and 1A2 in blood lymphocyte

with the liver enzyme. The factors that affect CYP1A 1 and 1A2 activity in liver also influenced its expression and activity in blood lymphocytes. With these and other studies evidence was produced that blood lymphocyte CYP1A isoenzymes could be used as a surrogate for studying tissue expression of CYP1A enzymes. The CYP2E1, also expressed in lymphocyte, is involved in the activation of a variety of compounds including procarcinogens such as N-nitrosamines and toxic, low-molecular weight chemicals like benzene, styrene, etc. It is also involved in the metabolism of certain drugs, for e.g. acetaminophen, ethanol and anesthetic agents. CYP2E1 gene has been shown to contain several restriction fragment length polymorphisms (RFLP) that may affect functional activity of the expressed protein. Individuals with variant CYP2E1 genotypes were found to be at increased risk to lung cancer, nasopharyngeal carcinoma (Swedish Study), though in other studies, no differences were observed in the frequency of variant genotype between lung cancer patients and controls.

To assess the usefulness of blood lymphocytes CYP2E1 as surrogate for monitoring tissue expression, we investigated the catalytic activity of CYP2E1 in blood lymphocyte and correlated it with the mRNA and protein expression. Our data demonstrated that CYP2E1 expressed in blood lymphocytes is catalytically and functionally active and reflects the *in vivo* activity of the liver enzyme. Similarities in the expression and functional activity of CYP2E1 in blood lymphocyte with the tissue isoenzyme suggest that lymphocyte CYP2E1 could be used to monitor tissue expression.

For monitoring human genotoxicity, newer and more powerful techniques such as the comet assay is being used which provide early information of exposure. Proteome profiling is also an upcoming area in health risk assessment and provides mechanistic, diagnostic and prognostic markers. These capabilities along with molecular metabolomics need to be developed and practiced in disease management.



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II

ABSTRACT OF
Platimun Jubilee Lecture

Structural Biology of Malaria Parasite Proteins: Insights and Implications for Drug Discovery

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Malaria continues to be a major cause of morbidity and mortality throughout the world. DNA sequencing of the most severe malaria-causing parasite, *P. falciparum*, has opened new vistas for understanding the parasite biology. Our laboratory uses multi-disciplinary techniques within modern biology - including biochemistry, bioinformatics, cell biology, parasitology and protein crystallography to unravel the mechanism of action of important parasite proteins. Over the past few years, we have made significant progress in understanding various parasite proteins involved in erythrocyte invasion, hepatocyte invasion and gametocytogenesis using various techniques in structural biology. We have solved crystal structures of many novel and crucial malaria parasite proteins from several stages of the parasite life cycle. We have also explored structure-based inhibitor discovery routines against several malaria parasite targets. We will discuss in detail insights into function of these proteins and their potential for development of anti-malarials.

PROF. S.S. KATIYAR ENDOWMENT LECTURE

Functional Validation of Novel Genes for Abiotic Stress Tolerance in Rice

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Abiotic stresses like, drought, salinity, temperature extremes etc. affect plant development and productivity. It is predicted that due to global climate change there will be an increase in average temperatures, higher sea levels and changes in rainfall patterns which can lead to depletion of fresh water and increase in saline areas. There is thus a need to enhance food production for growing population on less land with less water, or bring other areas like saline areas under cultivation. This is a challenge for science and technology. It is suggested that understanding genomic level changes in plants in response to stress can help develop strategies to engineer plants towards stress tolerance.

Abiotic stress leads to an enhanced expression of a number of genes and results in the modulation of various physiological and metabolic factors. The aim of our work is to identify genes and processes that are regulated under stress and move these genes into stress sensitive plants. We have identified a large number of stress up-regulated EST's from a saline tolerant pokkali rice. The expression of these genes in response to salinity stress has been checked by northern and RT-PCR. Gene expression data analyzed suggest that salinity tolerance of pokkali may be due to constitutive expression of a number of genes. A detailed work on some of the genes encoding components in the signal transduction, transcription factors and those involved in glutathione and ion homeostasis and trafficking has been undertaken.

In this lecture, we will present some of the work on two families of proteins

.One of the large family consists of proteins having CBS domain. A comparative RT-PCR profile between a sensitive and saline tolerant pokkali rice showed that many of these genes were expressed at very high level in the tolerant cultivar. A detailed analysis of one -CBS domain containing protein showed that it might have some role in plant development and stress tolerance.

Extensive work has also been done for genes encoding glyoxalase I and II. Glyoxalase pathway enzymes (Glyoxalase I and II) are required for glutathione based detoxification of methylglyoxal which is a potent toxic compound produced under stress. We have developed transgenic tobacco and rice that over-express both glyoxalase I and II which were found to tolerate higher levels of NaCl and were able to grow, flower and set seeds under stress conditions. The transgenic plants also showed tolerance to drought stress. We have found that rice genome contains 10 glyI genes and three glyII genes. One of the GlyI gene on chromosome 8 was found to be induced by MG and the encoded protein showed Ni dependent activity. Functional validation of this gene showed its role in salinity stress tolerance.

Our studies with glyoxalase pathway engineering and also with some other genes show that it may be possible in future to grow crop plants under unfavorable conditions like saline and arid regions.



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III

ABSTRACT OF
**Young Scientist
Award Programme**

Development of Light Sensitive Protein Tools for Clinical Application

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Rhodopsins are light sensitive retinal proteins. Recently, it has been shown that light-gated ion conducting rhodopsins (Channelrhodopsins) are useful in particular to control transmembrane ion fluxes and / or membrane potential of animal cells in culture, tissue or living species, simply by illumination (Optogenetics). The application of Channelrhodopsins in neuroscience and medical biotechnology has sparked the search for the new rhodopsin (s) with different spectral properties, ion selectivity and enzymehodopsin. This research paper reports the characterization of novel enzymehodopsins and hannelrhodopsins from newly sequenced algal genome (Chalamydomonas, Volvox, etc) databases. The principle, potential and challenges of these rhodopsins will be discussed in detail.



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IV

ABSTRACTS OF
Symposium and Invited Lectures

GENOMICS IN HEALTH AND DISEASE

Chairman : S.S. Parmar

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Key Note Lecture

Genomics in Health and Disease

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1. Isolation and Application of Human Brain Derived Neural Stem Cells to Study Neurotropic Virus Infections

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Keywords: Neural stem cells, Virus infections, JCV, HIV1

Cell cultures of human brain derived, multipotential progenitor cells can be directed to lineage commitments using growth factors in the cell media. If the progenitor cells are infected with neurotropic viruses such as JCV or HIV-1 moved to differentiate into astrocytes, viral infection proceeds rapidly. If the same population of infected cells is directed to become neurons, then viral

infection is abruptly altered and diminishes. Such a culture system allows investigation of cellular factors that support or block viral infection and determines not only cell fate in differentiation but also fate of infection. The temporal relationship between duration of infection and timing of differentiation can be followed also using cell identification by flow cytometry of infected cells.

2. A Biological Framework of Suicide

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Keywords: Suicide, Postmortem brain, Prefrontal cortex (PFC), Serotonin (5HT), Hypothalamic pituitary adrenal (HPA), Corticotropin releasing factor (CRF), CRF binding protein (CRF-BP), Glucocorticoid receptor (GR), Cytokines

Suicide is a major public health concern. Teenage suicide is an even greater concern as it is the second leading cause of death in the adolescents; however, its neurobiology is not clear. Abnormalities in serotonergic mechanisms have been implicated in suicidal behavior. There is a strong interaction between serotonin (5HT), the hypothalamic pituitary adrenal (HPA) axis and immune function. There is also an interaction between HPA axis and the immune function, which communicates with the brain through cytokines.

We have investigated the role of serotonin, HPA axis and the immune function in teenage suicide by determining the 5HT_{2A} receptor expression, components of the HPA axis, and the levels of pro- and anti-inflammatory cytokines in the postmortem brain of teenage suicide victims and matched control subjects. We observe that protein and mRNA expression of 5HT_{2A}

receptors is increased in the prefrontal cortex (PFC) and hippocampus of teenage suicide victims compared with normal controls. The protein and mRNA expression of the components of the HPA axis, such as CRF, CRF-BP, and GR are also altered in the PFC of teenage suicide victims compared with normal control subjects. We have also observed an increase in the levels of pro-inflammatory cytokines and a decrease in the levels of anti-inflammatory cytokines in the PFC of suicide victims compared with controls. Based on these observations, we are proposing a biological framework for suicide involving the abnormalities of serotonin, HPA axis and the cytokines, which communicate with each other and also affect and interact with each others functions. This biological framework for suicide will be presented and its implications discussed.

(Supported by NIMH RO1 48153) and Distinguished Investigators grant from the American Foundation for Suicide Prevention)

3. Therapeutic Potential of Sequence Specific 10-23 DNAzyme

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Keywords: HCV, INOS, TNF- α , Apoptosis

Gene-targeting using small molecule nucleic acid-based strategies is introducing prevailing possibilities to increase understanding of the regulatory mechanisms involved in the pathogenesis of disease as well as opportunities for therapeutic intervention. Our recent approaches to explore the therapeutic potential of 10-23 DNAzymes designed to cleave pathogenic RNAs in diverse systems indicate DNAzymes as promising candidates for drug development

in a wide range of diseases. The site specific cleavage of hepatitis C virus (HCV) genomic RNA, and its internal ribosome entry site (IRES) and RNA dependent RNA polymerase (RDRP/NS5B) coding regions of HCV genomic RNA by specific DNAzymes in HepG2 cells can be used in controlling the replication of HCV RNA. DNAzymes targeting inducible nitric oxide synthase (iNOS) mRNA effectively suppressed nitric oxide (NO) production and subsequent inhibition of tissue inflammation. In murine model of LPS induced lethal systemic inflammation intraperitoneal intervention of iNOS DNAzymes significantly improved the survival and inflammatory responses. DNAzymes mediated co-silencing of TNF- α receptors R1 and R2 in LPS stimulated THP-1 macrophages reduced the autocrine production of TNF- α and consequently induces apoptosis through caspase pathway indicating their roles in autoimmune diseases associated with TNF- α over-production. Among numerous nucleic acid-based biopharmaceuticals DNAzyme appears to be more versatile and potential therapeutics.

4. New Insights into Maternal Age and Origin of Trisomy 21 Down Syndrome

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Keywords: Trisomy 21, Altered recombination, Telomere, Nondisjunction, Maternal age

Down syndrome or trisomy 21 is one of the most frequent live born aneuploidy in human. We have investigated the effect of maternal age on the association between reduced meiotic recombination and nondisjunction in Indian sample of Down syndrome population.

We examined 138 families from the eastern part of India, each with a free trisomy 21 child. We genotyped each family by a set of microsatellite markers spanning along 21q from centromere to telomere, using PCR based method.

The stage of origin of nondisjunction and the recombination pattern of maternal chromosome 21 during oogenesis were characterized.

Our sample contains 107 maternal meiosis-I and 31 maternal meiosis-II errors and we subsequently stratified them with respect to maternal age and the number of detectable crossover events. We observed an association between meiosis I nondisjunction and recombination in the telomeric 5.1 Mb of chromosome 21. By contrast, in meiosis II cases, we observed preferential pericentromeric exchanges covering the proximal 5.7 Mb region, with interaction between maternal age and the location of the crossover. Overall reduction of recombination irrespective of maternal age is also evident in meiosis I cases. A genetic map was constructed, using maternal meiotic I nondisjunction data which was restricted to 21q. The distribution of chiasma showed a difference throughout the length of chromosome arm (21q) with more recombination towards telomeric end in comparison to control data. The telomeric exchange was found to be a significant risk factor for meiotic I nondisjunction, irrespective of the age of the mother. The linkage map of 21q (39.58cM) was significantly shorter than that of control female, indicating an overall reduction of recombination.

5. Impact of leishmania Glycosome and Host Peroxisome to Search for a New Chemotherapy Against Visceral Leishmaniasis

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Keywords: Leishmaniasis; Kala-azar; Glycosome; Peroxisome; Chemotherapy

Visceral leishmaniasis (VL) is one of the life threatening parasitic diseases caused by *Leishmania donovani*. Female sand flies are responsible to infect humans of the Mediterranean and adjacent countries with this pathogen in the form of flagellated promastigotes. However, aflagellated amastigotes are found inside and outside of the affected reticuloendothelial cells. Most of the affected countries are found to be located in the areas of tropic and subtropics but more than world's 90 percent cases of VL are reported to occur in India, Bangladesh, Nepal, Sudan and Brazil.

At this time there is no definite antileishmania agent, which may be used confidently to treat VL for a permanent cure. Drugs of choice available in the market, are being taken up selectively by trial and error methods. Most of them are too toxic to cause severe secondary infections and at the same time very costly to afford. Moreover, drug resistance is one of the acute problems to provide guarantee for a complete cure of VL. As a result there is an urgent need to explore for new drug development against this parasitic disease, which is lethal, if untreated.

Strategy to design an ideal new chemotherapeutic agent against any disease is to be oriented towards finding a definite target within the cellular network of the microorganism. Keeping this view in mind leishmania glycosome has been thoroughly investigated to show its uniqueness compared to the host cells. It has also been shown that host peroxisomes, which play a significant

role to monitor normal cellular properties, must be taken into account for new drug development against these dangerous protozoa. Again, prospective drug should be nontoxic to the host cells and must be of low cost to use. This requirement has been considered to evaluate a novel organic compound, which may be used for potential chemotherapy to treat VL commonly known as Kala-azar. Supportive documents in this regard will be presented to discuss.

6. Molecular Mechanisms of Drug Resistance in *Leishmania*

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Understanding the molecular mechanism of drug resistance is important not only for the development of molecular probes to monitor resistance in the field but also for a rationale drug design for the treatment of resistant cases. Resistance to Sodium Antimony Gluconate (SAG), first-line drug for Visceral Leishmaniasis, is a major cause of failure to therapy in over 60% of patients in India. Elemental requirement now is to explore the determinants of antimony resistance and protect the new drugs against development of resistance. Most of our understanding stems from work on laboratory mutants while the mechanism of resistance to antimony in the field is unknown. Membrane transporters including MRPA, AQP-1 and Thiols (GSH) have been shown to play a role in antimony resistance. Development of resistance to Miltefosine (MLF), the new oral drug for kala azar, seems a strong possibility due to its long half-life. Studies with lab generated MLF resistant parasites indicate the requirement of a novel P-type ATPase LdMT and its putative beta subunit LdRos3 for the drug translocation.

Our studies with field isolates of *L. donovani* revealed that the *in vitro* susceptibility to SAG correlates with the clinical response. Interestingly, drug susceptibility of the isolates to SAG, MLF, and Amphotericin B, significantly correlated with one another raising the possibility of cross-resistance. Gene expression analysis of LdMT and LdRos3 were suggestive of alternate mechanisms operative in the field. Transcriptome profiling of RNA from SAG

resistant and sensitive *L. donovani* revealed higher expression of genes encoding PSA-2, Histones (H1, H2A, H4), and MAP-kinase-1 in antimony resistant parasite. Investigations in a large number of field isolates revealed consistently high expression of both PSA-2 and H2A in all resistant parasites. Over-expression of PSA-2 or H2A in *L. donovani* resulted in conversion of SAG sensitive parasite into resistant phenotype. Further, *Leishmania* mutants over-expressing PSA-2 or H2A showed significantly lower susceptibility towards both Amphotericin-B and MLF pointing to the possibility of cross-resistance mechanism operative in the field.

7. Transcriptional Regulation of Ovarian Genes by the Homeodomain Transcription Factor Pitx2: its Implications in Ovarian Disorders

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Keywords: **Transcription factor, Gene expression, Pitx2, Transcriptional network**

Pitx2, a bicoid related homeodomain transcription factor, transcriptionally regulates gonadotropin genes in pituitary and plays a substantial role in cell growth and proliferation. But adequate information on Pitx2's expression and function in ovarian tissue is not available. We have shown that Plod2 gene, which encodes the key enzyme in collagen biosynthesis, is notably reduced in hypothyroid ovary. It is accompanied by less collagen biosynthesis. Our studies have implicated that Pitx2 is expressed in rat ovary and binds to Pitx2-specific bicoid element in Plod2 proximal promoter, suggesting a plausible Pitx2-Plod2 interaction in this tissue. By luciferase assay we have found increased level of Plod2 promoter activity having additional bicoids in SK-OV3 ovarian cancer cell line. Apart from Plod2, Pitx2 is likely to regulate various genes to execute its multiple functions and hence we have chosen ChIP-Chip as a viable means to identify Pitx2 target genes. Primary results of ChIP-Chip assay suggests

that Pitx2 binds to the cell cycle and growth regulatory genes, such as cyclin D1, -D2, cMyc and also genes that are linked with Wnt signaling in ovarian cell line. Our study also suggests that Pitx2 is expressed in different stages of ovarian development and interacts with other transcription factors in sex-specific manner. Presently we pursue our study about these interactions to get an idea of Pitx2 transcriptional circuitry present in ovary and to explore different ovarian disorders, including cancer.

8. Cytochrome P450s: A Biomarker for Predicting Chemical Exposure and Susceptibility

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Keywords: Cytochrome P450s; Biomarker; lymphocytes; Susceptibility; Expression

Cytochrome P450s (CYPs), involved in the metabolic activation and detoxification has long been used as a biomarker to predict susceptibility to environment induced diseases. Our studies have shown that variant genotypes of polycyclic aromatic hydrocarbon (PAH)-responsive CYP1A1, 1B1 and glutathione S-transferases (GSTs) modify the risk to tobacco-induced squamous cell carcinoma of head and neck (HNSCC) and lung. Our data demonstrated the importance of gene-gene and gene-environment interaction in determining the risk to these malignancies. To determine if the susceptible genotype changes are associated with phenotypes of increased resistance or susceptibility, studies were initiated to identify the expression and regulation of CYP isoenzymes in freshly prepared rat peripheral blood lymphocytes (PBL). Semi-quantitative and RT-PCR studies demonstrated constitutive and inducible mRNA expression of polycyclic aromatic hydrocarbon (PAH)-responsive CYP1A1, 1A2, 1B1 and

the associated transcription factors, aryl hydrocarbon receptor (AhR) and AhR translocator (ARNT) and ethanol regulated CYP2E1 isoenzymes in freshly prepared PBL. Absolute quantification using RT-PCR revealed several fold lower basal expression of CYP1A1, 1A2, 1B1 and 2E1 in PBL when compared to the liver enzyme. The increase in the mRNA expression was found to be associated with an increase in the protein expression of CYP1A1, 1A2 and 2E1 isoenzymes in PBL. Further, factors which modify these CYPs in animal tissue were also found to alter the activity in PBL. These studies were further extended to humans and it was found that lymphocytes CYP1A1 levels were increased in the lung cancer cases. The variant genotypes of CYP1A1 were found to be associated with much higher increase in the expression of blood lymphocyte CYP1A1 when compared to those with wild type genotypes. These studies have led us to conclude that blood lymphocyte CYP gene expression and activity could be used as a biomarker of exposure and biological effects. Associating and integrating expression and functional studies with genetically susceptible (sub) population can thus allow more precise identification of biomarkers that may help to identify disease and true risks of exposure.

Symposium on **Recent Advances in Biomedical Sciences:** **Emphasis on Heart and Lung Diseases**

Chairmen: P.K. Seth (India)¹ & Hari S. Sharma (The Netherlands)²

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1. Natural Products as Potential Treatments for the Human Metabolic Syndrome Culminating Cardiovascular Diseases

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Keywords: **Metabolic Syndrome, Cardiovascular Diseases**

The metabolic syndrome as the clustering of abdominal obesity, dyslipidaemia, hyperglycaemia, a prothrombotic state and hypertension increases the risk of cardiovascular and liver disease in humans. Animal models that mimic the range of pathophysiological changes seen in humans diagnosed with the metabolic syndrome are necessary for understanding the initiation and progression of the metabolic syndrome and the development of new treatments. Diets rich in carbohydrates and saturated fats contribute to

hypertension, cardiac damage, insulin resistance, metabolic defects, excess body weight, lipid abnormalities and the development of pre-diabetic or diabetic states. The metabolic changes and the structure and function of the heart and liver were characterized in young adult male Wistar rats fed a high carbohydrate/high fat diet for 16 weeks. These rats developed many of the chronic signs of the metabolic syndrome in humans including hypertension, impaired glucose tolerance, increased oxidative stress markers, plasma lipid abnormalities, excess deposition of abdominal visceral fat, increased waist circumference, ventricular dilatation with hypertrophy and decreased contractile function, impaired conduction, increased inflammatory cell infiltration into the myocardium and liver resulting in fibrosis, an increased stiffness of the heart, endothelial dysfunction as a decreased response to acetylcholine and increased plasma concentrations of liver enzymes. Further, treatment with curcumin, rutin or olive leaf polyphenols attenuated or reversed these structural and functional changes. Thus, this dietary-induced model of the metabolic syndrome in rats may provide a realistic and useful experimental tool to evaluate the development of symptoms and their reversal by potential treatments such as natural products.

2. Therapeutic Angiogenesis: Treating the Coronary Vessel Disease with Genes and Gene Products

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Keywords: Ischemic Heart Disease, Myocardial Angiogenesis, Vasculogenesis, Arteriogenesis

Inspite of the improvements in its medical and surgical management Ischemic Heart Disease (IHD) is still the leading cause of morbidity and mortality in the Western world. Over the past few years the better understanding

of the biology of vasculogenesis, angiogenesis and arteriogenesis has significantly increased the use of growth factors in the treatment and/or prevention ischemic heart disease by inducing blood vessel formation and thereby restoring / enriching the blood flow. Therapeutic angiogenesis makes use of the administration of a drug or angiogenic growth factor protein or gene to promote the development of endogenous collateral vessels in ischemic myocardium. Although growth factor therapy, primarily with VEGF and FGF has demonstrated great success in animal models, clinical trials have shown limitations in this treatment modality. Gene transfer and protein therapy are the two main approaches that have been used to achieve therapeutic angiogenesis. Most recently, interest has grown in the potential of cell-based gene transfer to induce myocardial angiogenesis. It is a promising new strategy, but still under its nascent stages, that uses autologous bone marrow cells or cultured stem cells transfected with a transgene of interest to express it *in vivo*. Although cell therapy holds promise for myocardial angiogenesis, many apprehensions still exist with regard to the methods currently available to track the transplanted cells, the capacity of these transplanted cells to phenotypically and functionally differentiate into cells of interest and whether they induce neovascularisation through secretion of growth factors. The right time / stage for the therapy, optimal gene delivery of the ideal angiogenic agent, appropriate design for the clinical trial and assessment of the outcome are still relevant questions that have to be answered. Science is quickening its pace towards better ideas in every field it has stepped into, particularly in the field of medicine. A time may not far when we might be able to effectively cure IHD. But, “prevention is always better than cure”.

3. Molecular Mechanisms of Angiogenesis in Heart and Lung

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Keywords: Myocardial Angiogenesis, Vascularisation, Vascular Endothelial Growth Factor, Fibroblast Growth Factor

Right ventricular hypertrophy and failure are common features in patients with tetraogy of Fallot (TF). Myocardial angiogenic gene expression profile was examined in patients with right ventricular hypertrophy and failure in order to assess myocardial vascularisation. DNA microarray analysis on right ventricular biopsies from TF-patients operated for primary corrective surgery (TF-1, n=8, mean age 0.5 year) and age matched controls was validated by Northern hybridization and RT-PCR. Employing immuno-histochemistry and video image analysis expression of vascular endothelial growth factor (VEGF), vascular density (by α -SMA and CD31 staining) and myocyte cross sectional area (Gomori's reticuline staining) were assessed in TF-1 and adult patients (TF-2, n=8, mean age 30 years) who underwent surgery for pulmonary regurgitation and compared the data with age matched controls. DNA microarray analysis revealed altered expression pattern for several genes including angiogenic factors, VEGF, flt-1, flk-1 angiopoietin-2, FGF-2, FGF-R1, PDGF-A, whereas, flt-4, Tie, TGF- β , TGF- β 3R showed decreased expression in TF patients. Northern blot analysis verified VEGF expression whereas, flk-1 expression remained unaltered in both TF-1 and TF-2 patients. VEGF staining in cardiomyocytes was increased in TF-1 as compared to TF-2. Video image analysis revealed enhanced vascular density with unchanged wall thickness and enlarged myocyte cross sectional area in both TF-1 and TF-2 as compared to respective age matched controls. Right ventricular hypertrophy is associated with an altered gene profile for a number of genes where the VEGF/VEGF-R system plays an important role in enhanced myocardial angiogenesis with limited vascular remodeling.

In another set of experiments, mechanisms of angiogenesis were studied using human Cell culture based in vitro models. We have shown that different

growth factors and cytokines result in differential gene expression and secretion of various proinflammatory cytokines and vascular endothelial growth factor (VEGF), an angiogenic molecule in cultured human ASM cells. To assess the role of airway smooth muscle (ASM) in bronchial angiogenesis and remodeling, we investigated the production of VEGF in ASM cells in relation to mediators of asthma, such as, IL-1 β , TNF- α , TGF- β , ANG II and ET-1. Time dependent release of VEGF protein in the conditioned medium was observed which in its turn induced proliferation and growth of pulmonary artery endothelial cells. We further investigated the effects of nitric oxide (NO) pathway on the pro-inflammatory cytokine; Interleukin-1 β (IL-1 β) induced expression and secretion of VEGF and PlGF. Serum deprived (for 48h) ASM cells were stimulated with IL-1 β (5 ng/ml), IL-1 β + N^w-nitro-L-arginine methyl ester (L-NAME), IL-1 β + L-arginin and IL-1 β + L-NAME + L-arginine for 4 and 24 h. NO synthase inhibitor (L-NAME) was used 1h prior to IL-1 β incubation in all experiments. IL-1 β induced expression (1.8 fold vs control) of VEGF mRNA (quantitative RT-PCR) was attenuated by L-NAME and augmented by L-arginine. L-NAME inhibited the secretion of VEGF and PlGF in conditioned media of IL-1 β treated PASMC at 4 and 24 h, respectively. By restoring NO pathway (L-arginine treatment) in L-NAME treated cells led to elevated (2.2 fold) expression of VEGF. Taken together, our findings suggest that a cytokine cascade involving mainly IL-6, IL-8 and VEGF operates in hyper contractile human ASM cells where NO pathway may modulate VEGF signaling during airway inflammation and subsequently contributing to bronchial angiogenesis and airway remodeling in patients with asthma and COPD.

4. Cardiac Regeneration

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**Keywords: Cardiac regeneration; Stem cells; Myocardial ischemia;
Cardiac failure**

Cell transplantation (cellular cardiomyoplasty) to repair or regenerate injured myocardium is the new frontier in regenerative therapy for heart diseases, and is a rapidly growing domain involving multiple enabling technologies. Over the years, a number of studies in experimental animals have shown that transplantation into the damaged heart with cells of various types-endothelial progenitor cells, embryonic stem cells, stem cells from cord blood and bone marrow, and skeletal myoblasts- can lead to effective angiogenesis and regeneration of ischemic and infarcted myocardium. Cardiac regeneration is expected to have an important impact also on the treatment of heart failure, a major cause of disability and death. Strategies currently being evaluated for regeneration of the damaged myocardium in heart failure are aimed at 'reviving' existing malfunctioning cells, repopulating the myocardium by new cells from exogenous or endogenous sources, altering the extracellular matrix, or increasing blood supply by enhancing vasculogenesis. Several small clinical trials have reported varying degrees of functional improvement.

The future of cellular cardiomyoplasty will depend on conducting carefully controlled, randomized clinical trials with appropriate selection of end points. Controversies exist over the specific cells to be used, the dosages needed for tissue repair, route of administration and how the transplanted cells would affect the electrical activity of the myocardium. Whether the cells after transplantation can improve myocardial function over long term is also not yet clear. The challenge in regenerative therapy in cardiac diseases is not simply to arrest cardiac dysfunction but is to achieve cell engraftment with electromechanical integration in to the heart, arrest adverse myocardial remodeling and improve contractility of the diseased heart.

Symposium on Innovative Technologies

Chairmen:

P.K. Seth¹ & Sandeep Saxena²

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1. FUNGISOME – An Innovative Nanosomal Amphotericin B: Optimizing Drug Effect and Minimizing Toxicity by Synergy of Liposome- and Nano-Technologies

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Amphotericin B is the most potent and broad-spectrum antifungal of all the drugs discovered in more than a century of global efforts. The drug, however, has dual reputation emanating from the experience of half a century of its clinical use; on one hand owing to its merits it earned the status of a Gold standard while the dose limiting and frequently fatal toxicities predominantly nephrotoxicity labeled it as *Ampho-The-Terrible*. Despite the nephrotoxicity in two thirds of the patients using Amphotericin B, hitherto no better drug could be discovered to replace it.

The only dosage form of Amphotericin B that could be formulated is an intravenous infusion presently known as “conventional Amphotericin B” - a

Sodium Deoxycholate- Amphotericin B colloidal suspension in 5% Dextrose. Decades of studies unraveled the ironic fact that each of the three constituents of the formulation contribute to the range of adverse effects including potentially fatal nephro-, cardio-, and neuro-toxicities.

Extremely limited solubility of Amphotericin B that prevented deviation from the conventional Amphotericin B recipe could be addressed to by the advent of Liposome Technology in mid 1960s. Through the decade of 1980s a novel Liposomal formulation was developed by replacing Deoxycholate of conventional Amphotericin B with a strategic mix of lipids that formed the matrix of the drug carrier Liposomes. The shift from conventional to Liposomal formulation helped improve efficacy from 33% to 76% and bring down nephrotoxicity 60% to 20%. However, much remained to be desired. In the decade of 1990s two other Liposomal/Lipid formulations with different lipid combinations and compositions were developed but remained short of expectations on efficacy and toxicity.

FUNGISOME developed in India by combining Liposome- and Nano-Technologies along with strategic changes in composition have given a Nanosomal Amphotericin B i.e. most broad-spectrum, potent at lower dose and yet nephrosafe at high dose making it the best antifungal ever developed anywhere in the world for the treatment of systemic as well as superficial fungal infections.

2. Lasers in Ophthalmology

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Ophthalmic lasers are presently used clinically for both diagnostic and therapeutic purposes.

Diagnostic lasers are based on a principle called optical coherence and are used to diagnose diseases of the retina, optic nerve and cornea and to calculate the power of the intraocular lens to be placed during cataract surgery. Laser

interferometry is now mandatory for power calculation in accommodative intraocular lenses used for presbyopic correction.

We and some others have been using in wave front guided LASIK, an aberrometer is used to calculate pre-existing errors in the human eye using a collimated laser beam. We observed that Wave-front guided LASIK increased visual acuity and contrast sensitivity in more than 70% of patients and 50% of the patients obtained more than 20/20 vision.

Therapeutic lasers use a large portion of the electromagnetic spectrum ranging from the ultraviolet portion through the visible wavelengths and into the near and far infrared areas. The interaction of specific laser emission of different wavelengths with various ocular tissues causes distinctly different tissue changes, which include laser photocoagulation, photodynamic therapy, photovaporization, photodisruption and photoablation.

Laser photocoagulation using the Argon or frequency doubled YAG laser, is most widely used in the management of diabetic retinopathy. We observed that in large clinical trials timely photocoagulation can prevent blindness in more than 90% of diabetics, and is playing a major role in preventing blindness among the millions of diabetics in India.

Laser photodynamic therapy, done in conjunction with intraocular injection, has completely revolutionized the management of macular degeneration. Clinical trials show that it stabilizes or improves vision on more than 80% of patients with age-related macular degeneration.

In our studies involving 150 patients we observed that collagen cross-linking using an ultraviolet laser light prevents progression of Keratoconus in 100% of the patients where a progressive thinning of the cornea leading to severe decrease in vision.

The presentation will include some of our experiences with this new technology in bringing benefits to the patients.

3. **Stem Cells in Degenerative Disease: A Move Forward**

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**Keywords: Mesenchymal and hematopoietic stem cells,
Cryopreservation, Autologous and allogenic sources,
Neurodegenerative diseases.**

Stem cells therapies- A new dawn in modern medicine, though still known as experimental therapy, it can promise a better quality of life for patients suffering from degenerative diseases.

The stem cells are called master cells of human body. They can give rise to more stem cells (self renewal) and to different cells of various systems (differentiation) hence they play vital role in repair and rejuvenation. In normal functioning human body damages are healed by these stem cells. They are normally circulating in blood and are attracted towards damaged area as they can sense the cytokines secreted from such damaged parts and also oxygen depleted area. In severe or chronic diseases the damaged tissue and number of stem cells circulating does not match so the repair is not as expected.

The new advancement in modern medicine is stem cells therapy. Though still known as experimental therapy it is a promising therapy with no documented adverse side effects. In stem cells therapy these stem cells are harvested from rich sources in the body and are injected at the site of injury. Since they are injected in more number they help in faster repair. The three commonly used rich sources in an adult human body are umbilical cord blood, bone marrow and peripheral blood. There are mainly two types of stem cells found in these 3 sources mesenchymal and hematopoietic stem cells.

These separated stem cells are stored at -196°C known as cryopreservation. This storage maintains the viability of cells. Till date StemOne Biologicals Pvt. Ltd., Pune has successfully stored 3000 units of stem cells from various sources.

Recently StemOne has started giving autologous bone marrow derived stem cells therapy. Till date we have successfully given therapy for thirty degenerative diseases. To name a few cerebral palsy, spinal cord injury etc. We have done total 250 infusions so far. We are associated with ten hospitals in and around Pune for cell based therapies.

We have done 68 cases of Neurodegenerative diseases. Enriched autologous BMMNCs were given to these patients intrathecally. They are showing on average 25 % recovery. For all these patients the dosage and count of cells were different dependent on their condition but they all are showing good results.

Other public stem cells banks & institutes are using allogenic sources of stem cells. These can be mainly used for genetic disorders like Thalessemia, DMD etc.

4. Personalized Medicine, Selecting the Right drug and the right dose based on the Genetic Profile of a Patient

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Keywords: Pharmacogenomics, Genetics, Genes, Diagnostics, SNPs, Personalized medicine, Cancer, Cardiology

We all are different because of our genes. These differences lead to difficulties in medical management of the disease in terms of selecting a drug and prescribing the write dose for the patient. Some patients benefit from a drug, while other show severe side effects. This can be avoided by performing

a genetic test on patients before prescribing strong drugs for diseases like Cancer, AIDS, asthma, diabetes, TB, CNS and cardiovascular disorders. In recent years we have witnessed a drastic drop in genetic testing prices and now such tests are affordable to a common man. Uses of such tests are of inestimable advantage to the treating physicians as they enable him to select an effective and safer drug for treatment at affordable cost. Further it saves the patients of unnecessary side effects and complications of getting exposed to ineffective drugs.

We initiated studies in this direction in 2006 and have invested close to 2 years in literature survey, developing protocols, internal and third party validation, quality control, and market analysis.

Acton Biotech is the first and only company in India to offer these tests even today. We initiated our studies with cancer, as therapeutic agents for this unfortunate disease are not only expensive but are toxic associated with severe deleterious toxic side effects with a narrow therapeutic index. We are undertaking tests for drug metabolizing enzymes, drug targets, transporters and receptors. Currently we are offering tests to identify patients who are at greater risk of developing severe toxicity from commonly used chemotherapy and targeted therapy. We are testing for commonly reported mutations in EGFR, KRAS, TPMT, DPD, MTHFR etc. using simple RFLP based PCR technology in patient samples. Recently the company has launched tests for Cardiology, Haematology, organ transplant and immuno disorders. Samples are collected from all over India and analyzed them in our laboratory in Pune. Our mission is to make medicines safer and effective. Gradually these services will be extended for other complicated diseases like Asthma, Diabetes, TB, AIDS, and CNS disorders.

5. Therapeutic Potential of Novel Natural Biomaterials in Preventive and Regenerative Wound Care

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Several advancements have been made in the area of wound care in last few decades, however pre-hospital wound care and continuous wound care are still underserved globally. Most pre-hospital trauma wounds are unable to be treated with conventional interventions, which lead to loss of life before reaching a clinical facility. Where as in continuous wound care, infections like MRSA poses serious survival issues.

With advent of certain novel biomaterials coupled with advanced bio-engineering processes, provides better alternative and efficient solutions in wound care. These active natural biomaterials include but not limited to Alginates, Hyaluronic acid, Chitosan, Carageenan and Silk fibroin. Availability from natural sources and properties like non-immunogenicity, biocompatibility, charge density, biodegradability, mucoadhesiveness and no risk of viral infections makes them desirable for various biomedical applications. These characteristics make them ideal candidates to be used in tissue engineering, drug delivery, permeable membrane systems, tissue sealants etc.

Axio develops wound dressing scaffold from high molecular weight natural biomaterials. Fabricated into 2D scaffolds with interconnected pores, it serves as a robust external hemostat, anti-microbial dressing, burn cover and aid in tissue regeneration. It exhibited high hemostatic efficiency on rabbit model where arterial bleed hemostasis was achieved in less than 4 minutes thus sealing the wound. Successfully tested for Cytotoxicity and Skin irritation, these scaffolds were also being used for stem cell seeding, cell culturing for regenerative therapy and found to be an excellent delivery matrix for stem cell based tissue engineering.

SPECIAL LECTURE**1. Molecular Biodiversity of few Bacterial and Fungal Strains from North East India and their Functional Properties****B. G. Unni*, Yelena Kakoty, & Basab Rani Devi***Biotechnology Department, North-East Institute of Science & Technology (CSIR),
Jorhat 785 006, Assam, India*** Email: bgunni@rrljorhat.res.in***Keywords: Biodiversity, Bacteria, Fungi, RAPD**

Soil constitutes the major habitat of terrestrial microorganisms. Among these, bacteria are most abundant followed by fungi. The diverse physiography and eco-climatic conditions of North East India has made it one of the mega biodiversity hotspot, bestowed with a wide range of variety of microorganisms. The conventional methods for species-specific identification and characterization of microbial strains are time consuming and prone to errors. Therefore for rapid and accurate identification and characterization of wide variety of microbes, PCR based diagnostic tools are used. Bacterial strains were isolated from selected spots from the southern Brahmaputra corridor of Assam, among which 20 strains are α -amylase producing bacteria. RAPD was done from the genomic DNA extracted from selected bacterial strains. Based on the RAPD result, cluster analysis was generated. One novel strain of *Staphylococcus sp.* was isolated, using consensus primers, the ~1.5 kb 16S rDNA fragment was amplified and the PCR product was sequenced. Sequenced data was analyzed to find the closest homolog of the bacteria. The extracellular protein (coagulase) extracted from *Staphylococcus basab R.* culture was further subjected to molecular separation techniques and studied its properties. Eight strains of *Fusarium sp* were also isolated from wilt affected tomato, potato, cabbage and ginger plants, from different parts of North East India. For rapid identification and characterization of the *Fusarium* isolates, PCR-based

molecular techniques have been used. Based on RAPD profile, phylogenetic tree of the *Fusarium* strains has been generated and the antifungal properties of extracts of *Piper betel* L will also be presented and discussed.

Acknowledgement:

Authors are thankful to Dr. P. G. Rao, Director, North-East Institute of Science & Technology (CSIR), Jorhat, Assam and the Director, Defence Research Laboratory (DRDO), Tezpur, Assam, for encouraging the projects. The authors are also thankful to Department of Science & Technology (DST) and Defence Research and Development Organization (DRDO), Govt. of India for financial support.

2. Curcumin: A Neuroprotectant with Multiple Biological Activities

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A great impetus has been given on curcumin, a polyphenolic diketone present in turmeric in recent years due to its multiple pharmacological properties. Reports that curcumin crosses the blood brain barrier suggested its use to investigate its neuroprotective potential in experimental models of neurodegenerative disorders and neurotoxicity. Studies were carried out to investigate the neuroprotective efficacy of curcumin in rat model of middle cerebral artery occlusion (MCAO, ischaemia) and arsenic induced neurotoxicity. Pre- and post-treatment with curcumin (100 mg/kg, p.o., 5 days prior and 3 days after middle cerebral artery occlusion) in ischaemic rats was found to improve rota-rod performance and grid walking, decrease oxidative stress and reduce intracellular calcium levels in corpus striatum and frontal cortex as compared to ischaemic rats. Simultaneous treatment with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) and curcumin (100

mg/kg body weight, p.o., 28 days) caused an increase in locomotor activity and grip strength and improved the rota-rod performance in comparison to arsenic treated rats. Binding of striatal dopamine receptors and TH expression increased while arsenic levels and oxidative stress decreased in brain regions of rats simultaneously treated with arsenic and curcumin as compared to those treated with arsenic alone. A significant protection in behavioral, neurochemical and immunohistochemical parameters in curcumin treated ischaemic rats and those simultaneously treated with arsenic and curcumin suggests the neuroprotective efficacy of curcumin.

3. Industrial Enzymes – the tools for Bioprocesses: Role and Significance of Microbial Cellulases in Biorefineries

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The elaborate networks of reaction brought about by ‘Enzymes’ are basis for the continuity of the living world that has evolved over the course of time to ensure the survival and reproduction of organisms and diversity of life. The enzyme specificity is one of the most important biological phenomenon, without which ordered metabolism of living matter would not exist and life would be impossible. They offer efficient biocatalytic conversion potentials to technologies in diverse industries. Enzymes are generally intracellular (or extracellular). Later are usually robust and tolerate range of conditions compared to intracellular ones. Robustness of the immobilized enzymes enabled them to be used for various purposes ranging from environmental cleanup to industrial biocatalysis.

An important aspect of the biotechnological research and development in this regard is targeted towards the advanced understanding of the structure and function of the enzymes to broaden its application insight. Advances in the tools such as the genetic engineering have made it possible to manipulate

the microorganisms at genetic level to produce the enzymes in high titers with desired properties. The largest segment of the markets for enzymes is in food applications and therapeutic enzymes are the fast growing segment in enzyme industries. Enzyme technology is still a developing technology with great potential in diverse industries.

Cellulases are the second most important industrial enzyme following amylase; sharing a major part of the enzyme market. Among its vast application in textile, detergent and paper industries; bio-ethanol production from lignocellulosic biomass has boost up the research in this direction. Cellulases are enzymes which hydrolyze cellulose (b-1, 4-D-glucan linkages) and produce as primary products glucose, cellobiose and cello-oligosaccharides. Cellulases are produced by a number of microorganisms and comprise several different enzyme classifications. In the hydrolysis of native cellulose, it is known that there are three major types of cellulase enzymes involved, namely Cellobiohydrolase (CBH or 1,4- b-D-glucan cellobiohydrolase, EC 3.2.1.91), Endo-b-1,4-glucanase (EG or endo-1,4-b-D-glucan 4-glucanohydrolase, EC 3.2.1.4) and b-glucosidase (BG-EC 3.2.1.21).

Considerable amount of work has been done on fungal cellulases, especially with resurgence of interest in biomass-ethanol and concept of bio-refineries. The biological aspects of processing of cellulosic biomass become the crux of future researches involving cellulases and cellulolytic microorganisms. Significant information has also been gained on the basic biology of organisms producing cellulases, and in process development for enzyme production and biomass saccharification.

The problems which warrants attention is not limited to cellulase production alone, but a concerted effort in understanding the basic physiology of cellulolytic microbes and the utilization of this knowledge coupled with engineering principles to achieve a better processing and utilization of this most abundant natural resource. A major limitation of most of the cellulolytic system is the relatively lower amount of β -glucosidase and its feedback inhibition by glucose, which necessitates development of cocktail for efficient hydrolysis of lignocellulosic biomass. There could be several approaches to address this particular problem. Few workers have tried to drive the expression of the *BGL* gene and the secretion of the protein product by employing *CBHI* promoter of *Trichoderma reesei* and xylanase secretion signal, resulting in some dramatic increase in the enzyme yield. This strategy probably could help to reduce the amount of cellulases needed for saccharification, but further improvements are needed in increasing the glucose tolerance of beta glucosidase.

4. Marine Chemical Ecology: An Insight into Discovering drugs from the Sea

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In the marine environment, biodiversity in coral reefs is higher than that of tropical rain forests. Many marine organisms living in the highly competitive ecological niche are soft bodied and have a sedentary life style necessitating various means of defense. Apart from mechanical or behavioural adaptations, there are a great number of marine invertebrates that produce or sequester secondary metabolites. Secondary metabolites from marine organisms have been hypothesized to function as Toxins, Feeding Deterrents, Antifouling Agents, Antimicrobial Agents, Settling Cues, Morphogens and compounds that mediate Competitive Interactions between organisms. In the coral reef environment, many invertebrates, especially sponges, coelenterates, bryozoans, molluscs and tunicates employ secondary metabolites for such interactions. In addition some of the natural products isolated from marine invertebrates have been shown to be of microbial origin. Marine microorganisms, whose immense genetic and biochemical diversity is only beginning to be appreciated. Research into the ecological value of marine natural products has shown that many of these compounds function as chemical weapons and have evolved into highly potent inhibitors of physiological processes in the prey, predators or competitors of the marine organisms that use them.

Over the past five decades, about ~21,000 compounds from various marine sources have been reported and about 50 compounds have entered clinical trials. One of the breakthroughs in drug discovery was the use of mechanism-based screening for bioassay-guided fractionation. Through continual improvement of screening formats, reagent production, robotics, and data management, mechanism-based screening has since become the mainstay of high-throughput screening (HTS). The large array of bioactive natural products derived from marine organisms are Molecules targeting ion channels, Compounds targeting enzymes, Microtubule-interfering agents, DNA-interactive agents, Oxidative stress inducers, Immunostimulatory agents, Calcium-binding protein antagonists etc., which indicates that the organisms

synthesize these diverse physiologically active secondary metabolites for their effective survival. The evidences from in situ evaluation to ascertain the chemical defense which is indicative of unique molecules and in vitro mechanism-based screening suggest that, in the future, the oceans will become an important source of novel chemical classes not found in the terrestrial environment. The success rate of finding a new active chemical is 500 times higher in marine organisms than from terrestrial sources.

In recent years, marine natural product bioprospecting has yielded a considerable number of drug candidates. Most of these molecules are still in preclinical or early clinical development but some are already in the market. The promising marine derived drug candidates are ecteinascidin **ET743** (**Yondelis**TM) derived from *Ecteinascidia turbinata* being marketed in the European Union for the treatment of soft tissue sarcoma and licensed to Zeltia S.A and Ortho Biotech Products L.P.; **Æ-941 (Neovastat)** derived from shark is under clinical trial Phase II/III (cancer) and exhibits antiangiogenic activity as well; **Ziconotide (Prialt**TM) from *Conus magus* is a non-opioid and non-NSAID analgesic (neuropathic pain) licensed by Elan to Warner Lambert; **GTS-21** (aka **DMBX**) modification of a Nemertine worm toxin in Phase II (Alzheimer's) licensed to Taiho by Univ. Florida; **HTI-286** (hemiasterlin derivative) from *Cymbastella* sp. in Phase II (cancer) made by Univ. British Columbia and licensed to Wyeth; **kahalalide F** derived from *Eylsia rufescens/Bryopsis* sp. is under Phase II (cancer) licensed to PharmaMar by Univ. Hawaii; **Aplidin**[®] is currently in phase II clinical trials for solid and haematological malignant neoplasias like multiple myeloma; **discodermolide** derived from *Discodermia dissoluta* under Phase I (cancer) licensed to Novartis by Harbor Branch Oceanographic Institution; **bryostatin1** from *Bugula neritina* under Phase II (cancer) now in combination therapy trials, licensed to GPC Biotech by Arizona State Univ.; **IPL-576,092** (aka HMR-4011A) derived from *Petrosia contignata* is under Phase II (antiasthmatic) is a derivative of contignasterol, and licensed to Aventis., **Pseudopterosin A-methyl ether** derived from *Pseudoptero-gorgia elisabethae* and licenced to Terosin Group Inc./Univ. of CA in the Clinical trial Phase II for Wound Healing, to name a few.

A multidisciplinary approach to drug discovery through chemical ecology, and involving the generation of truly novel molecular diversity from natural product sources, combined with total and combinatorial synthetic methodologies, and including the manipulation of biosynthetic pathways (so-called combinatorial biosynthesis), manipulating functional genes of marine microbial symbionts, aquaculture of prospective marine organisms will provide the best solution to the current productivity crisis facing the scientific community engaged in drug discovery and development.

5. Lectinomics and Mass Spectrometry – A New Systemic Approach for Diagnosis of Diseases

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Keywords: **Diagnosis of Diseases, Lectins, Mass Spectrometry**

The carbohydrate specificities of different lectins offer affinity approach in the biological systems that complement existing mass spectrometry technique and retain automated throughput options. Serum samples from autoimmune diseases, liver diseases, prostate cancer and liver cancer provided along with suggested experimental strategies for integration of lectin-based methods into clinical fluid expression strategies. The present topic discusses the necessity of early validation in biomarker discovery using an immunoaffinity-based targeted analytical approach that integrates well with upstream discovery technologies.

6. Transplanted Embryonic Stem Cells Repair and Regenerate Injured Myocardium

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Keywords: **Stem cell therapy, Myocardium regeneration,
Embryonic stem cell**

Stem cell therapy holds a potential for the treatment of heart diseases, and we tested the capacity of the transplanted embryonic stem (ES) cells to engraft and regenerate myocardium following myocardial infarction (MI) in mouse hearts. Mouse ES cells were delivered via direct injections into the heart. We examined evidences of engraftment and newly differentiated heart cell types of transplanted ES cells and their effects cardiac remodeling (apoptosis, fibrosis and hypertrophy).

We also determined if released factors from transplanted stem cells stimulate endogenous cardiac resident stem cells and enhance cardiac regeneration. In fact, transplanted ES cells differentiate into all three major heart cell types such as cardiomyocytes, vascular smooth muscle cells and endothelial cells. A significant decrease in cardiac apoptosis and fibrosis was also obvious in the ES cells transplanted groups compared with control group. Moreover, factors released from stem cells stimulated cardiac and vascular progenitor cells and differentiated into cardiac myocytes and enhanced neovascularization. Furthermore, cardiac function determined with M-mode echocardiography revealed an improvement in function. Mechanisms of regenerated myocardium were also examined by producing simulating cell culture model. Using cell culture model, we demonstrated that factors released from ES-cells inhibit apoptosis. We identified released factors and observed that mechanisms of inhibited apoptosis are mediated through cell survival and Akt pathway. Our data suggests that factors released from stem cells as well as differentiated ES cells following transplantation can regenerate the injured myocardium and also demonstrate cardiac repair by decreasing apoptosis, fibrosis and hypertrophy resulted into improved cardiac function.

7. Metabolic Control of Angiogenesis Through Modulation of VEGF

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Angiogenesis involves complex interactions among different types of cells, particularly endothelial cells, extracellular matrix and soluble growth factors which lead to endothelial cell proliferation, migration and tube formation. Neovascularisation contributes to the progression of disease in a number of pathologies such as tumour growth, diabetic retinopathy, rheumatoid arthritis, while other conditions such as ischemic disease, infertility are associated with insufficient angiogenesis. Vascular Endothelial Growth Factor (VEGF) is a key endothelial specific mitogen that regulates the process of angiogenesis. The VEGF activates endothelial cells by signaling through specific VEGF receptors that are located on vascular endothelium and are up regulated during angiogenesis. Expression of VEGF is regulated at transcriptional and posttranscriptional level by a number of factors including the oxygen status. Hypoxia inducible factor and other transcriptional factors such as NFkB regulate VEGF gene expression. The focus of this presentation is the post translational modification of VEGF, particularly related to our findings on poly ADP ribosylation which modulates the angiogenic potential of VEGF. Metabolites such as lactate which accumulate in tumour cells and basement membrane protein such as laminin promote angiogenesis through modulation of the angiogenic potential of VEGF by regulating PAR modification of VEGF.



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ABSTRACTS OF
Poster Presentations

1. The Effect of VAM on the Growth of Groundnut Crops in Ahmednagar District

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Key words: **VAM, Percentage infectivity-level, Micronutrients, Groundnut**

Vesicular-Arbuscular mycorrhizae (VAM) enhance plant growth through increased nutrient uptake, stress tolerance and disease resistance. Roots support a multitude of microorganisms that, in concert, can have profound influence on growth and survival of the plant. VAM-positive plants are known to exhibit resistance towards soil-borne and foliar pathogens. The purpose of this review is to discuss VAM infection in groundnut field in the areas of the Ahmednagar district and in the pot culture. Based on these observations, a working model is proposed to explain the VAM infection, Effect of VAM on the growth of plant, root and shoot length on the groundnut crop and VAM interaction under varied environmental conditions.

2. Downstream Process Development to Extract Biotransformed Colchicine from Fermented Broth

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Key words: **Downstream process, Colchicine, Biotransformation, Fermentation, Anticancer drug**

Colchicine is a natural alkaloid has an anti-mitotic property. Derivatives of colchicine i.e., 3-demethylatedcolchicine (3-DMC), and colchicoside are proven to be an effective anticancer drug. After a completion of 72 h, the fermentation was terminated; 3-DMC and 3-DMTC were extracted by polar solvents. Methanol was noticed to be best solvent in which more than 90 % product could be extracted. The process of purification was standardized, and alumina (acidic) with 300 mesh size was proven to be the most suitable material for column chromatography. The final product 3-DMC was noticed to be 4.3 g/l, when 7g/l colchicine used as substrates.

3. Optimization of Parameters for High-Cell-Density Fermentation and Extraction of CoQ10 by *Paracoccus Denitrificans* ATCC-1222

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Key words: **Fermentation, Downstream process, Ubiquinone-10, Paracoccus denitrificans.**

Ubiquinone-10 or CoQ10 is a naturally occurring co-enzyme, which helps in the transfer of electrons in the mitochondrial or cytoplasmic membrane. Ubiquinone-10 is also regarded as an essential component for ATP generation in oxidative phosphorylation process. Recent research on ubiquinone shows its many therapeutic uses ranging from anti-oxidants to treatment of heart disease. Among the various strains exploited for ubiquinone-10 production, we chose *Paracoccus denitrificans* for producing ubiquinone-10. Various parameters for medium, culture conditions and operational parameters viz., carbon and nitrogen concentration, pH, temperature, DO, OUR, aeration rate, back pressure were optimized for achieving maximum growth rate/ high-cell-density. Additionally, we have also optimized a suitable downstream process for maximum extraction of ubiquinone-10.

4. Preparation and Properties of Galactomannan Nanoparticles

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Key words: **Nanoprecipitation; Galactomannan; Guar gum;
Locust bean gum**

Nanoparticles from locust bean gum and guar gum were developed which are stable, neutral, non-toxic, bio-compatible, sterilizable and bio-degradable. The nanoparticles were prepared by nano precipitation and cross linking method. Nanoparticles act as potential carries for several classes of drugs and cosmetics. It was found that the formation of nanoparticles depends upon the molecular mass of the galactomannan, the solvent, the non-solvent, surfactant, cross linker and the agitation method. The particle sizes were analyzed by light scattering, scanning electron microscopy and transmission electron microscopy studies. Optimized method produced spherical nanoparticles of around of 40-100 nm with a polydispersity index in the range 0.1-0.4.

5. mi-RNA Target Prediction and its Testing using SVM in Plants (*Oryza Sativa* a Case Study)

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Keywords: mi-RNA, SVM Rice, mi-RNA Computation

MicroRNAs (miRNAs) are small noncoding RNAs, which play a significant role in posttranscriptional regulation. The function of plant miRNA is generally based on complementarity of their component. Although several computational miRNA target methods have been proposed, they still have limitations in revealing actual target gene sites. Identification is stage specific, so one cannot clone miRNA for every developmental stage simultaneously and cloning of small mi-RNA is very tedious, expensive and time consuming. Further, a differentiation between degraded probe of miRNA and miRNA is not possible. Hence the computational identification of miRNA is important to overcome all such experimental limitations. We carried out global computational analysis of rice (*Oryza sativa*) UTRs to generate a comprehensive list of putative miRNA targets and then tested it with SVM lite. Our predictions (500 unique transcripts) showed that rice miRNAs mediate regulation of diverse functions including transcription (38%), catalysis (20%) and binding (15%). It was observed that more than half (52%) of the targets were conserved between the other species of *Oryza*. In our work we implemented a support vector machine classifier for miRNA target prediction. The categorization is done on the basis of three broad features: structural, thermodynamic, and position-based feature. At current level we analyzed 27 miRNA. UTRs sequences for predicting miRNA target that gave 143 possible targets and the linear kernel gave 81.143% accuracy by testing through SVM.

6. Hypolipidemic and Hepatoprotective Effects of *Ficus Bengalensis* Aerial roots in Severely-diabetic Animals

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Keywords: Aerial roots, *Ficus bengalensis*; Hypolipidemic; Hepatoprotective; Moraceae; Severe-diabetic

The present study is an extension of our previous work carried out for evaluation of antidiabetic potential of *Ficus bengalensis* aerial roots, and it deals with further scientific exploration of hypolipidemic and hepatoprotective effects in STZ induced severely-diabetic animals. Severelydiabetic animals were treated once a day for one month with the most effective dose, identified in previous study and were found to reduce the fasting blood glucose (FBG) and urine sugar (US) from their pretreatment levels substantially. The same dose brought about significant fall in their lipid profile too by reducing total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels. However, there was a significant rise in high-density lipoprotein (HDL) level. Levels of hepatoprotective enzymes viz. serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALKP) were decreased significantly in addition to creatinine (CRTN) with the same dose. Increase in haemoglobin (Hb), total protein (TP) and body weight (bw) were additional observations. These results clearly indicate that the aqueous extract of aerial roots of *Ficus bengalensis* possess hypolipidemic and hepatoprotective effects in addition to highly significant antidiabetic effect in severely-diabetic rats.

7. Studies on Antimicrobial Substance Producer

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Now a days we use different Antibiotics for treatment of different diseases but problem of resistance strain is created. Microorganisms are getting adapted to various antibiotics. It is necessary that screening of new antimicrobial substance producer.

Present study was done on studies of antimicrobial substance producer from different soil samples. The primary and secondary screening was carried out for two isolates. In secondary screening Spot on lawn and Giant colony technique was carried out .The test organisms were purchased from NCL (National chemical Laboratory).

8. Studies on Cellulase Production Using Actinomycete

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In the world of Biotechnology different microorganisms are used for the production of cellulose. Cellulase is mostly used in industries. Cellulase is an extracellular enzyme. Cellulase acts on cellulose and converts it into monomer units of glucose.

Present study is done on cellulose producing actinomycete obtained from different soil samples. Soil isolates were screened for cellulolytic activity. One is selected for optimization of pH and fermentation time.

9. Poly (adp-ribosyl) Ation is not Critical for Secretion of Vascular Endothelial Growth Factor

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Angiogenesis is required for the establishment and maintenance of vascular supply to both normal and neoplastic tissues. Vascular Endothelial Growth Factor (VEGF) is one of the major factors, which induce angiogenic phenotype. One of the key regulatory mechanisms of VEGF is poly (ADP-ribosyl)ation (PAR modification). The objective of this study was to examine how PAR modification affects the production of VEGF. Increase in the angiogenic potential in CAM assay on inhibiting PAR modification of VEGF using 3-aminobenzamide confirmed that biological activity of VEGF depends on PAR modification. Inhibition of PAR modification in HUVECs did not affect the rate of secretion of VEGF, whereas inhibition of glycosylation by 2-deoxy glucose decreased the VEGF secretion. Thus it appears that the two post translational modifications of VEGF namely glycosylation and Poly (ADP-ribosyl)ation occur independently and poly(ADP-ribosyl)ation is not critical for secretion of VEGF.

10. Catecholamines: Role in Adipose Tissue Angiogenesis

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Adipose tissue angiogenesis, the formation of new blood vessels, is a major process in adipose tissue development. Developing blood vessels in adipose tissue represents a potential target for regulating adipose tissue development and thus obesity. Hormones particularly catecholamines appear to influence adipogenesis. To elucidate the role of adrenaline in adipose tissue angiogenesis, we studied its effect on epididymal white adipose tissue. Adrenaline stimulated endothelial cell sprouting and tube formation from the stromovascular fraction of epididymal fat pads. Further studies to elucidate its mechanism showed that 1) adrenaline stimulated cell-cell contact and tube formation in HUVECs, 2) stimulated the expression of angiogenic markers such as CD31 and E-Selectin, 3) induced Von Willebrand factor secretion. Together, these observations suggest that adrenaline has a regulatory role in adipose tissue angiogenesis and adipogenesis.

11. Elucidation of Primary Structure of an Adipokinetic Neuropeptide of the Coffee Locust, *Aularches Miliaris* L.

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Key words: Adipokinetic neuropeptides, Aularches miliaris, MALDI-TOF-MS/MS

In insects, the retrocerebral complex comprises the corpora cardiaca (CC) and the corpora allata (CA). The CC act as a neurohaemal organ, storing and releasing neuropeptides produced in the brain. The CC also contains intrinsic glandular cells that produce and release several neuropeptides including adipokinetic hormones (AKHs). The present investigation reveals the presence of adipokinetic neuropeptides in the coffee locust, *Aularches miliaris* Linnaeus. The corpora cardiaca extract was fractionated by HPLC, monitoring the eluants both at 210 and 280 nm. Fractions were collected, dried and tested for adipokinetic activity by a homologous *in vivo* bioassay. Three UV absorbance peaks were found to contain materials with significant adipokinetic activity. MALDI-MS analysis of the extract indicated that the molecular mass, 1167.16 Da, is similar to the already known AKH from the insect, *Phymateus morbillosus*. MALDI-MS/MS analysis confirmed that their primary structures are exactly similar to the structures reported for the Phymo-AKH-I (pE-L-N-F-T-P-N-W-G-S-NH₂). Since *A. miliaris* is a pest of economically important plants, the knowledge of primary structure of its AKH may be helpful in designing mimetic antagonistic and agonistic analogues of these peptides, which may be useful for the control of this pest as well as pests of related groups, employing biotechnological tools.

12. Role of Cardiac Specific 182 kda Protein in Differential Expression of Genes Induced by Cardiac Hypertrophy in Diabetes

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Diabetes mellitus is a world wide epidemic. Coronary heart disease (CHD) is the major cause of morbidity and mortality in diabetic patients. Cardiac adaptation via cardiac hypertrophy involves triggering of several quantitative changes, which include changes in gene expression profile. Studies have shown the involvement of a high molecular weight serum protein (182 kDa) in the development of cardiac hypertrophy. Real time PCR analysis of gene expression revealed the upregulation of genes like beta MHC, MLC-2 and skeletal actin and a down regulation of genes such as cardiac actin and alpha MHC in diabetic group and also in presence of 182 kDa protein.

13. Histomorphological Effects of Sublethal Concentration of Carbaryl in the Male Reproductive System of Plant bug, *Iphita Limbata* Stal. (Heteroptera: Pyrrhocoridae)

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**Keywords: *Iphita limbata*, Carbamates, Carbaryl,
Male reproductive system**

Carbamate compounds are preferred over other pesticides in the recent past because of their reversible inhibitory effect on acetylcholinesterase. Topical application of carbaryl (1-naphthyl N-methyl carbamate) to the plant bug *Iphita limbata* at a sublethal concentration (0.6 mg) showed histomorphological changes in the testes and mesadenes. The cytoplasm of spermatocyte cysts shows small vacuoles, loose arrangement of spermatids and clumped spermatozoa in the treated insects. The nuclei of the outer wall of the testes show vacuole formation and degenerating nuclei. The nuclei in the outer wall of the testes are deeply stained and not clearly visible in treated condition. Large number of vacuoles and enlarged nuclei in the epithelial wall of mesadenes indicates the degradation of nuclei after carbaryl treatment.

14. Bioflavonoid Quercetin Inhibits Oxidized LDL Induced Inflammation in Human Monocyte/Macrophages

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Key words: **Atherosclerosis, Inflammation, Flavonoid, Quercetin, Cytokines**

The inflammatory responses of monocytes/macrophages and the stimulation of lipid uptake into these cells by oxidized low density lipoprotein (oxLDL) are critical to the initiation and development of atherosclerosis. We investigated whether the dietary flavonoid quercetin inhibits the release of cyclooxygenase, lipoxygenases, PGE₂, IL6 and NF-κB in OxLDL stimulated monocyte/macrophages. Our results show that quercetin at 100 μM concentration significantly inhibits the activities of COX, LOX, PGE₂, IL6 and expression of NF-κB in CuSO₄ OxLDL induced inflammation in PBMCs. Quercetin possess antioxidant properties as well as anti inflammatory properties and may therefore have additional protective effect against the oxidized LDL induced atherosclerosis.

15. Triterpenoid Fraction from *Bacopa Monniera* (L.) Wettst Alleviates Arthritis in rats by Downregulating IL-6 Expression and Prostaglandin E₂ Production

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Key words: Arthritis, Interleukin-6, Prostaglandin, Inflammation, Bacopa, Cyclooxygenase

IL-6 and prostaglandin E₂ have been proven to play an important role in the development of arthritis. The present study aims at investigating the effect of a chloroform fraction rich in triterpenoids isolated from *Bacopa monniera* in downregulating the production of IL-6 and PGE₂ during adjuvant induced arthritis. Male Wistar rats were employed for this study. Arthritis induction significantly increased the paw volume of the rats of arthritic group compared to normal control. This was followed by upregulation of IL-6 in blood mononuclear cells, increased activity of cyclooxygenase and enhanced level of PGE₂ in the paw tissue of arthritic rats. Administration of triterpenoid fraction to arthritic rats significantly inhibited paw edema formation by 89%, decreased the production of PGE₂ and also downregulated the expression of IL-6. This study provides an insight into the mechanism underlying anti-arthritic effect of *Bacopa monniera*.

16. Metastasis Associated Gene 1 (MTA1): Role in Angiogenesis

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Key words: **Metastasis-associated gene 1(MTA1); Angiogenesis;
RNA interference (RNAi)**

Metastasis associated gene 1 (MTA1) is a recently identified candidate metastasis-associated gene that plays an important role in tumorigenesis and tumor aggressiveness, especially tumor invasiveness and metastasis. It has been found to be contained in the nucleosome remodeling and histone deacetylase complex. Although it has been reported that the expression of MTA1 is related to tumor progression and metastasis, it is still unclear how MTA1 is involved in angiogenesis. Here we analyzed and characterized the relationship between MTA1 expression and tumor angiogenesis. The MTA1 protein was produced recombinantly by baculovirus expression system and the biological protein was analysed for angiogenesis in various angiogenesis assays. MTA1 down regulation by RNA interference greatly reversed the malignant phenotype of the breast cancer cells MDA-MB 231 and affected other angiogenic genes. Studies *in vitro* showed that MTA1 promoted the metastatic ability of cancer cells. In conclusion, MTA1 over expression was found to be closely associated with higher tumor grade and increased tumor angiogenesis. These findings suggest MTA1 as a predictor of aggressive phenotype and a possible target molecule for anti-angiogenic drugs in breast cancer treatment.

17. Protective Effect of Coconut Sprout (Haustorium) on Isoniazid and Rifampicin Induced Hepatic Injury in Rats

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Key words: Coconut sprout, Isoniazid, Rifampicin, Hepatic injury, Hepatic marker enzymes, Lipid peroxidation, Antioxidants

Hepatoprotective effect of coconut sprout (coconut haustorium) was investigated on liver injury induced by antitubercular drugs viz Isoniazid (INH) and Rifampicin (RMP). Rats were treated orally with INH+RMP. For hepatoprotective studies, lyophilized coconut sprout administered orally before the administration of drugs for 30 days. Co-administration of coconut sprout along with these drugs significantly decreased the elevated levels of hepatic marker enzymes and lipid peroxides. Coconut sprout restored the serum protein level and antioxidant status. Histopathological studies of liver shows normal morphology in coconut sprout fed rats treated with INH+RMP. These results indicate that coconut sprout has a significant antioxidant effect and ameliorated the liver injury by antitubercular drugs.

18. Hunting of Peptide Antibiotics from Frogs: A Novel Approach

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Key words: Frog, Skin secretion, HPLC, cDNA, Peptide antibiotics

The emergence of strains of pathogenic bacteria with resistance to commonly used antibiotics has necessitated a search for novel types of antimicrobial agents. Amphibian skin is said to be a rich resource of antimicrobial peptides, which are generally cationic and hydrophobic in nature. These short peptide are thought to be a part of innate immune system and fight against wide range of micro organisms. The frog skin antimicrobial peptide serve potential as new therapeutic agents. In the present study we have isolated the peptide fractions by reverse phase HPLC, which show activity against both gram positive and gram negative bacteria. We also adopted a trascriptomic approach to isolate the antimicrobial clones from the c DNA library by RACE protocol and a BLAST search in data base revealed similarity of these new peptides with already existing antimicrobial peptides.

19. **Cardioprotective Properties of Haustorium from Germinating Coconut (*Cocos nucifera* L. seed) against Isoproterenol Induced Myocardial Infarction in Rats**

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Key words: Coconut haustorium, Isoproterenol, Myocardial infarction, Marker enzymes, Antioxidant enzymes, Cardioprotection

In the present investigation, protective effect of coconut (*Cocos nucifera* L. seed) haustorium against acute myocardial infarction induced by isoproterenol was evaluated in rat model. Decreased levels of marker enzymes (lactate dehydrogenase, creatine phosphokinase, alanine transaminase and aspartate transaminase) in serum, myocardial lipid peroxides, serum lipids and increased levels of endogenous antioxidants (superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) was observed in rats pretreated coconut haustorium orally for 30 days. Histopathological examination of heart sections from these rats showed almost normal tissue morphology. These results indicate that coconut haustorium possess significant cardioprotective and antioxidant properties during acute myocardial infarction.

20. ***Cocos Nucifera* (L.) Inflorescence - A Novel Material in Experimental Diabetes Mellitus**

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Key words: **Diabetes mellitus, Alloxan, *Cocos nucifera* Inflorescence, Biochemical effects, Antioxidant status.**

In *Cocos nucifera*, flowers and branched stalk are together called inflorescence. The present study was carried out to assess the biochemical effects of coconut inflorescence in normal and diabetic conditions *in vivo*. Diabetes was induced in rats using alloxan administration (150mg/kg). After 45days of feeding experiment, animals were euthanized and concentrations of fasting blood glucose, liver glycogen, and activities of metabolic key enzymes were assayed. Decreased glucose level, increased liver glycogen concentration and restoration of enzyme activities were observed in treated groups. Histopathological evaluation also confirmed these results. The results clearly indicate that coconut inflorescence possess beneficial effects on regulating the blood glucose level, liver glycogen content and antioxidant status in diabetic rats.

21. Bioinformatics based Analysis of Organic Anion Transport Protein (OAT2)

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Key words: Sequence Analysis, Phylogenetic analysis, Structure analysis

Transporter protein provides clues that could lead to efficient strategies for the use of transporter to deliver drug and to optimize lead compounds. A comprehensive list of drug transporter has become available and now information on the tissue distribution and the mechanism of these transporters is accumulating. However as the transport mechanism for the majority of drug still remains to be clarified, much work remains to be done.

Member of organic anion transporting polypeptide family are involved in the transport of various endogenous xenobiotic compounds, such as bile salt, steroids hormone, thyroid hormones, anionic oligopeptide, drugs and toxins.

Another aspect that demands careful consideration is drug-drug interaction, because the pharmacokinetics of drug that are transporter substrate might be influenced by co administered drugs that work as inhibitors or enhancer of the transporter functions.

22. Phylogenetic Tree Construction and Analysis of Different Species

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Keywords: Program, Phylogenetic tree construction

Phylogenetic is the study of the evolutionary history of living organism using tree like diagrams to represent pedigrees of these organisms. Phylogenetic is the study to know the relation between different organism, species, phylum, or between kingdom. Phylogenetic treats a species as a group of lineage-connected individuals over time taxonomy, the classification of organism according to similarity has been richly informed by phylogenetic but remains methodologically and logically distinct.

Phylogenetic also helps user to extract the relationship between species or organism in pictorial form, which is useful to easily understand phylogenetics is known as Phylogenetic Tree Analysis.

23. Biodegradation of Phenol using Microbial Fuel Cell

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**Keywords: Electricity generation, Microbial fuel cell, MFC,
Phenol degradation, Biodegradation**

Microbial fuel cells (MFCs) have been operated successfully by using a variety of readily degradable compounds. The near term application for MFCs was presumed to generate power from wastewater. In this study, MFCs using phenol or glucose–phenol mixture as the substrate (fuel) were designed to investigate the bio degradation of phenol. In an aqueous air cathode MFC using phenol (400 mg/L) as the sole fuel, electricity was generated during the degradation of phenol. The degradation rates of phenol in the MFC increased about 15% as compared to the open-circuit control. Further experiments were conducted by using graphite packed MFC with a ferricyanide cathode. When phenol served as the sole fuel, the peak voltage output was obtained, when 90% of phenol was depleted. A unique pattern of twin voltage peaks was observed when phenol–glucose mixture was used as the fuel. At the occurrence of the first and second voltage peaks, phenol was degraded by 20% and 90% respectively, suggesting a preferential sequence in substrate consumption. The maximal power densities were 9.1 and 28.3W/m³ for MFCs using phenol and glucose–phenol mixture as the fuel, respectively. Co-occurring with electricity generation, the degradation efficiencies of phenol in all the MFCs reached above 95% within 60 h. The results indicate that the MFC can enhance biodegradation of recalcitrant contaminants such as phenol in practical applications.

24. Metagenomic Approach Cloning of α Amylase Genes from a Fresh Water Ecosystem

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**Keywords: α amylase, Biocatalyst, Metagenome,
Function based screening**

The microbial ecology studies have revealed that the total number of prokaryotic cells on earth is approximately 30 million of which only 1.5 million species have been described and cultivated. Metagenome cloning is a powerful tool to exploit the biocatalytic potential of unculturable microbial communities for the discovery of novel biocatalysts. While much of the focus to date has centered on terrestrial and marine based microbial ecosystems, it is clear that the fresh water environment has enormous microbial biodiversity that remains largely unstudied. In this research work, we highlight the exciting potential that metagenomic based approaches offer us in gaining access to protein-coding genes with biotechnological potential from unculturable microorganisms in fresh water ecosystem. The analysis was performed using the metagenomic DNA isolated from pond water by a modified direct lysis method, digested using *Bam*H1 and cloned into a pUC 18 vector. Transformation of *Escherichia coli* JM 109 resulted in a metagenomic expression library. To estimate the functional diversity of the constructed library, it was screened based on functional heterologous expression, which resulted in identification of active clones capable of synthesizing amylolytic enzymes. The amylase screening was performed using starch as substrate and five clones gave positive clear zones. The putative amylase gene had 50°C and pH 5.6 as the optimum temperature and pH. The purified recombinant amylase gave a specific activity of 11.6U mg⁻¹, thereby revealing the utility of metagenomic approach in discovering industrially important enzymes. The recombinant plasmid isolated can be further investigated by restriction digestion and sequencing to determine the novelty of the identified amylolytic activity of the gene. The new approach

will bring into light myriad capabilities of microbial communities, thereby providing a new way of examining the as yet untapped fresh water microbial biodiversity resource.

25. Bioprocessing of Sugar Cane Waste for α -amylase Production by *Bacillus Subtilis*

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Keywords: **α -amylase, *Bacillus subtilis*, Sugar cane waste, Chickpea peel.**

Many microorganisms produce α -amylase. One most important and commonly used for laboratory production is *Bacillus* species. In the present study production of amylase under solid-state fermentation (SSF) by *Bacillus subtilis* utilizing sugar cane waste as carbon source has been investigated. Enzyme production parameter was optimized by varying carbon source concentration, incubation period, pH, temperature and nitrogen source. 20 gms of sugar cane waste in the inoculums media yields maximum enzyme production. Maximum enzyme production was also observed after 72 hours, which decrease with further incubation. Optimum pH and temperature of the culture medium for enzyme production was 7 and 35°C respectively. Nitrogen sources such as urea, peptone and casein were added to the media however supplementation with chickpea peel (as nitrogen source) enhances the amylase production.

26. Influence of Spent Wash Application on Protease and Amylase Activity in Germinating Wheat Seeds

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Keywords: Spent wash, Amylase, Protease, Wheat

Spent wash is a major waste product from alcohol distilleries. In India, total 279 alcohol distilleries are present that liberate up to 40 billion liters of spent wash annually. Discharge of such spent wash in water bodies or nearby land results in number of environmental, water and soil pollution including threat to plant and animal lives. But if applied in appropriate quantity to plants increases the uptake of Zinc, Copper, Iron and Manganese in Maize and Wheat as compared to control and highest uptake of this was found at lower dilution level than higher. However not much information is available on impact of spent wash on Amylase and Protease activity in germinating Wheat seed. Therefore, present study was carried out to investigate the impact of different doses of Spent wash (@ 100, 200, 300 pot^l) and enzymatic activity was checked after 24 hrs, 48 hrs and 72 hrs. The activity of these enzymes increased with increase in the dose of Spent wash.

27. Blood Sugar and Lipid Profile Changes during Pregnancy

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Keywords: Blood Sugar, Lipid Profile, Pregnancy

Study was carried out in the department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola. High Lipid profile level is the major factor behind atherosclerosis and hypoglycemic condition may lead to unconsciousness. To avoid such problems during pregnancy study was done to investigate the effect of pregnancy on blood sugar and lipid profile changes during all three trimesters.

Blood sugar and lipid profile changes were measured in 35 cases during each trimester of pregnancy and in control group of 30 cases of non-pregnant women. The study proves that blood sugar level in pregnant woman is within normal range but it is towards lower side. In case of lipid profile, total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol increased from trimester to trimester while high-density lipoprotein cholesterol decreased as compare to control group. The result reveals metabolic changes in blood sugar and lipid profile during pregnancy.

28. Co-relation of Minerals and Hemoglobin Level in Preschool Children with Pica

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Keywords: Pica, Minerals, Hemoglobin.

Pica is an eating disorder typically defined as persistent eating of nonnutritive substances for a period of at least 1 month at an age in which the behavior is developed mentally. Ingestion of non-nutritive substances includes dirt clay, laundry starch plastic, pencil, erasers ice, burnt matches, fingernails etc (5). There was a definite male predominance and majority of children belonged to 2-4 years age group. Cough, abdominal pain, poor appetite, abdomen fullness etc were other presenting features. A prospective study was undertaken to determine relation of hemoglobin and minerals in patients with pica. For this 50 children and 20 controls were matched for age, sex and nutrition in an observation case and control study. The study included estimation of plasma iron, calcium, zinc and hemoglobin and their relation in patients with pica as well as in healthy control groups. Our finding suggests that plasma iron level is lower than that in control. Similarly, low levels of zinc, calcium and hemoglobin were also found suggestive of hypozincemia with iron deficiency anemia and nutritional deficiency may be a possible cause of pica.

29. **Antibacterial Activity of Leaf Extracts of *Gloriosa Superba* L**

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Keywords: **Gloriosa superba L, Antibacterial activity, TLC**

Gloriosa superba L. is one of the important medicinal plants, which has been categorized in the endangered plant. The present study deals with antibacterial activity of the *Gloriosa superba* L. Acetone and hexane extract of plant leaves were tested against three bacterial strains viz. *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. As compared to hexane extract acetone treated extract shows more activity. The highest antibacterial activity was recorded in acetone extract when tested against *B. subtilis*, while the minimum zone of inhibition was recorded in the hexane extract, tested against *E. coli*. TLC of acetone extract revealed two spots with different R_f values and both of them showed marked antibacterial activity.

30. **Isolation and Characterization of *Salmonella* in and on Raw Tomatoes as Affected by Stage of Ripeness and Storage Temperature**

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Keywords: **Salmonellosis, S.typhi, Tomato**

Salmonella causes an estimated infection in around 20 million people worldwide each year and is responsible for several human deaths. *Salmonella*

have been isolated from all food animals and also found in the vegetables such as tomatoes, spinach etc. Several out breaks of Salmonellosis have been associated with the consumption of raw tomatoes. This is because of the fact that *Salmonella* attaches to the surface of tomatoes and also present in the interior due to geotropic transmission via contaminated soil and irrigation with contaminated water. A total 200 sample of tomatoes were analyzed out of which 10 sample were found to contain the *Salmonella*. All the 10 isolates were then subjected to the antibiotic susceptibility testing.

31. Incidence of Urinary Tract Infections among Hospitalized Patients from Akola City

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Keywords: Urinary Tract Infections (UTI's), Morbidity, Mortality

Urinary tract infection (UTI) is defined as the presence of multiplying microorganisms in the tract through which the urine flows from the kidneys via the bladder to the urethra and finally to the outside of the body. Urinary tract infections (UTI's) are one of the most common infectious diseases ranking next to upper respiratory tract infections, and are one of the important causes of morbidity and mortality in human. It has been found that 10-15% of human populations will experience once UTI during their lifetime and women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their lifetime moreover pregnancy also makes them more susceptible to infection. Bacteria are the major causative organisms and are responsible for more than 95% of UTI's cases. Recent studies suggested that infection result from a breakdown in local defense mechanism in the bladder that allows bacteria to invade the bladder mucosa and multiply. So the present studies aimed to gain knowledge about the type of pathogens responsible for

urinary tract infections and their resistance pattern which are isolated from urine samples of suspected cases of UTI's from different hospitals of Akola city.

32. Screening of Methicillin Resistant and Vancomycin Resistant *Staphylococcus Aureus* from various Clinical Samples

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Keywords: S.aureus, Methicillin, Vancomycin, Drug resistance

Humans have been prey to infections caused by virulent strains of *Staphylococci* and *Streptococci*, through out recorded history. *S.aureus* is one of the commonest causes of nosocomial infection especially pneumonia, surgical site infection, blood stream infection, etc. In addition it is now incipient community pathogen in many geographical regions. The bacterium has ability to rapidly acquire multiple drug resistance. Most of the *Staphylococcal* strains (almost 50%) were resistant to Penicillin and by late 1980s Methicillin resistant *Staphylococcus aureus* (MRSA) emerged as prevalent nosocomial pathogen. Just a decade later in 1990s *Staphylococcal* isolates with diminished susceptibility to Vancomycin were reported. A discernible increase in the use of Vancomycin during early 90s resulted in selective pressure that leads to the emergence of strain of *S. aureus* and other *Staphylococci* species with decreased susceptibility to Vancomycin and other glycopeptides. Keeping this in view the present study was carried out to screen Methicillin resistant *S.aureus* and Vancomycin resistant *S.aureus* from various clinical samples, so that hospitals can adapt a proactive approach towards it.

Isolation, identification and screening of MRSA and VRSA were made by

standard conventional method from various clinical samples viz., blood, pus, etc. Amongst the 50 isolates gained, more than 50% were Methicillin resistant and approximately 35% were resistant to Vancomycin. The obtained isolates were found susceptible to tetracycline and chloramphenicol.

The present work revealed the emergence of Vancomycin resistant *S. aureus* from Akola region and indicates the magnitude of antibiotic resistant, the major cause of which may be unawareness and indiscriminate use of broad-spectrum antibiotics.

33. Ribonuclease – A Biochemical and Morphological Study

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Keywords: RNase, Bacteria, Soil, Purification, Assay

Ribonucleases are the enzyme, which degrades phosphodiester bonds of ribonucleic acid (RNA). These enzymes have wide range of application. Very few organisms produce RNA extracellularly. Present study is done on RNase producing bacteria from soil. About 10 soil samples were chosen for study & screened on nutrient agar containing 0.2% RNA. After 24 hour of growth this was flooded by 0.1N HCL. The unprecipitated area indicate RNase activity where as precipitated area indicate unhydrolysed RNA because RNA on the reaction with Hcl gets precipitated. Two positive culture from 10 soil sample were chosen for morphological and biochemical characterization. Two efficient cultures were selected and assay of Ribonucleases was performed and compare with standard RNase.

The extracellular enzyme i.e. RNase was obtained by centrifugation at 13,500g for 30 min. This was used as source of RNase assay of this crude enzyme also performed the RNase was purified by SDS-PAGE. It was eluted

and was assay RNase prior. This assay of standard RNase was performed. The amount of RNA degraded in one minute was calculated the amount of RNA degraded correspond to the concentration of RNase.

34. Reutilization of Bacteriological Media

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Keywords: **Bacteriological media, Reutilization of media**

Now days a vast variety of Bacteriological media are being used for the cultivation of large number and different type of microorganisms. Due to the increasing demands of media the cost of media increased a lot and supplier are also unable to fulfill the demands of costumers. When microorganisms grown on that media, it does not completely utilize available nutrients, and still some amounts are left after the complete growth of microorganisms. The analysis was carried out to find out the amount of residual Proteins, Carbohydrates, Fats and Vitamins. It was noticed that more than 85% nutrients are unutilized which goes wasted.

It is noticed that most of media can be reused after the addition of small amount of ingredients and indicators. This helps us to reducing the cost of media, also found to be economically beneficial and is a need of present day. So by considering all beneficial aspect the present study of reutilization of media was carried out. In this work about 10 media were reutilized in which both solid and liquid media present, which shows good growth of microorganisms indicating a positive aspect towards the reutilization of media.

35. Comparative Efficiency of Chemical Fungicides and Phytoextracts against Mung-bean Pathogens

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Keywords: Antifungal activity, Sclerocium rolfsii, Colletotrichum capsici

Extensive use of synthetic chemical formulation in agriculture has resulted in environmental pollution and public health risk. So considering the present day need it is now necessary to find out alternative and eco-friendly remedy to maintain the environmental status by developing the eco-friendly antifungal agents from herbal plants.

The present study was therefore undertaken for evaluation of antifungal activity of two herbal extracts i.e. Kalami and Garlic and their comparative analysis with chemical fungicides was done against the fungal isolates of Mung-been i.e. *Sclerotium rolfsii* and *Colletotrichum capsici* by poison food technique. Considerable antifungal activity was found in both medicinal plants tested.

36. Microbial Analysis of Hospital Waste in Akola City

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Keywords: **Hospital waste, Microbial analysis**

The hospital environmental waste contains large number of pathogenic organisms and are found to be important source of cross infections. The hospital waste i.e. not intended for further use such as clinical bandages, cotton, syringes, and disposable needle including pharmaceutical waste and foodstuffs.

To overcome the hazardous effects proper disposal of hospital waste is necessary. Similarly people must have adequate knowledge about disease producing organisms. The present work was done by collecting various samples from hospital waste and studied for their isolation, identification and antibiotic resistant pattern. Mostly *Staphylococci*, *Enterobacter*, *Pseudomonas* and *Klebsiella* were found to be predominant, which shows resistance towards antibiotics.

37. Studies on Antibacterial Activity of *Solanum Xanthocarpum* against Oral Microflora and Cariogenic *Streptococcus Mutans* with Special Reference to Quorum Sensing

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Keywords: Quorum sensing, Oral microflora, Streptococcus mutans, Cariogenic pathogens, Antibacterial activity, Solanum xanthocarpum

Bacterial species employ complex communication mechanisms termed Quorum sensing that link cell density with gene expression. In this process, diffusible signal molecules autoinducers like acyl homoserine lactones, accumulate in the extracellular environment, attain a critical concentration and trigger the response, which leads to gene expression.

Streptococcus mutans an obligate biofilm forming bacterial pathogen operate Quorum sensing system to form biofilms on tooth. Understanding of Quorum sensing in *Streptococcus mutans* will facilitate to develop a therapy against dental caries.

The aim of the study is to assess the antibacterial potential of extracts from *Solanum xanthocarpum* against oral micro flora and. The extracts were obtained by standardized protocol so as to obtain the enhanced antibacterial activity of the extract from seeds of *Solanum xanthocarpum*. The antibacterial activity is tested by agar well diffusion method. The extracts of *Solanum xanthocarpum* are found to be very effective in inhibiting the growth of bacterial pathogens. The antimicrobial activity tested against cariogenic pathogens i.e. *S. mutans* and four different isolates from decayed tooth. The Gram positive cariogenic bacterium *Streptococcus mutans* (40mm, 35mm) was more susceptible to inhibition by the extracts of *Solanum xanthocarpum* than the other 4 oral bacterial isolates.

38. Studies of Leaves and Stem of Three Lamiaceae Members

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Keywords: Hyptis suaveolens, Ocimum sanctum, Plectranthus mollis, Proximate analysis, Phytochemistry

Preliminary phytochemical and proximate analysis of leaves and stem of three lamiaceae members i.e. *Ocimum sanctum* L. *Hyptis suaveolens* Poit. and *Plectranthus mollis* L. was carried out. The preliminary phytochemistry reveals that, these plants are rich in phytochemical constituents like Alkaloids, Flavonoids, Tannins, Saponins and cynogenic glycosides. *H. suaveolens* showed maximum phytoconstituents amongst all three plants. The proximate analysis showed significant nutritional composition with high level of proteins, lipids, carbohydrate and fibers. Thus, apart from the ethnomedicinal uses, these plants can be use as source of dietary components.³

39. Parallel Substitution of Binding Sites in two Androgen Binding Proteins

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Key-words: **Binding sites, Androgen receptor, Aromatase, Molecular evolution**

The parallel evolution of proteins that are under a common selective constraint to bind a certain ligand is a good model for understanding the mechanisms of molecular evolution. Parallel evolution is detected in two non-orthologous androgen binding proteins, aromatase (CYP19) and androgen receptor (AR) during metazoan evolution. The androgen binding sites of AR and CYP19 are expected to play a key role in the evolution of these proteins in animals, and their amino acid sequence alignments of vertebrates with other species show very interesting patterns. The common substitution pattern observed between the AR and CYP19 binding sites is a single substitution involving conversion of a charged amino acid to a neutral amino acid and *vice versa* between vertebrates and invertebrates. The common pattern in amino acid substitution between vertebrates and invertebrates indicates parallel nature of evolution in AR and CYP19 binding sites.

40. Forensic DNA Profiling – A case of Rape Exclusion

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Key words: **DNA, Rape, STR' s, Y**

In this paper, a case of rape is reported which was solved by using DNA fingerprinting technique from the material recovered from the garment and the vaginal smear slide of the victim. A woman reported that she had been victim of sexual assaults by a person. She was able to identify the person. The alleged maintained that he was innocent. Therefore the clothes and vaginal smear slide of the victim and the referral blood sample of the alleged was received for DNA examination. Mixed male DNA profile was generated from petticoat and vaginal smear slide of the victim. In this case the allegation of rape was proved to be false. And also two different male DNA profile were found present on the petticoat of the complainant.

41. Anti inflammatory Effects of *Oryza sativa* Linn. (Njavara)

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**Keywords: Njavara, Anti-inflammatory, Antiarthritic,
Freund's adjuvant, Cyclooxygenase, Carrageenan**

Oryza Sativa Linn (Njavara) is a unique extra short duration rice cultivar indigenous to Kerala and is the only genotype used in ayurveda system of medicine, involving baths and massages for curing paralytic condition. The anti-inflammatory effect of Njavara was investigated on carrageenan induced acute model. The boiled Njavara (BN), which is most potent, was further assessed in chronic inflammatory models induced by adjuvant. The oral administration of boiled grain of Njavara (100 mg/kg) showed a maximum inhibition of about 83% in paw edema induced by carrageenan. In the chronic inflammation model BN showed maximum inhibition of 80% on the 20th day of treatment. On the basis of biochemical estimations of antioxidant enzymes like SOD, Catalase, Glutathione peroxidase (Gpx), Glutathione reductase (GR), lipid peroxidation product MDA and enzymes involved in inflammation like Nitric oxide synthase,(NOS), lipoxygenase and cyclooxygenase, myeloperoxidase(MPO) it may inferred that (Njavara) have antiinflammatory and antiarthritic effect. The results of the present study demonstrated the potential beneficiary effect of boiled Njavara on adjuvant-induced arthritis in rats.

42. Therapeutic Effectiveness of HEDTA along with Propolis against Beryllium Intoxication in Rats

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Keywords: Beryllium toxicity, HEDTA, Propolis, Hepatorenal dysfunction, Combined therapy

Intervention of chelating agent N-2-hydroxy ethyl ethylene diamine triacetic acid (HEDTA; 20 mg kg⁻¹, i.p.) in conjunction with propolis (honeybee hive product; 200 mg kg⁻¹, p.o.) was evaluated to encounter beryllium induced characteristic biochemical alterations following subchronic exposure to beryllium. Female albino rats were exposed to beryllium nitrate (1 mg kg⁻¹, intraperitoneally) daily for 28 days followed by treatment of above-mentioned therapeutic agents either individually or in combination for 5 consecutive days. Exposure to beryllium caused significant alterations in lactate dehydrogenase, α -glutamyl transpeptidase, albumin, bilirubin, creatinine and urea in serum; activity of acid phosphatase, alkaline phosphatase, adenosine triphosphatase, glucose-6-phosphatase, succinic dehydrogenase, triglycerides, cholesterol, protein contents, glycogen contents in liver and kidney. Monotherapy of propolis and HEDTA recovered some of the studied parameters moderately towards control. Interestingly, combination of HEDTA and propolis reversed the alterations of all the variables towards normal over mono therapy in attenuating beryllium induced systemic toxicity.

43. Hepatoprotective Efficacy of Aqueous extract of *Rosa Damascena* against APAP-induced Hepatic Damage in Albino Rats

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Keywords: Hepatoprotective; APAP; Oxidative stress; Rosa damascena

The present investigation was planned to evaluate the hepatoprotective activity of aqueous extract of *Rosa damascena* flower at different doses (acute - 250, 500 and 1000 mg/kg, *p.o.*, once only) against APAP (2 g/kg, *p.o.* once only) induced hepatocellular damage in rats. We determined a minimum effective dose of *Rosa damascena* through their therapeutic potential and further evaluated them individually against toxicant induced biochemical alterations and oxidative stress consequences in rats. The administration of toxicant altered blood biochemical variables and significantly increased the level of serum marker enzymes such as AST and ALT. Hepatic lipid peroxidation was increased significantly, whereas substantial depletion was observed in reduced glutathione level after toxicant administration. A slight elevation was found in protein content, while a noteworthy fall was observed in the activities of adenosine triphosphatase and glucose-6-phosphatase after induction of toxicity. The treatment with aqueous extract of *Rosa damascena* flower at higher doses (500 and 1000 mg/kg) significantly improved hepatic antioxidant status and suppressed malondialdehyde formation in liver. Administration of *Rosa damascena* significantly brought the values of studied parameters towards normal and also reversed the histopathological alterations by attenuating lipid peroxidation and enhancing antioxidant defense. Thus, it may be concluded that *Rosa damascena* flowers can be used to reduce the hepatic damage and may serve as an alternative medicine in hepatic etiology.

44. **Phylogenetic Studies on some Members of Family Ancyrocephalidae Employing Bioinformatics**

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Keywords: Monogenea, Ancyrocephalidae, Phylogeny, Bioinformatics

During present study a combination of different Phylogenetic tools of Bioinformatic softwares /algorithm were employed in inferring phylogenetic relationship between members of family Ancyrocephalidae. Different species of thirteen well known different genus, viz. *Ancyrocephalus*, *Bravohollisia*, *Cleidodiscus*, *Heteronchocleidus*, *Ligophorus*, *Onchocleidus*, *Quadriacanthus*, *Scutogyrus*, *Silurodiscoides*, *Tetrancistrum*, *Thylacicleidus*, *Trianchoratus* and *Urocleidus* of the family Ancyrocephalidae were selected and used in the study. Sequences such as ribosomal RNA, ITS etc. representing various species of these genus have been retrieved. *Dactylogyrus extensus* was used as outgroup taxa. Sequence alignment was done using Clustal-X. Phylogenetic Tree preparation was done using NJ as well UPGMA approaches which are widely used for the same purpose. The findings are supported with the help of self-explanatory trees. Present investigation brings about molecular evidences of the phylogenetic relationships among the selected members of the family Ancyrocephalidae.

45. Quorum Sensing, Pathogenesis, Prophylaxis and Evolutionary Strategy in Streptococci Group

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Keywords: Quorum-Sensing, Streptococci Group, Gene Family, Phylogenetics.

Quorum-sensing (QS) system is used to regulate ecologically and medically important traits. The involvement of QS in virulence of streptococci group is well known, but the widely distributed gene families LuxI/R of QS are not reported in Streptococci group. We retrieved sequences of LuxS proteins of 7 species, including *S.mutans* from GenBank and using PROML module of PHYLIP version 3.6, analyzed phylogeny. The LuxS protein of *S. mutans* shows the sequence homology with with *Vibrio* genera.

The genes underlying QS are distributed in a discontinuous manner among the bacteria, suggesting that they have been subject to loss or horizontal transfer. In this regard, gene phylogenies for the components of QS systems of different Streptococci sp. can provide evidence as to whether they are ancestral and lost in some species or have been acquired from distantly related lineages.

The present study will explore unknown quorum sensing mechanism behind biofilm formation in virulent streptococci group to get new potential drug targets, to know their co-evolution and horizontal gene transfer among them.

46. DNA Barcode of Life: the Species-specific DNA Sequences from East and Northeast Indian Biodiversity

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Keywords: DNA barcode, Flora, Fauna, Molecular phylogeny, COXI, d-loop, Mitochondrion DNA

The eastern region (including Sunderbans) and the north-eastern region (including Eastern Himalayas) of India having a wide variation in climates ranging from extreme hot to cold conditions grew as biodiversity hotspots of the breeds/strains of flora and fauna. The biodiversity and ecosystem stability are threatened by invasive alien species. In this scenario, investigation of the biodiversity and the genetic uniqueness based on mitochondrial DNA variation along with its identification, characterization and patenting of the unique genetic traits of animals and plants in this biodiversity hotspot is highly demanding. Sequence diversity in a 650 bp region near the 5' region of mitochondrial *Cytochrome oxidase* subunit1 (*COI*) gene provides a strong species level resolution for varied animal groups. We have already deposited DNA barcode sequences of several animals including Royal Bengal Tiger (National animal of India), Indian domestic Cat, Oysters, Shrimps, Cattle and Buffalo, etc. DNA barcodes of different groups of Fishes (including Ornamental fish), turtle and tortoise, mammals, birds, medicinal plants and other species found in East and North East Himalayan biosphere are underway.

47. Strategy for Identification of Species within *Bacillus Cereus* Group Using Whole Cell Proteome Comparison

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Bacillus anthracis, the etiological agent of anthrax, is responsible for a serious and often fatal disease of mammalian livestock and humans and is an important biological and toxin warfare agent. Although several phenotypes and biochemical tests can differentiate *B. anthracis* from *B. cereus* and *B. thuringiensis*, species delimitation is unclear. *Bacillus* sp. AKG was isolated from a hot spring in western Himalayas that exhibited 100% sequence identity at 16S rDNA level with *B. anthracis* and a few other members of *Bacillus cereus* group. Species-specific primers targeting *gyrB* gene identified the strain as *B. anthracis* within cereus-group. Cloning, sequencing and phylogenetic analysis of the partial *gyrB* sequence indicated that the strain possessed a novel *gyrB* gene sequence. It exhibited a close affiliation with *B. thuringiensis* strain Al Hakam that has been shown to be phylogenetically more closely related to *B. anthracis* and other “Branch F” *Bacillus* isolates than to many of the commercially important *B. thuringiensis* isolates. Phenotypic features indicated a non-anthrax affiliation for the strain AKG. Proteome comparison was used to establish the identity of this unknown environmental strain. Protein spots unique to each group and those showing match between the groups, in a pairwise comparison, indicated strain AKG as a member of *B. thuringiensis*. This strategy can be used to assign strains of *B. cereus* group to their respective species.

48. Study on Physicochemical and Biological Parameters of Sanjivani Lake Water Located near Wadval Belt of Latur District – A Case Study

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Keywords: Sanjivani Lake water, Physicochemical, Biological Parameters, Pollution profile.

Social, economical and political development of region is dependent on the quality and quantity of natural resources. So the management of natural resources and its monitoring is the primary task. The present study deals with the some physicochemical and biological parameters of Sanjivani Lake, located near Wadaval Bet of Latur District were assessed to determine their pollution profiles. Physicochemical Parameters such as pH, turbidity, total suspended solids (TSS), chemical oxygen demand (COD), dissolved oxygen (DO), sulphate, phosphate, chloride, bicarbonate, etc. and biological Parameters such as MPN and SPC were determined. Some of the parameters were above WHO standard for drinking water, so there is need for strict monitoring to ensure quality water supply for human health. Results are discussed in full length.

49. Quantitative Analysis of Nitrate and Nitrite Ion Concentration in Different Vegetables of Akola Markets

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Keywords: Vegetables, Nitrate, Nitrite, Tomato, Spinach, Carrot

Vegetables constitute the major dietary source of nitrate generally leafy vegetables. However, frequent consumption of large quantities of vegetables high in nitrate contents may prove health hazards, particularly to infants. In this study, the nitrate and nitrite contents of a total 30 samples of 5 different vegetables (tomato, carrot, spinach, cabbage, pumpkin) sold in Akola markets in Vidarbha region of Maharashtra state were determined. The nitrate contents in the vegetables were determined using phenolsulfonic acid method, while the nitrite contents were determined by the Nessler's reagent method. The highest content of nitrate was found in spinach and lowest level in tomato. Nitrite concentration was found higher under adversely post-harvested vegetables. The values of nitrate and nitrite were found slightly higher than the established Acceptable Daily Intake (ADI) values. In general, the leafy vegetables had higher nitrate content than root and fruit vegetables.

50. Evaluation of Serum Calcium Level during Pregnancy in Buldhana District

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Keywords: Pregnancy, Calcium

During pregnancy foetus skeleton is formed & mother provides calcium mineral, hence in mother's skeleton significant changes in calcium level may occur. To evaluate such changes study was carried out in the department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola. Calcium level was measured in 50 cases during each trimester of pregnancy & 40 Control groups of Non-Pregnant women by using kit method. Decreased level was observed during each trimester as compare to control group. Study reveals that decrease in calcium level is due to foetus skeleton development and inadequate dietary intake of calcium by mother during pregnancy.

51. Study of NADPH Cytochrome P₄₅₀ Reductase in Different Varieties of *Pigeon pea*

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Keywords: Cytochrome P450 reductase, NADPH

Higher plants are rich in Cytochrome P₄₅₀ reductase and some plants are known to catalyze monooxygenation reaction involved in diverse biosynthetic pathway concerned with formation of secondary metabolites such as lignin, flavonoids, sterols and alkaloids. Microsomal-bounded Cytochrome P₄₅₀ reactions are depended on transfer of electrons from NADPH by common flavoprotein, NADPH-Cytochrome P₄₅₀ reductase (E.C. 1.6.2.4). In the present study an attempt was made to assay the Cytochrome P₄₅₀ reductase activity in various varieties of *Pigeon Pea*. The Cytochrome P450 content was found in the range from 2.78 nmole to 5.02 nmole and the highest activity (5.02 nmole) was found in Tur-2 22498-3 variety.

52. Isolation and Characterization of Seb Producing Strains of *Staphylococcus Aureus* in Mutton, Milk and Pastry Samples and their Control by Natural Extracts and Spiking Studies on Mutton

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Keywords: **Staphylococcal Enterotoxin B, PCR, Spiking**

Staphylococcal food poisoning is one of the widely spread gastroenteric diseases in the world. *Staphylococcus* is a common pathogenic organism found in India and South East Asia. Staphylococcal enterotoxin B (SEB) affects the functions of the gastrointestinal tract hence said to be a potent food borne pathogen. Hence, we isolated *Staphylococcus* from different food sources and characterized by key biochemical tests. Primer designing for Enterotoxin B was done using Gene runner, BLAST and NCBI site. DNA extraction was carried out by phenol: chloroform method. PCR amplification of the SEB gene from the isolate was done by Master cycler gradient, Eppendorf thermocycler. Out of the 3 isolates Of *Staphylococcus aureus* one isolate from milk was found to be producing the endotoxin B. The bands that were formed on the gel were having same basepairs as that of SEB gene in the standard strain of *Staphylococci*; FRI22. Control of *S. aureus* in food items can be ensured by addition of preservatives. Various chemicals available commercially impart antimicrobial properties, which are used in various stored & processed foods. Research data in the present scenario shows that many of such chemicals used as preservatives are getting accumulated in the body of the consumer. Such chemicals are not properly assimilated in the body and impart carcinogenic properties. As an alternative to this, we have made an effort to study the effect of some natural extracts on *S. aureus*. *S.aureus* was spiked on to mutton chunks along with the effective concentration of natural extracts. The results obtained revealed the reduction in the count of *S. aureus*.

53. Prevalence and Antimicrobial Multi drug Resistance of *Staphylococcus Aureus* in the Hospitals of Gulbarga and Raichur Districts

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Keywords: *Staphylococcus aureus*, Methicillin, Multidrug resistance, Clinical samples, Antibiotics, Beta lactamase Coagulase test.

A study to investigate the multidrug resistance of staphylococcus aureus to conventional antibiotics being frequently used in these regions. Methicillin resistance staphylococcus is pathogenic and is an important nosocomial organism. We report the prevalence and antimicrobial susceptibility pattern of MRSA is major in Northern districts of Karnataka. We were collected 200 samples from various hospitals in Gulbarga Districts out of these 50 isolates were characterized and identified using standard microbiological techniques. 25 isolates are Coagulase positive staphylococcus aureus. Almost all isolates of MRSA 95% were resistant to ampicillin, 93.2% penicillin, 75% amoxicillin, 72% gentamycin, 70% erythromycin respectively. Multidrug resistance was observed and suggests possible abuse of these drugs poor hospital attendance & the need for better enlightenment campaign against the use of drug without prescription.

54. Application of STG in *Citrus* Sanitation, Quarantine and Certification at NRCC

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Keywords: STG, Citrus, Disease free, Certification

Major cultivars of the genus *Citrus* is confronted with several viruses like diseases which are widely distributed and cause tremendous losses to our *citrus* industry. Selection and indexing of existing cultivars being followed in budwood certification program is less efficient under our conditions because of massive infection of almost all cultivars. The only possibility is to obtain disease free plants from disease infected plants. From the available techniques thermo therapy is ineffective to eliminate some fastidious *Citrus* like exocortis and xyloporosis. The negative aspect of nucellar approach is juvenility and undesirable fruit quality parameters. Shoot tip grafting is recommended and widely followed worldwide or the elimination of all known major *citrus* pathogens, with thorough indexing before & after STG therapy to ascertain the disease freeness. *In vitro* STG has been effective in elimination & in production of true to type healthy certified planting material. STG is also adapted for introducing of *Citrus* species through the quarantine in countries like Spain, Brazil, USA, Australia etc. The technique developed by Murashige et.al. further refined by Navaro et.al. abroad and in India successfully implemented for *Citrus* sanitation, quarantine at NRCC by Vijayakumari & coworkers. The *in vitro* grafting is based on the fact that the apical meristem used for grafting is so minute (0.1mm-0.3mm) the virus and bacteria particles will not reach the extreme shoot tip, hence the resultant progeny plants are disease free. In this paper the comprehensive *Citrus* Improvement Programme at NRC for *Citrus*, Nagpur to clean, produce, maintain & large scale release of disease free *citrus* bud grafts through *in vitro* STG is detailed by the authoress. Through successful transfer of technology from lab to land NRCC, Nagpur so far distributed more than three lakh certified disease free quality bud grafts to *Citrus* growers / nurserymen which facilitated establishment of more than four hundred hectares elite healthy *Citrus* orchards in farmer's field thus paving the way for a sound and sustainable *Citrus* industry.

55. Biochemical Studies of some Medicinal Plants and their Antioxidant Activities

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Keywords: Biochemical evaluation, Antioxidant activity, Medicinal plants, *Andrographis paniculata*, *Asparagus racemosus*, *Desmodium gangeticum*.

The Indian conditions provide immense scope and potential for carrying out biochemical studies on plants of medicinal importance as only little information is available on them. The phytochemical and biochemical studies on the under utilized parts of medicinal plants may bring to light their nutraceutical potential leading to their utilization in the modern system of medicine (Anonymous 1969). This paper is described systematic biochemical evaluation and antioxidant activity of under-utilized parts of some medicinal plants such as *Andrographis paniculata*, *Asparagus racemosus* and *Desmodium gangeticum*.

Andrographis paniculata (Acanthaceae) 'Kalmegh' a bitter tonic is used in 'Ayurveda' for liver disorders, it also find use to remove toxins, infectious fever causing diseases, diabetes, tonsillitis, pharyngitis, pneumonia and tuberculosis etc. *Asparagus racemosus* (Liliaceae), 'Satavari' is used as galactagogue, appetizer, tonic, aphrodisiac, astringent, useful in tumors, inflammation, eye diseases, throat complaints, leprosy and epilepsy. *Desmodium gangeticum* (Fabaceae) 'Shalaparni' roots of the plant are used as febrifuge, bitter tonic, antipyretic, digestive, anticatarral, alterative, tonic, expectorant, diuretic, aphrodisiac and in the treatment of typhoid biliousness. Such studies may be of some help in better utilization of our natural resources effectively leading to sustainable use of the country's plant wealth.

56. Protective effect of Ginger (Rhizome of *Zingiber officinale* Roscoe) against Lornoxicam induced liver Injury in Rats

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Key words: **Lornoxicam; Zingiber officinale; Oxidative stress; NF-kB**

Ginger (rhizome of *Zingiber officinale* Roscoe) is a widespread herbal medicine mainly used for the treatment of gastrointestinal (GI) disorders. This study was designed to evaluate the hepatoprotective activity against Lornoxicam (LOR) induced acute liver injury in rats and to determine its mechanism of action. Oral administration of ZO at doses of 75, 150 and 300 mg kg⁻¹ for 30 days significantly reduced the elevated relative value of liver weight, serum transaminases (alanine aminotransferase and aspartate aminotransferase) and the hepatic morphological changes induced by (LOR) in rats. In addition, ZO markedly inhibited LOR induced lipid peroxidation and enhanced the activity of the antioxidant enzymes; superoxide dismutase and glutathione peroxidase. Moreover, pretreatment with ZO suppresses nitric oxide production and nuclear factor- κ B activation in LOR treated rats. The results suggest that ZO has significant hepatoprotective activity and its mechanism is related, at least in part, to its antioxidant properties. Further research is required to investigate the detailed mechanism of the protective effect of ZO on liver injury.

57. Effect of Cadmium Exposure on non –non-Specific Host Defense Mechanism in Swiss Albino mice

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Key words: **Cadmium, Chemotaxis, Phagocytosis, Immunomodulation, Splenic macrophages**

The role of heavy metal like cadmium (cd) as environmental toxins is well established. However, the exact mechanism of its effect on activity of immunocompetent cells is not well-known. To elucidate immunomodulatory effect of cadmium, cell-function studies like phagocytosis, chemotaxis, intracellular killing and myeloperoxidase release were performed on splenic macrophages isolated from cadmium-treated and control group of mice. The objective of our study was to demonstrate the toxic effect of cadmium on splenic macrophages towards the impairment of proper phagocytic engulfment of pathogens both *invivo* and *invitro*, in an experimental mouse model. Prolonged exposure to heavy metals diminishes the functional capacities of splenic macrophages, which can also be correlated, to the lowered ingestion capacity of splenic macrophages, since more bacteria are cultured from blood and spleen of cadmium exposed mice. Our findings have shown a significant alteration of macrophage function like phagocytosis, chemotaxis, intercellular killing and enzyme release in cadmium-treated group of mice as compared to control. The results obtained in the present study may also be helpful to establish a causal relationship between intercellular survival of *S. aureus* and the altered cell functions.

58. Vascular Exudates as Biochemical Markers in Cucurbitaceae

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Key words: **Exudates, phloem sap, cucurbitaceae, proteins**

Vascular exudates has been used in many studies on solute transport in plants addition to small molecules like sugars and amino acids, phloem sap of higher land plants contains proteins that can accumulate up to higher concentrations. Although the knowledge about the identities of these phloem sap proteins is increasing, the functions of most of them are still poorly understood. Since many phloem sap proteins have predicted roles in wound and defense responses, they constitute a class of compounds that can potentially influence plant-insect interactions. The present study aims to reveal vascular exudates a chemotaxonomic marker character in Cucurbitaceae. The organic and inorganic solute content of phloem and root pressure exudates from cucurbits was examined. The range of values obtained for the solutes in phloem sap indicated that cucurbit phloem sap was dissimilar in a number of respects from the phloem saps of other known plant species. It has a high protein content and low total sugar content, and also contains a high proportion of the simple sugars. SDS-PAGE of phloem sap protein revealed bands ranging in molecular mass from 10 to 200 kDa. The phloem exudates also have features typical of other phloem saps, e.g. high pH and K⁺ concentration. These data provide an indication of which solutes are translocated, providing a quantitative basis for further studies on photo assimilates loading and translocation in cucurbits and similarly the role of phloem sap proteins on insect feeding or vice-versa.

59. Radio Toxicity after Radioiodine-131 (¹³¹I) Therapy in Graves' Hyperthyroidism- Chromosomal Aberrations and Micronucleus as Biomarkers

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Key words: ¹³¹I therapy, Graves' disease, Chromosomal aberrations, Micronucleus induction

Genotoxic study was carried out on the presence of chromosomal aberrations (CA) and micro molecules (MN) in peripheral blood lymphocytes of 19 Graves' disease patients who received ¹³¹I sodium iodine orally. Blood samples were obtained before treatment, 7th and 30th day after treatment. The results indicate a relationship among dose, CA and MN frequency. A significant increase in 7th day post therapy compared to pre therapy and a decrease in mean levels of CA and MN of 30th day post therapy was observed compared to the 7th day therapy sample suggesting that low dose ¹³¹I cytogenetic damage is minimal and reversible.

60. Assessment of Erythrocyte Arginase as a Marker in South Indian Breast Cancer Patients

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Key words: **Breast cancer, Erythrocyte arginase, Urea, Tumor marker**

To assess the level of erythrocyte arginase as a marker in breast cancer patients, a study was conducted among 30 subjects (15 breast cancer patients + 15 normal). Packed RBC was washed and activity of erythrocyte arginase was estimated. The breast cancer patients showed a mean value of 15.10+ 4.49 (7-20U/L). In the controls, the enzyme activity levels were estimated to be 5-6.9 U/L with a mean of 5.54+ 0.69. The significant increase in the activity of breast cancers compared to normal suggests that the enzyme may be used as a marker to detect early cancers.

61. Induction of Micronuclei in Buccal Mucosa of Liquid Mosquito Repellents Users

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Key words: **Micronucleus test, Chromosomal aberration**

In this study micronucleus and other nuclear abnormalities were investigated in exfoliated buccal mucosa of 75 mosquito repellents users and 75 control individuals with ages varying between 29-46 years. The frequency of micro nucleated cell (MNC) was significantly higher in exposed groups than in

controls. The frequency of total MNC and other nuclear abnormalities were increased with duration of exposure compared to control group. We determined the frequency of binucleated cells (BNC) and broken egg cells (BEG) amongst exposed and control individuals. This is the primary study that gives a data regarding the genetic risk of repellent usage.

62. Expression Profiling and Sequencing Analysis of ω 3 Fatty Acid Desaturase Gene (*GmFAD3C*) from Indian Cultivar Soybean JS385

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Key words: Omega3 Fatty acid desaturase, Sequence analysis, Linolenic acid, RT-PCR; q-PCR; Soybean

Omega 3 fatty acid desaturase gene (*GmFAD3C*) is the key player for the conversion of the polyunsaturated fatty acid linoleic acid to linolenic acid. Two full-length cDNA sequences (EU678358 and EU908063) for omega 3 fatty acid desaturase gene (*GmFAD3C*) were cloned and sequenced from leaf and seed of soybean (*Glycine max*) Indian cultivar JS385. Phylogenetic analysis revealed that both EU678358 and EU908063 belonged to *GmFAD3C* group. The relative quantitation of *FAD3* gene in seed, leaf and stem of soybean showed the expression difference of the gene in various plant tissues. q-PCR indicated that the mRNA expression of *GmFAD3* in seeds was five fold higher than that of leaves.

63. Inhibitory Potential of Ginger Extracts Against Enzymes Linked to type 2 Diabetes and Inflammation

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Key words: **Ginger, α -glucosidase, α -amylase, COX-1**

In the current study, ginger extracts were screened to determine the variations in phenolic-linked antioxidant activity and the potential to inhibit key enzymes relevant to type 2 diabetes and inflammation. Polyphenol rich extracts from ginger was tested for their ability to inhibit pancreatic α -amylase, α -glucosidase and cyclooxygenase. From all the extracts, ethyl acetate extract showed higher activity compared to others. These studies indicate that ginger had the best potential for inhibition of α -glucosidase and α -amylase relevant for type-2 diabetes management and COX-1 inhibition for inflammation.

64. Anti-ulcer Activity of *Solanum Xanthocarpum* Scrad and Wendle (Solanaceae), 70% Methanolic Extract

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Key words: **Glutathione, Glutathione peroxidase, Superoxide dismutase, Lipid peroxidation**

Solanum xanthocarpum Scrad and Wendle (Solanaceae), a well known ingredient of Dasamoolarishtom with wide spectrum of medicinal properties

was evaluated for its ability to prevent ethanol induced ulceration and gastric mucosal damage in Wistar strain albino rats and its comparison with standard antiulcer drug Ranitidine. The activity may be due to the inhibition of acid and pepsin secretion or their *in vitro* ability to bind this or scavenging free radicals. Pretreatment with *Solanum xanthocarpum*, methanolic extract (250mg and 500mg/Kg) and standard reference drug Ranitidine reduced the ulcer index. Further the activities of reduced glutathione, glutathione peroxidase, superoxide dismutase in gastric mucosa were increased whereas lipid peroxidation was found to be decreased. The results were more significant in rats administered with 500mg/Kg body weight. This indicates the presence of antiulcer properties of *Solanum xanthocarpum*.

65. Exploration of Molecular Targets and QSAR Studies for Betulinic Acid

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Key words: **Betulinic acid, docking, QSAR, anti-inflammatory, anti-HIV, anti-malarial, anti-neoplastic**

Betulinic acid is a naturally occurring pentacyclic triterpenoid found in the bark of *Betula alba*, *B. pubescens*, *Ziziphus mauritiana* etc. It has been reported as anti-retroviral, anti-malarial, anti-cancer & anti-inflammatory agent. Keeping these pharmacological activities in mind, we explored potential target for each. Structure activity relationship was studied through QSAR. Docking experiments showed that Human's receptors *viz.*, Serum albumin, TNF, Dihydropterolate Synthase-I and DNA Topoisomerase-I, are potential targets for Betulinic acid against anti-inflammatory, anti-HIV, anti-malarial and anti-neoplastic activities respectively. The QSAR studies indicate that hydroxyl group chemical descriptor is responsible for anti-neoplastic ($rCV^2= 0.75$ & $r^2=0.9872$) and anti-HIV ($rCV^2 = 0.59$ & $r^2=0.57741$) activities, while logP

(Octanol/Water Partition coefficient) and shape index (basic kappa, order 2) chemical descriptors found responsible for anti-malarial activity ($r_{CV}^2=0.68$ & $r^2=0.999449$). On the other hand logP (Octanol/Water partition coefficient) and conformation minimum energy chemical descriptors found responsible for anti-inflammatory activity ($r_{CV}^2=0.7$ & $r^2=0.99$).

66. Factors Influencing *Lactobacillus Plantarum* as a Probiotic and it's *in vitro* Cholesterol Reducing Properties

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Key words: Lactobacillus, Probiotics, Bile salts, Cholesterol assimilation, Co-precipitation

This study focuses on factors influencing the usage of *Lactobacillus plantarum* as a probiotic and to evaluate its cholesterol up taking capacity. Several *in vitro* tests were conducted to screen its probiotic properties such as resistance to gastric acidity, bile tolerance, bile salt hydrolase activity, anti-microbial activity, antibiotic resistance and metabolic activities were also examined. *In vitro* cholesterol assimilation was studied by using different concentrations of Phosphotidyl Choline- Cholesterol (PC-C) vesicles in MRS broth. The strain was able to assimilate cholesterol at varying levels. Possible mechanisms involved in cholesterol uptake such as assimilation and co-precipitation was investigated.

67. Herpatoprotective and Immunomodulatory Properties of Aqueous Extract of *Curcuma Longa* in CCI_4 Intoxicated Swiss Albino Mice

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Key words: Immune system, Swiss albino mice, Curcuma longa, Immunotherapeutic agents, SGOT, SGPT, Bilirubin

The goal of the present study was to evaluate the hepatoprotective and immunotherapeutic effect of aqueous extract of turmeric rhizome in CCI_4 intoxicated Swiss albino mice. The mice of average weight 20g were taken and divided into four groups. Each experimental group has three mice. One group of mice received CCI_4 treatment at a dose of 0.25ml/kg b.w. (i. p.) for 7 days. Second group was fed orally the aqueous extract of turmeric at dose of 50 mg/kg b.w. for 15 days. The third group was given both the turmeric extract (for 15 days, orally) and CCI_4 (for last 7 days, i.p.). The fourth group was kept as a control. To assay the liver function, the serum level of transaminase enzymes viz, SGOT and SGPT and bilirubin level were determined. The activities of transaminase enzymes as well as bilirubin level were raised in serum in cells treated animal. The aqueous extract of turmeric reduced the level of SGOT, SGPT and Bilirubin in CCI_4 intoxicated mice. To evaluate the immunotherapeutic properties of *Curcuma longa* (turmeric), non specific host response was studied for each group and parameters like phagocytosis index, intracellular killing assay, morphological alteration and myeloperoxidase release were studied in peritoneal macrophages. The results suggest that the *Curcuma longa* has immunotherapeutic properties along with its ability to ameliorate hepatotoxicity.

68. Evaluation of Gold Nanorods as a Novel Cancer Therapeutic-in vitro and in vivo Studies

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Key words: **Gold nanorods, monocytes, cytotoxicity, biodistribution, ICP, AES, TEM**

Gold Nanorods (GNR), a new class of gold nanoparticles has shown high potential for photo thermal therapy and imaging. Assessment of biocompatibility and biodistribution of GNR is necessary before going into clinical use. For *in vitro* studies cultured monocytes were treated with different concentrations of GNR and exposed for 48h and evaluated their cellular morphology (Light microscopy), mitochondrial function (MTT), mitochondrial leakage (LDH) and inflammatory response (Lipoxygenase) under control and exposed condition (48h). Results were similar to that of control. The *in vivo* biodistribution of GNR in Swiss albino mice was analyzed by UV-VISIBLE – NR Spectrophotometry, ICP AES and TEM. This pilot study confirms that the maximum organ distribution of GNR occurs at 3h post injection and this time interval can be optimized for further biomedical applications such as drug delivery, cancer cell diagnostics, cell imaging and therapeutics.

69. **Cardioprotective Effect of Cuttlefish Liver Oil in Isoproterenol Induced Myocardial Infarction**

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Key words: Cardioprotective, Cuttlefish liver oil, Myocardial infarction, Eicosapentaenoic acid, Docosahexaenoic acid

Feeding 1% cuttlefish (*Sepia pharaonis*) liver oil for a period of 45 days to male albino Sprague Dawley rats significantly prevented the isoproterenol-induced elevation in the levels of serum marker enzymes. The fatty acid analysis of the cuttlefish liver oil showed that among PUFAs, ω -3 PUFAs were 7.6% whereas ω -6PUFAs were 1.5% of the ω -3 PUFAs, EPA was 4.9% and DHA 2.5%. PUFAs, viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have powerful antithrombogenic effect and are suggested to act by inhibiting the conversion of arachidonic acid (AA) to thromboxane A₂ (TXA₂) and facilitating production of prostacyclin (PGI₂), an inhibitor of platelet aggregation. The findings indicate that feeding CFLO at a dose of 1% offered protection against induction of myocardial infarction.

70. Anticancer Property of Purified Fraction of Cuttlefish Ink on Cervical Cancer Cells

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Key words: **Cervicalcancer, HeLa, Caski,apoptosis, Peptidoglycan fraction C2**

Cuttlefish and squid form a major marine fishery resource of India and they are exploited mainly for export. The ink sacs form the waste material in squid and cuttlefish processing industry. Attempts are currently being made to isolate bioactive substances from these waste materials for biomedical research. In the present study we report the anticancer property of a peptidoglycan isolated from the ink of cuttlefish, *Sepia pharaonis* Ehrenberg. The ink from cuttlefish, *Sepia pharaonis* was first extracted using Tris-HCl and fractionated using ion exchange and gel filtration chromatography. Further analysis showed that the fraction C₂ was a uronic acid rich peptidoglycan (molecular mass 10KD) and it is made up of five aminoacids namely aspartic acid, serine, threonine, glutamic acid and alanine. Purified fraction C₂ of cuttlefish, *Sepia pharaonis* showed a significant anticancer activity through inducing typical morphological characters of apoptosis like chromatin condensation, membrane blebbing and DNA damage on cervical cancer cells *in vitro*. These findings suggest the profound anticarcinogenic activity of purified fraction of cuttlefish ink on cervical cancer cells and thus render itself as a potential chemotherapeutic drug for the treatment of cervical cancer.

71. SDS-PAGE Analysis of Globulin and Albumin Fraction of Seed Storage Proteins in Mungbean

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Key words: *Vigna radiata*, Protein content and SDS-PAGE

Pulses are the richest source of proteins. About 23 million hectares of land (17% of the total cultivated area) have been under cultivation of pulses with an annual production of about 12 million tones. Mungbean (*Vigna radiata* (L) Wilczek) is one of the most important pulse crops. It is grown in almost all parts of the country. Mungbean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snack and many other preparations. Ascorbic acid (Vit. C) is synthesized in sprouted seeds of Mungbean. The amount of riboflavin and thiamin is also increased. In order to analyze the protein content of the two popular varieties grown in Maharashtra the present work was undertaken. For the study Vaibhav and Kopergaon -1 cultivars were selected. In the biochemical estimation, amount of total protein estimated by Lowry's method whereas the fractions of globulin and albumin proteins were prepared using SDS-PAGE method of protein separation. Now a day's proteins are used as molecular marker for the identification of different genotypes. Banding pattern of the varieties compared to show the difference among the studied varieties them.

72. Association of ACE(I/D) and ApoE (HhaI) Gene Polymorphisms Among the Adult Asian Indians with Indian Diabetes Risk Scores: A Population Based Study from Kolkata, India

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Keywords: ACE, ApoE, Diabetes, Asian Indian

Both angiotensin converting enzyme (ACE) gene insertion/ deletion and apolipoprotein E gene (ApoE) HhaI polymorphisms are well identified potential risk factor related to cardiovascular health including diabetes. In the present study the association of the ACE and ApoE gene polymorphisms on individuals with high and low Indian Diabetes Risk Score (IDRS) was investigated. A total of 350 (184 males & 166 females) adults participated in the study. Anthropometric measures, lipid profiles, fasting blood glucose and blood pressure measures were obtained from participants. Individuals were then scored according to the conditions as mentioned in IDRS and divided into two groups as high (≥ 60) and low (< 60). A sample of 139 individuals was selected randomly for genotyping. The association of ACE (I/D) and ApoE (HhaI) gene polymorphisms among individuals with high and low groups were analyzed. It was found that individuals with ApoE e4 and ACE*D combination had significantly high IDR score as compared to their counterpart indicating genetic susceptibility among the people of Indian origin (PIO)

73. Spermicidal Action of a Purified Protein from *Ricinus Communis*: An *in vitro* Study

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Key words: *Ricinus communis*, Sperm immobilization, Antifertility, *In vitro*, Acrosin

We isolated a 62kDa protein from *Ricinus communis* (Rp) having sperm immobilization activity and studied its effects *in vitro*. There was also a significant reduction in sperm viability and hypo-osmotic swelling in the treated groups. In the proteins treated groups the number of acrosome reacted cells were found to be high. The activity of membrane bound 5' nucleotidase and acrosomal acrosin were reduced. The spermicidal action was reversible as revealed by the sperm revival test. In short the protein Rp disturbs the seminal plasma membrane integrity and caused spermicidal action *in vitro*.

74. **Beneficial Effects of Sida Cordifolia L. on Quinolinic Acid Induced Neurotoxicity**

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Key words: Sida Cordifolia; Quinolinic acid; lipid peroxidation; Oxidative stress; scavenging enzymes; antioxidant

This study aimed at assessing the effects of ethanolic extract of sida cordifolia roots on quinolinic acid induced neurotoxicity in rat brain. Results revealed that the lipid peroxidation products malondialdehyde, conjugated dienes and hydroperoxides decreased and the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase increased significantly in the brain of rats administered quinolinic acid along with the extract. There was also a concomitant increase in the activity of acetyl choline esterase. In short, the study reveals that 50% ethanolic extract of sida cordifolia has got potent antioxidant activity and it alters the membrane fluidity.

75. Derangements in Carbohydrate Metabolism of Ethanol Administered Diabetic Rats

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Key words: Diabetes, Gluconeogenesis, Glycogen metabolism, Glycolysis, Citric acid cycle, Glucose incorporation

This study was aimed at revealing the derangements in carbohydrate metabolism of ethanol administered diabetic rats. Sprague Dawley rats were made diabetic using streptozotocin and were administered ethanol for 30 days. Marker enzymes of glycolysis, gluconeogenesis, citric acid cycle and glycogen metabolism were assayed along with the incorporation of labeled glucose to hepatic glycogen store. Results revealed that ethanol administration to diabetic animals reduced blood glucose levels by inhibiting gluconeogenesis along with a concomitant increase in glucose utilization via the glycolytic, citric acid cycle. There was also increase in hepatic glycogen store.

76. Tumor site- Targeted Induction of Oxidative Damage by BIS (3, 5-Diiodo 2, 4, 6-Trihydroxyphenyl) Squaraine Mediated Photodynamic Therapy

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Key words: Photodynamic therapy, Squaraine dye, Photosensitizer, Skin tumor, DMBA

Photodynamic therapy (PDT) of cancer involves the administration of a photosensitizing drug, which preferentially localizes within the tumor, followed by illumination of the tumor region with light of required wavelength. The mechanism of tumor photodestruction by bis (3, 5-diiodo-2, 4, 6-trihydroxyphenyl) squaraine- a newly developed photosensitizer- is evaluated using skin tumor-induced mice models. Since PDT kills tumor tissues through the generation of reactive oxygen species, we have checked whether the oxidative stress is confined only to the tumor site. The results demonstrate that squaraine-PDT elicits oxidative stress only in the tumor region keeping the normal metabolic organs like liver and kidney unaffected.

77. Chemopreventive Potential of *Saraca Asoka* Against DMBA Induced Skin Carcinogenesis in Mice

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Key words: Chemoprevention, DMBA, *Saraca asoka*, Skin Tumor

This study investigates the chemopreventive activity of flavonoid fraction of *Saraca asoka* (flowers) on DMBA induced skin cancer in swiss albino mice. Single topical application of DMBA (100 µg/ 50 µl of acetone) followed by three times a week treatment of croton oil (1% in acetone) after two weeks for 20 weeks resulted in tumour induction. The application of *S. asoka* 30 minutes prior to the application of croton oil thrice weekly for 20 weeks caused significant reduction in the number of tumours per mouse, percentage of tumour-bearing mice and delayed latency period for appearance of first tumour. In plant treated animals there was a significant increase in the levels of reduced glutathione, catalase and protein in skin compared to the untreated animals. Conversely, there was a significant decrease in the lipid peroxidation levels. These findings are suggestive for the chemopreventive activity of flavonoids fraction of *S. asoka* on two stage skin carcinogenesis.

78. Toxicity Evaluation of Tri Ethylene Glycol Capped Gold Nanoparticles on Experimental Models

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Key words: AuNP, *In vitro* toxicity, Cell viability, Biodistribution

This study was undertaken to address the cellular response to gold nanoparticle exposure. The study evaluated the toxic effects of AuNPs for its future use in cancer therapy. Here we report the interactions of Gold nanoparticles (AuNPs) on *in vitro* and *in vivo* experimental models. For *in vitro* toxicity evaluation, Trypan blue cell viability assay, cellular morphology (light microscopy), mitochondrial function (MTT assay), lysosomal membrane integrity (Neutral Red Uptake assay) were assessed under control and nanoparticle exposed conditions. *In vivo* biodistribution and toxicity studies revealed the compatibility of AuNPs in animal systems. Overall, the *in vitro* and *in vivo* studies revealed that AuNPs were non-toxic in both the systems and can be used for future clinical trials.

79. Probing the Cytotoxicity of CdSe Quantum Dots in SiHa Cell Cultures

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Keywords: Quantum Dots, Cytotoxicity, Cell Viability, Cervical cancer, Silica overcoating

In this report, we evaluated the cytotoxicity of a series of quantum dots i.e., silica overcoated CdSe QDs and TOPO capped CdSe QDs, with systematically on human cervical cancer cell line (SiHa). TOPO capped CdSe QDs were synthesized and rendered water soluble by overcoating with silica, using APS as silica precursor. Cytotoxicity studies were conducted by exposing cells to freshly synthesized QDs as a function of time (0-72 h) and different concentration by following various methodologies such as MTT assay, NRU assay, trypan blue exclusion method and morphological examination of cells using phase contrast microscope. Results showed that surface modification with silica could lessen the cytotoxicity of CdSe QDs, while surface modification with TOPO showed significant cell damage.

80. *Moringa Oleifera* Modulates Oxidative Stress and Opacification in Selenite Cataract

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Key words: **Antioxidants, Flavonoids, *Moringa oleifera*, Oxidative stress, Selenite cataract**

Purpose

To study the efficacy of flavonoid from *M oleifera* (FMO) in maintaining lens antioxidant status in selenite cataract.

Methods

In vivo studies were carried out on Sprague-Dawley rat pups and divided into G I- Normal; G II - Selenite-induced; G III- Selenite + FMO treated (2.5 µg/g body weight). Cataract was induced by single subcutaneous injection of sodium selenite (4µg/g body weight) on the 10th day and treatment from 8th to 15th day. *In vitro* DPPH and reducing power assays in FMO and antioxidant status, levels of lipid and protein oxidation were carried out in lens.

Results

In vitro assays indicate FMO possess excellent antioxidant property. *In vivo* studies, near normal levels of antioxidant status, lower levels of lipid and protein oxidation were observed in G III compared to G II.

Conclusion

These results indicate the promising antioxidant and therapeutic potential of FMO as an anticataractogenic agent.

81. Comparative Molecular Typing of *Aeromonas* Species using Eric-pcr, Box-pcr and Rapd Techniques

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Key words: *Aeromonas hydrophila*, Molecular typing, ERIC-PCR, RAPD-PCR

Aeromonas species are Gram-negative, non-spore-forming, rod-shaped bacteria and occur ubiquitously and autochthonously in aquatic environments. However historically the *Aeromonas* genus belongs in the family *Vibrionaceae*, but later placed in family, the *Aeromonadaceae*. The genus includes at least 13 genospecies, among which are the mesophilic *A. hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, and *A. schubertii*, and the non-motile, psychrophilic *A. salmonicida*. The mesophilic species have been associated with a wide range of infections in humans (Janda & Abbott, 1996). Although members of the genus have classically been divided into three biochemically differentiated groups (typified by *A. hydrophila*, *A. caviae*, and *A. sobria*), these contain a number of genospecies, to which new species have been added (Carnahan & Altwegg, 1996). Currently the genus is made up of 17 DNA hybridization groups representing a range of genospecies and phenospecies.

Attempts have been made for prompt identification, classification and characterization of *Aeromonas* species by application of molecular techniques. Different molecular genomic methods like polymerase chain reaction (PCR), Randomly amplified polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Ribotyping etc. are now commonly used to identify and characterize this pathogen. RAPD-PCR, as developed by Williams *et al.*, (1990) and Welsh and McClelland (1990), has been proved useful for identification and classification of *Aeromonas* species. Three categories of conserved repetitive sequences are used for bacterial typing, the enterobacterial repetitive intergenic consensus sequences (ERIC), the repetitive extragenic

palindromic sequence (REP), and the BOX element. These techniques have wide scope of application in characterization of this pathogen. In the present paper, application of above molecular techniques in genomic characterization of *Aeromonas* species have been elaborated.

82. Ameliorative Effects of Tender Coconut Water in Hypertensive Rats

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Key words: Tender coconut water, Fructose, Hypertension, Lipid profile, Plasma insulin

In the present study we examined the effect of Tender Coconut Water (TCW) in hypertensive rats on Systolic blood pressure, serum cholesterol, HDL, LDL, Triglyceride, Plasma glucose and insulin. Male Sprague *Dawley* rats were fed fructose – rich diets treated with TCW (4ml/100 g of body weight) by gastric intubation for 3 weeks. Systolic blood pressure was measured every three days using the indirect tail cuff method. Systolic blood pressure was higher in hypertensive rats (145±2 mm Hg, $p < 0.05$) compared with the controls (119±2 mm Hg), and was lower in TCW treated groups (126±3 mm Hg). Administration of TCW also reduced serum total cholesterol, triglycerides, LDL cholesterol levels and increased HDL cholesterol. Plasma insulin and glucose were higher in fructose fed hypertensive group compared to controls, which on TCW treatment were decreased. These results suggest that, TCW treatment could prevent and reverse high blood pressure induced by the diet rich in fructose probably by improvement of lipid profile and plasma insulin levels.

83. Antidiabetic Effect of *Costus Igneus* (N. E.Br.) in Experimental Animals

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Key words: Hypoglycaemic, Antidiabetic, *Costus igneus* (N. E. Br.), chloroform extract of *C. igneus* (CEC), Diabetes mellitus (DM), Streptozotocin (STZ), Blood glucose, Serum insulin, Glycosylated haemoglobin (HbA1c), Fructosamine, Glucose tolerance test (GTT)

Antidiabetic activity of the chloroform extract of *Costus igneus* (N. E. Br.) (CEC) in streptozotocin (STZ) - induced was studied. After 30 days of treatment of diabetic rats with 50mg/kg BW of CEC (CEC₅₀), there was 66.78% fall in fasting blood glucose (FBG) level, significant enhancement of liver glycogen and serum insulin level. Activities of various enzymes were also brought to normal levels. Glycosylated haemoglobin (HbA1c) and the fructosamine were decreased. Single dose of CEC₅₀ enhanced serum insulin during GTT. Incubation of pancreatic islets of diabetic rat with column fraction enhanced insulin release. *C. igneus* had potential inhibitory effect on albumin glycation also.

84. Antibacterial Effect of Selected Medicinal Plants used in Traditional Medicine Against Meningitis Causing Bacteria

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Key words: **Meningitis, Antibacterial activity, Phytomedicine, Medicinal plants, Bacteria, Brain infections**

Meningitis is a particularly dangerous infection because of the delicate nature of brain. Medicinal plants are important elements of traditional medicine. Phytomedicines have shown great promise in the treatment of incurable infectious diseases including various bacterial and viral infections. Antimicrobial activity of 11 traditional medicinal plants against four meningitis causing bacteria was demonstrated. Results showed immense antibacterial potentials for *A. sativum*, *F. religiosa* and *C. asiatica* that will help to discover new chemical classes of antibiotics that could effectively treat bacterial meningitis other than or along with the conventional antibiotics used for its treatment. Group B *Streptococci* and *Streptococcus pneumoniae* were susceptible to almost all the plant samples tested whereas *Pseudomonas aeruginosa* and *Escherichia coli* were susceptible only to the crude extract of *Allium sativum*. The study may help in the development of new pharmaceutical preparations that may address unmet therapeutic needs in the treatment of brain infections.

85. Inhibitory Effects of some Anti-inflammatory and Anti-hypertensive drugs on Purified Goat Brain Aminopeptidase B

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Key words: Goat brain aminopeptidase B, Anti-inflammatory drugs, Anti-hypertensive agents

Aminopeptidase B, an exopeptidase of cytosolic origin has been reported to be involved in inflammation as well as in angiotensin metabolism. It was, therefore, thought proper to study the *in vitro* inhibitory effects of some common anti-inflammatory and some anti-hypertensive agents on purified goat brain aminopeptidase B. Out of the anti-inflammatory drugs, analgin, diclofenac, nimesulide, paracetamol, piroxicam and hydrocorticosterone, only analgin, diclofenac and nimesulide showed strong competitive inhibition. Among the anti-hypertensive agents, losartan, amlodipin besylate, atenolol, labetalol and analprin studied, the three, amlodipin besylate (a calcium channel blocker), atenolol (β -androgenic blocker), labetalol (α - and β -androgenic blocker) were strong competitive inhibitors of aminopeptidase B.

86. *In vitro* Antimicrobial and Phytochemical Investigation of the Leaves of *Diospyros ebenum*, L. – A Less known Folk medicinal plant practiced by Tribal people at Biligirirangana Hill, Karnataka, India

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Key words: *Diospyros ebenum*, Ethno-medicinal plant, Infectious diseases, Antimicrobial activity, Phytochemicals

In the present study, *Diospyros ebenum*, L. belongs to family, *Ebenaceae* a less known ethno-medicinal plant practiced by tribal people of 'Biligirirangana hill' a historical religious hillock of Karnataka, India. The plant leaves contain a number of medicinally important compounds so, an attempt was made to evaluate the phyto-chemical analysis for the quantity of Protein, total sugar, ascorbic acid, phenolic compounds and the presence of a key secondary metabolite *i.e.*, flavonoid respectively. The antibacterial activity of the leaf extracts of *Diospyros ebenum* against pathogenic bacteria like gram positive (*Pseudomonas aeruginosa* and *staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria by *in vitro* agar well diffusion method. The plant aqueous leaves extract showed pronounced inhibition than Methanolic leaf extract. Interestingly, the purified Leaf extract *D. ebenum* showed more inhibitory action on *Pseudomonas aeruginosa* followed by *Escherichia coli*. In conclusion, the presence of active phytochemicals and flavonoid indicates that, the plant possessing antimicrobial properties against pathogenic bacteria and justifies the practice of the plant against infectious diseases by tribal people at *Biligirirangana Hill* area. Further, this can be recommended as a potential antimicrobial drug to the authority of drug development based on the active phytochemicals present in the above plant.

87. Sound Production by the Marine Fish, *Etroplus Suratensis* (Bloch, 1785) of Family Cichlidae

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Key words: Fish bioacoustics, Pharyngeal apparatus, Swimbladder, *Etroplus suratensis*, Cichlidae

Fishes produce a variety of sounds using different mechanisms and for different reasons. Sounds may be intentionally produced as warnings to predators or competitors, to attract mates or as a fright response.

An advanced technology was used in the underwater sound recordings with the help of the imported hydrophone Reson TC-4032-1 model and the software RT Pro Dynamic Signal Analysis software Photon II. The data were analysed in MATLAB 7.0 Software. The experimental study was undertaken at a rectangular tank (length 426 x width 226x depth 122 cm) designed for acoustic experiment in the seawater circulation system in our centre. Seven days before beginning the experiment, the fish were allowed to establish territories. The study was carried out day and night with ambient water temperature (25-29°C). A typical sound class in series of low frequency pulses often entitled 'br-r-r' or growls, which have been produced by the pharyngeal apparatus and then amplified by the swim bladder. Chewing and thump sounds are rarely produced by the above fish.

Sounds of *E. suratensis* showed a low frequency at a bandwidth from 50Hz to 3 kHz and 50 to 600Hz. Oscillograms and Sonograms were plotted from the recorded sounds. The mean dominant frequency was seen at 500 Hz (ranged from 450 to 900Hz). The mean number of pulses was 12 and ranged from 4 to 20 and the mean call duration level of 85 ms ranged from 50 to 120ms. The mean pulse ratio of 14.6 observed ranged between 10 to 18. In the present study an interesting observation of colour change at the time of aggressive interactions was noted.

88. Studies on Nucleus / Cytoplasmic Ratio and Mitotic Index in Chilli (*Capsicum Frutescens* L.) Induced by Chilli Mottle Virus (CHIMV) Disease

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Key words: **Chilli (*Capsicum frutescens* L.), ChiMV, Carnoy's fluid, N/C ratio and Mitotic index**

The diseased plants of *Capsicum frutescens* L. show symptoms like dark green mottling adjacent to main leaf veins, reduction in leaf area and distortion virus combination. Generally cytoplasm depends upon nucleus for energy supply and also for its replacement. The power nature of the nucleus envelop permits the ready passage of materials in other direction. During the study, the root-tip of *C. frutescens* L. were fixed in carnoy's fluid of ratio 1:3 (one part glacial acetic acid and three part ethyl alcohol) for 6-14 hours in acetic acid. The present investigation revealed that the nucleus / Cytoplasmic ratio (N/C) of healthy and diseased root meristem cells of *C. frutescens* L. were 0.114 and 0.190 respectively. On the other hand cytoplasm also depends upon nucleus for its replacements possibly through RNA, which is formed in the nucleus and passed to the cytoplasm. Mitotic index (MI) in the meristem cells of healthy root-tip was recorded as 25.13 while the diseased was 8.96. The increase in the percentage value of mitotic index of diseased calls was low in comparison to the healthy cells of test plants.

89. Differential Antioxidative Effect of Fruits

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Key words: Oxidative stress, Antioxidants, Flavonoids, Superoxide dismutase and DPPH

Phytochemicals, including phenolics and flavonoids, are suggested to be the major bioactive compounds contributing to the health benefits of fruits and vegetables. Among these grapes have been found to result almost double the amount of recommended total antioxidant capacity needed to counteract the deficit associated with consuming 1000 calories of food. In present study varieties and species based antioxidant capacity using biochemical means and marker assistance has been investigated. The enzymatic antioxidants such as superoxide dismutase, ascorbate peroxidase, glutathione reductase were found to be less effective in exhibits varietal antioxidative effect than the non-enzymatic antioxidants.

Black and green grapes were found to possess higher antioxidative potential (measured by % of DPPH reduction – black grapes-80, green grapes-78, red apple-60, green apple-55 and cucumber-12), total flavonoids measured spectrophotometrically at 285nm- black grapes-0.1, green grapes-0.09, red apple-0.05, green apple-0.05 and cucumber-0.01) and UV absorption capacity (measured at 305 nm black grapes-0.20, green grapes-0.19, red apple-0.15, green apple-0.12 and cucumber-0.04). It was interesting to note that though total antioxidative potential (including enzymatic and non-enzymatic) varied in different species, no variation was found in enzymatic antioxidant Superoxide Dismutase status (all the fruits had value 0.12 U/mg of total protein). Variations were found in biochemical and genomic studies related to non-enzymatic antioxidants viz. flavonoid. The FSAR study of flavonoids suggest that the tolerance to UV exposure and the scavenging action of ROS is due its ability to exhibit keto enol tautomerism and high redox potential and subsequent conformational changes (δ to δ^*). The Agarose gel electrophoresis also substantiated highest antioxidative capacity of the grapes, followed by apples and cucumber. The results suggest usefulness of grapes in the prevention of diseases caused by ROS activity.

90. Effects of Deprenyl on T47D Human Breast Cancer Cell Lines and MAPK Signalling Pathways

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Key words: T47D, Stress, Deprenyl, MAPK

Breast cancer remains the second leading cause of cancer mortality among women. Several factors influence breast cancer, stress and aging are major of them. The focus of the study was to investigate neuroendocrine-immune modulation in breast cancer. T47D cells were treated with different doses of Deprenyl, monoamine oxidase inhibitor, for different time interval and it was noticed that 10^{-6} M concentration showed more viability at 72 hour incubation. Western blotting analysis demonstrated Deprenyl activated MAPK at 10^{-3} M concentration and activation level increased with more incubation period at same concentration.

91. Analysis of the Anti-Microbial Properties of Cardiovascular Drug - *Amlodipine Besylate*

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Antibiotics are among the most commonly used drugs till date. Hence, they have become one of the drugs to be commonly misused by physicians. Such injudicious over usage of antibiotics has led to the emergence of antibiotic resistant pathogens, thus resulting in a serious threat to global health. A renewed effort is being made to seek newer antibacterial agents, preferably in the form of anti-microbial chemotherapeutics (Non-Antibiotics) effective against pathogenic bacteria resistant to current antibiotics. Conventionally, a drug is designated by its dominant or first recognized function. However, after prolonged use, some other effects, not necessarily toxic effects, were discovered by systemic research. This multifunctional nature of most drugs has prompted the investigation into the possible anti-microbial property of drugs that have been used for a long time but have not been pharmacologically categorized as antibiotics. Among the Non-Antibiotics, phenothiazines demonstrated the maximum duality of action. An analysis of the structure activity relationship studies of various phenothiazines have revealed that the presence of three benzene rings in their structure, along with one or more halogen moieties, were the prime factors responsible for the characteristic anti-microbial action. The present study has been designed to investigate the possible antimicrobial action in cardiovascular drugs having two or more benzene rings in their structure. In this study, a particular cardiovascular drug called amlodipine besylate has been used and its antimicrobial property has been tested against a large number of bacterial pathogens belonging to both Gram positive and Gram-negative genera *in vitro*. Results have shown that this drug can arrest bacterial growth at concentrations 10-25 mg/ml. The antibacterial potentiality of the experimental drug was further enhanced by combining it synergistically with known antibiotics like penicillin, chloramphenicol, streptomycin, tetracycline and non-antibiotics like diclofenac, methdilazine, promethazine, trifluoperazine

and other chemotherapeutic agents. The ultimate aim of this investigation is to discover means to join the worldwide battle against drug resistance created by the excessive and indiscriminate applications of conventional antibiotics, and thereby search for newer alternatives in the form of newer anti-microbials.

92. Identification of Molecular Markers Linked with Bruchid Resistance in an Indian Sublobata (*Vigna radiata* var. Sublobata)

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The wild relatives and the primitive cultivars of grain legumes in general and *Vigna* in particular, constitute a reservoir of gene pool. Genetic resistance to bruchid was identified in an Indian collection, *V. radiata* var. *sublobata*, the progenitor of mungbean. Bruchid resistance assay of F₂ seeds revealed that 'bruchid resistance' is a dominant character which is governed by a major gene with a few modifier genes. On the basis of insect feeding assay, homozygous resistant and susceptible seed lots were identified. Twelve pairs of SSR primers of *V. radiata*, thirty eight pairs of SSR primers of *Vigna angularis*, two pairs of common bean SSR primers and two pairs of STS primers of a wild mungbean genotype ACC41 were employed to assess the polymorphism between the selected parents. STS 1 amplified 225 bp fragment from the resistant parent Sub-2 and all 85 resistant segregants where as the same fragment was absent in susceptible cultivar B-1 and 25 susceptible

segregants. The tightly linked marker validated in this study may be useful for generating superior genotypes with durable bruchid resistance by marker assisted selection in relatively shorter time span with accuracy.

93. Modelling of Inhibitors of Neuraminidase(NA) Proteins of Influenza B Virus

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Key words: Influenza, Influenza Virus B, Neuraminidase, Drug designing

This study is directed towards the Neuraminidase (NA) protein of the Influenza B Virus. First, NA sequences have been obtained from different continents and compared to identify the universal consensus sequence. From the 3D-structure of the consensus, attempt has been made to identify common regions/domains that remain fairly conserved in all the sequences. The domains thus identified, different existing drugs and a few new drugs are docked and their scores are compared. Hence, any drug/vaccine can be more effective if it is designed keeping in view these conserved domains, and can decrease the severity/mortality of the disease, and will probably prove to be a better weapon to combat mutated strains.

94. Different Molecular Identification Strategies for the Conservation of Endangered Indian Snakes

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Key words: **Snake species, Molecular identification, Cytochrome *b*, 16S rRNA, Cytochrome oxidase I, Multiplex PCR**

Survival of snake species like *Python molurus*, *Naja naja*, *Ptyas mucosus* and *Xenochrophis piscator*, is threatened due to uncontrolled illegal trade and poaching. Effective species identification techniques from seized materials are essential to ensure law enforcement. We describe the development of species identification methods based on the variations in certain regions of mitochondrial DNA in snake species. Partial sequences of three different genes were utilized for development of novel techniques i.e. cytochrome *b* for PCR-RFLP, 16S rRNA for multiplex species-specific PCR and cytochrome oxidase I for DNA mini-barcoding. The restriction fragment pattern generated by the PCR-RFLP allowed effective discrimination between the target species. Similarly, the species amplicons generated in the multiplexing approach and the high sequence similarity scores obtained through DNA mini-barcoding enabled unambiguous identification of endangered snake species of India.

95. Mitochondrial and nuclear genes: insights in to the phylogeny of recently evolved taxa

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Keywords: Mitochondrial genes, Nuclear markers, Crocodylia, Phylogenetic reconstruction, Recent evolution, Intrageneric relationships

The DNA sequences contain enormous phylogenetic information, which is helpful in revealing the evolutionary history of species. Two kinds of DNA sequences, mitochondrial and nuclear, are generally utilized to study the various levels of relationships between species. However, there is no consensus regarding the choice of mitochondrial or nuclear genes as the appropriate marker to resolve the phylogenetic relationships of recent radiations. Therefore the current study compares the utility of mitochondrial and nuclear markers by reconstructing the crocodylian phylogeny as the order Crocodylia consists of both ancient as well as recently evolved species. In this study three mtDNA genes, 16S rRNA, CO I and ND2 and three nuclear genes, C-MYC, Neurotrophin and DMP 1, were extensively analyzed using four different phylogenetic methods, Neighbour Joining, Maximum Parsimony, Maximum Likelihood and Bayesian inference. The results show that the mitochondrial markers are more efficient to study the phylogeny of recently evolved taxa than nuclear genes. Therefore the current study suggests the use of mitochondrial genes/ whole genome to examine the recent evolutionary events.

96. In Vitro Assessment of Lipid Peroxidation of Synthetic Pyrimidinone and its Analogues in Rat Liver Tissue

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Key words: **Synthesis, Pyrimidinone, Antioxidant, Lipid peroxidation**

Pyrimidinone and its derivatives have wide occurrence in nature being part of several biologically active molecules like vitamin B₂, folic acid, DNA-RNA nucleolus bases etc. Biological importance of pyrimidinones has been further established by anticancer, antiviral, angiotensin II antagonist, central stimulant, calcium antagonist etc. activities of a number of pyrimidinones. The present work utilizes chalcones as the starting compound. These chalcones are reacted with urea and thiourea in hydrochloric acid to obtain the corresponding pyrimidinones and thiopyrimidinones. These synthesized heterocyclics are characterized by standard tests and spectral data. These compounds show appreciable lipid peroxidation inhibiting activity in rat liver tissue.

97. Curative Effects of Garlic Oil & Onion Oil Fractions as Compared to Vitamin E on Rats Orally Fed with Lead Acetate Solution

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Lead is one of the ubiquitous pollutants. Daily feeding of lead acetate solution (10 mg \Kg\day) to rats for a month produced certain deleterious effects such as changes in the parameters of blood, serum & tissues, viz; RBC,

WBC, Hb, lead salt, proteins, lipids, oxidized lipids (TBARS), Vitamin C & E levels, glutathione content & in activities of AST, ALT & anti oxidant enzymes viz; catalase, GR, GPx & SOD. Simultaneous feeding of polar & non-polar fractions of garlic & onion oils & vitamin E (100mg \Kg \ day) to the rats fed with lead acetate for a month counteracted its deleterious effects significantly.

98. Free Radical Injury Status in Chronic Cervicitis, Cervical Intraepithelia Neoplasia and Carcinoma Cervix

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Key words: MDA, SOD, Free Radicals, Cervix Carcinoma.

Cancer of the cervix is the most frequent occurring disease after the breast cancer. Free radicals have been implicated in mediating vascular and tissue damage associated with several diseases. The present study was undertaken to evaluate the role of free radical injury in woman with cervicitis, Cervical Intraepithelial neoplasia and carcinoma cervix on 100 subjects (29 controls and 71 cases).

The mean plasma MDA levels in control group was found by 2.16 ± 0.16 nmol/ml whereas in the study group it range from 2.63 ± 0.09 in chronic cervicitis to 3.204 ± 0.306 in CIN to 4.295 ± 0.481 in carcinoma cervix patients ($p < 0.001$).

Mean SOD level in the control group was 2.352 ± 0.298 mU/mg and in the study it varied from 2.047 ± 0.157 in chronic cervicitis, 1.614 ± 0.192 in CIN and 0.924 ± 0.154 in carcinoma cervix patients ($p < 0.001$).

The above data revealed that the reduction of SOD and elevation in MDA level in patients with cervical pathology as compare to healthy subject.

99. Effect of Triethylamine on Chromosomal Changes in jute (*Corchorus olitorus* 1. Variety jro-632)

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Key words: **Triethylamine, *Corchorus olitorius* L., Root tip, Chromosome**

The present investigation was undertaken to study the cytological effects of Triethyl amine on jute (*Corchorus olitorius* 1. Variety JRO-632) obtained from Jute Agricultural Research Institute, ICAR, Barrackpore, West Bengal. Presoaked seeds of jute were treated with 0.5% Ethylamine for 6 hours. Chromosome analysis from root tip cells revealed a number of aberrations like fragments, clumping, random grouping of chromosomes, condensed chromosomes, erosion of chromosomes, stickiness, depolarization and polyploidy in metaphase stage and fragment, lagging, early and late separations were observed in anaphase stages. Micronuclei were also visible. The principal objective of this study lies in the fact that in mutation studies with jute if chromosome aberrations and sterility percentages could be of great significance and commercial importance.

100. Chromosomal Changes During In vitro Growth in Jute (*Corchorus olitorius* L. Variety Jro-632)

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Key words: **Leaf Tip, Chromosome, *Corchorus olitorius*, Auxins, Cytokinins**

The leaf tips of jute (*Corchorus olitorius* L. variety JRO-632) were grown in invitro cultured aseptically on 20 ml solid nutrient medium Murashige and Skoog's (1962) (MS) and Schenk and Hildebrandt's (1972)(SH) media were tried with various combinations and concentrations of different auxins (NAA, IAA, IBA and 2,4-D used separately 0.0170 mg/L to 0.3500 mg/L) and cytokines (BAP, Kn used separately 0.1400 mg/L to 3.3000 mg/L, coconut milk 10-35% (V/V). It was observed that the callus growing in medium containing NAA revealed different degrees of ploidy and mitotic abnormalities such as stickiness, clumping, diplo-chromatids and spindle disturbances from the early stage. The frequency of chromosomal abnormalities gradually increased with the age of the callus tissues. A comparison of the cytology of the callus growing in NAA and the regenerating callus growing in IBA revealed difference in the rate of division and mitotic abnormalities. Callus growing in NAA showed a comparatively lower rate of division and higher rate of mitotic abnormalities, while that growing in IBA exhibited just the opposite effect. It appears that NAA has a distinct role to play in influencing Karyological instabilities and mitotic rate of cells and also observed the role of different hormones in inducing karyological changes during in-vitro growth. The present study was aimed to study the role of different constituents of the medium in controlling in vitro growth and the karyological changes induced by them during organogenesis and callus formation of jute explants (*Corchorus olitorius* L. Variety JRO-632).

101. Chromosomal Changes in Jute (*Corchorus Olitorus* 1. Variety Jro-632)

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Key words: Ethylamine, Corchorus olitorius L., Root tip, Chromosome

The present investigation was undertaken to study the cytological effects of Ethylamine on jute (*Corchorus olitorius* 1. Variety JRO-632) obtained from Jute Agricultural Research Institute, ICAR, Barrackpore, West Bengal. Presoaked seeds of jute were treated with 0.5% Ethylamine for 6 hours. Chromosome analysis from root tip cells revealed a number of aberrations like fragments, clumping, random grouping of chromosomes, condensed chromosomes, erosion of chromosomes, stickiness, depolarization and polyploidy were observed in metaphase stage and fragment, lagging, early and late separations were observed in anaphase stages. Micronuclei were also visible. The principal objective of the above mentioned study lies in the fact that in mutation studies with jute if chromosome aberrations and sterility percentages could be of great significance and commercial importance.

102. An Investigation on the Effect of Napthalene Acetic Acid on Epicotyl Derived Callus Tissues of Jute (*Corchorus olitorius* I. Variety Jro-632)

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Key words: *Corchorus olitorius* L., Epicotyl Explant, Chromosome, Indole Acetic Acid.

Jute seeds (*Corchorus olitorius* L. Variety JRO-632) were grown in vitro in White's medium. The epicotyls explants were collected from in vitro growing seedlings and were cultured aseptically in both Murashige and Skoog's (MS) and Schenk and Hildebrandt's (SH) media with different combinations and concentrations of auxins and cytiokinins whereas in other cases no auxins was utilized. Callus tissues were obtained from the epicotyls explants in MS basal medium supplemented with Inodole Acetic Acid (IAA) and coconut milk. When the concentration of Indole Acetic Acid (IAA) was gradually increased vigorous growth of the callus tissues was observed. Indole Acetic Acid (IAA) also enhanced rapid growth of the callus tissues. Cytological analysis revealed that the callus tissues are mixoploid, diploid, polyploidy in nature. The present investigation indicates that Indole Acetic Acid (IAA) plays an important role in in- vitro culture of epicotyls callus tissues.

103. Effect of Tannic Acid Concentration on Percent Conversion of Tannic Acid to Gallic Acid by a *Penicillium Granulatum* pm-33 Strain Isolated from Kanpur Tannery Soil

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Key words: Tanneries, Tannase, Gallic acid, Percent conversion, Bioremediation, *Penicillium granulatum* PM-33 strain

Kanpur is one of the largest industrial metropolis of U.P. having more than two million population. About 480 leather industries are situated in the Jajmau area along the river Ganga at the downstream of the city. The tannery effluents are flown in the river Ganga. These effluents are highly toxic and contain tannin in addition to chloride, sulphide, chromium, etc. In the present work tannin tolerant fungal strain, *Penicillium granulatum* PM-33 has been isolated from tannery soil and evaluated for its tannase activity.

The strain has the potentiality to produce the enzyme Tannin Acyl Hydrolase (E.C.3.1.1.20) in presence of the substrate tannin as carbon source in the medium. Minimal medium containing 0.2% tannic acid as the sole carbon source was used for the isolation of the fungal strain. Tannase is an inducible and regulatory enzyme. With the increase in the concentration of tannic acid in the culture both the production of the enzyme tannase by the *P. granulatum* PM-33 strain is accordingly enhanced. The enzyme tannase (TAH) catalyses the hydrolysis of ester and depside bonds of hydrolysable tannins to liberate gallic acid and glucose. Gallic acid has undisputed commercial importance. It is a phenolic compound (3, 4, 5, trihydroxy benzoic acid) and is used mainly in pharmaceutical industries for manufacturing trimethoprim (TMP). The percent conversion of Tannic acid to Gallic acid increases with the increase in TA concentration in the medium upto 100 mgml⁻¹. At this concentration the percent conversion is 84. Any further increase in TA concentration results in

the decline of percent conversion. This may be either due to toxic nature of the tannic acid or due to end product repression. High tannin tolerant *Penicillium granulatum* PM-33 strains may be developed either through isolation and selection or through genetic manipulation and they may be used in the biotechnological processes like industrial production of gallic acid from the hydrolysable tannins. They can also be used for bioremediation of tannins from tannery effluents and tannin contaminated soils. Immobilization of TAH has added benefit.

104. Molecular Characterization of a Begomovirus Causing Leaf Curl Disease in Chilli (*Capsicum annuum*)

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Survey was conducted at chilli growing tract of Lucknow district, U.P., India to detect the incidence, severity and distribution of the leaf curl disease of chilli. Several fields had chilli plants (varieties/cultivars) exhibiting symptoms typical of begomovirus infection, with moderate to high disease incidence with varying degrees of severity, including puckering, crinkling, upward and downward curling of leaves, and stunting of plants. The top leaves of these plants were sampled and brought to laboratory for biological and molecular detection of the causal begomovirus including few apparently asymptomatic plants. Whitefly (*Bemisia tabaci*, Gennadius) transmission test of the disease dually appeared in chilli and some other solanaceous test plants also showed leaf curl symptoms. Total genomic DNA was isolated in accordance with the method prescribed by Dellaporta *et al.* (1983) from 100 mg leaf tissues of naturally infected as well as few apparently asymptomatic chilli samples. Polymerase Chain Reaction (PCR) amplifications were carried out with total DNA isolated from chilli samples using begomovirus DNA-A

(CP gene), DNA-B (MP gene) and DNA- β specific primers which resulted in positive amplification of DNA fragments of ~771bp, ~875bp and ~850bp, respectively, revealing the virus isolate under study to be a bipartite begomovirus associated with a satellite DNA- β . No such amplicons were obtained with asymptomatic plant samples taken as negative control. PCR amplified fragments (CP and MP genes) were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and the purified DNA fragments were sequenced from Bangalore GeNei (GeNei Pvt. Ltd., India). The nucleotide sequences of both the CP and MP genes were analyzed and data was submitted in GenBank database under accession nos. GQ284842 and GQ284844, respectively. Basic Local Alignment Search Tool (BLAST) of both the genes of the virus isolate shared highest sequence identity (97-98%) with various strains of *Tomato leaf curl New Delhi virus* (ToLCND). On the basis of highest sequence identities, the virus isolate has been identified as a strain of ToLCNDV.

105. Codon Optimization of the Matrix Genes of Diverse Strains of Influenza A Virus

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Highly pathogenic influenza A virus represents a growing threat for an influenza pandemic globally. Matrix proteins play an important role in determining host specificity. We have used the matrix gene for codon optimization in *E. coli*. Gene expression levels depend on several factors, such as promoter sequences and regulatory elements. One of the most important factors is the adaptation of the **codon** usage of the transcript gene to the typical **codon** usage of the host. Therefore, highly expressed genes in prokaryotic genomes under translational selection have a pronounced **codon** usage bias as they use a small subset of **codons** that are recognized by the most abundant tRNA species. Total 20 different strains of influenza virus were used and the codon adaptation index was found to be higher than the wild type of DNA

sequences. These artificial DNA can now be used for over expression in *E. coli* due to sufficient t-RNA in host cells. Wild type of DNA was not expressed properly due to codon bias. These finding may help to improve the heterologous expression system and also produce sufficient quantity of matrix based vaccines candidate.

106. Phylogeny of the Genus *Flavivirus* Using Complete Structural Protein of Mosquito Borne Viruses

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Keywords: Flaviviruses, Homology, Nucleotide sequence, Amino acid sequence

Several human pathogenic viruses such as Dengue, Yellow Fever, Japanese encephalitis, West Nile, Murray Valley encephalitis, and St. Louis encephalitis viruses belong to the genus *flavivirus*. These viruses are mostly transmitted by the arthropod vectors viz. mosquitoes and ticks. Vaccines for few of Mosquito-borne flaviviruses have been developed but no chemotherapeutic agents are available for the treatment of diseases caused by this group of virus.

The study aims to examine conserved regions in the flavivirus proteome that spell out the vector specificity. Sequences with mosquito-borne flaviviruses were comparatively analyzed using different bioinformatical software programs. Phylogenetic study to establish the genetic relationship among the viruses of the genus *Flavivirus* showed that these viruses can be classified into clusters, clades, and species. Such study may help in the identification of conserved regions which could be appropriate target facilitating development of possible drugs against Mosquito Borne virus infection.

107. Dynamics of Chromosome Engineering for Designing Wheat Genotypes for the Changing Climates

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Keywords: Chromosome elimination, Doubled haploid, GISH & FISH, Molecular cytogenetics, Alien introgressions, Bread wheat

Abrupt changes in the climate of various regions of north-west Himalayas has generated a challenge for the plant breeders, geneticists and molecular biologists to mitigate the vagries and design crops for such situations. Molecular Cytogenetics & Tissue Culture Lab of the CSK HP Agricultural University, Palampur has been credited for developing wheat x *Imperata cylindrica* system as an innovative chromosome elimination-mediated approach of doubled haploid (DH) production in bread wheat and utilization of novel tools like genomic *in situ* hybridization (GISH) and fluorescence *in situ* hybridization (FISH) of molecular cytogenetic approach for physical mapping of the targeted alien introgressions. By employing the chromosome elimination approach of DH breeding, new genotypes of bread wheat associated with high level of drought tolerance, cold tolerance and disease resistance have been reconstituted and screened for the detection of targeted alien chromatins introgressed from a related plant species, *Secale cereale* in this Lab. Besides, these novel techniques have also been utilized for tracing the chromosome elimination process and understanding the phenomenon at cytological and molecular level. The chromosome elimination approach has accelerated the crop improvement programme by developing the complete homozygous lines instantly whereas, the molecular cytogenetic approaches enhanced the precision of selection through physical mapping of the alien introgressions and detecting the target with minimum linkage drag.. Combination of both of these modern tools can open new horizon for wheat and other crops for developing genetically upgraded ideotypes for changed climatic situations and varied farming systems in an accelerated and précised manner.



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