RESEARCH

Proceedings of an International Conference on Trends in Biochemical and Biomedical Research: Advances and Challenges

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ABSTRACT

Biochemistry is fundamentally connected to multidisciplinary areas of research. Many breakthrough discoveries in biochemistry have contributed comprehensively to understand the fundamental biological processes. The subject helps in understanding the pathogenesis of several chronic diseases including but not limited to cancer, diabetes, obesity, mental disorders, arthritis, cardiovascular, pulmonary, and infectious diseases. To discuss the existing concepts, recent findings, and challenges associated with the applications of Biochemistry in modern biology, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, India organized an international conference on ***Trends in Biochemical and Biomedical Research: Advances and Challenges*** from February 13-15, 2018. Over 350 delegates including scientists, clinicians, research students, and industry representatives across the globe participated in the conference. Innovative talks by the experts of the global scientific community were included. The conference also provided a platform for sharing experiences, ideas and latest developments, and in establishing future collaborations. This issue is comprised of the abstracts of different talks presented by the speakers and participants of the conference.

KEYWORDS: Biochemical, biomedical, research, cancer, diabetes, obesity, mental disorders, arthritis, cardiovascular, pulmonary, and infectious diseases.

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Targeting inflammatory pathways linked to chronic diseases by agents designed by Mother Nature: Haldi is healthy

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Of 91 new therapies approved for solid tumors between 2002 and 2016, the median overall survival benefit was little more than two months. Yet the annual price tag per patient now regularly exceeds $100,000 (Workman P, Draetta GF, Schellens JH, Bernards R. Cell. 2017; 168:579-583). What is the solution to the problem is topic of our talk. Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of cancer. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long- term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF-B and STAT3, two major pathways for inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF-B and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF-B; fifth, chemotherapeutic agents and gamma irradiation activate NF-B and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF-B and STAT3; seventh, suppression of NF-B and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF-B and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. We will discuss the potential of nutraceuticals derived from spices and from traditional Indian medicine in suppression of inflammatory pathways and their role in prevention and therapy of cancer. (DOI:10.9777/rr.2018.1001)

Genetic engineering for healthy security

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The essential requirements of health security (i.e. healthy and disease free life) are met by adequate food, vaccines for disease prevention and therapeutics for disease cure. Current technologies have ensured enough food and good health but our population is anticipated to reach 171 crore from the current 135 crore by the year 2050. The food production is static for the last five years and the land use is optimum. Anemia, vitamin A deficiency blindness and micronutrient deficiencies are on the rise. There is an urgent need to produce more nutritious food rich in iron, vitamin A and other micronutrients as also to bring dry and salty land under food production by the use of genetic engineering technologies in order to avoid famine-like situation of mid 1960s. The health care sector is ensuring prophylactic and therapeutic remedies. Vaccines against emerging infections to contain and prevent their spread and therapeutics against newer infections, life style diseases, metabolic and degenerative disorders

and cancer are the demand of the day. Majority of the available vaccines are live attenuated and some killed and all of them are nearly a century old; they need cold chain for transport. Sub-unit vaccines are few but DNA and genetically engineered vaccines are not yet on the horizon. Indian pharma has ensured availability of good quality small molecule drugs, vaccines, protein therapeutics and monoclonal antibodies to meet not only our needs but is also exporting them. The therapeutics is generics and biosimilars but we are still to produce innovative drugs. The year 2017 has been a land mark year for cell and gene therapy since personalized genetically engineered immune cell therapy for blood cancer and gene therapy for retinal eye disorder have been approved for clinical use. This is the beginning of gene therapy and engineered immune cell therapy. The field is now open for the academia, agri- and pharma industry to invest their intellectual synergy to make breakthroughs in both the high end science areas to ensure adequate nutritious food as also preventive and curative therapeutics for health security of the society. (DOI:10.9777/rr.2018.1002)

Gut microbiome regulation of cancer stem cells and colon carcinogenesis

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Colorectal cancer (CRC), whose incidence increases markedly after the age of 50 years, is a multi-step process resulting from accumulation of mutations during progression from normal epithelium to carcinoma. Loss or inactivation of the tumor suppressor gene in adenomatous polyposis coli (Apc) initiates genomic instability that is thought to produce the phenotypic appearance of an adenoma. Increasing evidence suggests that pluripotent cancer stem cells (CSCs) are involved in the development and progression of many types of malignancies, including CRC. Earlier, we reported that patients with ≥3 adenomas (High-risk for CRC) exhibit a marked increase in CSCs in the colon than those without adenomas. Although the regulatory mechanisms for this increase in CCSs are poorly understood, we have suggested a role for secondary bile acids in the intestine, specifically deoxycholic (DCA) and lithocholic (LCA) acids, bio-transformed by gut microbiota, in regulating this process. Indeed, we observed a marked rise in Fusobacterium nucleatum and Enterobacterium (both are associated with CRC) in High-risk CRC patients. An opposite phenomenon was noted for the anti-inflammatory Bifidobacteria and for probiotic Lactobacillus acidophilus. Observations similar to those noted for High-risk for CRC were also seen for African Americans who exhibit high incidence of CRC. The secondary bile acids, DCA and LCA are thought to be the most significant with respect to the development of CRC. Interestingly, we found the levels of DCA and LCA in the colon of High-risk (HR) CRC patients to be markedly higher than those at lower risk (LR) for CRC. Interestingly, we found DCA and/or LCA to induce not only mutations of CRC initiating genes such as β-catenin but also CSCs in normal human colonic epithelial cells, as evidenced by increased colonosphere formation and elevated expression of several CSC markers as well as MMP-2, accompanied by an induction in drug exclusion and increased expression of multiple drug

resistance (MDR) transporters ABCB1 and ABCG2. Our observations suggest that alterations in specific gut micro-organisms resulting in increase in DCA and LCA that induce stemness in colonic mucosal cells where CRC-initiating genes are mutated are responsible for the development of sporadic CRC. (DOI:10.9777/rr.2018.1003)

Role of Lipocalin-2 in the development of human oral squamous cell carcinoma

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Oral cancer is the most prevalent cancer in India which kills approximately 80,000 people annually. Currently, there is no effective standard chemotherapeutic agent being used in the treatment of oral cancer. Recent studies have reported that Lipocalin-2 (LCN2), also known as neutrophil gelatinase- associated lipocalin (NGAL), a secreted glycoprotein is known to be upregulated in various Oral cancer is the most prevalent cancer in India which kills approximately 80,000 people annually. Currently, there is no effective standard chemotherapeutic agent being used in the treatment of oral cancer. Recent studies have reported that Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), a secreted glycoprotein is known to be upregulated in various tumours.However, the role of LCN2 in oral cancer is poorly understood. In our study we found that LCN2 was found to be significantly downregulated in primary malignant and metastatic tissues of oral cancer compared to normal tissues. The downregulation of LCN2 was strongly correlated with the degree of differentiation of the tumours, stage (I-IV)and grade (I-III) of the tumour and can serve as a prognostic biomarker for oral cancer. The tobacco carcinogens were also found to be involved in the downregulation of LCN2. Therefore, to understandthe role of LCN2in oral cancer tumorigenesis, we silenced the mRNA of LCN2 using shRNA. It was observed that knockdown of LCN2 increased the proliferation, survival,invasion and metastases of oral cancer cells. Moreover, silencing of LCN2 upregulatedNF-κB and its target proteins survivin, cyclin D1, Bcl-2, MMP-9,and. CXCR-4, which are well known to regulate cancer cell proliferation, survival, invasion and metastases. Thus, from our study it is evident that downregulation of LCN2 activates NF-κB and its target proteins and helps in the progression of oral cancer. Taken together, our study suggests that LCN2 can serve as a prognostic biomarker and therapeutic target in the effective management of this disease. (DOI:10.9777/rr.2018.1004)

Central role of transcription factor, ap-1 in cervical carcinogenesis: from controlling viral transcription to maintenance of cancer stemness.

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Cervical cancer poses a formidable health problem in women belonging to poor socioeconomic developing countries in general and Indian women in particular. Persistent infection of high-risk human papillomavirus (HPV) is causally linked to development of cervical cancer. In a sizable number of advanced cases treatment fails due to onset of chemo/radio-resistance. Transcription

factor AP-1 plays a central role in HPV-mediated cervical carcinogenesis. AP-1 has also been implicated in chemo-/radio- resistance but the mechanism(s) remained unexplored. In the present study, cervical cancer stem-like cells (CaCxSLCs) isolated and enriched from cervical cancer cell lines SiHa and C33a demonstrated an elevated AP-1 DNA-binding activity in comparison to non-stem cervical cancer cells. The enriched CaCxSLCs were highly tumorigenic and did recapitulate primary tumor histology in nude mice. CaCxSLCs also showed differential overexpression of c-Fos and c-Jun at transcript as well as in protein level. The loss of AP-1 activity and expression was accompanied by decrease in cell viability and proliferation in UV irradiated non-stem cancer cells. Interestingly, CaCxSLCs treated with neutraceutical curcumin prior to UV irradiation abolished AP-1 activity and a concomitant reduction in SP cells leading to abrogation of sphere forming ability, loss of proliferation, induction of apoptosis and the cells were poorly tumorigenic. Curcumin pre-treatment abolished the expression of c-Fos and c-Jun but upregulated Fra-1 expression in irradiated CaCxSLCs. Thus the study suggests a critical role of AP-1 protein in the manifestation of radioresistance and targeting with curcumin helps in radiosensitizing cancer stem cells through upregulation of Fra-1, which can potentially improve the treatment outcome. (DOI:10.9777/rr.2018.1005)

The human placenta: A unique organ with central role in fetal growth restriction

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Fetal growth restriction (FGR) is the most common cause of neonatal morbidity and mortality. The incidence of poor fetal growth is unacceptably high in India. Apart from the immediate consequences of poor fetal growth on neonatal health, it also forms the foundation for increased risk of developing non- communicable diseases in adulthood according to the 'fetal-origins' hypothesis. However, the pathophysiology of FGR is poorly understood. This has delayed progress in the development of effective interventional strategies for prevention of FGR. The human placenta at birth is a chronicle of fetal intrauterine life providing access for detailed examination of molecular-morphological adaptations of the fetus to intrauterine exposures. Dysfunctional placental development, maturity and function have been speculated to mediate the effect of maternal exposures such as diet during pregnancy, on fetal growth. Nevertheless, we barely understand the tipping point when the placental adaptation becomes pathophysiological leading to fetal growth restriction. We have developed methods for at-scale and systematic examination of human placentae executable in a public health setting. We are using these methods to routinely collect and preserve placentae from the St. John's mother-baby cohort. Using 400 placentae collected from our birth cohort, we are now addressing questions related to the transcriptional and epigenetic control of genes in critical regulatory pathways in the placenta to understand the etiology of fetal growth restriction. Recent findings from our group's work indicate that the placental transcript abundance of an imprinted gene, growth receptor binding protein 10 (GRB10) and of the de novo methyltransferase (DNMT1), are associated with human fetoplacental growth, interestingly, in a

gender- specific manner. The vision of our group is to systematically engage with the mechanistic basis of adaptation to nutritional and environmental exposures, in the seemingly intractable problem of low birth weight in India, with the eventual goal of informing sensible preventive strategies. (DOI:10.9777/rr.2018.1006)

Venomics and antivenomics of Indian cobra *Naja naja* venom: Failure to immuno-recognition of low molecular mass venom proteins by commercial polyvalent antivenom

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Indian cobra (Naja naja) is one of the major venomous, category-I medically important snakes in Indian subcontinent. The venom of N. naja, which is an extracellular secretion, is a mixture of pharmacologically active proteins and polypeptides. The composition of snake venom varies depending upon the geographical origin of the snake and this result in differences in severity of pathogenies following snakebite. This necessitates to unravel the complex venom proteome composition Indian spectacled cobra (Naja naja) from different regions of India as well as to assess the potency of commercial polyvalent antivenom to recognize the major toxins of this venom. We have performed the tandem mass spectrometry analysis of venom of N. naja obtained from different geographical locations of India. The SDS-PAGE and MALDI-TOF-MS analyses revealed that the bulk proportion of N. naja venom were low molecular mass (<18 kDa) proteins. The ESI-LC-MS/MS analysis revealed that approximately 70% proteins were non- enzymatic whereas the remaining ~30% proteins are enzymes. In general, irrespective of the geographical origin of Indian cobra, the three finger toxins and phospholipase A2s were the most abundant families of non-enzymatic and enzymatic proteins, respectively. The correlation between N. naja venom proteome composition and its in vitro and in vivo pharmacological/pathophysiological properties was demonstrated to explain the major features of clinical manifestations post cobra bite. The immunological cross-reactivity analysis proven the least immuno-recognition of most toxic, low molecular mass N. naja venom proteins such as 3 FTxs by commercial polyvalent antivenoms (PAVs) which is a sever concern for efficient hospital management of cobra bite patients. Our study suggested the requirement of well- recognized immunological protocols for the production of commercial antivenom for effective snakebite treatment. (DOI:10.9777/rr.2018.1007)

Neuro AIDS: An upcoming health issue

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Active researches across the globe are still ongoing to find a permanent cure for HIV infections as only a limited success has been achieved in controlling aggressive HIV replication in seropositive population. Anti-retroviral therapy (ART) is used nowadays to control morbidity and mortality in HIV seropositives. These advancements ART have proven to be a double

edged sword for HIV-seropositives. On one hand improved ART has helped HIV seropositives to lead a better and longer life; while on the other hand, it has shown its effect in a really unexpected manner. As times pass by, a significant rise in new clinical symptoms started showing up among HIV-seropositives. HIV-seropositives who are under effective ART slowly developed numerous neuropsychiatric complications. A wide spectrum of such neuropsychiatric complications reported in HIV seropositives are now collectively grouped as Neuro AIDS. These new complications have added new woes to the life of HIV-seropositives. Certainly addition of new clinical complications to a patient suffering from a incurable infectious diseases like HIV is a matter of serious concerns for policy makers, clinicians, scientist, etc. The saddest part of evolution of symptoms of Neuro AIDS is that it afflicts its patients in their prime of life i.e., ~30-40 years of age. Even though the prevalence of HIV infections is very low i.e. 0.3%, but total number of HIV-seropositives are astounding because India is the second most populous nation on this globe. As per one of the estimates, epidemiologically India is the 3rd largest nation with reference to HIV-seropositives. In the recent past, intensive efforts by Indian government, NACO, UNAIDS and other NGO's have provided better access as well as access for improved antiretrovirals to HIV patients, which helps to improve life expectancy among HIV seropositives in India, too. Another factor which should be accounted for here is the high pathogenicity associated with HIV clade prevalent in India. These two confounding factors may be reasons for serious concern for its impact on Neuro AIDS. There is significant decline in new infection, but possibilities of Neuro AIDS among HIV-seropositives remains very high. All these facts put together, definitely suggest that it is a high time to initiate appropriate measures for better understanding and directing control measures for this upcoming health issue in Indian scenario. (DOI:10.9777/rr.2018.1008)

Applications of 'biomarkers' responses in environmental risk assessment: linking human and environmental health

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Biological systems are the ultimate recipients of pollutant-induced damage. Consequently, our traditional reliance on analytical tools is not enough to assess the health of an ecosystem. Historically, a large number of studies using aquatic organisms have provided a wealth of information for basic biomedical research and to elucidate the underlying mechanisms of human diseases. Given that a large number of stress related genes are highly conserved, advancement in 'omics' approaches are now also realising the potential of these organisms to correlate human and environmental health. Furthermore, despite the fact that qualitatively similar biological responses are observed in natural biota and in humans, relatively little importance has been given to applications of these biomarkers in environmental risk assessment (ERA) until recently. New legislations around the world are however emphasizing the need for biological effects of contaminants as the criteria for ERA. Inthis context, wehave attempted to develop and implement a range of biological responses at different levels of biological organisation in several ecologically relevant species. The broader aims have been to determine the relative sensitivity of the biomarkersandthespecies followingexposuretoa

rangeofcontaminants. Linking'toxicokinetics' with 'toxicodynamics' processes, the studies compliment the observed responses with bioavailability and body burden data using analytical tools. The synthesized information provide added value when obtaining information through traditional bioassays, chemical and ecological measures, in order to adopt a preventive approach for human health and environmental sustainability. We however need to focus on the challenges which hinder applications of biological tools from being more readily incorporated into regulatory frameworks. (DOI:10.9777/rr.2018.1009)

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1RT) catalysed misinsertion and mispair extension and drug resistance

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In addition to several catalytic properties possessed by human immunodeficiency virus type 1 reverse trancriptase (HIV-1 RT), the unique one is its high propensity for misinsertion and misincorporation of deoxyribonucleotide triphosphate (dNTP) in the growing chain's 3' terminus. An analysis of the three dimensional crystal structure of this enzyme reflects that the interaction of the side chain of K154 in HIV- 1RT with the penultimate nucleotide of the template may be crucial in determination of fidelity of proviral DNA synthesis. This hypothesis was tested by steady-state kinetic studies using wild-type HIV-1 RT and five K154 mutants. These mutants contained replacement of positively charged side chain of Lysine with two amino acids' with hydrophobic side chains and two amino acids with negatively charged side chains. In one of the mutants, the positive charge of Lysine was retained but the side chain was enlarged by one carbon atom while replacing it with Arginine. The results indicated that the HIV-1 RT mutants with negatively charged side chains displayed significant decrease in enzyme activity; the values being only 10- 20% of the wild type enzyme. Other mutants exhibited enzyme activities almost comparable to the wild type, It was observed that excepting the mutants with negatively charged side chains which displayed higher fidelity than wild type, all other mutants registered enhanced levels of misinsertion and mispair extension; K154R being the most prominent. Using dideoxy nucleotides, it was demonstrated that dCTP was the most favourable nucleotide to be added in the growing chain against dATP on the template during misinsertion and mispair extension. All of these mutant derivatives of HIV-1 RT when tested for their response to 3TC, an FDA approved antiHIV-RT agent, displayed significant resistance to this nucleotide analog when compared to wild type enzyme. The mechanism of drug resistance would be explained in the light of 3D crystal structures of apoenzyme, binary and ternary complexes of both the wild type and the mutant HIV-1 RTs. (DOI:10.9777/rr.2018.1010)

Applied Biochemistry: What are needs in the orthomolecular medical Praxis?

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I'm heading one of the leading orthomolecular medical practices of Germany since ten years. Despite the fact, that the diagnostic techniques and instrumentation has been widened during this years, there are still many areas, where better

techniques are missing up to now. This limits the clinical diagnostic of the cellular- or organic- or body-biochemical status and thereby the specific individualized therapy. This short communication shall shed some light onto needs for better biochemical analytical tool, applicable in a medical-routine-laboratory. Thereby, the emphasis is on applicability of such techniques in a routine laboratory at reasonable costs. The understanding, diagnosis, and therapy of the mitochondrion get more and more in the center of interest for the therapy of a wide number of chronic diseases, including overweight, metabolic syndrome, type-2-diabetes, cancer, and so on. The measurement of central co- factors, metabolites, and enzyme activities are essential for a functional-based medicine. The present talk shall exemplify some of the so far missing or inappropriate analytical tools. (DOI:10.9777/rr.2018.1011)

The garlic compound ajoene targets vimentin and disrupts filament formation in MDA-MB- 231 breast cancer cells

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Vimentin is a major constituent of the intermediate filament family of proteins and is ubiquitously expressed in normal mesenchymal cells where it is important in the maintenance of cellular integrity. Vimentin is overexpressed in many epithelial cancers and its overexpression correlates well with accelerated tumour growth, invasion, angiogenesis and poor prognosis. By virtue of its overexpression in cancer, and its association with cancer progression, vimentin serves as an attractive intervention and therapeutic target for cancer. Garlic is a traditional medicine with cancer preventative properties. The bioactivity of garlic is attributed to the sulfur-rich compounds which are found in crushed cloves of which ajoene is one of these compounds. Chemically, ajoene is thought to target and S-thiolate cysteine residues in redox sensitive proteins resulting in inhibition of enzyme activity or modification of protein function. We have previously synthesised a dansyl-labelled ajoene probe which we have used to identify the ajoene targets in cancer cells. Using this probe, vimentin was isolated and identified as one of the ajoene targets in MDA-MB-231 breast cancer cells by 2D gel electrophoresis. Vimentin as an ajoene target was validated using the recombinant protein which was found to become S-thiolated at Cys328 by western blot and proteomics. To investigate the apparent reactivity of the cysteine residue we found by visual and computational analysis that the cysteine residues in vimentin are exposed at the terminus of the vimentin tetramer. One of the four cysteines is situated to readily interact with a neighboring glutamic acid which may assist in increasing the thiol nucleophilicity through general base catalysis. S-thiolation of vimentin by ajoene was found to interfere with its ability to form filaments in an in vitro filament forming assay and was also observed in MDA-MB-231 cells by immunofluorescence. It is proposed that ajoene may exert its antimetastatic activity in

MDA- MB-231 cells by targeting vimentin. (DOI:10.9777/rr.2018.1012)

Novel NSAIDs: Colorectal cancer prevention and treatment

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Colorectal cancer (CRC) is one of the most common cause of cancer related deaths world-wide and estimated over 1.4 million cases and about half of (0.7 million deaths) were reported annually. Importantly, recent trends show that developing countries had increased CRC incidence rates and even developed countries show increase in the incidence in younger age groups. Evidence from randomized clinical trials and observational studies suggest that among individuals who took nonsteroidal antiinflammatory drugs (NSAIDs) more frequently associated with lowered risk of developing various cancers including CRC from~20% to >50%. Recent evidence further supports the usefulness of aspirin and other NSAIDs better survival of cancer patients. Experimental evidence from laboratory studies also support cancer protective effects of Aspirin and other NSAIDs. Mechanistic data support that Aspirin/NSAIDs blocks activity of COX-1 and COX-2, lynchpins in the tumor's inflammatory microenvironment. In spite of large body of evidence of Aspirin/NSAIDs as cancer preventives, several important questions need to be answered, including: a) Safety/side effects of Aspirin/NSAIDs; b) What dose provides most protection against the cancer; c) Approaches in establishing lowest risk of serious side effects; d) who is most likely benefit; and e) what is current status of safer NSAIDs for future clinical trials. To address above important issues we and other have carried number of preclinical and clinical experiments. We will present recent results on approaches to minimizing risk of side effects, improving efficacy safety of NSAIDs and development of new generation NSAIDs with safety and efficacy. (DOI:10.9777/rr.2018.1013)

Mismatch repair system and female fertility: A tale from *Drosophila melanogaster*

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The involvement of mismatch repair (MMR) proteins (mutS and mutL) in DNA repair and meiotic recombination are well documented. Different MMR components are highly conserved across animal taxa, ranging from small organisms to humans. The mutation in genes belonging to different MMR components has been shown to exhibit various gametogenesis defects leading to infertility/subfertility in mutant organisms. Studies on the role of MMR proteins in non-vertebrate organisms are limited in the context of repair and meiosis. A transcriptomic study from this laboratory reported earlier revealed misregulation of MMR genes against xenobiotic exposure. Hence, the role of MMR gene, mlh1 (mutL homologue), in the fertility of Drosophila was analyzed. Mlh1 mutation has been shown to cause female mediated decline in fertility and various defects in the process of oogenesis leading to

embryonic lethality. Homozygous mlh1 mutation leads to the generation of increased double strand breaks (DSBs) and decreased synaptonemal complex formation resulting in increased apoptosis and thereby, reduced number of mature oocytes in the ovaries of mutant females. The eggs from these females had abnormal anterior-posterior and dorso-ventral patterning along with abnormal embryonic development such that these eggs never hatch. The female biased adverse effect of mlh1 mutation in Drosophila is in contrast to the mlh1 null mice which show male and female infertility while mlh1 mutant zebra fish exhibits male infertility. Taken together, our study for the first time reports the role of mlh1 in meiotic crossing over as well as female fertility in Drosophila. (DOI:10.9777/rr.2018.1014)

Perturbation of spindle microtubule dynamics: A powerful approach for cancer chemotherapy

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The assembly dynamics of spindle microtubules plays an essential role in the proper separation of the genetic materials. A perturbation of the assembly and stability of spindle microtubules stop the progression of the mitotic cells and ultimately induces cell death. The suppression of spindle microtubule dynamics also influences chromosome dynamics. I will talk about our current understanding of the regulation of microtubule dynamics and how we can effectively kill tumor cells by inhibiting mitosis. We have recently discovered highly potent antitumor agents and also developed an effective strategy for delivering antitumor drugs. (DOI:10.9777/rr.2018.1015)

Anticancer activity of Withaferin-A: Bioinformatics and experimental insights into the role of NFκB as a key regulator

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NFκB comprises the family of transcription factors, aberrant or constitutive expression of which is observed in most of the cancer types. This marks it as apotential therapeutic target for development of anticancer drugs. Withaferin-A (Wi-A, a withanolide extracted from medicinal herb Withania somnifera) has been shown to possess considerable anticancer activity. However, its action mechanism(s) and cellular targets have not been fully resolved. Role of Wi-A in NFκB inactivation through inhibition of activity of its upstream regulator, IκB kinase (IKK) complex, has been reported in many experimental studies but the underlying mechanism is not yet clear. Molecular docking and dynamic simulations were performed to target IKKβ-Nemo association domain of IKK complex to unveil the effect of Wi-A on conformation of the complex. Moreover, the effect of Wi-Aon DNA-bindingaffinity of NFκBdimer was explored. Experiments were performed to check the effect of Wi-A on IKK complex activity and downstream effectors of NFκB. Computational analysis revealed that Wi-A neither disrupt interaction of IKKβ with the Nemo that regulate NFκB activity nor NFκB-DNA interactions. However, it caused moderate change in the conformation of IKKβ-Nemo interaction domain that may affects its activity.NFκB-DNA

binding affinity study revealed that Wi-Ainduced decrease in DNAbinding affinity of NFκB. Effect of Wi-A on IKKcomplex activity was monitored by checking the level of phosphorylated IκBα and localization of NFκB in cells. Increased level of phosphorylated IκBα in Wi-A treated cells was observed, suggesting anactivation of IKK complex that was supported by nuclear translocation of NFκB. Decrease in NFκB-DNA binding affinity and instability of NFκB-DNA interactions were supported by downregulation of NFκ effectors, Cyclin D and Cyclin E. **Conclusion:** Further experimental analysis for effect of low doses of Wi-A on normal and cancer cells revealed an activation of DNA damage and oxidative stress response in both cells. Wi-A- induced senescence and growth arrest was observed by upregulation of Cyclin D and CyclinE regulators, namely, CARF(CollaboratorofARF), $p21^{WAF1}$, $p16^{INK4A}$. (DOI:10.9777/rr.2018.1016)

Naturally occurring enzyme inhibitors as potential drugs

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Almost half of the 877 small molecules introduced as pharmaceuticals between 1991 and 2002 are of natural products origin. Recent estimates indicate that approximately 50 % of commercially available antitumor and anti-infective agents are of natural products origin, and 25 % of these pharmaceuticals are of plant origin. A key aspect of drug discovery is the identification of small molecules with enzyme- inhibiting activities. Enzymes are essential to human life, mediating biochemical processes including metabolism, cellular signal transduction, cell cycling, and development. Malfunction in these biochemical systems often leads to disease, the root cause of which can often be traced to the dysfunction, overexpression, or hyperactivation of the enzymes involved. An understanding of diseases at the molecular level has led to the discovery of effective enzyme inhibitors that are used in clinical practice. Over 25 years, Liver Resarch Lab (FAB-Lab) at Mansoura University, Mansoura, Egypt had developed several naturally occurring enzyme inhibitors as potentials drugs: (i) aldose reductose inhibitors from Olive and Ginkgo (Catarcact), (ii) tyrosinase inhibitors from Flavonoids (Melasma), (iii) topoisomerase inhibitors from betulinic acid analogues (breast cancer), (iv) alpha amylase Inhibitors from fruits (obesity and diabetes). (DOI:10.9777/rr.2018.1017)

Role of osteopontin in tumor microenvironment: A new paradigm in cancer therapy

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Cancer is a complex disease and most cancer treatments are limited to chemotherapy, radiation, and surgery. Substantial advances in cancer treatments have resulted in significant decrease in mortality. However, existing cancer therapies often result in high toxicity and nonspecific side effects. Therefore, better targeted delivery and increased efficacy of drugs are crucial to overcome these effects. Osteopontin (OPN), a chemokine like protein plays crucial role in regulating the oncogenic and angiogenic potential of various cancers including breast. Several groups have demonstrated the role of OPN in regulating the cell signaling that ultimately controls tumor progression and metastasis covering all the

hallmarks of cancer. During last several years, we have demonstrated that both tumor and stroma-derived OPN regulate tumor growth and angiogenesis through induction of pro-angiogenic and metastasis associated genes. Our data also revealed that OPN regulates p70S6 kinase dependent ICAM-1 expression and JAK/STAT3 signaling leading to breast tumor growth. Our recent data showed that OPN controls HIF-1alpha dependent VEGF expression in response to hypoxia and breast tumor angiogenesis. Thus targeting OPN and its regulated signaling network could be novel therapeutic strategies for the management of breast and other cancers. Moreover, role of CD133+ cancer stem cells in regulation of melanoma progression and lung metastasis and effect of druggable candidate, andrographolide in suppression of these processes will be discussed. (DOI:10.9777/rr.2018.1018)

The molecular mechanisms for the antitumorigenic effect of a small molecule Piperlongumine

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Natural products have been used from ancient time for various purposes. Piperlongumine, is a natural alkaloid has shown potent anticancer activity, it was identified to exhibit it's effects by targeting ROS signaling. Inourstudies weobserved that Piperlongumine induces apoptosis viadownregulation oftumor cell-survival pathways, sensitizes drug resistant-cancer cells, and synergizes apoptosis with different chemotherapeutic drugs. Additionally piperlongumine inhibits colony formation and migration of variouscancer cells. Recentlyweshowedthatpiperlonguminesynergizest o TRAILtopromotethe deathof prostate cancer cells. Treatment with piperlongumine resulted in the upregulation of TRAIL receptor or death receptor (DR)-5, which potentiated TRAIL-induced apoptosis. Angiogenesis is the process of generating new blood vessels from pre-existing vessels and is considered essential in many pathological conditions including cancer. Inchoriallantoic membran model, piperlongumine decreased angiogenesis in a concentration-dependent manner and triggered cell death in umbilical vein/vascular endothelium cell line. These results provide evidence that a natural compound, piperlongumine, inhibits angiogenesis and induces cell death with various ways. It may be used in treatment of the diseases those pathologic bases are aboutangiogenesisanduncontrolledcell proliferationsuchas cancer. (DOI:10.9777/rr.2018.1019)

The role of inflammation in the metabolic syndrome

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The Metabolic Syndrome (MetS) is a common global problem that comprises the cardio-metabolic cluster and predisposes to both diabetes and cardiovascular diseases. Although the pathogenic mechanisms have not been elucidated, both increased inflammation and insulin resistance play a pivotal role. It appears that both monocyte/macrophages and adipose tissue conspire to accentuate both the pro- inflammatory state and increased insulin resistance. Whilst there is scanty data on visceral adipose tissue (VAT) and epicardial adipose tissue (EAT) biology, there is data on subcutaneous AT (SAT) dysregulation.

There is a significant increase in macrophages and crown-like structures in SAT of patients with MetS. With respect to adipokines there is increase in plasma leptin, plasminogen activator inhibitor-1, retinol- binding protein -4 (RBP-4), chemerin, serum amyloid-A, C-reactive protein , interleukin -1, -6, -8, lipopolysaccharide , Fetuin A and decreases in adiponectin and omentin-1. All of the abnormalities in plasma were also confirmed for SAT secreted adipokines except for adiponectin and RBP-4 which derive largely from VAT. Since many of these biomediators correlate with both insulin resistance and increased inflammation, we can posit that dysregulation of SAT is detrimental and contributes to both the pathogenesis of MetS and its sequalae. Furthermore, as future directions, much work is needed with respect to VAT/EAT biology, autophagy, sirtuins, the gut microbiome, browning of AT, to further elucidate this common syndrome and identify potential therapeutic targets to forestall its serious complications. (DOI:10.9777/rr.2018.1020)

Challenges and opportunities in drug discovery for neurodegenerative diseases

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Neurodegenerative diseases have become the most common cause of dementia among the elderly. There were 36 million people living with dementia worldwide in 2010, increasing to 66 million by 2030 and 115 million by 2050. In 2010, the global cost of dementia was $604 billion. This is 1% of global GDP and it is likely that these costs will increase in proportion to the number of people with dementia. Gene therapy has been identified to possess a broad potential for the treatment of numerous neurological diseases, including Alzheimer's disease (AD). AD is a progressive neurodegenerative disease and the most common form of dementia caused by accumulation of toxic amyloid-β (Aβ) peptides in the brain, in which the development of effective therapies have been desired. However, the major challenge in the field of gene/drug therapy is the design of safe vectors that can cross the blood brain barrier (BBB). It has been found that the transferrin receptors are present on the surface of brain endothelial cells. The liposomes, lipid based nanoparticles, were surface modified with transferrin (Tf) protein for targeting the brain endothelial receptors and conjugated to cell penetrating peptide (CPP) for improving their internalization into brain by overcoming receptor saturation. Thus, we designed near neutral, PEGylated liposomal nanoparticles encapsulating gene and drug and modifying the surface with Tf and CPP. The nanoparticles were characterized for size, surface characteristics, zeta- potential, biocompatibility and cell toxicity. Further, in vivo biocompatibility and biodistribution of nanoparticles were studied in animal model. Results and The nanoparticles were < 200 nm in size, biocompatible and did not demonstrate any hemolytic activity up to 600 nM phospholipid concentration. In vivo, nanoparticles crossed the BBB and accumulated into brain parenchyma. Nanoparticles showed biocompatibility, high cellular uptake, and efficient transport of gene/drug molecules across brain endothelial barrier into brain parenchyma. (DOI:10.9777/rr.2018.1021)

Exploring new chemical entities from *Hydnocarpus wightiana* blume for anti-cancer potential

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Medicinal plants have demonstrated their potential as a repository of active bio-active molecules with promising therapeutic potential and represent an important pool for the identification of novel drug leads. Hydnocarpus wightiana Blume is a popularly known medicinal plant and its phytochemical investigations on acetone extract decipher the excellent free radical scavenging property with high total phenolic and flavonoid content. Hydnocarpin (Hy), which has been isolated and purified from the acetone extract, promotes moderate cytotoxicity on cancer cells. In an attempt to increase the efficiency of Hy as an anticancer agent, we have synthesized of a series of Hy containing isoxozole and isoxazolone analogues in one-pot synthesis through multi component reaction (MCR) methodology. The cytotoxic potential of hydnocarpin as well as its new derivatives were assessed on lung and melanoma cancer cell lines (A549 and A375) and a human fibroblast cell line (WI-38) over a wide range of concentrations. Apoptosis assays along with insilico docking studies, molecular dynamics simulations and ADME-Tox profiling of these derivatives showed better cytotoxic effect and selective inhibition towards lung and melanoma cancer cells which prompted us for further study towards the development towards lead generation. (DOI:10.9777/rr.2018.1022)

Understanding immune evasive strategies of *Mycobacterium tuberculosis*

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Tuberculosis caused by Mycobacterium tuberculosis (M. tb) results in mortality and morbidity on a global scale. Over the last several years our lab has been working on understanding the nuances of host- pathogen interactions to identify possible mechanisms that the pathogen uses for immune evasion. Our initial studies identified an array of proteins/antigens secreted by the pathogen that tweak the activation of the dendritic cells (DCs) by inducing their differentiation from precursor cells. These antigen differentiated DCs downmodulate pro-inflammatory responses from interacting T cells. One major mechanism that is involved in this process is remodeling of calcium dynamics of the infected cell that appreciably determines M. tb survival. We identified Voltage Gated Calcium Channels (VGCC) to play a critical negative role in regulating immune responses to pathogens. Inhibiting the VGCC increased calcium influx that upregulated expression of genes favoring protective responses in infected macrophages and effectively enhanced killing of intracellular M. tb. Calcium channel blockers promoted phagosome-lysosome fusion and initiated apoptosis and autophagy in infected macrophages that significantly reduced M. tb loads in mice. Conversely, activating VGCC induced suppressor responses. Recently we have identified cellular pathways that are modulated by M. tb for its advantage. These include Neddylation and SUMOylation. Furthermore, M. tb has in its genes that mimic key signaling molecules. Incubation of such proteins with macrophages neutralizes protective responses by scavenging these molecules. Put together our results indicate the

presence of diverse immune evasive strategies of M. tb and its antigens that help it create a niche inside macrophages for persistent infection. (DOI:10.9777/rr.2018.1023)

Overview of tocotrienols in cancer prevention

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Natural compounds with possible health benefits have become attractive targets for research in areas pertaining to human health. In the field of anti-cancer research, the toxicity factor associated with synthetic drugs has turned the attention toward natural compounds as the primary focus of interest as anticancer agents. Furthermore, due to the involvement of multiple genes and various pathways, current drugs that target single genes have not been effective in providing a therapeutic cure. On the other hand, natural products target multiple genes and therefore have better success compared to drugs. In the past two decades, research focus on vitamin E has had a shift from saturated tocopherols to the unsaturated tocotrienols (T3). Despite sharing structural similarities with tocopherols, T3 strive to gain scientific prominence due to their anticancer effects. The discovery of the antiangiogenic, antiproliferative, and pro- apoptotic effects of tocotrienols, as well as their role as an inducer of immunological functions, not only reveals a new horizon as a potent antitumor agent but also reinforces the notion that tocotrienols are indeed more than antioxidants. Preclinical results of these physiological functions in anti-cancer research were translated into clinical trials gaining global attention. (DOI:10.9777/rr.2018.1024)

Targeting inflammation to reverse cancer progression

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Resistance to systemic therapy (refractory tumors) and metastasis (recurrent tumors) pose major clinical impediment to successful treatment of cancer. Many recent reports have supported the presence of a small and elusive subpopulation of cancer stem cells (CSC) in tumors that drive the disease recurrence and drug resistance. Therefore, identification of proteins that are essential for CSC survival may offer promising therapeutic targets for effective control and treatment of cancer. In this context, we have identified a stress-response protein, transglutaminase II (TG2) whose expression is abundantly overexpressed in many drug resistant and metastatic tumors and tumor cell lines. Importantly, high expression of TG2 in tumor samples is associated with shorter remissions and poor survival rates in patients. Using mammary epithelial cells as a model we showed that stable expression of TG2 initiates a comprehensive program of inflammatory cell signaling networks that play fundamental roles in promoting cell survival and metastatic abilities. Moreover, chronic expression of TG2 induced epithelial- to-mesenchymal transition (EMT) and conferred stem cell traits in epithelial cells. At molecular level, its expression constitutively activated the pro-inflammatory transcription factor NF-κB via a non-canonical pathway. Hypoxia-induced factor-alpha (HIF-1α) was identified as one of the direct downstream targets of TG2 activated NF-κB. TG2-dependent increase in HIF-1 accumulation resulted in transcriptional regulation

of many repressor genes like Snail, Zeb-1, and Twist. Moreover, TG2 expression rendered epithelial cancer cells resistant to chemo- and radiation-therapies and increased their propensity to metastasize. Conversely, inhibition of TG2 reversed the sensitivity of cancer cells to chemotherapy and inhibited their invasiveness. Strategies to block TG2-regulated cell signaling in anticipation to reverse refractoriness of cancer cells to chemotherapy and to prevent their progression to metastatic disease will be discussed. (DOI:10.9777/rr.2018.1025)

Colistin resistance: What we know and what is new?

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Antibiotic resistance is a global healthcare problem, especially in severe infections caused by carbapenem-resistant superbugs. Colistin is re-introduced as the last resort to treat such infections resulting in its excessive use and emergence of mobile colistin-resistance (mcr-1) gene. Total 1000 clinical Gram-negative bacterial isolates were subjected to colistin-susceptibility tests. All colistin-resistant isolates were analysed by PCR for mcr-1 and common carbapenem-resistant genes. Localisation study for mcr-1 was done by Southern- blot and clonality by pulsed field gel electrophoresis. mcr-1 was probed for ISApl1 element in K. pneumoniae. Colistin-resistant was detected in 40 (4%) isolates; 12 (30%) of them harboured mcr-1 and their distributions were as follows: Klebsiella pneumoniae (n=4), Pseudomonas aeruginosa (n=4), Providencia rettgeri (n=3) and Myroides odoratus (n=1). Southern-blot analysis revealed mcr-1 on chromosome of K. pneumoniae and P. aeruginosa; blaNDM-1 was present in one isolate each of mcr-1 positive K. pneumoniae and P. aeruginosa. Three each K. pneumoniae and P. aeruginosa isolates were clonality-related. ISApl1 element was absent in mcr-1. Chromosome-mediated intrinsic and adaptive colistin- resistance through two component systems (PhoPQ and Pmr AB) is well known. Plasmid-borne colistin- resistance through mcr-1 has recently been reported. The present study first-time reports chromosomal mcr-1-mediated colistin-resistance in K. pneumoniae and P. aeruginosa. Absence of ISApl1 element on mcr-1 in K. pneumoniae suggests alternative mechanisms of transmission. There are still strains with colistin-resistance but exact mechanisms remain unexplored. Emergence of mcr-1 along with blaNDM is a bad news for medical fraternity. Since data on colistin-resistance is scarce, further studies are required to know different resistance mechanisms, their clinical impact, contributions of colistin use in veterinary practices for emergence of resistance and mode of transmission. (DOI:10.9777/rr.2018.1026)

In vitro and *in vivo* studies suggest that COX-2/EP2-EP4/-catenin signaling regulates patulin-induced intestinal cell proliferation and inflammation

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Patulin (PAT), a secondary metabolite of fungi, is a natural contaminant of fruits and fruit juices but also occurs in high concentrations in commercially available juices and food stuffs. It is known to cause glutathione depletion, oxidative DNA damage and cell proliferation. Recently, in vitro studies have indicated that PAT can increase intestinal epithelial permeability, modulate tight junctions and decrease transepithelial electrical resistance. To evaluate the mechanisms involved, Wistar rats were orally treated with 100 µg/kg b.wt of PAT either alone or along with 100 mg/kg b. wt of celecoxib for 3 days. PGE2 levels in serum and intestinal tissue, histopathological and immunohistochemical analysis for COX-2, cyclin D1 and -catenin were performed. We found that PAT exposure led to significantly higher levels of PGE2 in serum and intestinal tissue and high expression of COX-2, cyclin D1 and -catenin compared to controls. Interestingly, our results showed that celecoxib treatment could decrease PAT-induced PGE2 and reduce expression of COX-2, cyclin D1 and β-catenin. We also used normal rat intestinal epithelial cells (IEC-6) treated with non-toxic concentrations (100 nM, 250 nM and 500 nM) of PAT for different time intervals. We found that PAT exposure caused enhanced proliferation, higher expression of COX-2, increased PGE2 levels and enhanced expression of the prostanoid EP2 receptor. We also observed that PAT exposure caused enhanced Akt expression, which in turn inhibits GSK-3β and stabilizes β-catenin. Thus, we suggest that the COX-2/EP2-EP4/β-catenin signaling cascade is involved in the regulation of PAT-induced intestinal cell proliferation and inflammation. (DOI:10.9777/rr.2018.1027)

Numtogenesis as a mechanism involved in development of cancer

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Colorectal adenocarcinomas are characterized by abnormal mitochondrial DNA (mtDNA) copy number and genomic instability, but a molecular interaction between mitochondrial and nuclear genome remains unknown. Here we report the discovery of increased copies of nuclear mtDNA (NUMT) in colorectal adenocarcinomas, which supports link between mtDNA and genomic instability in the nucleus. We named this phenomenon of nuclear occurrence of mitochondrial component as numtogenesis. We provide a description of NUMT abundance and distribution in tumor versus matched blood-derived normal genome. Whole genome sequence data were obtained for colon adenocarcinoma and rectum adenocarcinoma patients participating in The Cancer Genome Atlas, via the Cancer Genomics Hub, using the GeneTorrent file acquisition tool. Data were analyzed to determine NUMT proportion and distribution on a genome-wide scale. NUMT suppressor gene was identified by comparing numtogenesis in other organisms. Our study reveals that colorectal adenocarcinoma genome

on average contain up to 4.2-fold more somatic NUMTs than matched normal genomes. Women colorectal tumors contained more NUMT than men. NUMT abundance in tumor predicted parallel abundance in blood. NUMT abundance positively correlated with GC content and gene density. Increased numtogenesis was observed with higher mortality. We identified YME1L1, a human homolog of yeast YME1 (yeast mitochondrial DNA escape 1) to be frequently mutated in colorectal tumors. YME1L1was also mutated in tumors derived from other tissues. We show that inactivation of YME1L1 results in increased transfer of mtDNA in the nuclear genome. Our study demonstrates increased somatic transfer of mtDNA in colorectal tumors. Our study also reveals sex based differences in frequency of NUMT occurrence and that NUMT in blood reflects NUMT in tumors suggesting NUMT may be used as a biomarker for tumorigenesis. We identify YME1L1 as first NUMT suppressor gene in human and demonstrate that inactivation of YME1L1 induces migration of mtDNA to the nuclear genome. Our study reveals that numtogenesis plays an important role in development of cancer. (DOI:10.9777/rr.2018.1028)

Targeting PD-L1 in the neuroblastoma tumor microenvironment: A novel therapeutic approach?

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Even with aggressive treatments, high-risk neuroblastoma (NBL) remains one of the most difficult pediatric cancers to treat. MicroRNAs (miRNAs) are small noncoding RNAs with gene expression regulatory functionsand are dysregulated in almost all human tumors, including Neuroblastoma. Studies have shown that extracellular microvesicles, called exosomes, transfer miRNA from one cell to another, and affect tumor microenvironment of NBL. However, how exosomic miRNAs contribute to the development of drug resistance in the context of the tumor microenvironment has not been previously described in NBL.Our preliminary data indicate that: (i)Neuroblastoma cells secrete miRNA-21 in exosomes; (ii) up-regulate miRNA-21, NF-κB pathway activationand PD-L1 in MSCs after co-culturing with Neuroblastoma cells; (iii) acquire resistance to immunotherapy agent, dinutuximab antibody- mediated cell death when co-cultured with MSCs; (iv) Co-injection of Neuroblastoma cells with MSCs induces tumor growth, tumor volume, tumor weight and metastatic dissemination of cancer cells in Neuroblastoma xenografts. These data indicate a unique role of exosomic miR-21 in the cross-talk between NBL cells and BMMSCsin the resistance to immunotherapy. More in vivo studies are warranted on the therapeutic role of anti PD-L1 in NBL. (DOI:10.9777/rr.2018.1029)

Aβ, a risk factor for Parkinson's pathogenesis: Mechanisms and prevention

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Amyloid beta (Aβ) aggregation is generally associated with Alzheimer's onset. We have demonstrated that incubation of dopaminergic

SH-SY5Y cells with an Aβ peptide fragment (an 11-mer composed of residues 25–35; Aβ (25–35)) results in elevated intracellular nitrosative stress and induces chemical mutation of protein disulfide isomerase (PDI), an endoplasmic reticulum-resident oxidoreductase chaperone. Furthermore, Aβ (25–35) provokes aggregation of both the minor and major biomarkers of Parkinson's disease, namely, synphilin-1 and α-synuclein, respectively. Importantly, fluorescence studies demonstrate that Aβ (25–35) triggers colocalization of these Parkinsonian biomarkers to form Lewy-body- like aggregates, a key and irreversible milestone in the neurometabolic cascade leading to Parkinson's disease. In addition, fluorescence assays also reveal direct, aggregation-seeding interactions between Aβ (25–35), PDI and α-synuclein, suggesting neuronal pathogenesis occurs via prion-type cross-transfectivity. These data indicate that the introduction of an Alzheimer's-associated biomarker in dopaminergic cells is proliferative, with the percolative effect exercised via dual, independent, Parkinson- pathogenic pathways, one stress-derived and the other prion-like. The results define a novel molecular roadmap for Parkinsonian transfectivity via an Alzheimeric burden and reveal the involvement of PDI in amyloid beta induced Parkinson's. We have also explored the ability of phytochemicals to intercept Aβ- driven Parkinson's pathogenesis via multiple mechanisms. Results from these studies will be discussed. (DOI:10.9777/rr.2018.1030)

Targeting tumour microenvironment for multiple for myeloma therapy

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Multiple myeloma (MM) represents 1% of all malignancies, and approximately 10% of hematological malignancies. MM remains uniformly fatal owing to intrinsic or acquired drug resistance and the median survival time is 3 to 5 years. One of the major factors that lay the foundation for MM relapse is the intimate relationship of myeloma cells to bone marrow microenvironment. This interaction of myeloma to bone marrow not only required for growth, it activates several key signaling pathways playing important role in survival, migration, and chemo-resistance. Therefore, targeting myeloma cells trafficking to bone marrow and its adhesion would be a novel therapeutic strategy. It has been established that stem-like MM cells (MMSCs) play critical role in refraction and relapse. Recent studies have illustrated that Bruton's tyrosine kinase is highly expressed in MMSCs and regulates many vital signaling pathways playing critical role in cell proliferation, survival, migration, and resistance. Our laboratory focuses on targeting the trafficking of myeloma cells to bone marrow as well several signaling pathways contributing in the survival of MM. Recently we showed that targeting of BTK and CXCR4 inhibits the survival of myeloma cells and bone loss. This contribution in this regard is significant because it is expected to have broad translational importance in the treatment of MM. (DOI:10.9777/rr.2018.1031)

The lipid peroxidation biomarkers for detection of diseases

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Oxidative stress is implicated in the underlying mechanisms of several disorders and diseases, such as diabetes mellitus, cardiovascular diseases, cancer, neurodegenerative diseases, and aging. Researchers have focused their attention on lipid peroxidation products to elucidate the mechanism of lipid oxidation, its involvement in disease pathogenesis, and to develop specific and practical biomarkers for diagnosing diseases and evaluating therapies. Lipid peroxidation products have received considerable attention as indices for oxidative stress since lipids are the most susceptible to oxidation in vivo. However, lipid peroxidation yields numerous products, which makes it difficult to measure the extent of lipid peroxidation in vivo. We recently proposed the measurement of hydroxyoctadecanoic acids (HODEs) as a biomarker of oxidative stress in vivo. HODEs are the lipid peroxidation products from linoleic acid. HODEs are formed by a radical-mediated oxidation mechanism consist of four isomers: 13-hydroxy- 9(Z),11(E)-octadecadienoic acid (13-(Z,E)-HODE); 13-hydroxy-9(E), 11(E)-octadecadienoic acid (13-(E,E)- HODE); 9-hydroxy-10(E), 12(Z)-octadecadienoic acid (9-(E,Z)-HODE); and 9-hydroxy-10(E), 12(E)- octadecadienoic acid (9-(E,E)-HODE). 13-(Z,E)-HODE are also formed by enzymatic oxidation via lipoxygenase. Singlet oxygen oxidizes linoleic acids by non-radical oxidation to form 13-(Z, E)-HODE), 9- (E, Z)-HODE, 10-hydroxy-8(E), 12(Z) -octadecadienoic acid (10-(E, Z)-HODE), 12-hydroxy-9(Z), 13(E)-octadecadienoic acid (12-(Z,E)-HODE). In this case, 10- and 12-(Z, E)-HODEs are specific oxidation products of singlet oxygen. Recently, we reported that 10- and 12-(Z,E)-HODE highly correlated with clinical values for diabetes and these are suitable biomarkers for the evaluation of the early stages of diabetes. In my talk, I will explain about the lipid peroxidation in some diseases, especially in diabetes. (DOI:10.9777/rr.2018.1032)

Targeting skeletal muscle metabolism to control obesity and diabetes

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Obesity is taking the form of a pandemic and increasing at an alarming rate throughout the globe and in India. Obesity is a major contributing factor for Type 2 diabetes and develops due to higher caloric intake, and physical inactivity overtime. Skeletal muscle constitutes ~40% of body mass and is a major consumer of metabolites, including glucose and fat. Increasing fuel utilization in muscle is an effective strategy to control obesity and Type 2 diabetes. Our goal is to find effective ways to increase fat burning and less deposition of fat in muscle and adipose tissue to control obesity. Our research focuses on the cellular mechanisms that activate fat burning in order to reduce fat storage in the body. We have identified a muscle protein, namely sarcolipin (SLN), which plays an important role in muscle thermogenesis and metabolism. We found that mice lacking SLN protein were poor in metabolizing fat and became obese, whereas overexpression of SLN in skeletal muscle led to an increase in oxidative metabolism and was resistant to high fat diet-induced obesity. An important finding was that SLN overexpression in muscle led to an increase in mitochondrial biogenesis and oxidative metabolism. In addition, mice overexpressing SLN performed better during

endurance running test, ran longer and were less fatigued compared to control mice. Our data suggests that SLN signals mitochondria to increase ATP production and promotes better adaptation of muscle during metabolic stress states including cold, exercise and caloric excess metabolism. Our long-term goal is to develop novel therapies that promote energy expenditure in muscle. (DOI:10.9777/rr.2018.1033)

Searching a target to treat metabolic syndrome: Brown fat or the muscle?

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Metabolic syndromes including obesity and type II diabetes are becoming more and more common all over the world. It is widely accepted that this trend is due to dysregulation in the energy homeostasis of an individual. In an animal body, energy enters in the form of food and exits as work and heat. The body tries to store the unspent energy and this activates several metabolic remodeling of various organs, which is a major cause of initiating the program leading to the metabolic disorders. However, mammalians have ability to resist excessive weight gain due to energy surplus which has been termed as "Diet Induced Thermogenesis or DIT". Ever since the discovery of DIT in 1970s, researchers have been focusing to delineate mechanisms that contribute to this process. Early studies established the role of uncoupling protein 1 (UCP1) in the inner mitochondrial membrane of brown adipose tissue (BAT) in DIT. BAT is the major organ of cold-induced nonshivering thermogenesis (NST) in eutherian mammals. We have shown that the other site of NST in mammals, the skeletal muscle, is also activated during both cold and DIT. Recently, a third mechanism called "beiging" has been proposed to be involved in DIT, which can be based on either UCP1 dependent or independent processes. Now, there is a hot debate in the field as to which of these three mechanisms of DIT can be the best to target pharmacologically for countering obesity (and/or diabetes) in humans. Targeting mitochondria for obesity brings the whole concept into question due to side effects. Muscle NST being based on futile cycling of calcium ions and not on mitochondria directly, seems to be a safer route to increase energy expenditure and reduce energy surplus thereby countering obesity. Therefore, it is necessary to carefully define the details of mechanism involved in activation and maintenance of muscle NST. (DOI:10.9777/rr.2018.1034)

Transplantation of bone marrow stem cell: A promissing cell therapy for hemophilia A disease

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Hemophilia A (HA) is an X-linked recessive bleeding disorder caused by the deficiency of clotting Factor VIII. Currently, treatment of hemophilia consists of factor replacement using fresh frozen plasma, cryoprecipitate, or Factor VIII concentrate. The transdifferentiation of bone marrow cells (BMCs) into hepatocytes has created

enormous interest in applying this process to the development of cellular medicine for degenerative and genetic diseases. Liver transplantation cures hemophilia A, demonstrating that the liver is a major site of factor VIII synthesis. We hypothesize that the partial replacement of mutated liver cells by healthy cells in hemophilia A (HA) mice could ameliorate the severity of the bleeding disorder. We perturbed the host liver with acetaminophen to facilitate the engraftment and hepatic differentiation of lineage-depleted (Lin^-) enhanced green fluorescent protein (eGFP)- expressing BMCs. Immunohistochemistry experiments with the liver tissue showed that the donor-derived cells expressed the markers of both hepatocytes (albumin and cytokeratin-18) and endothelial cells (von Willbrand factor). The results of fluorescent in situ hybridization and immunocytochemistry experiments suggested that differentiation was direct in this model. The BMC- recipient mice expressed FVIII protein and survived in a tail clip challenge experiment. Furthermore, a coagulation assay confirmed that the plasma FVIII activity was maintained at 20.4 ± 3.6% of normal pooled plasma activity for more than a year without forming its inhibitor. Overall, this report demonstrate that phenotypic correction in HA mice with conditioned liver is possible by transplantation of BMCs. Thus, BMC therapy is a potential alternative approach to managing HA. (DOI:10.9777/rr.2018.1035)

MICRO-managing cardiac autophagy to ameliorate diabetic cardiomyopathy

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Impaired autophagy (an intracellular lysosomal degradation process required for cellular homeostasis) and differential expression of miRNAs (non-coding regulatory RNAs) are hallmarks of diabetes mellitus (DM) hearts. We have reported that lack of miR-133a, the most abundant miRNA in the human heart, contributes to diabetic cardiomyopathy. Using left ventricular tissue from heart failure patients, we demonstrated that DM upregulates autophagy markers but downregulates miR-133a in the heart. Deregulated autophagy is detrimental to the heart. However, it is unclear whether miR-133a directly involved in the regulation of autophagy in DM hearts. Aim and hypothesis: To investigate the role of miR-133a in regulation of autophagy in DM hearts. We hypothesized that reduced levels of miR-133a deregulates autophagy in DM hearts that compromises degradation of abnormal organelles leading to cardiomyopathy. We used Akita (spontaneous T1DM) and its sibling WT mice to determine the impact of DM on miR-133a and autophagy markers. To investigate the specific role of miR- 133a on autophagy in DM hearts, we used cardiac-specific miR-133a transgenic mice (miRTg) and Akita/miRTg (generated by crossbreeding Akita and miRTg) mice. At in vitro level, we used HL1 cardiomyocytes and treated them with high glucose with or without miR-133a mimic. We measured autophagic flux, and levels of LC3B-II, p62 and LAMP2. We found that miR-133a overexpression improved autophagic flux in diabetic Akita and hyperglycemic HL1 cardiomyocytes. Due to increased clearance of cellular cargo, the accumulation of LC3B, p62 and LAMP2 were decreased in diabetic Akita/miRTg and miR-133a mimic-treated hyperglycemic cardiomyocytes and their cellular homeostasis was

restored. Our hemodynamic study showed improved cardiac function after miR-133a overexpression in diabetic Akita hearts. We conclude that increasing the levels of miR-133a in the DM heart could be a novel approach to micromanage autophagy and ameliorate cardiomyopathy in DM hearts. (DOI:10.9777/rr.2018.1036)

Advancements in FTIR imaging and how it helps to understand micro chemical environment of tissues and cells in the field of disease (like cancer) diagnostics

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Fourier transform infrared (FTIR) microscopic imaging uses a combination of an FTIR spectrometer with a microscope and Focal Plane Array (FPA) detector. The method has been recognized as a powerful and versatile imaging tool in many disciplines, ranging from biomedical research through to materials science, art conservation and forensics. The Agilent Cary FTIR microscopes and chemical imaging systems represent the latest in cutting-edge performance, delivering unparalleled spatial resolution and sensitivity. When coupled with the wide range of options available. Present study focuses on recent advancements in FTIR imaging and how it helps to understand micro chemical environment of tissues and cells in the field of disease (like cancer) diagnostics. (DOI:10.9777/rr.2018.1037)

Impact of molecular signatures in management of oral cancer patients

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Oral cancer is major health hazard in India. There is no significant improvement in the success of anticancer treatment and survival of oral cancer patients in last decade. Invasion and Metastasis are the major strenuous problems in successful cancer treatment, and it is believed that they begin in the growth of the primary tumor. Hence, we have studied vascular epithelial growth factors (VEGF), matrix metalloproteinases (MMPs), phosphorylated epidermal growth factor receptor (pEGFR), truncated E- cadherin, c-Jun protein in oral cancer patients. The results revealed significant revealed significant VEGF183 and VEGF165 were significantly downregulated in malignant tissues as compared to adjacent normal tissues. VEGF183 and VEGF189 were significantly associated with tumor differentiation and tumor size. VEGF165 was significantly higher in recurrent early stage tumors. Serum VEGF levels were significantly higher in cases as compared to the controls and were associated with tumor differentiation. Serum VEGF levels were significantly higher in patients with recurrent advanced stage tumors. Further, patients with high levels of VEGF165 and serum VEGF levels had worst prognosis. Significantly high expression of pEGFR, truncated E-cadherin and c-Jun protein, MMP2 and MMP9 in malignant oral tissues as compared to adjacent normal tissues was observed. Plasma pro, active and total MMP-2, MMP-9 as well as TIMP-1 and TIMP-2 levels were significantly higher in oral cancer patients as compared to the controls. An increase in the levels of pEGFR and truncated E-cadherin protein was observed in advanced and metastatic disease. Further, elevated expression of pEGFR, truncated E- cadherin, c-Jun protein, active MMP-2, pro

MMP-9, total MMP-2 and total MMP-9 were associated with reduced overall survival. A positive correlation was observed between truncated E-cadherin, MMPs and c-Jun protein. Further, pEGFR was positively correlated with truncated E-cadherin protein. The data suggested that VEGF, pEGFR, truncated E-cadherin, c-Jun protein and MMPs, the major molecular signature of the disease play a prominent role in management of oral cancer. Therefore, the combination therapies either by pharmaceutical or nutraceutical products targeting these molecular signatures might help in combating oral cancer. (DOI:10.9777/rr.2018.1038)

Silibin in targets tumor suppressor p53 in its efficacy against skin cancer

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Ultraviolet B (UVB) portion of sunlight has been implicated as the major etiologic factor for non-melanoma skin cancer (NMSC), and p53 mutation is considered an early step in the molecular events of UVB-induced skin carcinogenesis. Novel nontoxic phytochemicals that target or prevent the occurrence of UVB-induced pre-neoplastic lesions as well as NMSC are currently emerging as attractive mechanism- based agents for skin cancer chemoprevention. Silibinin, a flavolignan isolated from milk thistle extract, has long been used as an anti-hepatotoxic agent. However, its cancer chemopreventive potential became a highlight in last two-decades and several studies have already been published showing promising anti- cancer efficacy of silibinin in both in vitro and in vivo cancer models. With regard to skin cancer chemoprevention studies with silibinin, recently we found that silibinin protects against UVB-induced squamous cell carcinoma (SCC) in SKH1 hairless mice, along with prominent effect on cell cycle modulators. Moreover, in short term tumor initiation studies, silibinin reduced the number of cyclobutane pyrimidine dimer (CPD) positive and apoptotic cells in mouse skin epidermis induced by single UVB irradiation. In all these studies, silibinin further upregulated p53 protein expression in UVB-irradiated epidermis as well as in tumors. To further dissect the molecular mechanisms of silibinin efficacy, we employed mouse epidermal JB6 cell culture model, where we observed that silibinin pretreatment inhibits apoptosis induced by moderate doses of UVB (30-50mJ/cm^2). This protective response was accompanied by a gradual reduction in UVB-induced cyclobutane pyrimidine dimer (CPD) levels, in a time dependent manner. Mechanistically, compared to UVB irradiated cells, silibinin pretreated cells showed a further upregulation of phospho (Ser 15) and total p53, as well as GADD45α, a downstream target of p53 that is involved in cell cycle arrest and DNA repair. Next we used p53-siRNA to identify the precise role of p53 in the cell cycle events, and observed a reduction in UVB-induced S phase arrest in p53-knock down cells and that silibinin pretreatment failed to reverse S phase arrest by UVB. In addition, both UVB and silibinin failed to induce GADD45α levels in p53 knock down cells indicating that GADD45α is regulated in a p53-dependent manner in JB6 cells. These studies suggest that the protective response of silibinin against UVB- induced damage is mainly through cell cycle delay, where p53 and GADD45α play important roles. Moreover, in in vivo studies using p53 knockout SKH1 hairless mice, the indispensable role of p53 in silibinin's protective response against UVB-induced DNA damage and

skin tumorigenesis was further established. Together, our completed studies suggest silibinin efficacy in pre-clinical SCC models by targeting p53, and form the basis for its evaluation in clinical SCC chemoprevention and therapy models. (DOI:10.9777/rr.2018.1039)

Heart failure is that brain or heart condition?

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Novel magnetic resonance imaging procedures and analytical methods over a unique opportunity to assess the brain structural, metabolic, hemodynamic, blood brain barrier, resting-state functional connectivity, and functional responses to autonomic challenges status in heart failure (HF) subjects. In a series of experiments in patients with heart failure, we characterized brain injury, abnormal metabolites, hemodynamics, resting-state functional connectivity, and abnormal functional responses to autonomic challenges, in autonomic, mood, and cognitive control sites, functions that are deficient in the condition. Also, potential pathological mechanisms, including compromised cerebral blood flow and blood brain barrier function, contributing to brain damages in HF subjects will be discussed. In addition, various analytical procedures will also be described that can be used to other brain conditions. (DOI:10.9777/rr.2018.1040)

Exploiting host-tumor interactions in targeting bone metastasis

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Our long-term goal is to improve the therapeutic strategies for osteolytic bone metastasis in breast cancer with a better understanding of the biology of bone metastasis. Breast cancer is the most common cancer and the second leading cause of cancer-related death in women in the United States. Breast cancer cells show a strong predilection for metastasis to bone and most complications of breast cancer are attributed to bone metastasis. Bone metastases in breast cancer are predominantly osteolytic, leading to pathological fracture, intractable bone pain, nerve compression and hypercalcemia. These complications are not only potentially lethal but also decrease the quality of life. Current therapies aimed at improving the quality of life, symptom control and prolongation of survival in breast cancer with bone metastasis are not effective, and often associated with severe toxicity. Therefore, it is critical to developing novel treatment strategies with no or limited toxicity for effective management of advanced breast cancer patients with the metastatic bone disease. Understanding the cellular and molecular changes in the bone microenvironment is essential for developing novel therapeutics. We identified a set of critical genes using a unique mouse model and tested their functional role in experimental and spontaneous osteolytic bone metastasis in a syngeneic setting. Up-regulation of Cathepsin G is one of the key regulators of tumor- bone interaction. We have defined the functional role Cathepsin G in osteolytic bone metastasis through the generation of soluble RANKL. Additionally, we have developed osteolysis-targeting nanomedicine to deliver Cathepsin G inhibitors and docetaxel. In summary,

these studies provide proof of concept for development of innovative approaches to inhibit tumor-induced osteolysis and malignant cell proliferation in the bone microenvironment using our novel delivery system for development of highly effective therapy for osteolytic bone metastasis with minimal toxicity. (DOI:10.9777/rr.2018.1041)

Expression of interferon genes is influenced by sex hormones in SLE

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Lupus (SLE) is a life-threatening autoimmune disease with female to male ratio of 9:1.Environmental factors, genetic defects, miRNA dysregulation, and hormonal factors can influence susceptibility to SLE. Estradiol has been shown to increase the expression of interferon genes and pathways and thus could promote susceptibility to the disease in women. Recent evidence suggests the existence of a nexus between inflammatory pathways and 17b-estradiol, resulting in increased interferon stimulated genes (ISGs), autoantibodies and dysregulation of immune cells in SLE. The mechanisms by which 17b-estradiol interacts with IFN and various immune cells in SLE is poorly understood. Women may be more susceptible than men to SLE and other autoimmune diseases in part because many healthy women have higher interferon regulated genes. We found that plasma estradiol levels positively correlated with levels of serum/plasma IL-6, and 21/IL-23 in SLE patients. Plasma 17b-estradiol levels are significantly increased in female SLE patients compared to healthy females ($p<0.01$). Furthermore, we found that estradiol increases proinflammatory cytokines, and level of estradiol positively correlated with expression of miR21, 25, and 186 in SLE patients. Expression of miR21, miR25 and miR186 are positively correlated with SLEDAI score in SLE. Conclusions: We found that expression of interferon genes is influenced by sex hormones in SLE. Understanding the role of 17b-estradiol in the regulation of pro-inflammatory pathways, ISGs, and microRNAs will further advance our understanding of SLE disease pathology and may identify additional novel therapeutic targets. (DOI:10.9777/rr.2018.1042)

A radiosensitizer for radiotherapy of cancer

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Radiotherapy is one of the common treatmentsfor the solid tumors in cancer patients. It is often limited by its high dose-related toxicity to normal cells and tissue, and the development of therapeutic resistance in cancer cells. We observed radiosensitizing effects of a small molecule, silibinin in in vitro and pre-clinical tumor xenograft models of prostate cancer. This effect was selective to cancer cells. Results were encouraging and revealed that the desired tumor growth inhibition can be achieved at lower doses of radiation treatment when it is combined with oral administered silibinin. Many parameters including clonogenic potential, cell survival, cell cycle, DNA damage and apoptosis were measured. Importantly, silibinin exhibited preferential radiosensitization of cancer cells and protected normal cells and tissuesfrom radiation toxicities. In animal studies, it protected various hematopoietic parameters from radiation-induced toxicities. At

molecular level, radiosensitization mechanism involved the inhibition of nuclear translocation of EGFR and DNA-PK. This indicated its likely effect on DNA repair signaling. The silibinin concentration used in cell culture study can be physiologically achievable in patients; therefore, these findings have the translational significance for the radiotherapy for prostate cancer patients. (DOI:10.9777/rr.2018.1043)

Protective effects of curcumin on allergic asthma exacerbations and airway remodelling

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Curcumin or diferuloylmethane, a yellow pigment present in turmeric (Curcuma longa) has been shown to exhibit numerous biological activities including anti-inflammatory and anti-asthmatic. Present study has focussed on anti-asthmatic and immunomodulatory potential of curcumin after intranasal administration, mainly asthma exacerbations affecting histamine release, IgE response, inflammatory cytokines, airway hyperplasia and fibrosis. We also have investigated its effects on LPS exposure and asthma exacerbations leading to airway remodeling. Airborne LPS is often present at high concentration in organic dusts, air pollutions and household dusts activates cells of the respiratory innate immune system causing asthma exacerbations leading to airway remodelling. LPS, a major component of the cell wall of gram-negative bacteria binds to Toll like receptor (TLR-4) initiating recruitment of inflammatory cells is known to be a culprit for the production of reactive oxygen species (ROS) and pro-inflammatory cytokines mainly IL-1, IL-6, TNF-α and IFN-γ, resulting in a sustained chronic inflammation of the airways that triggers bronchoconstriction and structural changes in the airways (i.e., airway remodeling). Balb/c (4-6 weeks) mice were sensitized on 1st and 8th day with ovalbumin (OVA) and challenged with 1 % OVA (aerosol) from 9th to 15th day. Mice were exposed to LPS on 2nd day an hour before OVA challenge and curcumin (i.n.) was administered 2 hr before each OVA challenge from 9th to 15th day. For the first time it is being reported that Matrix metalloproteinases (MMP-9) activity has altered extracellular matrix (ECM) after LPS exposure which is detected by higher hydroxyproline level and MMP-9 expression by gelatin zymography. Expression of MMP-9 was studied by histology where masson trichome stained lung sections have shown MMP-9 activity suppression after intranasal curcumin treatment. It has suppressed exacerbated asthmatic condition by lowering serum IgE and cytokine (IL-5 and IFN-λ) levels and recruitement of inflammatory cells (eosinophils and neutrophils) to the lungs.The present investigations suggest that intranasal route is effective for curcumin administration and could be better alternative in near future. (DOI:10.9777/rr.2018.1044)

Mortalin matters in carcinogenesis: Its management by herbs

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Mortalin/mthsp70 is a member of HSP70 family of proteins that was first discovered in 1993 in our laboratory. It is an essential protein and possess multiple functions including chaperoning, intracellular trafficking of proteins, mitochondrial import, and is involved in control of cell proliferation, ROS production and apoptosis. Enriched in all cancer cells, it has been shown to promote carcinogenesis, EMT and cancer cell stemness by multiple pathways including inactivation of tumor suppressor protein-p53, activation of telomerase, hnRNP-K, and factors involved in epithelial-mesenchymal transition (EMT). Consistently highly aggressive and metastatic cancers have been shown to possess high levels of mortalin. Mortalin siRNA, on the other hand, compromised growth of cancer cells and caused tumor regression in vivo. Furthermore, mortalin knockdown was associated with reversal of EMT induction and cancer cell stemness suggesting it is a strong candidate for cancer therapy. We performed a screening for anti-mortallin natural compounds and found thatherbs including Withania sominifera, Helicteres angustifolia, and propolisharbor anti-mortalin bioactives. These include Withanolides (Withaferin-A and Withanone), Cucurbitacin B and Caffeic Acid Phenethyl Ester (CAPE) and Artepillin. We present evidence that these compoundsare capable of disrupting mortalin-p53 complexes resulting in reactivation of tumor suppressor activities of p53 in cancer cells. Furthermore, downregulation of mortalin and several other key regulators of cell migration accountable for its anti-metastasis activity were detected, and were supported by in vivo tumor suppressor assays. In light of these data and to promote the use of these herbs for cancer therapeutics and health benefits, we have generated Active Ingredient Enriched Ashwagandha extracts and PP-Propolis (Pleasant and Premium Propolis) that possess high stability and lack repulsive odor. We propose these as NEW (Natural Efficient and Welfare) anti-cancer drugs with anti-mortalin activities. (DOI:10.9777/rr.2018.1045)

NRP1 Interacts with GIPC1/Syx to activate p38 MAPK Signaling to Drive Cancer Stem Cell Survival

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Epidermal cancer stem cells (ECS cells) comprise a limited population of cells that form aggressive, rapidly growing and highly vascularized tumors. VEGF-A/NRP-1 signlaing is a key driver of ECS cell surival and aggressive tumor formation. However, relatively less is known regarding the downstream events following VEGF-A/NRP-1 interaction. In the present study, we show that loss of VEGF-A, NRP-1, GIPC1, Syx or RhoA reduces p38 activity leading to reduced reduced ECS cell survival, spheroid formation, matrigel invasion and angiogenic factor production. p38 knockdown also reduces these responses. Moreover, expression of constitutively-active RhoA in NRP1-knockout cells restores p38 activity and spheroid formation and matrigel invasion. In addition, pharmacologic inhibition of VEGF-A/NRP-1 interaction with EG00229 or NRP-1 knockout reduces p38 MAPK activity leading to reduced tumor vascularity and growth. These studies suggest that NRP-1 forms a complex with GIPC1, Syx, RhoA and p38 MAPK and that this triggers p38 MAPK activity to enhance the ECS cell phenotype and tumor formation. Moreover, this

data suggest that these proteins are potential therapy targets. (DOI:10.9777/rr.2018.1046)

Evaluation of natural products as chemosensitizers

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A plethora of research studies indicate that, anti-cancer principles isolated from natural products are excellent chemotherapeutic lead molecules. Our lab focuses on isolation, identification and characterization of bioactive principles from natural resources and studies pertaining to their anti-cancer potential. The major limitation of almost all these compounds is their insolubility in aqueous medium and low bioavailability under in vivo conditions. Most of these compounds are available only at sub toxic concentrations in vivo since they are, either excreted immediately or not being absorbed. Our lab is interested in studying whether the sub toxic concentrations of these compounds can chemosensitize sub- toxic concentrations of conventional chemotherapeutic drugs, to elicit a synergistic therapeutic effect so that the toxicity and cost of the treatment can be minimized. Our team has evaluated the natural products, curcumin, resveratrol, tryptanthrin and heteronemin for their chemosensitization potential in various cancers using in vitro experiments and animal tumor models. We found that these compounds can counteract various survival signals, which act as the key players of chemoresistance. Curcumin exhibited exceptional chemosensitizing efficacy in paclitaxel chemotherapy for cervical cancer and 5-FU chemotherapy for breast cancer. Our in vitro studies indicates that resveratrol, a potent bioactive principle from grapes, is a promising candidate to be evaluated as an effective chemosensitizer in docetaxel chemotherapy against breast cancer, especially in Her-2 over-expressing cells. Tryptanthrin, an indoloquinazoline alkaloid which we isolated in the lab from the leaves of Wrightia tinctoria, displayed significant chemosensitization potential towards dacarbazine chemotherapy against malignant melanoma, as assessed by both in vitro and in vivo studies. We also found that heteronemin; a sesterpene isolated from marine sponges can elicit chemosensitization in acute myeloid leukemia, one of the most aggressive hematological malignancies, towards cytarabine chemotherapy. Thus, our studies indicate that, anti-cancer principles isolated from natural sources are promising chemosensitizers, which can bring about excellent therapeutic benefit and emerge as a boon for the successful management of cancer in the near future. (DOI:10.9777/rr.2018.1047)

Immune suppressive networks in the tumor microenvironment: implications for cancer immunotherapy

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Cancer immunotherapy has recently achieved remarkable success in treating late stage tumors but a substantial fraction of patients failed to respond. A detailed understanding of how cellular and molecular interactions within the tumor micro environment sculpt the activities of innate and antigen specific immune cells is important. Oral tumour microenvironment is characterized by chronic inflammation represented by infiltrating

leukocytes and soluble mediators that results in local and systemic immune suppression. Myeloid derived suppressor cells (MDSCs) and Regulatory T cells (Tregs) suppress T cell activation and dampen anti-tumour immunity.In the present study we investigated how MDSCs and Regulatory T cells (Tregs) maintain the immune suppressive tumour microenvironment in oral cancer (OC) and contribute to tumor progression. Higher levels of MDSC were observed in OC patients that correlated with tumor stage. Non-classical MDSCs were prevalent in periphery while granulocytic subset was dominant in the tumor compartment. MDSCs suppressed lymphocyte proliferation, decreased CD3-ζ chain expression and IFN-γ production. The levels of MDSCs also inversely correlated with CD3-ζ chain expression in T cells. MDSCs expressed higher levels of COX-2, Arginase 1, and pSTAT3. IL-6 induced pSTAT3 regulates PDL1, C/EBPα and IL-10 expression in MDSC. IL-10 secreted by MDSCs facilitates crosstalk between MDSCs and Tregs. Increased Tregs observed in OC patients correlated with stage of the tumor and IL-10 levels produced by MDSCs. Increased frequency of MDSCs, Tregs and decreased expression of CD3-ζ chain results in T cell tolerance and induce a chronic inflammatory state facilitating tumor growth. These cells could be viewed as therapeutic targets and may have important implications in dictating outcomes of cancer immunotherapy. (DOI:10.9777/rr.2018.1048)

Leadership in Science

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Science is a life-long pursuit, and scientists the practitioners of this art. While science teaches us to remain objective and let data drive our conclusions, successful scientists need many more skills that do not come naturally to us. These have to be understood, learned and cultivated if you are to be a leader. Choosing the right problems to study as our career progresses is critical to our success. Are there rules governing this? How should we choose the right problem as a PhD student, a postdoc or a professor? Funding agencies are increasingly seeking short-term investments. There is a push for translational and applied research over fundamental research. Should one be favored over the other? What is the recipe for a good mix for institutions and governments to follow? Leadership involves many soft skills for which we are ill equipped. What are these and how can PhD students learn those skills while practicing the art of their science? Having stopped doing "bench science" after being at it for 30 years, all I can do is wonder about such things. And share my experiences. (DOI:10.9777/rr.2018.1049)

RNA-Based therapy for the treatment of myotonic dystrophy type 1 using antisense technology

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RNA-based therapeutics have great potential to becomesuccessful commercial products upon approval of drugs by the FDA, notable among them are Kynamro for hypercholesterolemia,Eteplirsen for Duchenne muscular dystrophy, and Spinraza for spinal muscular atrophy. Some of the well-studied approaches to target RNA include small interfering RNA, antagomiRs, aptamers and antisense oligonucleotides (ASO) for a variety of diseasesin metabolic, cancer and CNS areas. ASO technology

is one of the most advanced RNA targeting modalities for neuromuscular diseasescaused by a gain-of-function mutation. Myotonic Dystrophy Type 1 (DM1) is themost common adult-onset muscular dystrophywhich affect skeletal and cardiac musclesas well as neuronal functions. To date there is no effective therapy available to treat this disease. DM1 is caused by a gain-of-function mutation in the Dystrophia Myotonica Protein Kinase (DMPK) gene withan expansion of thetriplet CTG repeats in the 3'untranslated region (UTR). This results in the retention of the mutated pathogenic expanded CUG (CUG^{exp}) RNA in the nucleus in the form of foci. This alters the splicing machinery function of MBLN1 and CELF resulting in the misregulation of several alternatively spliced genes in the muscle tissues of DM1 patients. The Elimination of CUG^{exp}toxic RNA by targeting DMPK RNA is a viable therapy to treat DM1. In pre-clinical studies, targeting the RNA with ASOs, which favors RNase H1-dependent degradation of the transcript, has been shown to effectively reverse thephenotype in the mouse models of DM1.Systemic delivery of ASOsinto mice reduces RNA levels, corrects mis-spliced genes and improves muscle histopathology and function.In addition, phase 1 clinical trials reveal that systemic delivery of ASO was found to be safe. In conclusion, ASO-mediated reduction of pathogenic toxicRNA is a promising therapeutic strategyfor the treatmentof DM1. (DOI:10.9777/rr.2018.1050)

Maximizing target clearance by design of pH-dependent target binding antibodies with altered affinity to FcRn

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Antibodies with pH-dependent binding properties to both target antigens and neonatal Fc receptor (FcRn) provide an alternative tool to conventional neutralizing antibodies, particularly for therapies where reduction in antigen level is challenging due to high target burden. However, the requirement for optimal binding kinetic framework and extent of pH dependence for these sweeping antibodies to maximize target clearance from circulation is not well understood. We show in vivo that pH-dependent binding to the antigen alone is not sufficient for effective antigen removal from circulation, but requires Fc mutations that increase antibody binding to FcRn. Specifically, mouse-derived IgGs with different antigen binding affinities and natural dissociation discrepancies in neutral and acidic solutions were paired with Fc variants with altered FcRn binding affinities, and evaluated for their ability to reduce target circulation in cynomolgus monkeys. Affinity-increased FcRn binding with double-digit nM at pH 7.4 and single-digit nM at pH 6 provided the highest benefit when combining with similar pH-dependency for target binding in opposite pH directions. Sustained target clearance below the baseline level was achieved 3 weeks after single-dose administration at 1.5 mg/kg. With the established mechanistic model, we further demonstrate the essential kinetic interplay between target turnovers, antibody-antigen and antibody-FcRn interactions during the intracellular trafficking process, as well as the requirements for the optimal design for the sweeping antibodies to maximize in vivo benefits to improve dosing convenience to the patient. (DOI:10.9777/rr.2018.1051)

Targeted disruption of PI3K/Aktand its antitumor effects in oral cancer

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The Phosphoinositide-3-kinase (PI3K) pathway is the frequently altered in human cancers. This has led to the development and study of novel PI3K inhibitors for targeted therapy and also to overcome radiotherapy resistance. The anti-tumor effects of PI3K inhibitors (PI-828, PI-103 and PX-866) in terms of cell proliferation, colony formation, induction of apoptosis, cell cycle arrest, invasion, autophagy, and pNF- κB/p65 translocation in SCC-4, SCC-9 and SCC-25 cells was studied by performing MTT, clonogenic, DAPI staining, propidium iodide staining, annexin –V binding, matrigel invasion, acridine orange staining and immuno-fluorescence assay. Alteration in expression of various proteins was studied by western blot assay. PI-828 and PI-103 treatment exhibited significant ($p<0.01$) dose-dependent anti-tumour activity through the inhibition of growth, proliferation and colony formation potential of OSCC cells with a concomitant induction of apoptosis, altered cell cycle regulation and decreased invasiveness. PX-866 induced apoptosis, cell cycle arrest and a significant decrease in the invasive ability in oral cancer cells. These compounds significantly reduced expression of COX-2, Cyclin- D and VEGF in the treated cells besides cytoplasmic accumulation of pNF-κB/p65 protein as compared to the untreated tumor cells. PI-103, PI-828 and PX-866 PI3K inhibitors may be developed as potential chemotherapeutic agents for effective treatment of OSCC patients associated with hyper activation of the PI3K/Akt pathway. (DOI:10.9777/rr.2018.1052)

Telomere dynamics as determinant in cancer research and treatment

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The chromosomes that control the genetic machinery of cells are guarded at both ends by a special substructure called – telomere. Telomere, which is composed of protein and DNA complex, serves as a "mitotic clock" for somatic cells and determines whether the cell after certain replication cycles will age, cease to divide, and undergo apoptosis, or after the crisis, recover as cancerous out-growth due to the reactivation/up-regulation of telomerase, an enzyme that adds nucleotides at the end of chromosomes. Cancer is a group of genetic diseases of old age (cellular and not the biological age). Genetic instability is one of the many hallmarks of cancer development, metastasis and resistance to treatment. Attrition of telomeric repeats (TTAGGG)n promotes genomic instability and mitotic catastrophe, another hallmark of cancer cells. Additional hallmarks of cancer are inflammation, proliferative signaling, and resistance to apoptosis, acquiring replicative immortality, inducing angiogenesis and activating migration to distant organs. Genetic instability, which is responsible for generating genomic diversity (formation of multiple clones), is the crucial step which fosters these multiple hallmark activities. Telomeres, considered to be the

guardian of individual chromosomes, undergo severe alterations and play an important role not only in the development but also in acquiring metastatic phenotypes. The chromosomes with shortened (dysfunctional) telomeres recombine forming rings and dicentrics and tend to become unstable, leading to cell death. In a series of publications, our group has shown amplification of telomeric repeats in human and murine metastatic cancers of different histologic origins. We have also observed amplification of telomeric DNA in the peripheral blood lymphocytes of those cancer patients who were first diagnosed with metastatic disease. This observation has significant implications in the identification of cancer patients whose tumor may have metastatic potential and then can be treated aggressively. Telomere dynamics can be helpful in the identification of cancer-prone individuals who could be predisposed to a particular type of malignancy. Data on telomere biology will be presented on cancer initiation, metastasis, resistance to treatment and other areas of its implications. In addition, our hypothesis on the production of resistant cancer cell population will also be presented. Cancer stem cells that are known to have more telomeric DNA compared to the differentiated somatic cells should be the target of cancer therapy. Only by targeting cancer stem cells and developing new anticancer drugs against such cells, oncologists will be able to treat cancer patients successfully. (DOI:10.9777/rr.2018.1053)

Mycobacterium tuberculosis, the TB bacterium, adopts intelligent strategies for its survival, virulence and pathogenesis

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Mycobacterium tuberculosis (M.tb), the causal organism of the dreaded disease tuberculosis (TB), takes one human life every 15-20 seconds globally. My lab has been focusing on the functional biology and epidemiology of this pathogen with a view to design innovative interventions against TB. We also identified and characterized several virulent proteins of M.tb that help in intracellular survival by epigenetic modification of the host cellular machinery. The M.tb PE_PGRS subfamily displays unusually high levels of intrinsically disordered stretches compared to any other family in the proteome and was highly enriched in average number of ANCHOR binding sites, eukaryotic linear motifs (ELMs). These family members also have highly biased amino acid composition rich in disorder promoting alanine and glycine residues and play roles in molecular mimicry. One member of this protein family causes activation of Unfolded Protein Response as evident from increased expression of GRP78/GRP94 and CHOP/ATF4, leading to disruption of intracellular Ca2+ homeostasis and increased NO and ROS production. The consequent activation of effector caspase-8, resulted in apoptosis of macrophages. In other series of investigations, comparative proteomic and genomic analyses revealed the exclusive presence of 'Signature sequences' in M.tb genome, some of which have potential utility in TB diagnosis based on limited clinical validation. Hypothetical proteins coded by one such 'Signature sequences' was found to be a functional S-adenosyl dependent DNA methyltransferase and binds DNA non-specifically and protects DNA

from oxidative stress by scavanging iron thereby, preventing generation of free radicals and also by physically binding DNA and providing a physical barrier. Using drug re-purposing strategies we also identified existing US FDA approved drugs that inhibit M.tb by virtue of disrupting the pathogen's biofilm forming ability and thus have the potential to act as a new TB drug and also to reduce the duration of treatment. My presentation will cover some of these findings from our group. (DOI:10.9777/rr.2018.1054)

Sprouty2 is an oncogene in colorectal cancer

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Background & aims: Four family members of Sprouty (SPRY) gene (SPRY1-4) have been identified. SPRY2 appears to be ubiquitously expressed whereas other family members show organ and tissue specificity. SPRY proteins appear to function as tumor suppressors in many human cancers, whereas we reported that SPRY2 acts as an oncogene in colorectal cancer (CRC) by increasing cMet expression and epithelial-mesenchymal-transition (EMT). The mechanism by which SPRY regulates EMT in CRC is investigated. **Methods:** Human CRC cDNA arrays and tissue microarrays (TMA) were employed to assess mRNA and protein levels of SPRY2, AKT2 and E-cadherin. SPRY2 regulated microRNAs (miRs) were identified by microarray profiling. Effects of SPRY and miRs were assessed by transient transfections, immunoblotting, real time PCR, luciferase reporter assay, cell migration-invasion assay, and confocal microscopy. Mouse embryonic fibroblasts (MEFs) derived from Spry1 and Spry2 floxed mice were utilized to dissect role of SPRY in mesenchymal cells. Results & **Discussion:** SPRY2 mRNA transcripts were significantly higher in human CRC. Treatment with SPRY2siRNA increased E-cadherin expression while EMT-inducing transcription factors were significantly reduced. SPRY2 negatively regulated miR-194-5p in CRC cells. MiR-194 directly interacts with AKT2 3'UTR. AKT2 and several EMT-inducing transcription factors are the targets of miR-194. An inverse expression pattern between AKT2 and E-cadherin was demonstrated in human CRC. Tamoxifen treatment of MEFs from Spry1 and Spry2 floxed mice demonstrated decreased cell migration, while acquiring several epithelial markers.Our results have identified that suppression of SPRY leads to reduction in EMT and enhancement in a phenotype that favors epithelial features. **Conclusion:** Studies indicate a role of SPRY in sustaining processes important to malignancy in CRC and suppression of SPRY may inhibit EMT in CRC patients. SPRY2 could serve as a biomarker of poor prognosis in CRC. (DOI:10.9777/rr.2018.1055)

HIV-1 Tat-mediated upregulation of miR-34a activates NF-κB-mediated microglial inflammation via targeting the 3′-UTR of NLRC5

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Background: Although the advent of combination antiretroviral therapy (cART) has dramatically increased the life expectancy of people living with HIV-1, paradoxically the prevalence of HIV-1-associated neurocognitive disorders in people treated with cART, is on the rise.It has been well-documented that despite the effectiveness of cART in suppressing viremia, CNS continues to harbor

viral reservoirs with persistence of low-level virus replication with sustained presence of cytotoxic early viral protein, HIV-1 Tat. **Aim:** The aim of the present study was to investigate the epigenetic mechanism(s) by which HIV-1 Tat activates NF-κB-mediated microglial activation. **Methods:** Protein levels of NLRC5, p- NF-κB, NF-κB, and IKKγ were determined by western blotting in HIV-1 Tat exposed mouse BV-2 microglial cells. Gene expression studies were determined using TaqMan qPCR assays. Ago2 immunoprecipitation was used for NLRC5 3′-UTR target validation. miR-34a mimic and miR-34a inhibitor oligos were used for overexpression and blocking experiments. Findings from in vitro experiments were validated in the brains of HIV-1 transgenic rats as well as in the archival brain samples from SIV-infected rhesus macaques. Results and **Discussion:** Exposure of mouse microglia to HIV-1 Tat upregulated miR-34aexpression while concomitantly also downregulating the expression of NLRC5 (a negative regulator of NF-κB signaling). Using Ago2 immunoprecipitation assay NLRC5 was identified as a novel 3′-UTR target of miR-34a. Transfection of mouse microglia with miR-34a mimics significantly downregulated NLRC5, resulting in nuclear accumulation of NF-κB p65. In contrast, transfection of cells with miR-34a inhibitor notably upregulated NLRC5 levels.Gene silencing approaches to block either NLRC5 or NF-κBdemonstrated that HIV-1 Tat-mediated microglial activation involved sequential downregulation of NLRC5 with concomitant activation of NF-κB. **Conclusion:** Overall, our findings demonstrate a novel mechanism of HIV-1 Tat-mediated activation of microglia via upregulation of miR- 34a, leading ultimately to downregulation of NLRC5 expression with a concomitant activation of NF-κB signaling. (DOI:10.9777/rr.2018.1056)

Novel approaches for prevention of breast cancer

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Breast cancer, which is a heterogeneous disease broadly grouped into different sub-types including luminal-type (luminal A and B), Her2+, basal-like (mostly triple-negative), and normal like, is a leading cause of cancer-related mortality among women worldwide. Chemoprevention represents a sensible strategy for reducing the morbidity and mortality associated with breast cancer. A safe and efficacious intervention for chemoprevention of estrogen receptor negative (ER-) breast cancer is still lacking. Constituents of Ayurvedic medicine, which has been practiced in India for thousands of years, continue to draw attention for identification of novel small-molecules potentially useful for prevention of chronic diseases including cancer. This presentation summarizes efficacy data, molecular targets, and biomarkers of mammary cancer prevention by a small molecule steroidal lactone (withaferin A; WA) derived from an Ayurvedic medicinal plant (Withania somnifera). Specifically, we demonstrate chemopreventive efficacy of WA against Her2+ (ER-) breast cancer in a transgenic mouse model (MMTV-neu) as well as against luminal-type breast cancer in a rat model. Strikingly, the mechanisms underlying breast cancer chemoprevention by WA are common in both models, including reversal of Warburg effect (inhibition of glycolysis) leading to suppression of cell proliferation and apoptosis, and inhibition of breast cancer stem cell population. (DOI:10.9777/rr.2018.1057)

A grip on arresting gastric ulcer by neem leaf flavonoid via metalloprotease inhibition

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Neem (Azadiractaindica) is widely used in traditional medicine because of high therapeutic potential. Different parts of neem tree e.g. leaf, seed, bark, flower, fruit and root show various biological activities. Bioactive constituents in neem leaf include several flavonoids, polyphenols, isoprenoids, sulphurous and polysaccharides that are effective for therapeutic function. Neem leaf extracts exhibit significant anti- inflammatory, anti-mutagenic and antioxidant properties. Although the antiulcer activity of neem leaf was reported but the mechanism of action and the lead compound is unknown. Crude methanolic extract of neem leaf was evaluated for anti gastric ulcer activity. We for the first time purified and characterized by IR, NMR and ESI-MS a new compound, tamarixetin 3-O-β-D-glucopyranoside (T3G) from methanolic extract of neem leaf. The gastroprotective activity of T3G was tested in rodent model of gastric ulcer. Dose dependent inhibition of ulcer scores by T3G was observed in indomethacin-induced ulcerated animal. Previous studies from our laboratory had established higher activity of matrix metalloproteinase (MMP)-9 in gastric tissues of indomethacin-induced ulcerated animal. Interestingly, T3G compound in a dose dependent manner showed protection against gastric ulcer by inhibiting MMP-9 activity. In addition, in silico and in vivo binding studies of T3G with MMP-9 showed IC50 <100 nM. In summary, T3G compound in neem leaf could be a lead therapeutic agent for arresting gastric ulcer via MMP-9 inhibition pathway. (DOI:10.9777/rr.2018.1058)

Targeting pancreatic tumor microenvironment for effective pancreatic cancer treatment

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Pancreatic cancer (PanCa) management is exceptionally difficult due to the extremely poor response to available therapeutic modalities and lack of effective therapeutic strategies. Highly desmoplastic (excessive fibrosis and extracellular matrix deposition) microenvironment in pancreatic tumor causes suboptimal drug delivery and increases chemo-resistance. In addition to tumor cells, presence of stromal components such as tumor associated fibroblasts (TAFs), pancreatic stellate cells (PSCs), cancer stem cells (CSCs) and tumor associated macrophages (TAMs) also play a pivotal role in induction, progression and metastasis of PanCa. At the onset of cancer induction and progression various oncogenic signaling molecules such as Mucin 13 (MUC13), KRas, NF-κB, and Sonic Hedgehog (SHH) play critical roles. In response to aberrant expression of these oncogenic molecules, cancer cells and stromal cells secrete various growth factors which are involved in reciprocal cross-talk in between tumor and stromal cells. This creates desmoplastic tumor microenvironment (TME) to facilitate PanCaprogression and metastasis. Thus, targeting components of TME along with oncogenic signaling pathways by non-toxic agents/ drugs will be effective therapeutic approach to combat this lethal disease. In this talk, we will discuss how components of pancreatic TME and oncogenic

signaling pathways play an important role in PanCa progression and metastasis. We will also discuss new strategy and molecular mechanisms to suppress components of pancreatic TME by non-toxic nutraceuticals. Our lab has identified novel nutraceuticals (curcumin, α-Mangostin, and plumbagin) whichinhibit expression of fibroblast cells marker (α-SMA, and CYGB) and oncogenic CXCL12/CXCR4/Shh signaling pathways in human TAFs and PanCa cells. Moreover, we have developed novel nano-formulations of these nutraceuticals which showhighsignificant therapeutic and chemosensitization potentialin orthotopic xenograft mouse model involving co-injection of PanCa cells along with TAFs. In, addition these agents also inhibit the growth of pancreatic CSCs via targeting CSCs marker (Nanog and CD44). These nutraceuticals could be a potential therapeutic modality in near future for the prevention and treatment of human PanCa. (DOI:10.9777/rr.2018.1059)

Ropinirole promotes neurological recovery by restricting mitochondria mediated ischemic cell death

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Dopamine D2 receptor agonist, Ropinirole has been found to promote neuroprotection in Parkinson and restless leg syndrome patients. Recent evidences have shown that ropinirole mediates its neuroprotection through mitochondria. Assuming this, we examined the possible mitochondrial role of ropinirole in promoting the neuroprotection in ischemic stroke of rat. Male Wistar rats underwent transient middle cerebral artery occlusion and then received the ropinirole (10 mg and 20 mg/kg b.w.) at 1 h, 6, 12 and 18 h post occlusion. The panel of neurological tests and infarct size were performed after 24 h of the ischemia. Flow cytometry was used to detect the mitochondrial membrane potential, ROS and Ca^{2+} respectively. Mitochondrial bioenergetics was analyzed by oxygraph. Western blotting was used to analyze the expression of various proteins such as Bax, Bcl-2 and cytochrome c. Ropinirole induces the neurological recovery as shown by the panel of neurobehavioural tests and 2, 3, 5-triphenyltetrazolium chloride staining. Post stroke treatment with ropinirole reduced the mitochondrial ROS and Ca^{2+} in tMCAO rats. Ropinirole elevated the mitochondrial membrane potential and mitochondria bioenergetics. Western blotting has shown that ropinirole inhibited the translocation of cytochrome c from mitochondria to cytosol. Post stroke administration of ropinirole induces the neurological recovery through mitochondrial pathways in ischemia. (DOI:10.9777/rr.2018.1060)

Leukaemia: hematopoietic pathophysiology and ayurvedic intervention

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Leukaemia is “blood cancer”, characterized by unconstrained production of the hematopoietic precursor cells which don't differentiate into mature blood cells and eventually accumulate as leukemic blasts and subsequently get released into the peripheral circulation. It arises due to

irreversible alteration in bone marrow hematopoietic stem/progenitor compartment as well as in microenvironment. The present study deals with the characterization of N-N' Ethylnitrosourea (ENU) induced mouse model of leukemia by peripheral blood hemogram, bone marrow cytology, histology, short-term bone marrow explant culture, cytochemical staining, scanning electron microscopic study and above all flow cytometric analysis. We also observed aberrant cell cycle regulation in leukemic bone marrow which enabled leukemic cells to proceed through indefinite numbers of cell cycle, escape from check point surveillance and become resistant to cell death and stroma-dependent progression of leukemic hematopoiesis at the expense of normal steady-state hematopoiesis. Flow cytometric approach was taken to study the alteration in conventional and hematopoiesis specific p53 pathways and associated molecules in leukemia bone marrow. Ayurveda is an ancient medical science developed in India more than five thousand years ago. Derived from its ancient Sanskrit roots 'ayus' (life) and 'veda' (knowledge) – offers a unique blend of science and philosophy that balances various components necessary for holistic health. The principle of Ayurveda is to prevent and treat diseases through natural therapies. In recent years, numerous studies have reported therapeutic potentials of Withania somnifera commonly called Ashwagandha for its anti- cancer properties and we have tried to reveal the therapeutic potentials of Ashwagandha against hematological disorders specifically in case of Leukaemia. In addition developmentally important Hedgehog signaling pathway plays a fundamental role behind leukemia pathogenesis by up-regulating PTCH, GLI-1 etc. expression and it represents an attractive target to intervene the disease. In our study we have seen Ashwagandha as a hedgehog pathway modulator which may open new avenues for therapeutic advancements regarding Leukemia in future. (DOI:10.9777/rr.2018.1061)

Structural and functional changes in the Sebox diguanylate cyclase from *Vibrio cholera* induced by site-directed mutagenesis

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Vibrio cholerae, responsible for seven pandemics over three centuries, has the ability to switch between two lifestyles – the sessile, non-pathogenic form and the motile, infectious form in human hosts. One of the manifestations of these changes is in the formation of surface biofilms, when in sessile aquatic habitats. The cell-cell interactions within a V. cholerae biofilm are stabilized by the production of an exoploysachharide (EPS) matrix, which in turn is regulated by the ubiquitous secondary messenger, cyclic di-GMP (c-di- GMP), synthesized by proteins containing GGD(/E)EF domains in all prokaryotic systems. The Sebox3 protein, a diguanylate cyclase from V. cholerae, displays enhanced biofilm forming ability with cellulose production as quantified and visualized by multiple assays, most notably using FEG-SEM. This has also been corroborated with the lack of motility of host containing Sebox3 in semi-solid media. . The structure of the active domain of Sebox3, too has revealed typical GGEEF architecture in its active site, exemplified by the typical helix-sheet architecture. Site-directed mutants of Sebox3 have been created at the central residues of the GGEEF domain in the wild type protein. These have

distinct reduction in their ability to form surface biofilms, as well as in the synthesis of cyclic-di-GMP from GTP (the core activity of a diguanylate cyclase). However, motility in these mutant strains is much enhanced as compared to the wild type. This difference has been traced to the structure of two of the mutants of Sebox3, further named as Sebox5 and Sebox 6. In Sebox5, the second glycine has been replaced by an arginine, and in Sebox 6, the first glutamate was replaced by a lysine. The wild type Sebox3 has an eminent GTP-binding pocket, where the glutamate forms extensive contacts with an incoming GTP. In the mutants, the shape of the pocket is altered due to the arginine or the lysine side chain which interferes with the entry of the GTP, and thereby leads to a loss of activity. The understanding of the activity of the diguanylate cyclase and the mechanism of steric inhibition in the mutants can go a long way in the design of functional mutants in *V. cholerae*. (DOI:10.9777/rr.2018.1062)

Therapeutic potentials of Ashwagandha: Biology to biotechnology

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Ashwagandha (Withania somnifera: family Solanaceae) is a widely admired herb intraditional Indian home medicine. It has been used for a variety of remedies. However, laboratory evidence and molecular mechanisms of its actions are only beginning to be determined. We initiated search on bioactivities in the leaves of Ashwagandha and found that both alcoholic (i-Extract) and water extracts (WEX) of Ashwagandha leaves possess considerableanticancer activities. Bioactives for anticancer activity were identified as withanolides, withanone (Wi-N) and withaferin-A (Wi-A) in the i-Extract, and triethylene glycol in the WEX. Using multiple experimental and bioinformatics approaches, we demonstrated that the two kinds of extracts possess different bioactive constituents and work through independent pathways. Most recently, we reported thatWi-A is cytotoxic not only to the telomerase plus, but also to telomerase negative (ALT) cancer cells, suggesting its value as a powerful anticancer drug. Based on these data, we have formulated a combination of Wi-N and Wi-A that exhibited potent anti-metastasis activity. Furthermore, we found that methoxyWi-A does not possess anticancer activity, but protects the normal human cells against the cytotoxicity of other components of the extract. In view of these findings, we have developed technologies to obtainActive Ingredients Enriched (AIE) Ashwagandha by manipulating itsenvironmental conditions. We demonstrate, for the first time,(i) field raised i-Ashwagandha leaves with high proportion of active withanolides as compared to the roots,(ii) hydroponically raised i-Ashwagandha and characterization of its bioactives, and (iii) method of extraction with enriched bioactive components that may serve as an economic anticancer drug especially when modern medicine is either not available or limited by severe side effects. (DOI:10.9777/rr.2018.1063)

Mutually exclusive post-translational modifications: advances and challenges

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The most commonly occurring PTMs involve similar residues in proteins suchas acetylation, ubiquitylation, methylation, and sumoylation at the lysine residue and phosphorylation and O-GlcNAcylation at serine/threonine residues. Thus, the possibility of modificationsites where two such PTMs may occur in a mutually exclusive manner (ME-PTM) is much higher than known. A recent surge in the identification and the mapping of the commonly occurringPTMs in proteins has revealed that this is indeed the case. However, in what way such ME-PTMs are regulated and what could be their relevance in the coordinated network of protein function remains to be known.To gain such potential insights in a biological context, we analyzed the data sets of two most prevalent PTMson the lysine residue by acetylation and ubiquitylation along with the most abundant PTM in proteins by phosphorylation among enzymes involved in glucosemetabolism. Similarly, we analyzed data sets of O- GlcNAc modified and phospho proteins. The analysis of the PTMdata sets has revealed two important clues that may be intrinsically associated with their regulation and function. First, the most commonly occurring PTMs by phosphorylation, acetylationand ubiquitylation are widespread and clustered in most of the enzymes involved in glucose metabolism; and the prevalence of phosphorylation sitescorrelates with the number of acetylation and ubiquitylation sites including the ME-modification sites. Second, the prevalence of ME-acetylation/ubiquitylationsites is exceptionally high among enzymes involved in glucose metabolism and have distinct pattern among the subset of enzymes of glucose metabolism such as glycolysis, TCA cycle, glycogen synthesis, and the irreversible steps of gluconeogenesis. Importantly, prevalence of tyrosine phosphorylation is exceptionally high in proteins with ME-PTMs. We hypothesize that phosphorylation including tyrosine phosphorylation playsan important role in the regulation ofME-PTMs (acetylation/ubiquitylation as well as serine/threonine phosphorylation and O-GlcNAcylation), and their similar pattern among the subset offunctionally related proteins allows theircoordinated regulation in the normalphysiology. Similarly their coordinated dysregulation may underlie the disease processes such as reprogrammed metabolismin cancer, obesity, and type 2 diabetes. Ourhypothesis provides an opportunity to understand the regulation of ME-PTMsin proteins and their relevance at the network level and is open for experimental validation. (DOI:10.9777/rr.2018.1064)

Structural basis of antibacterial action of innate immune proteins and their applications as protein-antibiotics

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Considering the alarming rise in the incidence of bacterial resistance to known antibiotics, there is a desperate need to develop bacterial resistance-free antibiotics. The proteins of the innate immune system provide the first line of defense against infecting microbes. These proteins recognize the conserved motifs that are present on the cell walls of bacteria. Thus the success of the innate immune system depends on the affinity of the proteins of innate immune system towards the bacterial cell wall molecules. The conserved motifs of microbial cell walls are called pathogen associated molecular patterns (PAMPs) that include the well known peptidoglycans (PGN) and lipopolysaccharides (LPS) of Gram-negative bacteria, PGN and

lipoteichoic acid (LTA) of the Gram-positive bacteria and mycolic acid (MA) and other fatty acids of Mycobacterium tuberculosis. These PAMPs are classified into two groups: (i) those which contain glycan moieties such PGN, LPS, LTA etc. and (ii) those that are derivatives of fatty acids such as MA. Therefore, there should be two independent binding sites for the two different types of PAMPs. The PAMPs are specifically recognized by innate immunity molecules which are historically known as peptidoglycan recognition proteins (PGRPs).These proteins bind to PAMPs with significant affinities and neutralize the infecting pathogens through a variety of actions. There are four types of PGRPs in mammals including humans, PGRP-L (MW = 90kDa), PGRP-Iα and Iβ (MW = 45kDa) and PGRP-S (MW = 21kDa). PGRP-S represents the domain that has the binding site for PAMPs. The binding affinities of PGRP-S and structures of unbound and bound PGRP-S from various species showed that the protein from camel has considerably higher affinity than those of other animals including humans. The epidemiological data indicate that the camels have the lowest rates of infections. Structurally, PGRP-S from camel exists in the form of a dimer whereas the human protein acts as a monomer. There are only a few sequence differences in the proteins from two species which are responsible for dimerization of camel protein. As a result of dimerization, a deep binding cleft is formed in the camel protein whereas only a shallow cleft is present in the case of human monomeric protein. Because of dimerization, the potency of camel protein is much higher than the same protein from other species. Thus if camel protein is used or a suitably mutated human protein is prepared and used, the fight against bacterial infection will improve. The mechanism of action of PGRP-S involves an effective sequestration of bacteria which results in the killing of bacteria. Since PGRP-S interacts with bacterial cell wall, the kinetics of bacterial cell death appears to be similar to those antibiotics which inhibit the biosynthesis of PGN. Due to this similarity, PGRP-S is suggested to be termed as "protein antibiotics" and since they bind to bacterial cell wall molecules the issues of side effects and resistance will not arise and if the potencies are high, the invading bacteria can be tackled rapidly. (DOI:10.9777/rr.2018.1065)

Fine tuning gene expression in physiology and pathophysiology: Implications in therapeutics

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The highly ordered nucleoprotein structure in the eukaryotic nuclei is referred as chromatin. Though DNA sequence is the template for gene expression, the fine tuning of this process is mediated by the epigenetic machinery. Broadly, epigenetics refers to the modification of DNA, and the proteins that help to organize the eukaryotic DNA. The protein component of chromatin undergoes several reversible post- translational modifications such as acetylation, methylation, phosphorylation etc., which occur on specific amino acid residues. We have found that lysine acetylation and arginine methylation of histones are essential for regulating the synthesis of mRNA as well as microRNA with functional consequences. The acetylation of proteins assisting transcription activation has also been found to be essential for the activation of gene expression. In

pathophysiological conditions such as cancer, AIDS and in neurodegenerative disorders, homeostasis is affected, and the state of these modifications also undergoes alteration. Hence, enzymes responsible for these modifications could be target for new generation epigenetic therapeutics. We have discovered a few small molecules which specifically target these enzymes and eventually suppress tumor growth, viral multiplication in AIDS and enhance cognate brain function in a model of neurodegeneration. Besides therapeutic implications, these efforts significantly contribute to our understanding of fundamental disease biology. (DOI:10.9777/rr.2018.1066)

Antitumor effects and the underlying mechanism of naringin combined with 5-fluorouracil in human breast cancer cells

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Antimetabolite has proven successful as therapeutics for advanced-stage breast cancers, but is often accompanied by severe side effects that can limit treatment regimens. 5-Fluorouracil (5-FU), an antimetabolite that inhibits cell proliferation, has served an important role in standard chemotherapy protocols for a variety of solid tumors. Although reasonable response rates have been reported for 5-FU, continued investigation is necessary to improve clinical outcomes and reduce cytotoxic side effects that are an inherent problem for chemotherapeutic interventions. Because of its diverse anti cancer properties, we explored whether by combining the natural product naringin with 5-FU, synergistic improvements in preventing breast cancer cell proliferation and/or provide protection against 5-FU- induced cytotoxicity could be achieved. Naringin and 5-FU inhibit DNA synthesis in MDA-MB-231 cells using MTT assay, however, combined treatment showed synergistic improvement. We next established the cytotoxicity profile for 5-FU in MDA-MB- 231 cells using a trypan blue based cell viability assay and obtained an IC 50 value of 80 µM. When 5-FU incubations were repeated with the addition of naringin, the IC50 value increased to 40–300 lM, representing a 7–10-fold protection by naringin against 5- FU cytotoxicity. These findings suggest that the addition of naringin as an adjuvant therapy during 5-FU treatment might enhance the chemotherapeutic effectiveness of 5-FU by protecting normal cells from reduced viability and thus permitting higher dosing or longer treatment times. This would be especially important to those individuals who are plagued with severe cytotoxicities and require frequent interruptions, or even early termination of their treatment regimens. (DOI:10.9777/rr.2018.1067)

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Mechanical properties of silk exceed all natural polymer and synthetic materials. Silk is biocompatible as biomaterial and has been used commercially as sutures. Silk suture that already established in the market is a product base on the species of Bombyx mory, a species of silk which is

only consume one type of leas of mulberry leaves. In this research a type of silk suture is being developed from species from Indonesian source of wild silkmoth of Attacus atlas to obtain more biocompatible sutures. Attacus atlas consume not only single type of leaves so that yield variety type of cocoon fiber that can be arranged for the purpose of better biocompatible comparing commercial silk suture that already established in the market. The Attacus atlas was domesticated by feeding with herb Cananga odorata. The released fiber from cocoon indicates high biocompatibility that is promising as biocompatible suture. A high composition of calcium (Ca) is identified in the fiber. Small amount of kalium (K) is also detected as an affect of feeding with Cananga odorata. (DOI:10.9777/rr.2018.1068)

Microbial production of active vitamin D3 intention, innovation and industry

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Vitamin D3 (VD3) is a fat-soluble prohormone in mammals that plays a crucial role in bone metabolism, immunity, and the control of cell proliferation and differentiation. VD3 is initially converted to 25- hydroxylatedVD3 (25(OH)VD3) in the liver, and then 25(OH)VD3 is further converted to a functionally active form of1α,25(OH)2VD3 in the kidney. 25(OH)VD3, which is the circulating form of hormone and clinically used to establish and monitor the VD3 status. Many studies have revealed that low serum 25(OH)VD3 concentration is closely linked with osteoporosis, numerous cancers, and chronic diseases. Recent meta-analysis and epidemiological data also suggest that low vitamin D concentration is associated with depression and an increased risk of dementia and Alzheimer disease. Therefore, the serum 25(OH)VD3 concentration appears to be important in maintain good health. 1α,25(OH)2VD3is industrially produc e d by s ingle - s te p biotra ns forma t ion proc e s s with a n a c t inomyc e te s t ra in Pseudonocardiaautotrophica. Vitamin D3 hydroxylase (Vdh) is a cytochrome P450 monooxygenase that catalyzes two-step hydroxylation of vitamin D3 and produces 25-hydroxyvitamin D3 (25(OH)VD3), a promising intermediate for synthesis of various hydroxylated VD3 derivatives, and 1α,25(OH)2VD3. By using the expression system using Rhodococcuserythropolis as a host cell, we successfully obtained 25(OH)VD3 by expression of Vdh (vitamin D3 hydroxylase) and redox partner proteins. To improve biotransformation activity, we created a highly active T107A mutant of Vdh (VdhT107A) by engineering the putative ferredoxin-binding site. Crystallographic and kinetic analyses indicated that T107A increased binding affinity with ferredoxin. An efficient biocatalytic synthesis of 25(OH)VD3 was carried out by using the nisin-treated. Rhodococcuserythropoliscells containing VdhT107A. Nisin is a small antimicrobial peptide and improve permeability of VD3 into the cytoplasm.The gene encoding glucose dehydrogenase-IV from Bacillus megaterium was co-expressed in R. erythropolis in order to avoid the exhaustion of NADH in a cytoplasmic space during bioconversion. As a result, approximately 573 μg/mL of 25(OH)VD3 was successfully produced after 2-hr bioconversion. (DOI:10.9777/rr.2018.1069)

Protein redox modification dictates Parkinson's disease pathology

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Nitric oxide (NO) is a unique redox molecule which exerts its effect via covalent addition of NO to a cysteine thiol group on the target protein called protein S-nitrosylation. NO plays an important role in neurotransmitter release and reuptake, neuro development, synaptic plasticity, and regulation of gene expression. However, excessive production of NO due to mitochondrial dysfunction or enhanced nitric oxide synthase activity is linked to neurotoxicity and neurodegenerative conditions. Increased NO leads to aberrant S-nitrosylation reactions and promotes protein misfolding, mitochondrial fragmentation, synaptic dysfunction, apoptosis or autophagy. Ubiquitin C-terminal Hydrolase-1 (UCHL1) is a neuron specific deubiquitinating enzyme which constitutes up to 2% of the soluble brain protein and plays a key role in Parkinson's disease (PD). We have shown that UCHL1 undergoes S-nitrosylation in vitro and rotenone induced PD mouse model. We have also shown that extracellular α-synuclein oligomers induce NO release and nitrosylate key proteins like Actin, DJ-1, HSP70 UCHL1, Parkin, and GAPDH that alter cytoskeletal network, protein folding machinery, ubiquitin proteasome system inducing apoptosis. (DOI:10.9777/rr.2018.1070)

Understanding the molecular link between obesity and asthma

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Epidemiological studies suggest a positive association between obesity and asthma, yet the molecular mechanism is not well established. Cysteinyl leukotrienes (CysLTs), the potent lipid inflammatory mediators found increased in obese asthmatics, may be a potential molecular link between obesity and airway epithelial cells inflammation. Although CysLTs is known to constrict airway smooth muscle cells in lean asthma patients, its role in airway epithelial cell inflammation is not known. We investigated the role of CysLT leukotriene D4 (LTD4) in human airway epithelial cell inflammation and remodelling. Small airway epithelial cells (SAECs) cells were incubated with different concentrations of LTD4 and Trypan blue and MTT assays were performed to assess cell viability, Western blotting, inflammation array, RT-PCR, immunocytochemistry (ICC), and H&E and PAS staining in air- liquid interface (ALI) culture experiment were performed to examine the effects on airway epithelial cells. LTD4 induced the airway epithelial cell proliferation, activation of NALP3 inflammasome complex, and increased expression of inflammatory markers which are indicator of increased airway inflammation in response to LTD4 stimulation. Further, LTD4 caused modulation in proteins such as E- cadherin, vimentin and mucin5A/C in airway epithelial cells suggesting changes in its epithelial nature. Furthermore, stimulation of differentiated into ciliated epithelial cells at ALI with LTD4 transformed them into mucus-filled goblet cells, caused degradation of cilia and mucin5A/C hypersecretion which suggested epithelial cell remodelling by LTD4. LTD4 is reported to be overexpressed in obese

asthmatics, however the link between increased inflammatory lipid mediator during obesity and asthma is not known. We observed that LTD4 may be involved in induction of airway epithelial cell inflammation and remodelling leading to the development of asthma like symptoms in obese. LTD4 caused airway epithelial cell inflammation through activation of inflammasome complex and caused airway epithelial cells remodelling indicating the plausible molecular link between obesity and asthma. (DOI:10.9777/rr.2018.1071)

Biomarkers of gall bladder cancer

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Gallbladder cancer is a relatively common in eastern part of Uttar Pradesh and Bihar. It is aggressive malignancy with a high mortality rate that shows widespread geographic and gender variation. It is the fifth most common cancer of gastrointestinal tract and the most common malignancy of the biliary tract with a less than 5% of overall 5-year survival. Complete surgical resection offers the only chance of cure, which is often not possible owing to the advanced stage of the disease at presentation. Thus, early diagnosis and resection of gallbladder cancer offers a chance of cure. It is therefore important to diagnose gallbladder cancer early. A biomarker is a substance used as an indicator of a biological state. It is a characteristic that is measured objectively as an indicator of normal biological processes, pathological processes, or response to a therapeutic intervention. Biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease. There is a lack of sensitive and specific biomarkers for gallbladder cancer for early detection of the disease. Biomarkers can also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages. There are two major types of biomarkers: biomarkers of exposure, which are used in risk prediction (Diagnostic Biomarker), and biomarkers of disease, which are used in screening and diagnosis and monitoring of disease progression (Prognostic Biomarker). Moreover, when these markers are used individually for the diagnosis of gallbladder cancer, inconsistent results have been obtained. This presentation aims to explore possible biomarkers of gallbladder cancer. Recently several serological and molecular biomarkers have been studied in gallbladder cancer. Molecular studies have demonstrated their association with the activation of dominant proto-oncogenes and inactivation of recessive tumor suppressor genes in gallbladder neoplasms. With the development of newer modalities of diagnosis and the discovery of molecular markers for the disease the search for possible subcellular molecules which can serve as early markers either for detection or for therapeutic intervention has intensified. Next-generation sequencing, genomic microarray, proteomic technologies have been employed in the biomarker discovery in gallbladder cancer. This will focus on the current research on gallbladder tumorigenesis, use of technological strategies in search of clinically relevant biomarkers for early diagnosis, and their limitations. (DOI:10.9777/rr.2018.1072)

Novel telomerase inhibitors

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While telomerase is recognized as a key target in cancer, telomerase inhibitors despite being good drugs are unsuccessful due to their side effects on stem cells. Unlike in stem cells, levels of telomerase catalytic subunit TERT are limiting in reconstituting telomerase activity in normal somatic cells. However, in 90-95 % of human cancers, TERT is transcriptionally reactivated and telomerase activity is reconstituted which is necessary for cancer progression. If TERT transcriptional reactivation in cancer cells can be blocked, telomerase reconstitution in cancers can be prevented. How TERT promoter is reactivated in cancers has been a fundamental unanswered question in cancer biology. The recent discovery of 2 prevalent somatic mutations - C250T and C228T in the TERT promoter in various cancers including 85% of melanomas and glioblastomas has provided insight into the plausible mechanism of telomerase reactivation in cancers. We have identified mechanisms by which mutant TERT promoters are reactivated. I will describe these mechanisms of TERT promoter firing and propose how we can selectively target TERT reactivation and hence telomerase activity in mutant cancer cells. (DOI:10.9777/rr.2018.1073)

Convergent synthetic approaches to biologically-inspired small molecules

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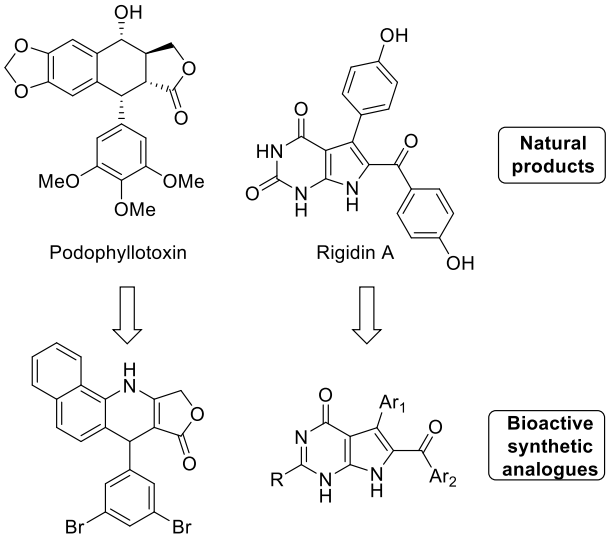
Small heteroaromatic and benzo-fused molecules have found much application as cytotoxic compounds, particularly in the area of cancer therapy research.[1] *In this field, natural products such as* podophyllotoxin and rigidin A (see structures below) have been seen as structural muses for novel molecules, as they induce apoptosis by the relatively well understood disruption of the cellular microtubulin assembly required during cell division.



Our research group has in collaborations over the last few years engaged in the area of biologically-inspired organic synthesis (BIOS), specifically to find novel tubulin polymerization inhibitors. In this talk, progress concerning the facile synthesis of podophylotoxin[3] and rigidin[4,5] mimics, by way of multi-component reactions, will be reflected on. (DOI:10.9777/rr.2018.1074)

A phase I study of docetaxel plus synthetic lycopene in metastatic, castration-resistant, and chemotherapy-naïve prostate cancer patients

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Preclinical study suggests that lycopene enhance anti-prostate cancer efficacy of docetaxel. A phase I trial (NCT0149519) was therefore conducted to identify an optimum dose of lycopene in combination with docetaxel and to evaluate its effect on safety and pharmacokinetics of docetaxel in men with metastatic, castration-resistant prostate cancer. Participants were treated with 21-day cycles of 75mg/m^2 docetaxel, plus lycopene at 30, 90, or 150 mg/day. A Bayesian model averaging continual reassessment method was used to guide dose escalation. Pharmacokinetics of docetaxel and multiple correlative studies were carried out. Twenty-four participants were enrolled, 18 in a dose escalation cohort to define the maximum tolerated dose (MTD), and 6 in a pharmacokinetic cohort. Docetaxel plus 150 mg/day lycopene resulted in dose-limiting toxicity (i.e. pulmonary embolus) in 1 out of 12 participants with estimated probability of 0.106 and thus is chosen as the MTD. The most common toxicities observed during this trial were fatigue (95.8%) and constipation (54.2%), while rate and severity of peripheral neuropathy (20.8%, grade I) were lower than expecteddocetaxel monotherapy. Lycopene increased the AUCInf andCmax of plasma docetaxel by 9.5 % and 15.1%, respectively. Levels of IGF-I, VEGF-A and circulating endothelial cells were suppressed by 30 mg/day lycopene, but not by 90 and 150 mg/day lycopene. Conclusions: The combination of docetaxel and lycopene is of low toxicities and favorable in pharmacokinetics. Thehormesis effects of lycopene on biomarkers suggest that the optimum dose for further trials may be determined by biochemical assays rather than by toxicity. (DOI:10.9777/rr.2018.1075)

Methylation of clock genes on cancer: Modulation of circadian rhythm

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Circadian rhythms function in behavior and physiology has an adaptive significance for living organisms from bacteria to humans and reflect the existence of biological clock. Eukaryotic engine of circadian rhythm is the transcription-translation feedback loop and is fine-tuned by epigenetic regulation in higher eukaryotes. We have elucidated the chromatin structure of the Bmal1 gene, a critical component of the mammalian clock system and have been investigating transcriptional regulation including DNA methylation. Disturbance of the circadian rhythms is linked to a variety of ailments and altered DNA methylation of clock genes is associated with many human diseases, especially cancer. DNA methylation itself is prevalent in various types of cancer and clock genes influence tumorigenesis; for example the methylation of clock gene promoters such as Clock and Pers contribute to cancer progression. Many tumor suppressors and oncogenes are under circadian control and Per genes function as tumor suppressors.Therefore, it is important for cancer treatment to regulate the circadian rhythms. Recently, we established an assay system based on NIH 3T3 cellscombined with the Bmal1promoter-driven luciferase gene to screen circadian modulators. Using this assay system we have succeeded to find components as a circadian modulator from herbal plants.It is hoped that these findings will help deepen the

understanding of cancerfrom the viewpoint of circadian rhythm. (DOI:10.9777/rr.2018.1076)

Development of bioluminescence-based functional quantitative assays

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In the post genome era, reporter assay systems are used widely to study promoters, interactions between promoters and transcription factors, signal transduction and other cellular activities. Reporter assays are also applied to drug and toxicity screening in vitro, in cellulo and in vivo. Of the reporter genes known to date, luciferases, enzymes that catalyze bioluminescence reactions, are used most frequently because their sensitivity and linear response range are superior to those of typical reporters including β-galactosidase, chloramphenicol acetyltransferase, β-glucuronidase and fluorescent proteins. Bioluminescence is a simple reaction that is triggered by the addition of luciferin solution, and the equipment for measuring light intensity is simple because it uses only a photomultiplier or a charge- coupled device (CCD) camera. So, luciferases are suitable reporter enzymes for the quantitative measurement of gene expression. In this section, I introduce our creating system using multiple bioluminescence probes. The multicolored reporter assay using beetle luciferases that emit various colors with a luciferin can observe the dynamics of three gene expressions in the cells. Namely, our tricolor reporter in vitro assay system in which three gene expressions are monitored simultaneously using green-, orange- and red-emitting beetle luciferases for gene expression analysis. The multicolored reporter cell line reveals the expressions change of two or three genes under the target chemicals simultaneously. Our technique is a unique and a powerful tool for the toxicity test. (DOI:10.9777/rr.2018.1077)

Precision oncology: The foundation of future cancer prevention and therapeutics

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Based on early evidence of fossilized bone tumors that were found in ancient Egyptian mummies, cancer is an ancient disease. The term “carcinoma” to refer to cancer was first used around 400 BC by Hippocrates. The understanding of cancer mechanisms began when John Bennett and Rudolf Virchow observed the abnormal accumulation of white blood cells in patients in 1845, which was one of the first cancers detected by microscopy. In contrast to the long history of the disease, diagnosis and treatment of cancer at a cellular or molecular level is a relatively new strategy. Although the field of oncology has developed and expanded dramatically, a single drug has not yet been discovered that can cure all patients, even those with similar cancer types. We now know that cancer is an extremely heterogeneous disease, which explains differences not only between cancer cells from different patients, but also between cancer cells within a single patient. Clearly, more effective strategies are critically needed to defeat the longstanding enemy known as cancer. The concept and practice of precision medicine is a methodical and systematic movement aimed at defeating diseases such as

cancer. Cancer is a major focus of the precision medicine initiative and developments in precise and effective treatments could benefit many other chronic diseases. The development of EGFR inhibitors enables oncologists to treat human lung cancer with a specific target. In the last few years our lab has discovered key pathways or targets for lung cancer therapy and prevention. By molecular modeling of the interactions of targeted proteins with the designed chemicals or nature compounds, we have provided knowledge for a better understanding of how these agents work and how to avoid or delay the development of drug resistance. Such knowledge will help the new clinical practice of precision medicine of cancer. (DOI:10.9777/rr.2018.1078)

Statistical optimization of xanthan gum production and its characterization using *pseudomonas aeruginosa ncim* (2948) in submerged fermentation (smf) process

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In the present study Pseudomonas aeruginosa NCIM 2948 was used to produce exopolysaccharide xanthan using Xanthomonas campestris NCIM 5028 as control strain and efforts have been made to optimize the variables viz carbon source, nitrogen source, carbon concentration, nitrogen concentration and agitation speed for enhanced xanthan gum production. Optimization of process variables was done by central composite rotatable design (CCRD) using design expert (DX 8.0.7) software. Shake flask cultivation performed under optimum condition viz; 55g/L carbon concentration, 5g/L nitrogen concentration and 200 rpm agitation speed gave xanthan yield of 17 g/L using sucrose as carbon source and soyabean casein hydrolysates as nitrogen source . Batch cultivation further performed in 7.5 L lab scale bioreactor (working volume: 3L) under optimized condition gave maximum cell biomass of 13±0.5 g L^{-1} with a xanthan content of17g/L after 72h of fermentation.Scale up study on bioreactor gave maximum xanthan yield.Xanthan yield (Yp/x) in terms of cell biomass produced was found to be 1.33 (g/g). Maximum productivity was found to be 0.24 $gL^{-1}h$ which is higher than previous reports under similar condition. Characterization of xanthan gum was done by FTIR. (DOI:10.9777/rr.2018.1079)

Overexpression of pyridoxine pathway gene in potato (*Solanumtuberosum*) leads to the higher accumulation of vitamin B6 and tocopherols content

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Vitamins are essential role in cells as a cofactor for several metabolic enzymes. In addition the vitamin B6 and vitamin E are required for all cellular organisms as they act a potent antioxidant molecule. In contrast to bacteria, fungi and plants, which have the ability to synthesize these vitamins de novo, animals have to take up these vitamins from their diet. Plants are the major source of vitamin B6 and vitamin E for animals. The recent identification of vitamins biosynthetic enzymes in plants makes it possible to regulate the biosynthesis of this important vitamin. The recent

identification of vitamin B6 biosynthetic enzymes PDX1 and PDX2 in plants makes it possible to regulate the biosynthesis of this important vitamin. In this study, we generated potato plants overexpressing the PDX1 and PDX2 gene and used a liquid chromatography / mass spectrometry / mass spectrometry method to determine the levels of different forms of vitamin B6 in these transgenic plants. It was found that expression of the PDX genes under control of the CaMV 35S promoter caused an increase in pyridoxine contents in potato tubers which is actually consumed as food. The added benefits associated with the enhanced vitamin B6 content as observed in our study was the higher vitamin E accumulation with enhanced biomass as well as resistance to various abiotic stresses caused by Methyl Viologen (MV), NaCl or mannitol, respectively. This study also suggested that increasing this essential micronutrient could be a valuable option to improve the nutritional quality and stress tolerance of crop plants. Our work demonstrates that it is feasible to enhance vitamin B6 content in seeds by metabolic engineering. (DOI:10.9777/rr.2018.1080)

Novel biogenic synthesis of AgNPs from seed extract of *Eugenia uniflora* L.: *in vitro* assessment of their antioxidant, antimicrobial and cytotoxic potential

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In recent years, researchers are interested in rapid development of nanotechnology processes for the synthesis of nanoparticles has been evolving into an important branch of green nanotechnology deals with the safe and eco-friendly methods uses in biomedicine, industry and agriculture field. The use of Eugenia Uniflora L. has a long history in folk medicine of many countries. E. uniflora fruits and leaves are used as an antioxidant, hypertensive, anti-inflammatory and hypoglycemic agent. With these evidences, this study was designed to synthesize AgNPs using aqueous E. uniflora seed extract and assessment of their antioxidant, antimicrobial and cytotoxic potential. AgNPs synthesis was done and confirmed by using different methods, which include; UV/visible spectra, XRD (X-ray diffraction) and SEM (Scanning Electron Microscope) analysis. And assessment of their antioxidant, antimicrobial and cytotoxic potential was evaluated. AgNPs was monitored by UV-visible spectroscopy which revealed intense surface plasmon resonance bands at 447 nm and X-ray diffraction were employed to identify various functional groups and crystalline nature of AgNPs. Scanning electron microscopy studies demonstrated that synthesized particles were crystalline in nature with average size of 78-100nm. In vitro antioxidant effects were analyzed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), which exhibited potent antioxidant activity there in the particles, could scavenge the stable free radical DPPH of 75% to that o positive control BHT. The value of 50% inhibition concentration (IC50) of BHT is 65.55 and E. uniflora is 38.63µg/ml. The antimicrobial activity of AgNPs displayed potent zone of inhibition against selected human pathogens. The present study also investigated the toxicity effect of AgNPs against human prostate cancer cells (PC-3) and the inhibitory concentrations (IC50) were found to be 6.25µg/ml, respectively. The present study is the first report green synthesis of silver nanoparticles by using E. uniflora seed extract. An uniflora seed has been to AgNPs and it has shown their more potent antioxidant, antibacterial and

cytotoxic potential. It is generally assumed that use of plant derived phytoconstituents may contribute to the stability in the direction of a sufficient antioxidant status. It could be concluded that E. uniflora seed extract AgNPs can be used efficiently for potential antioxidant, antibacterial and cytotoxic potential with potent biomedical applications. (DOI:10.9777/rr.2018.1081)

Brainwave entrainment using visual-auditory stimulation as therapy for sleep disorders

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Insomnia is a condition with difficulty in initiating and maintaining sleep, characterized by frequent awakenings or problems returning to sleep after awakenings. It may result in an increased risk of motor vehicle collisions, as well as problems faced in focusing and learning. Existing methods to treat insomnia are sleep hygiene, cognitive behavioral therapy and sleeping pills, that has strong side effects when taken for long time such as drug addiction, and also it is expensive. The main aim of developing this project is to provide drug-free treatment for insomnia through Audio-Visual Stimulus (AVS) technique by means of a user friendly and portable device that enables relaxation of brain and muscles. AVS consists of Audio & Visual unit, where audio unit produces audio signals at two different frequencies that is given for left and right ears and visual unit consists of an eye mask fitted with two red colored LED's (one for each eye). This system produces a binaural beat signal in delta band and aids the melatonin secretion thus inducing sleep. This stimulation is applied for insomnia (Test group) and Control group. By applying AVS for a short time, brain signals are recorded in the sleep laboratory. The result shows the increase of brain signal strength in delta band. The Test group took a longer time to enter into sleep state when compared with Controls. The results of the study demonstrates the ability of two different audio signal in producing a binaural beat at frequency equal to the difference between these two audio signal. This could be a reason for the participants feeling audio stimulation more effective than visual. This system is a simple with no hazardous power or radiation, if extended could be an alternative therapy for Insomnia. AVS is one way of influencing neural activity, thus producing brainwaves at different frequencies corresponding to the intensity and frequency of stimuli. Hence by selecting the right stimulating frequency, sleep can be induced thus producing delta wave. Hence this therapy could be non-invasive, least discomfort, cost effective and drug free treatment to the user. (DOI:10.9777/rr.2018.1082)

17-allylamino-17-demethoxygeldanamycin (17-AAG) on matrix molecules and angiogenetic factors in gastric cancer cells cultured on different substrates

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Migration, invasion, metastasis and angiogenesis associated with cancer depend on the surrounding microenvironment. Angiogenesis, the growth of new capillaries, is a regulator of cancer growth and a useful target for cancer therapy. We examined matrix protein interactions in a gastric cancer cell culture that was treated with different doses of 17-allylamino-17-demethoxygeldanamycin (17- AAG). We also investigated the relations among the levels of vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), endostatin (ES) and trombospondin-1 (TSP-1). Cytotoxity of 17-AAG was measured using the 3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We examined the behaviour of cells on laminin and collagen. I coated surfaces in response to the angiogenic effect of these matrix molecules. We examined the protein alterations of these matrix molecules immunohistochemically and measured the levels of VEGF, MMP-9, ES and TSP-1 using the ELISA test. We showed that application of 17- AAG to the gastric cancer cell line on tissue culture plastic, laminin and collagen I significantly decreased the VEGF, TSP-1, ES and MMP-9 protein levels. VEGF, ES and MMP-9 levels of gastric cancer cells were no significant change but, TSP-1 also was increased significantly when tissue was cultured on collagen I. Application of 17- AAG to cells on laminin coated surfaces significantly decreased all of the proteins except ES. An ES level was increased on the laminin covered surfaces. We demonstrated the beneficial effect of 17- AAG on a gastric cancer cell line including inhibition of proliferation and induction of some proteins that might be related to decreased angiogenesis. 17-AAG is a drug that has entered the works of phase-1 andphase-2; we thought that the usage of this drug can become a hope for gastric cancer treatment as a result of thephase-3 studies testing on more communities with patients. (DOI:10.9777/rr.2018.1083)

Novel phosphorylated prodrugs of abacavir and didanosine exerts antiviral activity against newcastle disease virus (NDV)

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Newcastle disease is one of the highly pathogenic viral diseases of avian species. Newcastle disease virus (NDV) is economically significant because of the huge mortality and morbidity associated with it. In this present study a set of novel analogues for abacavir and didanosine were designed using hyperchem and subjected to docking with fusion protein of NDV using glide maestro suite. The analogues with best docking score against NDV fusion protein were synthesized, characterized and evaluated its antiviral properties in vitro on DF-1 cell line and in vivo in day old chicken. Docking study demonstrated that of the novel analogues ABC-1, ABC-4, ABC-12, DDI-9, DDI-10 and DDI-13 has shown better binding affinity against fusion protein of NDV. Therefore these compounds were synthesized and assessed for antiviral activity in vitro on DF-1 cell line by plaque and cytopathic assays, in vitro antiviral assays has shown ABC-1 and DDI-10 has shown better antiviral activity with lower cytotoxicity. Therefore ABC-1 and DDI-10 was assessed for antiviral activity in vivo in day old chicken and evaluated the NDV induced pathogenesis. NDV is an economically important virus that still lacks the prophylactic treatment, though there are broad spectrum antiviral compounds were available these compounds has limitations like high cytotoxicity, low bioavailability,

low solubility and lack of target specificity, to overcome these disadvantages it is now focused to develop novel phosphorylated compounds which enhances the biological activity. In conclusion, ABC-1 and DDI-10 novel analogues have shown better antiviral activity against NDV both in vitro and in vivo assays compared to native compounds. Therefore these compounds might be considered as potent antiviral compounds against NDV in chicken. (DOI:10.9777/rr.2018.1084)

The role of gp07 in the developmental pathway of Phi11

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Temperate bacteriophages can adopt two modes of development-the lytic mode and the lysogenic mode-and also switch between them. In lambda, it has been reported that the switch from lysogenic mode to the lytic mode is mediated by a protein RecA. However, a handful of temperate bacteriophages such as Coliphage 186, P4 etc, have been reported to harbor an antirepressor gene whose product is involved in the lysogenic to lytic switch. In most of the temperate bacteriophages harboring antirepressor gene, prophage induction is effected by binding of the antirepressor protein to the corresponding repressor protein, thereby paving the way for prophage induction. Most importantly, it has already been shown that certain antirepressor proteins are actually toxic to the host bacterial cell Gp07 as well as its domains have been cloned, over-expressed and purified and the morphology of the host cells have been studied by Phase contrast as well as SEM. The interaction of Gp07 with the Phi11 developmental pathway proteins have been studied by Gel shift assay. The over expression of Gp07 as well as one of its domains were found to inhibit cell division of the host cells. Our Gel shift assay results indicate that Gp07 interacts with the Phi11 Cro protein and enhances its binding to the cognate operator. However we did not observe any interaction of Gp07 with the Phi11 CI protein. Thus Gp07 appears to regulate the developmental pathway of Phi11 by a novel mechanism which has not been reported for any other aureophage. The Gp07 of Phi11 is thus a key factor in its lytic-lysogenic switch. More interestingly, the protein plays an essential role in inhibiting cell division in the host cell. (DOI:10.9777/rr.2018.1085)

Red blood cell counting using embedded image processing techniques

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Background: Manual counting and automatic modern analyzers are used to count the erythrocytes in laboratories. Manual counting of blood cells for large samples are prone to human errors, tiresome and time consuming. The complexity and the cost of automatic modern analyzers are high. **Aim:** To count the red blood cells in the blood sample using embedded image processing techniques, as erythrocyte count gives information regarding the patient health status on hematological diseases. **Methods:** Subtracting the leukocyte segmented image using Ycbcr color conversion technique with the original blood sample image, the erythrocytes can be segmented out. After segmenting the erythrocytes from the

blood smear image, the overlapping and clumped erythrocytes in the segmented image are removed using watershed algorithm. Circular hough transform can be used to detect the presence of the circular objects in the images and to count the segmented erythrocytes. Using this technique objects that are not circular in shape are eliminated, thus increasing the accuracy of erythrocyte count. **Results:** The accuracy of Ycbcr color conversion and the combination of Ycbcr color conversion with watershed algorithm for erythrocyte segmentation were found to be 86.82 and 90.98 respectively. **Discussion:** Erythrocytes are segmented using the image processing techniques and can be counted using circular hough transform. **Conclusion:** Based on the count of leukocytes in blood smear image they can be differentiated into leukocytosis and leucopenia. Hardware implementation of leukocyte counting improves the counting efficiency of blood cell components, as it requires less power and complexity. (DOI:10.9777/rr.2018.1086)

Althea rosea: From ornamental to medicinal perspective

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Background: Phytochemicals with strong anti-inflammatory properties are known to modulate the process of carcinogenesis. An ornamental plant, Althea rosea (AR), has been an integral part of traditional medicine for curing inflammatory disorders. However, its potential as a chemopreventive agent in cancer needs to be evaluated using experimental models. **Aim:** The present study is designed to evaluate the antioxidant and chemopreventive potential of ethanolic extract of AR seeds in experimental colon carcinogenesis. **Methods:** The antioxidant potential of ethanolic extract of AR seeds was determined by measuring total phenolic content and free radical scavenging properties by using different methods. As the extract was found to exhibit high antioxidant potential, it was evaluated for chemopreventive properties in a murine model of colon cancer. **Results:** Histopathological examination of tissue sections revealed that AR extract inhibited the ACF counts, malignant transformation and inflammation rich regions. AR extracts promoted apoptosis through intrinsic pathway as evident from analysis of Bax, Bcl-2, cleaved caspase -3 and cleaved PARP expression. Furthermore, the extract treatment inhibited the expression of NF-kB, a central mediator of chronic inflammation. Administration of AR extract in the animals did not result in any toxic effects as shown by hepatic and renal function markers. The treatment with AR extract ameliorated damaging effects of oxidative stress by decreasing free radical generation and increasing the levels of enzymatic and non-enzymatic antioxidants. Interestingly, we also found that the extract was more potent at a lower dose of 100 mg/kg body weight than a higher dose (200mg/kg. body wt.) **Conclusion:** The results of the present study indicate that ethanolic extract of Althea rosea seeds have potential as chemopreventive agents and should be further evaluated for identification of the active principle. (DOI:10.9777/rr.2018.1087)

Differential regulation of platelet responses by AMP-activated protein kinase through RhoA-dependent signaling to cytoskeletal proteins

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Background: AMP-activated protein kinase (AMPK), a metabolic master regulator, has also been shown to regulate cell polarity and mitosis through modulation of cytoskeletal proteins. Extensive reorganization of cytoskeleton ensues upon activation of platelets. These changes underlie several responses including platelet shape change, aggregation, spreading and clot retraction. Although AMPK activity in platelets is known to be upregulated by agonists, it's significance to platelet cytoskeletal dynamics remains unexplored. **Aim:** To determine possible role of AMPK in regulating cytoskeletal changes in activated platelets, employing compound C, a specific inhibitor of AMPK. **Methods:** Platelets were isolated from peripheral venous blood of healthy volunteers by differential centrifugation method. Platelet aggregation, integrin activation and cell spreading on collagen were respectively analyzed by light transmittance aggregometry, flow cytometry and phase-contrast microscopy. The influence of AMPK on platelet cytoskeletal proteins was elucidated by immunoblotting and pull-down assays. **Results:** AMPK inhibition with Compound C (10 M) significantly restricted integrin activation, platelet aggregation and clot retraction induced by thrombin (0.5 U/ml). However, platelets pretreated with compound C exhibited greater spreading on immobilized collagen as compared to vehicle-treated platelets. Compound C (10 M) also brought about 39 % and 56 % decrease in thrombin-induced phosphorylation of myosin light chain (MLC) and MLC phosphatase (MYPT1), respectively. In consistence, it abrogated the spike in RhoA- GTP levels with thrombin stimulation. **Discussion:** Our findings suggest that AMPK activity in stimulated platelets promotes integrin activation, platelet aggregation and clot retraction, which are critical to thrombosis and hemostasis. However, AMPK restricts platelet spreading on immobilized collagen.Actomyosin association and contraction mediated by RhoA-dependent signaling possibly underlies this differential regulation of platelet responses by AMPK. **Conclusion:** An ideal antithrombotic drug should spare hemostatic responses that maintain vascular integrity while preferentially targeting responses that support thrombosis. AMPK-RhoA-ROCK-MLC axis could be a potential target for developing such antithrombotic therapies. (DOI:10.9777/rr.2018.1088)

Environmental signal in regulation of thyroid - reproductive events

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Phenomenon of seasonal reproduction is being regulated by changes in day length (photoperiod). The molecular mechanism underlying the event of photoperiodic regulation of testis and thyroid functions along with glucose uptake transporters has never been reported for golden hamster, M. auratus. The present study was performed to investigate the effect of photoperiod on the expression of key thyroid hormone receptor (TR-α), deiodinase-2 (Dio-2) and glucose uptake transporters (GLUT-1 & GLUT-4) in testicular germ cell and Leydig cells, and its correlation with the testicular androgen receptor (AR), germ cell proliferation factor (PCNA) and cell survival factor (Bcl-2) in testis of adult golden hamster. Hamsters were exposed to different photoperiodic regimes

i.e. critical photoperiod (CP), short day (SD) and long day (LD) for 10weeks. LD induces up regulation of thyroidal and testicular activity as evident by active thyroid gland and testicular histoarchitecture, peripheral total thyroid (tT3, tT4) and testosterone hormone profiles when compared with SD exposed hamsters. Further, LD increased the expression of testicular TR- α, Dio-2, GLUT-1, GLUT-4 along with testicular AR and glucose content thereby enhancing germ cell proliferation and survival as reflected by increased PCNA and Bcl-2 expression when compared to SD exposed hamsters. Thus, it can be suggested that testicular thyroid hormone status is being regulated by photoperiod and is possibly involved in seasonal adaptation to reproductive phenomenon of golden hamster. (DOI:10.9777/rr.2018.1089)

Comparative pharmacological studies on members of Acanthaceae used as the drug 'Sahachara' in Ayurveda

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Background: 'Sahachara' is an Ayurvedic drug used against rheumatism and neurological disorders. The official part of the drug is the root, a major ingredient of several Ayurvedic preparations. The identity of the species to be used as 'Sahachara' is controversial as several confusing Latin names have been assigned to 'Sahachara' in various Ayurvedic texts. The genuineness of the source plant has to be decided only after adequate pharmacological and clinical trials. Comparative pharmacological investigations are critical to find out which among them possesses curative properties and therapeutically more efficacious. Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for species authentication, quality evaluation and ensuring consistency of herbal drugs and their related products. **Aim:** To carry out pharmacological investigations of few plants of Acanthaceae used as the source plants of 'Sahachara' and their chemical fingerprinting by HPTLC. **Methods:** The plants used are Barleria cristata L., B. prionitis L., Ecbolium viride (Forssk.) Alston, Justicia betonica L., Strobilanthes ciliatus Nees and S. heyneanus. The roots were extracted with methanol. The antioxidant activities were evaluated using DPPH, ABTS, superoxide, and hydroxyl radical scavenging assays. Carrageenan induced acute inflammation and formalin induced chronic inflammation were employed to measure the anti- inflammatory effects. HPTLC analysis of the root methanolic extracts was performed using lupeol as the marker compound. **Results:** The highest antioxidant activity was shown by B. prionitis followed by B. cristata and S. ciliatus. The extracts of B. prionitis and S. ciliatus were demonstrated to be highly effective in suppressing inflammation in both acute and chronic models. Chemical fingerprints have been generated for each taxon using HPTLC. HPTLC profiles showed the presence of lupeol band in all the chromatograms, with the most prominent band in S. ciliatus. **Discussion:** The results of the study suggest that the taxa are good sources of antioxidants and support their role in treating inflammatory diseases. The oral administration of the extracts inhibits the release of free radicals during inflammation and contributes to reinforce the anti-inflammatory effects. S. ciliatus and B. prionitis showed the most prominent activity. HPTLC chromatograms revealed the unique profiles generated for each

taxon.Lupeol, used as the marker compound in the analysis, is considered as one of the pharmacologically important ingredients of the taxa investigated. **Conclusion:** The outcome of the study establishes that it is appropriate to use both the roots of B. prionitis and S. ciliatus as the source of 'Sahachara', as these plants were outstanding in scavenging free radicals and bringing down inflammation. HPTLC analysis led to the development of characteristic fingerprint profile for each taxon that can be used for identification and quality evaluation of the plants used as 'Sahachara'. (DOI:10.9777/rr.2018.1090)

Biological action of bis(3,5-diiodo-2,4,6-trihydroxyphenyl) squaraine as a photosensitizer for breast cancer"

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Background: Photodynamic therapy (PDT) is one of the emerging anticancer treatment modality via photosensitizer mediated oxidative cytotoxicity. Squaraines are a class of dyes possessing all beneficial characteristics of a photosensitizer and considered to be a potent candidate for clinical PDT. In this study we used iodo derivative of squaraine called Diiodo-squaraine (Bis (3,5-diiodo-2,4,6-trihydroxyphenyl) squaraine) which is well known for its tumor specificity but least studied for its molecular basis of action. **Aim:** In this study we aim at validating various molecular events initiated by oxidative stress in squaraine based PDT. **Methods:** To delineate various molecular pathways, we performed proteomic profiling followed by analysis using DAVID Bioinformatics Resources. Protein profiling with LC-MS/MS followed by bioinformatics analysis in breast adenocarcinoma. Further confirmation and role of key molecules involved was studied using Western blotting and Real time PCR and established relation oxidative stress an unfolded protein response (UPR) and endoplasmic reticulum(ER) stress. Confocal microscopy and Flow-cytometric analysis for oxidative stress, Mitochondrial Membrane Potential (MMP) and actin cytoskeletal changes demonstrate regulation of Reactive Oxygen Species (ROS) mediated cell death pathways. **Results:** MDA MB 231 cells showed activation of response to unfolded protein (RUP), cell redox homeostasis (CRH), actin cytoskeletal organization (ACO) and programmed cell death (PCD) pathways. **Discussion:** Squaraine PDT induces oxidative stress and it induces a series of pro and anti-apoptotic pathways which include cell redox homeostasis, response to unfolded protein response, regulation of programed cell death, actin cytoskeleton organization and cell redox homeostasis. We showed for the first time that squaraine PDT induces ROS mediated ER stress, which leads to loss of mitochondrial membrane potential which in turn induces a caspase cascade finally leading to apoptosis even in the presence of redox signaling. **Conclusion:** Here we demonstrated the death and survival signals in photodynamic therapy using squarine as photosensitizer. This will give rise to novel therapeutic strategies for enhancement of the effect of PDT. Modulation of redox signaling may enhance the efficacy of PDT. Molecular basis of squaraine PDT was identified and redox associated gens have a prominent role in PDT resistance so further studies will give rise to potent molecular

target for identifying redox mediated drug resistance. (DOI:10.9777/rr.2018.1091)

Exposure of endocrine disruptors on epigenetic changes and programming of early development

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Background: Endocrine disruptors such as BisphenolA (BPA) and its derivatives are widely exposed daily from contamination derived from consumer products and plastic substances. In utero exposure of low levels of BPA on development programming of endocrine related disease is not known. BPA is capable to mediate its effects directly or trans-generational effects through epigenetic modification such as DNA methylation, histone modification and production of non-coding RNA. **Aim:** To investigate the effects of low-dose BPA on early development of cell proliferation, uptake of long chain fatty acids (LCFAs) and epigenetic modification using trophoblast cells. **Methods:** Cell viability and proliferation was assessed by MTT and ^{3}H-thymidine assay. Cell migration and angiogenesis were performed by wound healing and Tube-formation assay. Gene expression was assessed by Real-time PCR and qRT-PCR-microarray. Epigenetics modification was analyzed by methylation specific changes at expression levels. Data were considered significant when p value <0.05. **Results:** Expression of vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA) and Intercellular Adhesion Molecule 1 (ICAM1) mRNAs were significantly down-regulated whereas, expression of 11-β-hydroxysteroid dehydrogenase 2 (HSD11β2) was significantly upregulated. BPA significantly altered promoter methylation patterns of key stress and toxicity genes. BPA (1nM) affected cell viability, growth and decreased proliferation as measured by ^{3}H-thymidine incorporation of trophoblast cells. Unlike VEGF, BPA (1nM) altered tube formation of HTR8/SV neo cells. Effects of BPA on ^{3}H DHA uptake and expression of a focused panel of genes involved in fatty acid metabolism are being investigated. Discussions: Our data shows that physiological levels of BPA exposure (0.01nM-1nM) affected cell viability and growth of the first trimester trophoblast cells in vitro. The mechanism of its effects on epigenetic changes will be discussed. Conclusions: Bisphenol A inhibit early placentation processes possibly by down-regulating expression of growth promoting factors VEGF, PCNA, ICAM-1 and affecting gene methylation in the first trimester trophoblast cells. (DOI:10.9777/rr.2018.1092)

In vivo antimutagenic assays and biochemical profiling of *Orthosiphon thymiflorus*

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Background: Orthosiphon thymiflorus (Lamiaceae) has been used in traditional medicines and folk medicines in India form historical times. Low toxicity was reported in vitro and plant model system, but no proper studies on the in vivo anti mutagenic potential of the plant. **Aim:** To incestigate the antimutagenic effect of ethanol and hexane extract of O. thymiflorus against the toxicity induced by malathion by conducting two

in vivo assays and biochemical profiling of the crude extract by GC-MS. Methodology: Male Swiss albino mice weighing 20-25 mg were subjected to micronucleus test described by Schmid and chromosomal aberration assay by the standard method described by Preston et al. Thousand polychromatic erythrocytes and corresponding monochromatic erythrocytes were scored for micronuclei formation. 100 well spread metaphase plates were examined for chromosomal aberrations and mitotic index were calculated. GC-MS was done in crude extract and quantification was done by percentage of peak area calculation and compound identification was done by NIST MS library search. Results: In vivo studies showed that the induction of micronuclei and chromosomal aberration in bone marrow cells of the mice induced by malathion were effectively reduced by both the extracts. The fall in PCE/NCE ratio was also improved in malathion post treated animal groups. Biochemical profiling revealed monoterpenoids, diterpenoids and sesquiterpenoids, alkaloids, vitamins, phenolics, steroids etc. in both the extracts. Discussion and Conclusion: Both extract showed a remarkable potential in decreasing the mutagenic effects of the pesticide. It is proposed that the synergistic effect of dismutagenic compounds present in the crude extract might have brought about the novel antimutagenic potential. . Thus the study establishes the genoprotective effect of O. thymiflorus and it's potential to develop into promising candidate plant for further isolation and characterization procedures in drug development research. (DOI:10.9777/ rr.2018.1093)

Effect of chemical elicitors on the metabolite profile of spinach plant (*Spinacia oleracea*)

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Background: Elicitors are chemical stimuli, capable of inducing physiological as well as biochemical changes in the plants. These changes provide protection to the plant and help in developing resistance against environmental stress. Elicitors are recently applied in crop protection against phytopathogens, as well as in the synthesis of neutraceutically and pharmaceutically important secondary metabolites. Aim: To study the effect of chitosan and salicylic acid elicitors on the metabolite content of spinach plant. Methods: Chitosan and salicylic acid of varying concentrations were applied on spinach leaves through infiltration or foliar spray. After elicitation, metabolites were extracted and subjected for biochemical analysis, including; total chlorophyll, total protein, total phenolics, total carbohydrate as well as enzyme activity of catalase, peroxidase and phenylalanine ammonium lyase. Direct analysis in real time mass spectrometry (DART-MS) was used to generate the metabolite profile of control and treated leaves. Results: A maximum of 2.7 fold increase in the total phenolics, a 2.4 fold increase in total flavonoid and a 1.7 fold increase in total protein were achieved with the treatment. A higher level of enzyme activity was observed with a maximum 4 fold increase in peroxidase and approximately 3 fold increases in catalase and phenylalanine ammonium lyase activity. DART-MS analysis revealed the activation of phenylpropanoid pathway by elicitor molecules, targeting the synthesis of diverse classes of phenolic compounds including phenolic acids and flavonoids as well as their glycosides. Discussion: Both the elicitors caused an increase in enzyme activity and phytochemical content of the spinach

plant, especially phenolic acids and flavonoids. These compounds are known to provide protection to the plants against environmental stress, and are also reported to contain a wide variety of health promoting effects. **Conclusion:** Chitosan and salicylic acid are capable of causing distinct changes in the metabolite profile of spinach plant. (DOI:10.9777/rr.2018.1094)

Genetic Polymorphism of tumor necrosis factor alpha (TNF-α) and tumor necrosis factor beta (TNF-β) genes and risk of oral pre cancer and cancer

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Background: In light of the recently found contribution of inflammation-related factors to oral cancer, the possible correlation of tumor necrosis factor alpha and beta genes (TNF-α and TNF-β) with risk of oral cancer was investigated. **Results:** A total 250 patients with Oral pre cancer & cancer and 250 healthy volunteers were genotypes for the TNF-α (-238) G/A and TNF-β (-252) A/G gene polymorphism. Genotypes were identified by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP). Genotype frequencies were evaluated by Chi-square test and Odds ratio (OR) relative risk. Compared to the GG genotype the GA genotype of TNF-α (G238A) polymorphism has been found to significantly increase the risk of oral disease (OR= 1.99) and especially the risk of Lichen planus and oral malignancy (OR= 2.805 and 5.790 respectively). The risk of overall oral disease, Lichenplanus and oral malignancy were also high with allele A compared to allele G of TNF-α (G238A) polymorphism (OR =1.88, and respectively). Similarly, the risk of oral disease was also more in the heterozygote (AG) than the common allele homozygote (AA) of TNF-β (A252G) polymorphism (OR= 1.483). Conclusions: We conclude that the TNF-α (-238) G/A, TNF-β (-252) A/G polymorphism were significantly associated with Oral pre cancer & cancer. (DOI:10.9777/rr.2018.1095)

Effect of aspirin on developing swiss albino mice

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Aims and Objective: Aspirin is a non-steroidal anti-inflammatory drug having antipyretic, analgesic and anti-inflammatory actions. It is also used in prevention of myocardial infarction. However its effect on the growth of developing fetus has not been explored yet. Material and **Methods:** Aspirin was given to pregnant mice in the dose of 100mg/kgbw. Control group was given equal amount of tap water. Fetuses obtained by uterotomy on 19th day of gestation were studied for any macroscopic changes. **Results:** On examination a decrease in fetal weight and length was observed. On gross examination the fetuses of treated group show hemorrhagic spots scattered over the body. **Conclusion:** Thus it can be hypothecated that aspirin in high doses has toxic effects on developing fetus. So this drug should be used with caution in pregnancy. (DOI:10.9777/rr.2018.1096)

The effects of coenzyme Q10 supplementation on experimental diabetes induced by streptozotocin

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Diabetes mellitus (DM), the most common endocrine disorder characterized by a state of chronic hyperglycemia and insulin resistance. Hyperglycemia is associated with absolute or relative deficiency in insulin secretion from the beta cells of pancreas and/or desensitization of insulin receptors. Sustained hyperglycemia results in glucose autoxidation and protein glycosylation which in turn leads to excessive production of reactive oxygen species (ROS). Hyperglycemia mediated oxidative stress and inflammation is involved in the development of diabetic complications such as heart disease, stroke, hypertension, retinopathy, nephropathy, and neuropathy. Antioxidants have played a considerable role in the management of diabetes and its complications. Coenzyme Q10 (CoQ10) is a fat-soluble, vitamin-like substance used in the treatment of a variety of disorders. CoQ10 is a key component in the electron transport chain of the mitochondria, serving as an electron transporter to transfer electrons from nicotinamide adenine dinucleotide (NADH), succinate, and glycerol-3-phosphate at complexes I and II to complex III in the process of adenosine triphosphate (ATP) synthesis. Its reduced form as ubiquinol is a potent lipophilic antioxidant that is found in most living cells in the body. CoQ10 has demonstrated activity in preventing lipid peroxidation as an antioxidant scavenger and regenerate other antioxidants such as vitamin E and vitamin C. It can be synthesized endogenously by cells or be obtained naturally from the diet. The effect of CoQ10 on oxidative stress-mediated damage in streptozotocin (STZ) induced diabetes modal will be discussed. (DOI:10.9777/rr.2018.1097)

Modelling of common carotid artery from MRA images for early cerebral stroke detection.

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Background: Cerebral stroke is a condition where the blood flow to the brain reduces, leading to cell infarction. This results in difficulty and impairment in cognitive actions. In general Magnetic Resonance Angiography is widely used technique to diagnose and localize this condition. It is also a non-invasive method to view venous and arterial blood vessels. However, the major constraint is that it is expensive and do not exhibit blood flow dynamics. **Aim:** To analyze vascular system of brain, a three dimensional (3D) visualization of blood vessel is required. Attempted here is to develop a 3D model of blood vessel which could show the hemodynamics. Tomography images obtained from MRA sequence is used to build the model. **Methods:** 3D model of cerebral blood vessel constructed using mimics software tool which takes MRA images as its input. The model thus constructed is analyzed by solving continuity and momentum equations. Suitable boundary conditions are chosen depending upon the structure and location of the blood vessel. The special flow behavior of blood with reference to its density, viscosity and the unique mechanical properties of walls of the blood vessels for different pressure condition will be factored in for analysis. **Results:** MRA brain images are obtained from a subject age39, with the help of these images and mimics tool a 3D model for common carotid artery is constructed. The subsequent output exported in Computational Fluid Dynamics (CFD) tool for flow analysis. Discussion and

Conclusion: Reaching out brain tissues is very difficult and this poses a series constraint in localizing any malfunction in the brain. Angiogram is a procedure where blood vessels are exhibited more clearly. Therefore these images are used to build a model with proper threshold and segmentation. However after flow analysis a correlation is need to be established with clinical information. This technique if extended more precisely early diagnosis based on symptom could be possible. (DOI:10.9777/rr.2018.1098)

Proteomics based identification of differential plasma proteins and changes in white matter integrity as markers for early detection of mild cognitive impaired subjects at high risk of Alzheimer's disease

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Background: Mild cognitive impairment (MCI) is an intermediate stage of cognitive decline and dementia. Currently, no specific diagnostic tests are available for early identification of MCI subjects vulnerable to Alzheimer's disease. **Aim:** The current study aims to find proteomics based change in plasma proteins and diffusion tensor imaging (DTI) based white matter changes in MCI for early detection of prodromal Alzheimer's disease. **Methods:** Fifty cases of mild cognitive impairment and age matched control between (55-75 yrs) were screened on basis of Mini Mental State Examination (MMSE). Two dimensional gel electrophoresis and DTI imaging was performed in MCI and age matched controls. **Results:** The MMSE score of MCI were in the range of (28±2 - 22.6±1) as compared with healthy control (28±2), DTI metrics apparent diffusion coefficient (ADC) and fractional Anisotropy (FA) has shown significant changes in fornix, corpus callosum, hippocampus, right temporal and right frontal lobe, left frontal lobe, forcep major of MCI subjects as compared with controls. The protein expression of keratin type-2 was up regulated and albumin was down regulated in MCI subjects as compared with control. **Conclusion:** The data from present study signifies that expression of Keratin type-2 and albumin along with white matter changes provides early signatures for identification of MCI at high risk of Alzheimer's disease. (DOI:10.9777/rr.2018.1099)

Circadian disruption due to chronic jet-lag/shift-work cause cognitive deficit in mice

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Circadian Rhythm Disorders (CRDs) causes marked decrease in efficiency, especially duringjet-lag/shift work, and they maycause life-threatening mishaps due to lapses in attention and imbalanceof homeostasis. Only few studies on circadian disruption and cognitive deficits have been reported, however, none of these studies are studied systematically. Therefore we, study the effects circadian disruptionon phase-resetting and on episodic memory. Mice were entrained to 12:12 h LD cycle and were divided into two groups (n=25 each). Group I (control), Group II (jet-lag).Animals were subjected to simulated chronic jet-lag/shift work conditions by advancing the

lighting cycle by 8-hour, after every three days for three consecutive cycles. Wheel-running circadian activity rhythm was recorded and data were analyzed with the help of Clocklab software.Novel object recognition (NOR) test was performed durig pre-,jet-lag and post-jet-lag conditions to explore cognitive impairment in miceperformed during the activity period of mice. Jet-lag mice showed differential phase–resetting to the newly shifted LD cycle. The rate of re-entrainment of activity onsets and offsets differ significantly during pre-, Jet-lag and post jet- lag conditions.The clock periodicity was highly variable and the percentage of night activity decreased significantly duringjet-lag compared to pre-and post-jet-lag condition, even though the onset of activity was entrained. Furthermore, during NOR task, mice significantly spent more timein exploring the novel object than the familiar object duringthe pre-jet-lag compared to that during jet-lag conditions. This may be due cognitive as a result of circadian disruption.Results suggest that even after attainment of the stable entrainment following jet-lagmost of the behavioural, physiological and metabolic parameters are still under transition sate. Therefore, selectingspecific time of drug treatment is essential for treating circadian rhtyhm disorders and increasing the efficiency of the industrial workforcethrough better compliance to the imposed shiftwithout clock disruption. (DOI:10.9777/rr.2018.1100)

Structure based drug discovery approaches against head and neck squamous cell carcinoma (HNSCC)

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Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cause of cancer worldwide. Around 500,000 patients are reported to suffer with the disease each year. There are few important drug targets identified in HNSCC with a dearth of knowledge related to compounds that can inhibit these targets. The mechanism of action of the currently known platinum compounds available against HNSCC remains unknown. Aim: Structure based rationale drug designing against Bcl-xL, a drug target upregulated in HNSCC. Determination of mechanism of action of existing platinum compounds against HNSCC using in silico approaches. Methods: We have used various in silico approaches such as virtual screening, docking, simulated annealing, chemical fingerprinting and molecular dynamics simulation to address our objectives. Results: We could successfully identify new potential lead compounds which are pentacyclic triterpenoid and piperidine derivatives that could potentially inhibit Bcl-xL in HNSCC. The binding site residues namely Ala93, Tyr101, Gly138, Phe97, Glu96, Arg100, Arg139, Arg103 etc. are important for protein-inhibitor interactions. Further determined the mechanism of action of cis platinum compounds in the disease. These compounds forms DNA adducts that binds to high- mobility group box 1 (HMGB1) proteins with Phe110 playing the crucial role. Discussion: The high specificity and binding affinity of the new inhibitors identified against Bcl-xL makes them distinct from the earlier known biarylacylsulfonamide antagonists. The classical Arginine finger formed by the conserved Arg100, Arg103 and Arg139 residues plays an important role in enhancing these attributes. Insights have been achieved on the mechanism of action of existing platinum compounds used in combination

with 5-fluorouracil against HNSCC. Interaction with HMGB1 involves formation of DNA adducts at the first place and subsequent interaction with the compounds. **Conclusion:** Potential lead compounds exhibiting identified herein paves way for experimental validation. Knowledge of mechanism of action opens the possibility of more effective drug designing. (DOI:10.9777/rr.2018.1101)

Efficacy and mechanism of anticancer activity of guggulsterone in prostate cancer: opportunities and prospects for chemopreventive and therapeutic drug approach

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Background: Research on guggulsterone in various health issues has started since early 1980s. The fact that GS is anti-inflammatory as well as targets steroid receptors accelerates the belief about the role of GS in hormone dependent tumors. Since prostate cancer is an early model to study GS mediated apoptosis majority of the studies were carried out on the cell line which have negligible expression of androgen receptor, as opposed to clinical scenario were 70% of cases maintains AR expression and activity in absence of hormone. Therefore, the information about the effects of GS on AR positive but androgen independent i.e. CRPCa is poorly documented. One of the earliest events in PCa initiation is inflammatory cytokines mediated loss of NKX3.1 proteinans expression and stability of NKX3.1 is crucial in PCa. **Aim:** (i) To investigate the effects of GS on both CRPCa cell lines, (ii) To identify GS mediated novel anticancer pathway based on its effect on functionality and stability of AR and NKX3.1, (iii) To study the effect of GS on cytokine induced NKX3.1 degradation. **Methods:** Proposed research is developed from analytical point of view to dissect the functional variations of androgen receptor and NKX3.1 in presence of GS. The proposed task will be accomplished by extensive use of various biochemical and molecular techniques as well as in-silico studies such as western blot, EMSA, luciferace reporter assays and Q-RT-PCR and In-silico molecular docking studies. **Results:** Recurrence of AR positive CRPCa indicates that certain selective pressure chooses to maintain cells with mutation/s in AR and specific mechanisms to restores AR functionality. Considering that it is reasonable to look for molecular drug, which suppresses AR activity in CRPCa, just like GS, that we plan to investigate which eventually will redefine the role of GS in managing AR for greater clinical efficacy.NKX3.1 protein loss being one of the earliest events in PCa initiation represents a novel anti-cancer drug target. This project will drive advances in the understanding of biochemical response pathway and effects of GS on it. **Discussion:** This project will provide an insight into details of anti-cancer mechanisms led by GS. Since ambition of this work is also to evaluate therapeutic efficacy of GS by exploring the possibility for its chemical modification. Since GS has anti-inflammatory properties as well as it is AR antagonist; GS can be used as bifocal strategy as both chemopreventive and anticancer agent for management of prostate cancer. **Conclusion:** An insight into anticancer mechanism led by GS could open new opportunities for therapeutic intervention. Furthermore, exploring its mode of action should safe guard the fast growing success of guggul as health supplement, which would be key to safety, and efficacy of GS as drug. (DOI:10.9777/rr.2018.1102)

Calcium sensor STIM1 regulates melanoma and pigmentation by activating two distinct signaling cascades

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Background: Skin pigmentation plays a vital role in protection against UV induced cancers. Skin cancers account for third highest number of cancer associated deaths worldwide. Further, perturbations in pigmentation pathways result in pigmentary disorders such as solar lentigo, melasma, and vitiligo. These disorders are considered as social stigma; impart long-term psychological trauma and are huge economical burden on the patients. The current therapeutic regimes are not efficient in alleviating pigmentary disorders. Therefore, it is critical to identify the novel molecular players regulating pigmentation and devise strategies to target them for calibrating pigmentation and for treatment of skin cancers. **Aim:** To identify novel targetable regulators of skin pigmentation. **Methods:** We performed unbiased microarrays for short listing the potential targets. Further, we performed biochemical assays, cell and molecular biology experiments, confocal microscopy, FRET and live cell calcium imaging. Finally, we validated our work in two animal models i.e. mice and zebrafish. **Results:** We have identified STIM1 (calcium sensor protein) as a novel molecular player that regulates both skin cancer and pigmentation. We demonstrated that the key mediator of UV induced pigmentation, MSH (Melanocyte Stimulating Hormone) activates STIM1 and that in turn regulates tanning responses. Interestingly, STIM1 activates two independent signaling modules (Calcium and cAMP) at ER-PM junctions for controlling tumor growth and pigmentation. Further using mice and zebrafish animal models, we validated STIM1's critical role in regulating melanoma and pigmentation. **Discussion:** STIM1 mediates above discussed diverse functions through its distinct regions and thus these can be individually targeted for better management of skin cancers and pigmentary disorders. Future studies with human pigmentary disorders biopsies will substantiate these findings in the clinical samples. **Conclusion:** Taken together, we have identified a novel regulator of pigmentation and skin cancer that holds high translational value. (DOI:10.9777/rr.2018.1103)

Tumor secreted exosomes regulate VE-PTP expression in vascular endothelial cells and induces metastasis

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Background: All Cancer cells secrete exosomes. Exosomes are small (30–100 nm), membrane-encapsulated vesicles, released into the extracellular environment. Exosomal content ranges from microRNAs, mRNAs, Transcription factors, proteins and lipids. Cancer-secreted exosomes and embedded cargo help in preparing metastatic niches at distant tissues. **Aim:** In this work, we have investigated; how Breast cancer secreted exosomes are internalized by vascular endothelial cells and post- transcriptionally regulate the VE-PTP expression. **Methods:** Human umbilical vein endothelial cells (HUVECs) were exposed to Breast cancer cells (MDA.MB.231) secreted exosomes. Endothelial permeability measured via ECIS assay, Overexpression and knockdown studies of miR-590-3p, Luciferase assay, Knock-down of ATF3 via siRNA, In vivo

injections of Breast cancer secreted exosomes into mice. **Results:** Breast Cancer secreted exosomes induced endothelial permeability was measured via ECIS assay. These exosomes decreases the miR-590-3p expression levels and increases the VE-PTP expression in endothelial cells. miR-590-3p binds directly to 3'UTR of VE-PTP gene and suppress the protein expression levels of VE-PTP in HUVECs. Overexpression and knockdown studies of miR-590-3p establishes the VE- PTP as a direct target of miR-590-3p. Blocking of VE-PTP expression by miR-590-3p mimic effectively combats the effect of cancer exosomes. Exogenous transfection of miR-590-3p also modulates overall endothelial permeability. Blockage of exosome release from cancer cell with inhibiter GW4896 showed the reversal of exosomal effect on endothelial cells. It normalizes the expression level of ATF3, VE-PTP and ultimately the endothelial permeability. Knock-down of ATF3 in resulted into less loading of ATF3 into exosomes and thereby reduces their ability to permeabilize the endothelial junctions. In vivo injections of Breast cancer secreted exosomes into mice also resulted into enhanced rate of metastasis as shown via standard metastasis assay (melanoma transmigration into lung) in B6 mice. **Conclusion:** Breast cancer secreted exosomes resulted into enhanced rate of metastasis both in biochemical studies as well as in vivo. (DOI:10.9777/rr.2018.1104)

Targeted deletion of MMP9 mitigates diabetes-induced cardiac necroptosis

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Background: Necroptosis is a RIPK3-dependent inflammatory cell death. Diabetes mellitus (DM) induces cardiac necroptosis and pro-inflammatory macrophages (M1Φ) that express matrix metalloproteinase-9 (MMP9), a collagenase that promotes pathological remodeling. However, it is unclear whether the MMP9 contributes to induction of necroptosis in DM heart. Aim and hypothesis: To determine the role of MMP9 in necroptosis of DM hearts, we created a novel strain of DM mice where MMP9 gene is knockout (Ins2+/-/MMP9-/- or DKO) by crossbreeding Akita (Ins2+/-, T1DM) and MMP9-/- (MKO) mice. We hypothesized that ablation of MMP9 mitigates DM-induced cardiac necroptosis. **Methods:** To test the hypothesis, we measured the protein levels of necroptosis markers (RIPK3, RIPK1, pMLKL, and MLKL), and macrophage markers (M1Φ- iNOS and M2Φ- CD206) in WT (sibling Ins2+/+), Akita and DKO hearts of 14-week male mice. **Results:** Our results revealed that necroptosis and pro-inflammatory (M1Φ) markers are upregulated in diabetic Akita hearts. However, targeted deletion of MMP9 (DKO) decreased the cardiac levels of necroptosis and pro-inflammatory markers but increased the cardiac anti-inflammatory M2Φ marker. The mean ± SE values of different necroptosis and macrophage markers are as follows: RIPK3 (WT/Akita: 0.4 ± 0.03/1.2 ± 0.12; Akita/DKO: 1.2 ± 0.12/0.3 ± 0.05), RIPK1 (WT/Akita: 0.51 ± 0.04/1.0 ± 0.15; Akita/DKO: 1.0 ± 0.15/0.4 ± 0.04), pMLKL/MLKL (WT/Akita: 0.92 ± 0.06/1.0 ± 0.10, Akita/DKO: 1.0 ± 0.10/0.4 ± 0.05), iNOS (WT/Akita: 0.24 ± 0.05/0.56 ± 0.08; Akita/DKO: 0.56 ± 0.08/0.16 ± 0.03), and CD206 (WT/Akita: 0.3 ± 0.03/0.14 ± 0.03). **Discussion:** These results suggests that DM increases pro-inflammatory M1Φ and decreases anti- inflammatory M2Φ that result into inflammation and MMP9 upregulation, which

in turn promotes necroptosis in the heart. This is the first report on MMP9-mediated regulation of necroptosis. **Conclusion:** We conclude that inhibition of MMP9 could be a novel approach to alleviate necroptosis in DM hearts. (DOI:10.9777/rr.2018.1105)

Proteometabolomic landscape revealed cultivar-specific acquisition of phytochemicals and nutrients in sweet potato

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Sweet potato has long been acknowledged as a significant contributor of global caloric needs, having remarkable economic value, and ranked seventh in terms of annual global production. Despite its agronomic merit, the knowledge of nutrient fluxes and phytochemicals in sweetpotato is still fragmentary. To explicate the molecular basis for nutritional diversity, and to exploit the natural genetic differences in sweetpotato, a comprehensive physiochemical and proteomics analyses were performed using two contrasting ecotypes, an orange-fleshed sweetpotato (OFSP) and a white-fleshed sweetpotato (WFSP). While carbohydrate, reducing sugar and total phenolic contents were found to be higher in cv. WFSP, augmented level of total protein, flavonoids, anthocyanins, and carotenoids was observed in OFSP. We aimed to develop proteometabolic profiles of both the cultivars to understand the role of proteins and metabolites for nutritional diversity and availability of phytochemicals. Comparative proteomic analysis by 1-DE coupled with mass spectrometry led to the identification of 1541 and 1201 proteins in cv. OFSP and WFSP, respectively, which might play a key role for their functional diversity leading to differential nutrient acquisition. The proteomic analysis further revealed cultivar-specific accumulation of proteins, besides evolutionarily conserved proteins. Metabolome profiling exhibited 148 and 126 metabolites in cv. OFSP and WFSP, respectively. Quantitative proteomic analysis using 2-DE revealed differential expression of 68 proteins in both cultivars, whereas 105 proteins were exclusive to cv. OFSP and 65 proteins to WFSP. Altogether, these results give new insights into molecular basis for differential nutrient and phytochemical availability in tuber crops in particular and plants in general. (DOI:10.9777/ rr.2018.1106)

Limbal stromal stem cell/ MSCs therapy for scars, burns and corneal pathologies and its molecular characterization

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Background: The current standard of care for most blinding corneal diseases is corneal transplantation Over the past decade and a half, human limbal derived stem cells (hLSCs) have manifested and proven their efficacy through various, well established surgical interventions of SLET, CLET etc

in restoring the corneal transparency, in patients with limbal stem cell deficiency, ocular burns and other corneal pathologies. Conventional surgical therapies for corneal pathologies like burns, ulcers and scars have several limitations. Pre-clinical studies have indicated that application of limbal stromal stem cells (LSSC) to the corneal surface promotes corneal stromal regeneration, prevents fibrosis and restores corneal transparency **Aim:** Present study explores the long-term safety and clinical efficacy of the stromal cells and its molecular characterization Methods & **Results:** Limbal stromal stem cells were isolated ex-vivo using a previously standardized technique. Control groups received the standard medical therapy along with debridement and fibrin glue but without the stem cells. Compared to controls, eyes receiving LSSCs (Limbal stromal stem cells), irrespective of the source, showed: (i) faster epithelization (ii) better corneal clarity, evaluated clinically (P=0.012) and on scheimpflug imaging (P<0.0001); (iii) greater improvement in best-corrected visual acuity (and lesser corneal vascularization (p<0.0001). Stromal cells were also characterized using NGS, IF and real time analysis. This study also shows that Human Limbus-derived Stromal/ MSCs obtained by low serum culture condition express greater stem cell characteristics. We also observe that genes related to the production and secretion of proteinaceous extracellular matrix, lipopolysaccharide receptors and membrane rafts are up-regulated **Discussion:** This technique of delivering autologous and allogenichLSSCs was effective in enhancing corneal epithelization; improving vision and corneal clarity; and reducing corneal scarring and vasularization in superficial corneal pathologies like burns, ulcers and scars **Conclusion:** This minimally-invasive technique of delivering allogeneic LSSC was effective in enhancing vision, improving corneal clarity, reducing corneal opacification and is safe where corneal transplantation is needed in eyes after burns or scars. (DOI:10.9777/rr.2018.1107)

MicroRNA-487b-3p inhibits osteoblast differentiation and *in-vivo* bone formation in ovariectomized osteopenic mice

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that have emerged as critical post-transcriptional regulators of gene expression. There is increasing evidence that miRNAs play an important role in osteoblast commitment and differentiation. **Aim:** The main aim of this study was to identify and characterize novel microRNAs regulating osteoblast functions and bone formation Methodology: MiRNA expression pattern in control and Medicarpin (Med) treated cells was analyzed by miRNA microarray and further validated by quantitative RT-PCR (qRT-PCR). Effect of mmu-487b-3p on osteoblast differentiation and mineralization was validated by transfection of mmu-miR-487b-3p and its anti-miR in mice calvarial osteoblast cells using biochemical assays and qRT-PCR. Luciferase reporter gene assay was performed to confirm mmu-miR-487b-3p target. Protein expression levels were determined by Western blotting and chemiluminescence. Further three groups of BALB/c mice were ovaractomized. After 1 month of overactomy, these groups 1, 2 and 3 were injected subcutaneously (one injection/week) for three weeks with miC, miR-487b-3p and Anti-miR-487b-3p respectively. On the fourth week, the

mice were sacrificed and femur bones were collected for analysis of trabecular microarchitecture by uCT. **Results:** MicroRNA profiling of calvarial osteoblasts revealed that mmu-miR-487b-3p was ~6.5 fold down regulated in response to Medicarpin treatment. This data was further validated by qRT-PCR in calvarial osteoblasts. Over-expression of mmu-miR-487b-3p inhibited osteoblast differentiation, whereas inhibition of mmu-miR-487b-3p function promoted osteoblast differentiation and mineralization. Target prediction analysis tools and experimental validation by luciferase 3′ UTR reporter assay identified Nrarp as a direct target of mmu-miR-487b-3p. Western blot analyses showed that transfection of mouse calvarial osteoblast cell (MCO) with mimic miR-487b-3p reduced the protein levels of Nrarp and RUNX-2 while the protein expression of Notch1 and Hes1 was up regulated. All these results were reversed when cells were transfected with anti-miR-487b-3p. Most importantly, in vivo treatment of miR-487b-3p inhibitor to BALB/c mice led to significant improvement in trabecular bone microarchitecture which was otherwise deteriorated in mimic treated mice. **Discussion:** Our preliminary studies suggest that miR-487b-3p functions as a negative regulator of osteogenesis by repressing Nrarp expression, which in turn, upregulates Notch1/Hes1 pathway leading to suppression of RUNX-2 expression resulting in the inhibition of osteoblast differentiation and invivo bone formation. **Conclusion:** We propose that therapeutic approaches targeting miR-487b-3p could be useful in enhancing the bone formation and treatment of pathological conditions of bone loss. (DOI:10.9777/rr.2018.1108)

Neuroprotective potential of piperine nanoparticle against transient focal ischemia brain injury rat model.

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Background and Aim: Piperine (PIP) is a phytopharmaceutical with reported neuroprotective potential against rat model of transient focal ischemia and reperfusion (I/R) brain injury. PIP exhibits various important pharmacological effects. The aim of present work was to investigate the piperine- nanoparticle (PIP-NP) against transient focal cerebral I/R injury in Sprague Dawley (SD) rats. Material and method: PIP was entrapped in 3-(hexadecyloxy)-1-chloropropan-2-ol dextran (HDD). PIP-NPs were prepared by the nanoprecipitation and HDD encapsulation. The surface of PIP-NP was determined by Transmission electron microscope (TEM). The transient occlusion of the right middle cerebral artery for 1.5 h was developed focal cerebral ischemia by using 4.0 siliconized monofilament (Doccol) followed by 24h reperfusion followed by brain dissection and estimation of infarct volume and edema volume of TTC stained four coronal sections. **Results:** The yield of the PIP-NPs was found to be ~67%. PIP encapsulation efficiency of 67% and the percent drug loading (%DL) was 3.4%. The reduction in % infarct and edema volume and neurological parameters for neuroprotective efficiency of PIP-NP compare to PIP bulk treatment in transient middle cerebral artery occlusion SD rat model. The physiological parameters such as PO2, CO2 and regional cerebral blood flow (rCBF) were measured before, and after 15min of ischemia by

ADInstruments. **Conclusion:**The overall results indicated that HDD entrapped PIP-NP was the crucial for their neuroprotective potential compare to bulk PIP in I/R brain injury SD rat model. (DOI:10.9777/rr.2018.1109)

Exploiting *Hydnocarpus wightiana* blume for the development of new chemical entities towards cancer therapy

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Background: Medicinal plants have attained vary good notice due to their potential as a repository of active biomolecules with promising therapeutic potential and still represent an important pool for the identification of novel drug leads. Hydnocarpus wightiana Blume is a popularly known medicinal plant and its acetone extract of the seed showed superior free radical scavenging property with a high total phenolic and flavonoid content. **Aim:** To develop Hit molecules from the compound hydnocarpin which was known to have moderate activity towards cancer by attaching potent biologically active heterocycles? Results and **Discussion:** Hydnocarpin (Hy), which has been isolated and purified from the acetone extract, shown to be a promising anti cancer agent as it promotes moderate cytotoxicity on cancer cells, viz. Lung and melanoma. Among the heterocycles, isoxozole and isoxazolone derivatives have shown good bioactivity1 and form the part of various pharmacological agents. We have extensively evaluated the synthesis of hydnocarpin containing isoxozole and isoxazolone moieties and cytotoxic potential of hydnocarpin as well as its synthetic analogues were assessed on cancer cell lines (A549 and A375) and a normal murine fibroblast cell line (3T3L1) over a wide range of concentrations Apoptosis assays along with in silico docking studies and ADME-Tox profiling of these derivatives which showed better cytotoxic effect and selective inhibition towards cancer cells, prompted us for further study towards the development of lead molecule. **Conclusion:** Developed a library of Hydnocarpin-isoxazole/ isoxazolone derivatives towards cancer therapy and the results obtained were promising to develop hit molecule from this library of compounds. (DOI:10.9777/rr.2018.1110)

Intranasal curcumin protects against LPS induced asthmatic structural changes by inhibiting TLR-4 and MAP kinases in mouse lung

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Background: Respiratory infections exacerbate asthma depending upon virulence and timing of exposure as well as immune status of the host. Bacterial infections have been reported to modulate asthma in several epidemiological studies. **Aim:** We evaluated protective effect of intranasal curcumin on lipopolysaccharide (LPS) induced asthmatic inflammation and structural changes in mouse lungs. **Methods:** Balb/c mice were sensitized with Ovalbumin (Ova) on 1st and 8thday and exposed to Ova-aerosol from 9th to14th day. LPS (0.1 µg, i.p) was given on 2nd day and an hour prior to Ova challenge, whereas curcumin was administrated through intranasal route 1 h before LPS exposure. We measured inflammatory cells infiltration in BALF by flow cytometry and reactive oxygen releases (ROS) by fluorescence spectroscopy. Eosinophil recruitment to lungs was

measured indirectly by immunofluorescence detection of major basic protein (MBP) as marker of asthmatic inflammation. MMP-9 activity was evaluated by gelatin zymography as well as mRNA expression of MMP-9, TIMP-1, TGF-β1, IL-13, Collagen-1 and TLR-4 were measured in lungs. Protein expression of MAP kinases (P-pERK, P-pJNK, P-p38), TLR-4, Cox-2, Lox-5 and Eotaxin were measured by western blotting. Collagen deposition in lungs was indirectly measured by hydroxyproline level and massion'strichrome staining of lung section. **Results:** LPS exposed groups had significantly higher lung inflammation as confirmed by MBP detection and MAP kinase expressions. Airway remodeling genes (MMP-9, TIMP-1, TGF-β1, IL-13, and Collagen-1) expression and MMP-9 activity were also higher in LPS exposed groups. These were significantly lowered in curcumin treated groups. **Discussion:** LPS exposure exacerbates airway inflammation and injury by inducing inflammatory cell infiltration, MAP kinases and ROS release via MAPK pathway. It also induces structural changes in lungs by increasing, collagen deposition and expression of gene involved in airway remodeling by affecting mediators. Intranasal curcumin pretreatment had significantly suppressed inflammatory and airway remodeling mediators. **Conclusion:** Our results strongly suggest that intranasal curcumin effectively protects from LPS induced airway structural changes in lungs which could be considered as adjunct medication. (DOI:10.9777/rr.2018.1111)

Histone modifications and their role in cell cycle phase dependent DNA damage and radio-resistance

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Background: Histone Post Translational Modifications (PTMs) are essential for modifying chromatin architecture and help in recruitment of DNA repair proteins on chromatin. Histone modifications change along with the phases of the cell cycle. Also, each cell cycle phase has differential intrinsic radio-sensitivity. **Aim:** Since histone modifications change along with the phases of the cell cycle, we question whether there is any epigenetic mechanism involved in conferring differential radio-sensitivity to different cell cycle phases? **Methods:** Radiotherapy is one of the treatment modalities for breast cancer, so we have conducted our study on MCF7 breast cancer cell line. Simultaneously, we have developed radio-resistant MCF7 cell line. Cells were synchronized in the G0/G1 and mitotic phase using serum starvation and nocodazole, respectively and subjected to ionizing radiation (IR). **Results:** Our group has reported thathistone PTM H3 Serine 10 phosphorylation (H3S10P) is de-phosphorylated after DNA damage and regains phosphorylation after repair exclusively in the G0/G1 phase of cell cycle. DNA damage induction in mitosis doesn't lead to any alteration of this PTM and cells remain blocked in M-phase. Phosphatases of H3S10P-MKP-1 and PP1 show chromatin recruitment in both G1 and M phase. However, unlike G1, levels of H3S10P kinase MSK1 steadily decline after radiation in mitosis. Inhibitor based disruption of H3S10P kinetics leads to increased cell death in G1 phase of cell cycle. Since H3S10P levels influence

cell survival post IR, we wanted to investigate how histone PTMs change as cells acquire radio-resistance. In 20Gy RR, we observe decrease in histone PTMs H3K27Ac, H3K9Ac, H3K56Ac, H3S10P and an increase of H4K16Ac. **Conclusion and Discussion:** H3S10P and its modifying enzymes follows cell cycle phase specific kinetics in response to DNA damage and could be an epigenetic determinant for cell cycle phase specific differential radio-sensitivity. (DOI:10.9777/rr.2018.1112)

Clonal propagation of endangered medicinal plant *Withania coagulans* Dunal *via* thin cell layer technique and withanolide production under *in-vitro* condition

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Background: In the present study, intense, fast rate shoot multiplication has been achieved utilizing thin cell layer explants for endangered important pharmaceutical plant Withania coagulans Dunal having high hypoglycemic potential. **Aim:** To develop an efficient protocol for rapid in-vitro propagation of W. coagulans. **Methods:** Stem node, shoot apical meristem (SAM), and transverse thin cell layer (tTCL) were used as explants and inoculated in different strength of MS medium supplemented with growth regulator. **Results:** The tTCL explant showed high frequency of shoot regeneration and was also affected by the concentration of plant growth regulators. The full strength solid MS medium was optimal for shoot regeneration (31.94 ± 1.39) and the highest percent of shoots (93.05 ± 2.77) were observed in MS medium fortified with 2.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. Rooting of the regenerated shoots was achieved best in half strength liquid MS medium supplemented with 2 mg l^{-1} IBA. Plants were successfully transferred to the field after acclimatization with a survival rate of 90.6 %. ISSR and RAPD markers were used to confirm the genetic fidelity of the in-vitro raised tTCL clones. Quantification of withanolides content through HPLC showed increase withanolides in in-vitro raised field transferred plant compared to wild plants. **Discussion:** In our finding tTCL was found as best explant, which gave highest number of shoots with better growth in full strength solid MS medium. Similar results were also reported for some other industrial and economic plants. **Conclusion:** This study provides an insight for rapid production of large numbers of genetically alike plants of W. coagulans utilizing tTCL as explants. It is also helpful in maintaining natural population of wild type W. coagulans for sustainable multiplication / production of biomass and withanolide extraction for commercial purposes. (DOI:10.9777/rr.2018.1113)

The anti-neoplastic potential of a chalconoid isolated from Chinese lacquer tree against human oral cancer

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Background: Oral cancer is one of the most prevalent cancers in North-East India, most particularly Assam and Meghalaya are the two states turning to be stock house of oral cancer as per the recent report. Despite this alarming

situation which can be attributed to various factors like high tobacco consumption, susceptibility of the population to long-term effects of tobacco, late detection etc., there is no such effective therapies available for the treatment of this disease. Consequently, there is an urgent need to develop effective yet safe regimens for its management. Butein, a chalconoid isolated from Chinese lacquer tree is one such compound with potent anti-inflammatory, anti-oxidative and pro-apoptotic effect. However, its anti-neoplastic potential in oral cancer has not been elucidated yet. Objective: To evaluate the anti- neoplastic effect of butein in oral cancer and the mechanism involved. Materials and **Methods:** Butein's effect on the proliferation, survival and cell cycle progression of oral cancer cells was evaluated using MTT, colony formation, PI-RNase-FACS and Annexin V/PI apoptosis assay. Further, its effect on the invasion and metastases of oral cancer cells was determined by wound healing and matrigel invasion assay. In addition, western blot was performed to decipher the underlined mechanism of action. Results and **Discussion:** To the best our knowledge, this is the first report showing the anti-neoplastic effect of butein against oral cancer. It exhibited potent anti-proliferative effect on oral cancer cells with IC50values less than 10 μM, cytotoxic effect at the concentration lower than 40 μM and anti-migratory effect at 10 μM. Further, butein inhibited the expression of gene products such as Cox-2, Cyclin D1, Bcl-2, Survivin, CXCR-4 and MMP-9 which are involved in proliferation, survival, invasion and metastases of oral cancer cells. **Conclusion:** Taken together, these results indicate butein to possess potential to serve as an effective strategy for the treatment of oral cancer. (DOI:10.9777/rr.2018.1114)

Translating resist in promoter associated genetic variants to causality and potential mechanisms of disease predisposition

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Introduction: Genome-wide association studies (GWAS) in the recent past have identified thousands of disease related genetic variants and trait associated polymorphisms. Recent advances in high throughput genotyping and gene expression analysis allowed scientific community for an extensive mapping of expressed quantitative trait loci (eQTLs). However, translating these associated genetic variants to causality and potential mechanisms of disease have been difficult. Hence, there is a need for targeted but yet comprehensive candidate gene based studies looking at various aspects of the potential disease gene from DNA to protein, also in pure populations of immune cells and/or tissues with appropriate stimuli and functional readouts. Materials/**Methods:** Genome-wide SNP array, transcriptomic analysis, EMSA/Supershift assay, Flow-cytometry, qPCR, CRISPR gene editing, CpG methylation, p50 inhibition, Haplotype promoter analysis, confocal microscopy and multiplex chemokine/cytokine array **Results:** Unpublished results from an ongoing project will be presented: The central goal of the project is an in-depth study of Resistin associated genetic variants which is one of the well characterized GWAS candidate in T2D (Type 2 Diabetes) and CAD (Coronary artery disease) case control studies. Using a combination of genetic analysis; whole blood, cell type specific eQTLs, SNP-SNP interaction analysis and molecular analysis mentioned in materials and

method section; we propose that the suppression of resistin promoter through p50/p50 homodimer a known suppressor and promoter methylation are the underlying mechanisms for genetic regulation of resistin expression in monocytes. Also a genetically defined multidimensional cohort based immune-phenotyping and plasma biomarkers analysis showed a significant modulation of immune cells and plasma biomarker; known to be associated with metabolic and inflammatory diseases. Significance: Thus; our study provides detailed understanding about the role of resistin associated genetic regulatory variants in the context of metabolic and inflammatory diseases. Also it would be helpful for the better understanding about gene-drug interaction and identification and design of new therapeutic targets. (DOI:10.9777/rr.2018.1115)

World's first systems level mechanistic depiction of human prostate cancer pathogenesis and its therapeutic resistance

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Background: Presently there is no effective molecularly targeted treatment strategy available for metastatic castration resistant prostate cancer. Currently, prostate cancer is the second leading cause of cancer in India and in 2016, more than 1.5 million new cases of prostate cancer has been detected in India, which is expected to reach nearly 3 million within 2020. Additionally, WHO (World Health Organization) warns that cancer (including prostate cancer) will become epidemic in India, which is the principal motivation for this current research. **Aim:** The current cancer research strongly suggests for identification of potential molecular targets and global molecular signaling map in the context of human prostate tumorigenesis and its progression. **Methods:** By an extensive manual extraction of the published biomedical literature, we have developed Human Prostate Cancer Hallmarks Map (HPCHM), a comprehensive database for human prostate cancer associated signaling and events. **Results:** Human Prostate Cancer Hallmarks Map (HPCHM) covers molecular signaling maps of most of the major cellular and pathogenic mechanisms implicated in prostate cancer progression, including 13 cancer hallmarks (10 classical & 3 prostate cancer unique hallmarks) and 11 cell biological feature based phenomenon. **Discussion:** Cancer hallmark capabilities, which are believed to be the fundamental organizing principle of human cancer centrally drives and influences every aspects of prostate cancer development, from its initiation to pathogenesis. Particularly it includes most of the prostate cancer related pathogenic processes including inflammation, angiogenesis, immune deregulation, tumor microenvironment, chemo/radiation resistance, castration resistance, epithelial mesenchymal transition (EMT), cell invasion and bone metastasis. **Conclusion:** Human Prostate Cancer Hallmarks Map (HPCHM) provides a major step towards first systems level mechanistic representation of prostate cancer which is of great interest for the implementation of future personalized cancer medicine. (DOI:10.9777/rr.2018.1116)

A novel nanoformulation with potentantitumorigenic effect against oral squamous cell carcinoma

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Background: Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in the world. The major limitation fortreatment of this cancer is that the currently available drugs are cytotoxic to the normal cells in addition to the target cells. The treatment of oral cancer thus demands therapies that specifically kill tumor cells without causing any toxicity to the normal cells. Dillenia indica or Elephant apple is a traditionally consumed fruit of the Northeastern region of India and it has been well known for its antidiabetic, antioxidant, antimicrobial, anti-inflammatory and analgesic properties. Green synthesis of gold nanoparticles being an emerging approach was used for the synthesis of gold nanoparticles using the leaf extract of Dillenia indica. **Aim:** To synthesize gold nanoparticles using Dillenia indica leaf extract (DI-GNPs) and decipher its anticancer potential against oral cancer cells (SAS). **Methods:** The DI-GNPs were synthesized using AuCl4and characterized through UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Transmission electron microscopy (TEM) and Zeta potential analysis. The anticancer activity of DI-GNPs was evaluated against SAS cells through MTT assay, live and dead analysis, Annexin V FITC assay, cell cycle analysis, and Western blot analysis. Results and Discussions: The DI-GNPs were crystalline, negatively charged, polygonal in shape, with size ranging from 1 to 20 nm. Cytotoxicity assays showed that DI-GNPs potentially inhibited the viability of SAS cells and did not show cytotoxicity to normal cells. The DI-GNP treated SAS cells also showed G1 phase arrest and change in morphology. Western blot analysis exhibited decrease in the levels of anti-proliferative, anti-survival, anti-angiogenic and anti-apoptotic proteins such as MMP-9, p-Akt, Cyclin D1, VEGF-A, CXCR4, Bcl-2 and survivin in DI- GNP treated SAS cells. Conclusions: Green synthesis of DI-GNPs was successfully achieved that exhibited strong antiproliferative and anti-survival activity against oral cancer cells. However, more studies are warranted to establish their anticancer potential. (DOI:10.9777/rr.2018.1117)

Drosophila based model for insulin resistance mediated tubular nephronic dysfunction

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Background: The prevalence of diabetes mellitus (DM) has increased in recent years due to changes in lifestyle, obesity and exposure to environmental chemicals etc. About one-third of the people with diabetes develop kidney diseases in later stages of life and it is the most common cause of end-stage renal failure. Underlying mechanism of glomerular dysfunction in DN is well established. However, tubular nephronic dysfunction is not well deciphered till date. **Aim:** To develop Drosophila as an alternate animal model for understanding underlying mechanism of insulin resistance mediated tubular dysfunction. **Methods:** Perturbations in the insulin signaling pathway was examined by the real time- PCR, western blotting and immunocytochemistry. Biochemical assays were performed to measure the ROS generation, antioxidant enzymes activity and uric acid level.

Cytoskeleton and tight junction protein expression were analyzed through immunocytochemistry. **Results:** Prolonged insulin resistance has led to perturbations in the insulin signaling pathway in the Malpighian tubules (MTs). Insulin resistance condition resulted in significant increase of ROS generation in MTs along with elevated antioxidant enzymes superoxide dismutase (SOD) and catalase. Oxidative stress caused apoptosis and structural deformities in the MTs of these flies. Impaired cytoskeleton and tight junction proteins were observed in the MTs of insulin-resistant flies. Uric acid was found to be increased in the insulin resistant flies. Average life span of insulin-resistant flies was significantly reduced by approximately 10 days that was nearly similar to age of flies having specifically disrupted insulin signaling in the MTs. **Discussion:** Current study shows the endpoints identified in MTs of insulin resistant flies are similar to the endpoints of the impaired tubular nephron of mammalian system in diabetic condition. **Conclusion:** MTs can be used as in-vivo tools for understanding underlying mechanisms of tubular dysfunction in diabetic condition. The identified endpoints in MTs can also be used for the pre-screening of molecules as potential drug for the disease. (DOI:10.9777/rr.2018.1118)

Effect of curcumin on Paraquat-induced epithelial to mesenchymal transition in A549 cells

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Background: PQ, is widely used as a potent herbicide, which generates superoxide anions and other free radicals, leading to cellular damage and severe toxicity in many organs. It can induce acute lung injury and fibrotic response. In animal model, PQ induces pulmonary fibrosis through the involvement of alveolar epithelial mesenchymal transition (EMT) which is characterized by increased number of myofibroblasts. **Aim:** The present study evaluates, time-dependent effect of curcumin on PQ induced EMT through TGF-β dependent mechanism, using A549 human alveolar epithelial cells **Methods:** A549 cells (alveolar epithelial cell line) were cultured and treated with curcumin (30μM) before 1hr and 3hr of PQ (700μM) intoxication. After 24hr of treatment, cell viability was assessed by MTT and determined level of reactive oxygen species (ROS). To study PQ induced EMT, measured the protein expression of epithelial cell marker E-cadherin and mesenchymal marker α-smooth-muscle actin (α-SMA) by western blot analysis and immunocytochemistry. Expression of TGF-β (Transforming growth factor beta) was quantified by RT-PCR. **Results:** Significantly reduction in the PQ induced cytotoxicity and intracellular ROS level were observed by curcumin treatment. Before 3hr of curcumin treatment prior to PQ toxicity, increases expression of E-cadherin, in contrast curcumin inhibits PQ induced expression of α-SMA. Curcumin pretreatment effectively inhibited the PQ induced TGF-β1 expression. **Discussion:** Increased expression of TGF-β1 showed its involvement in PQ induced EMT. Pretreatment of curcumin 30μm before 1hr of PQ toxicity in A549 cells was effective to inhibit the PQ induced cytotoxicity and intracellular ROS production but was not able to inhibit PQ induced transition of epithelial cells to mesenchymal cells but when pretreatment duration was enhanced from 1hr to 3hrs, then it was able to inhibit the PQ induced EMT. **Conclusion:** we conclude here that curcumin has some potential to suppress PQ induced EMT

by regulating the expression of TGF- β, when cells remained with i t for longer t ime. (DOI:10.9777/rr.2018.1119)

Distinct role of Akt isoforms in oral cancer and tobacco-induced aggressiveness

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Background: Oral cancer remains the global health concern and Akt kinase is found to be overexpressed. However, it has three isoforms and isoform-specific involvement is yet to be deciphered completely. **Aim:** To elucidate the isoform-specific role of Akt isoforms in oral cancer. **Methods:** Immunohistochemistry (IHC) was performed on oral cancer tissues. The genetic alterations data was retrieved from "The Cancer Genome Atlas" (TCGA) database of 530 patients of HNSCC. Role of Akt isoforms was analyzed through siRNA-mediated gene silencing by checking their effect on the expression of different hallmarks of cancer by western blotting. Also, FACS methods were utilized to study cell survival and cell-cycle arrest. The effect of tobacco on aggressiveness in terms of proliferation (MTT assay), clonogenic (colony formation assay), and migration (wound healing assay). The promoter sequence of the Akt isoforms was analyzed through in silico methods of Eukaryotic Promoter database and Genomatix program. To categorize the general Akt inhibitors and other natural inhibitors, computational docking method of Schrodinger software was used. **Results:** IHC on tissue microarray slides showed the overexpression of Akt1 and Akt2 isoforms but not Akt3 in cancer tissues. The TCGA data have suggested the maximum genetic alteration in Akt1 and Akt2 protein of HNSCC patients with worst clinical outcome. The knockdowns of Akt1 and Akt2 isoforms led to decreased cell survival and cell cycle arrest. Also, knockdown caused the reduced expression of molecular mediators involved in cancer progression such as MMP9, Cox-2, Bcl-2, cyclin D1, VEGF and Survivin. Furthermore, their knockdown significantly diminished tobacco-induced aggressiveness by decreasing the clonogenic and migration potential. The promoter sequences have shown significant variation. Based on the affinity towards the Akt isoforms selectively, the inhibitors were further classified and ranked. Discussions: Non-redundant functions of Akt isoforms in oral cancer were observed indicating differential clinical outcome. Akt1 and Akt2 knockdown decreased the expression of protein involved in cell proliferation, inflammation, anti-apoptosis, migration and invasion of cancer cells. Further, their knockdown reduced the action of tobacco- induced carcinogenesis. The promoter sequence variation might be responsible for differential expression of Akt isoforms. The ranking of general Akt inhibitors into isoform-specific inhibitors is a significant step towards developing precise chemotherapy against oral cancer. Conclusions: The present study is a preliminary step to understand the distinct role of Akt isoforms in oral cancer to develop Akt isoform- specific therapy to reduce the off-target toxicity and increase the therapeutic efficacy. (DOI:10.9777/rr.2018.1120)

Anti-cancer activities of Bharangin, a diterpenoid, against breast cancer: Evidence for the role of NF-κB activation and lncRNA expression

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Background: Accumulating evidence over the past several years has indicated that Mother Nature has been a "gold mine" for drug discovery. Premna herbacea is a medicinal plant that has been used for centuries against several chronic diseases including cancer. Bharangin is a diterpenoid derived from this plant. The efficacy of bharangin against breast cancer cells and the underlying molecular mechanism is poorly understood. **Aim:** The aim of this study was to examine the potential of bharangin against breast cancer cells, and to elucidate its mechanism of action. **Methods:** A number of breast cancer cell lines (MCF-7,T-47D,MDA-MB-231,MDA-MB-468,MDA-MB-453)were used during the study. The parameters used were MTT and clonogenic assay for cytotoxicity; cell cycle analysis, measurement of mitochondrial membrane potential, staining (AO/PI and DAPI) and DNA laddering for apoptosis.Western blotanalysis was performed for the expression of cell survival markers;immunocytochemistry for NF-κB activation;wound healing assay for cell migration; and real-time PCR for lncRNA expression. Results and **Discussion:** The exposure of breast cancer cells (MCF-7, T-47D, MDA-MB-231, MDA-MB-468, MDA-MB-453) to bharangin was associated with a dose and time dependent decrease in the viability of cells. At concentrations as low as 1 μM, bharangin inhibited the proliferation of cancer cells after 3 days of treatment. The diterpenoid inhibited the long-term colony formation of cancer cells. Barangin also suppressed the migration of MDA- MB-231 cells. Cell Cycle analysis revealed an accumulation of cells in sub-G1 phase after bharangin treatment. The diterpenoid was found to induce apoptosis features such as presence of fragmented nuclei, chromatin condensation, DNA laddering, caspase activation, PARP cleavage and suppression in the expression of cell survival proteinsin cancer cells. Concomitantly, the loss of mitochondrial membrane potential was also observed after bharangin treatment.Hydrogen peroxide and okadaic acid (OA) induced translocation of NF-κB-p65 from cytoplasm to nucleus that was significantly reduced by pretreatment with bharangin. The diterpenoid also induced expression of tumor suppressor lncRNAs (MEG-3, MHRT, NEAT, GAS-5), while down-regulating oncogenic H19 expression. The pro-inflammatory transcription factor, NF-κB is known to be regulated by H19 lncRNA.The suppression of H19 expression and NF-κB activation by bharangin may contribute to its anti-cancer activities. **Conclusion:** Bharangin exhibit potential against breast cancer. The modulation of lncRNA expression and inhibition of NF-κB activation by bharangin may contribute to its anti-cancer activities. Studies are underway to examine the in-depth molecular mechanism of bharangin's action. (DOI:10.9777/rr.2018.1121)

Role of AP-4 Complex Genes in Seed Development

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Background: The membrane-integrated proteins are transported in a living cell by vesicular trafficking mechanism in which a heterotetrameric AP-4 protein complex plays a major role. Very little information is available about this mechanism in plants. **Aim:** The aim of this study was to understand the molecular mechanism of vesicular trafficking in plants. **Methods:** The sequences of AP-4 complex genes and their promoters and protein products were characterized for introns, CDS, GC content, CpG islands, conserved motifs, cis regulatory elements (CREs) and SNPs. The expression of these genes was studied in Arabidopsis thaliana, Zea mays and Glycine max from the available transcriptomic data. **Results:** A high GC content was observed in the AP-4 complex genes of monocots. In each studied AP-4 complex, a conserved motif was found on the mu subunit for binding to the sorting signal of the cargo protein. Another motif in the beta subunit for binding to the accessory protein was also observed in each complex. In the beta subunit motif, three amino acids (tryptophan, tyrosine and glutamine) were found conserved. The promoters of AP-4 complex genes of A. thaliana, Z. mays and G. max contained 116, 122 and 136 CREs, respectively. Out of 300 SNPs observed in the AP-4 complex genes, more than half were present in the intronic regions. The transcriptomic analysis demonstrated more expression of the AP-4 complex genes in seed in comparison to other tissues. **Discussion:** Conserved motifs in mu and beta subunits identified in plants were like those reported in mammalian species. The transcriptomic analysis and identified CREs indicated the involvement of AP-4 complex genes in seed development, stress response, cellular development and hormonal regulation. **Conclusion:** The AP-4 complex seems to play a significant role in the seed development and stress responses. (DOI:10.9777/rr.2018.1122)

Extracts of semecarpus anacardium leaves induced cytotoxicity and apoptosis in human cancer cells

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Background: Semecarpus anacardium is an Indian medicinal herb which is described as Bhallataka in Ayurveda and its fruits have various activities like hypoglycemic, antiatherogenic, antimicrobial, antioxidant, anticarcinogenic, antiinflammatory, anti-reproductive, CNS stimulant, skin diseases and hair growth promoter. It is poorly understood the mechanism of anticancer activity of its fruit against human cancer while there is a lack of information about the medicinal value of its leaves. Therefore, this study is an attempt to investigate the anticancer activity of its leaves extracts on human breast carcinoma MCF-7 cells. **Methods:** The leaves of S. anacardium were shade dried at room temperature (25 ± 2°C) for three weeks and grinded into powder. The obtained powder (100 g) was macerated into 1 l petroleum ether, ethylacetate and methanol respectively for two days and this process was repeated thrice. After filtration, the filtrate was evaporated and dried at 50°C under reduced pressure using a rotary evaporator. The cytotoxicity of the extracts were evaluated using MTT assay whereas the basic mode of cell death was examined by AO/EB

double staining and DNA laddering assay against human breast adenocarcinoma (MCF-7) cells, human colon carcinoma (HCT-15) cells, mouse ascetic carcinoma (EAC) cells and mouse fibroblast (L929) cells. **Results:** The ethyl acetate extract of the leaves was found the most potent cytotoxic fraction (IC50 value 0.57 µg/ml) for human breast cancer, MCF-7 cells with less toxic for normal fibroblast, L929 cells (IC50 value 2.1 µg/ml). The ethyl acetate extract also revealed apoptosis inducing activity, which was confirmed with fluorescence microscopy and DNA laddering assay. **Conclusion:** The ethyl acetate extract of S. anacardium leaves induce apoptosis mediated cell death in MCF-7 cells. It may be used for future drug discovery against cancer treatment. (DOI:10.9777/rr.2018.1123)

Steroid hormone induced expression of LIF mediate vasoactive molecules during window of implantation and decidualization in golden hamster

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Embryo implantation is early and complex stage of pregnancy begins when competent blastocyst make a physiological attachment to receptive endometrium. Expressions of numerous molecules are essential for initiation of pregnancy. Leukaemia inhibitory factor (LIF) is essential cytokines required for priming uterus to make it receptive for implantation. In mice, the ovarian estrogen regulated expression of LIF is absolutely required for implantation. Golden hamster showed ovarian estrogen independent process of embryo implantation. Hence, the regulation of LIF in uterus of golden hamster during early pregnancy is still ambiguous. In this study, we explored the possible regulation of LIF by uterine factor and their spatio-temporal localization and expression in the uterus of golden hamster during early pregnancy and pseudopregnancy. We further demonstrated their ability to activate prostaglandin synthesizing enzymes to achieve successful pregnancy. We used immunohistochemistry, quantitative and semiquantitative PCR to achieve the objectives. We observed the expression of LIF in all the day of early pregnancy and pseudopregnancy in the uterus of hamster. Their m-RNA was found to be upregulated around the day of implantation and decidualization. LIF showed high expression in D3 pseudopregnancy. LIF was found to be regulated by estrogen in ovariectomized uterus and significantly reduced expression of LIF was observed in letrozole treated uterine horn. Downregulated expression of prostaglandin synthesizing enzymes was observed in anti-LIF antibody treated uterus. Together, these findings highlight that uterine factor regulated LIF mediate their action via activating prostaglandin synthesizing enzymes to make uterus receptive for successful early pregnancy in hamster. (DOI:10.9777/rr.2018.1124)

Bone marrow aplasia and deregulation of vital signaling components: a correlative study in experimental mice

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Background: Aplastic anemia is the bone marrow failure condition which is characterized by hypo-cellularity in both the marrow and peripheral

blood compartments. Inappropriate use of DNA alkylating drugs viz; busulfan, cyclophosphamide etc. is one of the major etiological factors for the dreadful disease. The detailed molecular mechanisms behind the hematopoietic catastrophe largely remained elusive till date. **Aim:** The present study aims in the mechanistic intervention of the pathophysiological conditionusing busulfan and cyclophosphamide mediated mouse model of the disease. **Methods:** The study involves the characterization of the disease by peripheral blood hemogram, bone marrow cytopathology, histopathology, cytochemical analysis, scanning electron microscopy, cell culture, cell cycle analysis, apoptosis profiling, etc. To gain deep insight into the mechanistic scenario of the disease, expressional analysis of mitotically important kinases and phosphatases viz; PKB, Gsk-3β, Chk-1, Plk-1, Aurora kinase A, Cdc25c, PP2A etc. were done in the bone marrow cells. **Results:** The study revealed that the accumulation of genomic insults in hematopoietic cells was associated with deregulation of the vital signaling axis involving the mentioned molecules which resulted in the mitotic catastrophe and cellular apoptosis. The damage repairing system was also found to be impaired. Discussion and **Conclusion:** The work established a significant correlation between the alteration of the signaling components and hematopoietic devastation during the aplastic condition that may be helpful for designing successful therapeutic modalities for the disease. (DOI:10.9777/rr.2018.1125)

SERS assisted profiling of molecular and DNA level damage during apoptosis induced by targeted three in one theranostic nanoprobe for metastatic melanoma

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Background: Despite of the tremendous growth achieved by modern medicine in cancer therapeutics, malignant melanoma continues to be a major cause of mortality all over the world due to its therapeutic resistance properties. Hence there is an alarming need for a combination therapeutic approach for complete eradication of melanoma. **Aim:** The aim of this work is to design and synthesize a nanorod based theranostic nanoprobe targeting melanoma by combining PTT, PDT and chemotherapy along with SERS imaging for the better treatment and effective follow up therapeutic response. **Methods:** Synthesized nanorod using direct method and surface coating is affected using BSA stabilized nanocluster. NIR absorbing quinaldene based squarine derivative was synthesized and coloaded to the nanoprobe with a melanoma specific chemotherapeutic agent dacarbazine. EDC NHS coupline method was adopted for the successful conjugation of anti DR5 monoclonal antibody for targeting A375 melanoma cells. Results and **Discussion:** A very easy and efficient strategy for addressing the toxicity issue of nanorod arising from the surfactant CTAB is optimized using BSA stabilized nanocluster coating on nanorod for better biocompatibility. A new NIR absorbing quinaldene based squarine dye ISQ was explored as a Raman reporter molecule as well as a photodynamic agent. The multiplexing potential of chemotherapeutic agent dacarbazine was explored for monitoring the drug loading and release profile using SERS platform. Targeting property of the nanoprobe was effected using the coupling to anti DR5 monoclonal antibody which

targets the death receptor 5 over expressed in melanoma cells. The therapeutic efficiency of various methodologies like PTT, PDT and chemotherapy was checked using different cell death assays like MTT live-dead assay annexin V assay and APOP assays and effectively utilized SERS platform for monitoring the molecular level changes upon apoptosis by monitoring the DNA fragmentation using SERS platform. We also checked the in vivo applicability of the nano probe using sub acute toxicity studies on BALB/c mice and realized that the nano-constructs designed is completely safe for administration. **Conclusion:** In short, we have designed and developed a non toxic multimodal theranostic nano platform for the effective treatment and follow up of malignant melanoma using PTT, PDT and chemotherapy as the therapeutic modalities and SERS platform for monitoring the therapeutic responses. (DOI:10.9777/rr.2018.1126)

Isodeoxyelephantopin, a sesquiterpene lactone exhibits anti-tumorigenic activities through modulation of nuclear factor-κB (NF-κB) activation

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Background: For centuries, medicinal plants have been used as the primary source of medicine. However, their active ingredient and underlying mechanism remains poorly understood. Elephantopus scaber Linn. is a medicinal plant that has been reported to possess anti-inflammatory activities. Several agents including isodeoxyelephantopin (IDET) and deoxyelephantopin (DET) have been isolated from this plant. **Aim:** The aim of this study was to examine if IDET and DET possess anti-tumorigenic activities. If so, whether these agents can modulate NF-κB activation in cancer cells? **Methods:** We used breast cancer (MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-453) and C6-glioma cell lines during the study. The proliferation and viability of cancer cells was examined by MTT assay. We also examined if IDET can sensitize breast cancer cells to doxorubicin. The ROS inducing potential of IDET was examined by DCF- DA staining. The other parameters used were AO/PI staining, DAPI staining and subG1 analysis for apoptosis, and western blotting for cell survival proteins. Results and **Discussion:** IDET was found to suppress the viability and proliferation of breast cancer (MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-453) and C6-glioma cell lines in a dose- and time-dependent manner. A comparison of IDET and DET revealed that former was more effective as compared to later. The IC50 of IDET was found in the range of 45μM to 3.6 μM after 24 hour to 48 hour treatment, respectively. The sesquiterpene induced apoptosis inbreast cancer cells. The sesquiterpene also suppressed expression of cell survival proteins in cancer cells. Furthermore, IDET was found to sensitize breast cancer cells to doxorubicin. The migration of MDA-MB- 468 was suppressed by IDET. Although okadaic acid induced NF-kB activation in breast cancer cells, the same was suppressed by the use of IDET. The sesquiterpene also induced ROS generation in breast cancer cells. **Conclusion:** Overall, these observations suggest that IDET is more potent as compared to DET against breast and glioma cells.

Further, the inhibitory effects of IDET on NF-κB activation may contribute to its anti-tumorigenic activities. Studies are underway to examine if ROS is required for NF-κB activation and anti-cancer activities by IDET in breast cancer cells. (DOI:10.9777/rr.2018.1127)

The early phase of acute paracetamol-induced liver injury in albino rats

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Background: Liver plays an important role in regulation of physiological processes, involved in several vital functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and xenobiotics and secretes bile that has an important role in digestion. Liver plays a central role in transforming, clearing the chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when administered within therapeutic ranges may injure this organ. **Methods:** The aim of our study was to investigate the relationship between liver antioxidant capacity and hepatic injury in the early phase of acute paracetamol intoxication in albino rat. A total number of 24 rats were included in the study and was divided into 2 groups.Group-I: Control group (n=6). Group-II: Paracetamol treated group(n=18) at a dose of 2gm/kg b.wt.At the end of the experiment rats were sacrificed by cervical dislocation at 6, 24, and 48 hours after paracetamol administration. **Results:** Animals were sacrificed 6, 24 and 48 hours after treatment. Oxidative stress parameters were determined in blood and liver samples spectrophotometrically. Liver and plasma malondialdehyde were significantly increased 6 hrs after paracetamol administration in comparison with control group. After this period MDA level showed a gradual increase and reached its highest value at 48 hours after treatment. Paracetamol induced a significant reduction in liver superoxide dismutase (SOD) activity at first 6 hrs. Our data showed that liver antioxidant capacity increases in first 6 hrs of paracetamol-induced liver injury. SOD activity in the serum was significantly lower in paracetamol group in comparison with control group. Similar activities were measured 24 and 48 hours after paracetamol administration. Serum activities of aminotransferases and alkaline phosphatase were significantly increased after paracetamol administration at all time intervals in comparison with control group. **Conclusion:** These effects are maintained for the next 48 hrs. On the other hand, antioxidant capacity of hepatocytes is increased within first 6 hrs. According to these findings, it can be estimated that antioxidant capacity should be administered as early as possible after paracetamol intoxication. (DOI:10.9777/rr.2018.1128)

L-Asparaginase enzyme production by *Bacillus subtilis*: Optimization through response surface methodology

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Background: L-Asparaginase is an important enzyme being used as a drug in the treatment of Acute Lymphoblastic Leukemia (ALL). It is also used to prevent acrylamide formation in heated food. **Aim:** Present study aims the Optimization of

the media components for the best production of L-Asparaginase enzyme by the isolate Bacillus subtilis, through Response Surface Methodology (RSM). **Methods:** The variables were primarily selected through one-factor-at-a-time method and the media components were further optimized by statistical method Placket Burmen Design (PBD) and Face Centered Central Composite Design (FCCCD). Results & **Discussion:** One-factor-at-a-time method has been used for various carbon, nitrogen and mineral sources in the initial study. Based on the results, the eight variables like L-Asparagine, yeast extract, glucose, galactose, potassium hydrogen phosphate and sodium hydrogen phosphate were employed in Plackett-Burman design to study the interactive effect of these variables on the activity of L-Asparaginase enzyme. L-Asparagine, yeast extracts and sodium hydrogen phosphate showed significant result among these eight variables. Further analysis was performed to find optimum values of selected parameters using FCCCD. The maximum activity of L-Asparaginase was produced from the optimized media that contained L-Asparagine - 0.984%, yeast extract - 0.484%, and sodium hydrogen phosphate -0.454%. The L-Asparaginase activity in the optimized media was 12.86 U/ml which was higher than that obtained in the unoptimized media (1.827 U/ml). Kenariet al (2011) had reported a tenfold enhancement in activity of L-Asparaginase from Esherichia coli after optimization through RSM. **Conclusion:** The present study shows that the endophyte Bacillus subtilisis a potent source of L-Asparaginase enzyme. A detailed study of this enzyme is required to establish its pharmaceutical as well as Industrial applications. (DOI:10.9777/rr.2018.1129)

V-shaped bis-allylpyridylhydrazone and its nanoaggregates in the recognition of metal ions

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Background: Conjugated organic molecules are demanding now-a-days owing to their range of functional applications, such as organic light-emitting diodes and organic film solar cells. Materials which are usually non fluorescent in the solution state become emissive in the aggregated/solid state by the restriction of their intramolecular rotation and non planar configuration. Due to the emissive nature of the materials, they are emerged as potential candidates in biological, and optoelectronic applications. These are also utilized in generation of nanoparticle and are also used for recognition of toxic metal ions, small molecules and explosives. **Aim:** Development of AIE active probe (bis-allylpyridylhydrazone, PYAL) and its nanoaggregate for the recognition of Cu^{2+} ions. **Methods:** Synthesized compound was analyzed using CHN analysis, IR, 1HNMR, 13CNMR, ESIMS, absorption, emission spectroscopy, XRD, SEM, TEM and AFM. **Results:** A V-shaped bis-allylpyridylhydrazone, PYAL is synthesized and well characterized using spectroscopic techniques and x-ray crystallography. The supramolecular architecture of PYAL shows stair case arrangement via H-bonding and CH-π interactions. It shows AIE at 90% water fraction and results in the formation of nanoaggragates which is well characterized by SEM, TEM and AFM techniques. Different shaped nanoaggregates are formed which further utilized to detect Cu2+ ions selectivity without interference from other cations. **Discussion:** Results shows that

the polarity of solvent plays a key role for deciding the shape of the nanoaggregates i.e. in THF, hexagonal whereas in DMF branch nanoaggregates are formed. The recognition of Cu^{2+} ions by these nanoaggregates are clearly explained by self assembly process. This self assembly was further supported by ESI-MS and TEM studies. **Conclusion:** In summary, bis-allylpyridylhydrazone (PYAL) forms nanoaggregates (nPYAL) in THF: water (10:90,v/v). These, nanoaggregates can be used to selectively detect Cu^{2+} ions. (DOI:10.9777/rr.2018.1130)

Analysis of GTPases carrying mutations at conventional GTP binding machinery

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Introduction: GTP binding proteins or GTPases participate in cell signaling cascade by specifically binding and catalyzing nucleotide hydrolysis. Ras, a prototype of G-proteins, utilizes highly conserved catalytic motifs, commonly known as G1-G5 motifs, for the GTP binding and hydrolysis. In many mutagenic experiments the variations in the conserved G-motifs have been shown to disrupt the GTPase reaction. Mutations in the P-loop residues, other than invariant residue of G1 motif, have been shown to lower the GTPase activity leading to cancer. **Aim:** We present an analysis of the GTPases with variations at invariant position of the conventional GTP binding machinery. The objective is to investigate and classify the variations and it's implication in the catalysis. **Methods:** The HMM and PSI-Blast based profile search was used to retrieve the GTPase sequences. Classification of the sequences was based on the variations on the G-motifs. Homology model was generated for the representative sequences using Modeller tool. Structural changes were analyzed by superposition of each of the representative model onto the Ras-GTPase as a reference. Results and **Discussion:** We have divided our dataset into following three categories: 1) G1/p-loop variation, 2) G-3 motif or swII variation and 3) G-4 motif variation. Substitution at the invariant position of the G1-motif was: Ala and Leu corresponding to the G15; His, Val, Glu, Met corresponding to K16; and Gly, His, Gln, Lys corresponding to S17 of Ras. The conserved and indispensible G-2 motif Threonine of switch1 is either substituted or finds a different structural position in most of the cases. Based on structural co-evolutionary analysis, we propose that the residues away from the catalytic site may replace the functions of variant residues. Recognition of these residue positions facilitates to classify the uncharacterized GTPases family and their catalytic capacity. (DOI:10.9777/rr.2018.1131)

Exploration of bioactive compounds from mangrove actinobacteria as cancer therapeutics: Insights into inhibition of phosphoinositide 3-kinase (PI3K) signaling

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Background: Investigation of molecular mechanisms involving marine natural products as cancer therapeutics has taken new dimensions. **Aim:** To explore the anticancer potential of bioactive compounds from mangrove actinobacteria and potential role in PI3K inhibition. **Methods:** To harness the crucial role of these extracted compounds as PI3K inhibitor, competitive ELISA of PI3K inhibition was assessed.

Cytotoxic potentiality of the compounds was evaluated against breast cancer cell lines (MCF-7). In order to explore the binding mode of compounds to PI3 Kinase, the compounds were docked into the active site of PI3 kinase p110α (PDB ID: 2RD0). **Results:** Excellent binding affinity (an average of -17.62 kcal/mol) along with affordable inhibitory constant values (lowest ki 96 nM for Cpd5) enforced to execute further biological evaluations. In the PI3 kinase inhibition studies, the calculated relative activity % was found as high as for compound Cpd5 (90 %) with an IC50 0.075 µM, which was excessively found with –OH (electron donor) and nitro (electron acceptors) substitutions to facilitate admirable medicinal value. MTT assay results revealed the potential of compounds Cpd1-9 with an average antiproliferative value of 82 % (Doxorubicin 78 %). Remarkably, the required IC50 was only found in the rage of <0.025µM. Subsequently, none of those testified compounds found as inactive for all the activities that are executed in this study. **Discussion:** The results ensure the anticancer potential and inhibitory activity of the compounds from actinobacteria. Cpd5 displayed extensive anticancer activity and PI3 kinase inhibition.SAR studies predicted the compound as potent PI3K inhibitors and thus as cancer therapeutics. **Conclusion:** Since the inhibitors of PI3 kinase can serve as putative potential cancer medications, a preliminary screening of PI3kinase selective inhibitors from the extracted samples of actinobacteria was done. (DOI:10.9777/rr.2018.1132)

Neuroprotective effects of brilliant blue G in 6-OHDA induced PD model

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Background: Parkinson's disease (PD) is one of the major progressive neurodegenerative movement disorders which occur due to death of approximately 50-70% of dopaminergic (DA) neurons in the substantia-nigra-pars-compacta (SNPC) of basal ganglia. This leads to loss of DA nerve terminals in striatum giving rise to symptoms like motor disturbances such as bradykinesia, resting tremor, rigidity and non-motor symptoms. The pathogenesis of PD remains obscure and current therapy involves use of drugs which could only provide symptomatic relief. **Aim:** Purinergic receptors are abundantly found in neurons, microglia and oligodendrocytes and activated by ATP throughout the brain and the spinal cord. It controls DA release, neuronal damage and striatal-related function. Their expression is increased in SNPC during PD pathology. Therefore, we investigated the neuroprotective role of selective purinergic receptor P2X7 antagonist Brilliant blue G (BBG) against 6-OHDA induced dopaminergic neurotoxicity in rats. **Methods:** BBG was administered by intra cerebroventricular (I.C.V.) injections half-an-hour before 6- OHDA-induced PD and animals were killed on 21st day. Behavioral parameters like spontaneous motor activity were performed at every week to observe motor deficits. At 21st day, striatal DA content was estimated for neurochemical measurement of PD. Mitochondrial membrane potential (MMP) was evaluated to assess permeability of mitochondrial membrane. **Results:** BBG increased locomotor activity and also increased striatal DA content in 6-OHDA PD rats. Mitochondrial integrity was maintained as BBG improved MMP in 6-OHDA

administered rats. **Discussion:** Role of purinergic signaling in DA neurotransmission was confirmed by present study as BBG augmented striatal DA content. Further it also maintained mitochondrial function by improving mitochondrial membrane potential in PD animals. **Conclusion:** Purinergic receptor antagonism is neuroprotective in 6-OHDA induced experimental PD model in vivo. (DOI:10.9777/rr.2018.1133)

Withania somnifera and its active chemical constituent Withaferin-A improves spermatogenesis in a hypo-spermatogenic rat model

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Background: Withania somnifera (WS), which is commonly known as Ashwagnadha, Indian ginseng or winter cherry, has been recommended and used for a variety of clinical conditions and health promoting effects. **Aim:** Evaluation of pro-male fertility activity and mechanism of action of WS and its active chemical constituent Withaferin-A (WFA) using a hypo-spermatogenic rat model. **Methods:** Hypo- spermatogenic rat model was obtained by oral administration of ethinyl estradiol for 14 days. These animals were further administered with WS root powder suspension and Withaferin-A in 0.5 % carboxymethyl cellulose for a period of 56 days and the data were compared with a vehicle treated auto- recovery group. **Results:** By the end of the treatment regimen, sperm count and motility were significantly improved. Reactive oxygen species, mitochondrial membrane potential, peripheral hormone levels, and testicular apoptosis, were normalized. DNA content and histo-architecture analysis showed actual improvement in spermatogenesis. The restorative effect of WS was much more pronounced in comparison to WFA. **Discussion:** WS improves spermatogenesis both quantitatively and qualitatively. Quantitative effects are attributed to its restorative impact on reproductive hormones and alleviation of oxidative stress that results in increased sperm production while qualitative improvements are primarily due to its beneficial effects on mitochondrial membrane potential, DNA integrity and motility. Use of pure compounds, such as WFA, limits its pro-fertility activity, perhaps due to the lack of a number of other active constituents present in WS root. **Conclusion:** WS and its chemical constituents Withaferin-A improves spermatogenesis. (DOI:10.9777/rr.2018.1134)

The promoter methylation of BRCA1 gene associated risk factors in sporadic breast cancer: a north Indian hospital based study

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Background: Breast cancer (BC) is a leading cause of cancer-related deaths among women worldwide, and this study further demonstrates that the women of Varanasi (north India) are not untouched by this fatal fact. During BC development, epigenetic part plays a key role for silencing the gene expression. Its widespread occurrence in cancer genome could inactivate many cellular pathways including DNA repair, cell cycle control, apoptosis, cell adherence, and detoxification. **Aim:** In this study, our aim was to determine the penetrance of BRCA1 promoter methylation and their correlation with pathological

and demographic factors in sporadic BC in Indian population. **Methods:** Our analysis included 127 patients, who were diagnosed with sporadic BC. Methylation specific PCR for BRCA1 promoter was used during the study and correlated with pathological and demographic factors. **Results:** Methylation of the BRCA1 promoter was detected in 8.7% (11/127) of the tumors. Correlation of promoter methylation with demographic factors and clinicopathological markers revealed the following data: (i) BRCA1 methylation was more frequently observed in tumor samples taken from premenopausal or perimenopausal women (P= 0.026), (ii) Methylation of BRCA1 promoter negatively correlated with estrogen receptor (P = 0.040), progesterone receptor (P =0.013), and epidermal growth factor receptor-2-2 (P = 0.002), (iii) the overall promoter methylation was higher in more advanced stage (P = 0.036) of the disease. Discussion & **Conclusion:** This study has immense implication in understanding epigenetic mechanisms in BC development. The result suggests that the epigenetic silencing of BRCA1 is uncommon and is associated with triple-negative phenotype. (DOI:10.9777/rr.2018.1135)

Determination of antioxidant property of flowers and fruits of *Sterculia alata* Roxb.

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Background: Sterculia alata Roxb. commonly known as Buddha's Coconut is highly medicinal tree. Leaves and barks show antioxidant properties. **Aim:** To determine the antioxidant property of flower and fruit extracts of S. alata. **Methods:** Flowers and fruits extracts were prepared. Ethanol, methanol and double distilled water were extraction solvents. The free radical scavenging activity of the extracts was determined by DPPH method and total phenolic content (TPC) by Folin–Ciocalteu assay. AlCl3 calorimetric method was used for total flavonoid content (TFC). Reducing potential of the extract was evaluated according to the method used by Terpenic et al. (2012). **Results:** Maximum TPC and TFC were found in methanolic and ethanolic extracts of fruits and flowers respectively. Similarly, ethanolic extract of fruit and methanolic extract of flower showed maximum scavenging activity. In both extracts, reducing power was found to be maximum in the ethanolic extract. **Discussion:** For the assessment of antioxidant activity, DPPH was used as it is easy, rapid and stable method and reduced by antioxidant. Methanolic extract of flower showed maximum scavenging activity. Polyphenolic compounds are secondary metabolites found widely in plants and possess scavenging ability due to their hydroxyl group. In the reducing power assay, the colour of the sample solution changes from yellow to various shades of green and blue colour according to sample concentration due to the reduction of Fe^{3+} to Fe^{2+}. In this investigation, both ethanolic plant extracts showed highest reducing potential. **Conclusion:** Extracts of S. alata are rich source of polyphenols like phenolics and flavonoids, thus showing its high medicinal importance. (DOI:10.9777/rr.2018.1136)

Antitumor effects of Vietnamese coriander on human oral squamous cell carcinoma

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Background: Oral cancer, the most common neoplasm of head and neck cancer is the sixth most prevalent cancer of the world. The poor prognosis of Oral Squamous Cell Carcinoma (OSCC) is due to aggressive local invasion and metastasis leading to recurrence. Persicaria odorata, commonly known as Vietnamese coriander or Phak-Pai has been used in traditional systems of medicine for the treatment of swelling, inflammation, excessive bleeding, sores, ulcers and wounds, stomach ailments, tumors etc. Inflammation has long been linked with the development and progression of cancer; here we investigated whether this plant has any anticancer effect on OSCC in vitro. Objectives: To investigate the antitumor effects of the leaves of Persicaria odorata in OSCC cells. Materials and **Methods:** The methanolic leaf extract of Persicariaodorata was prepared at room temperature. Experiments such as MTT assay, PI staining, cell cycle analysis and wound healing assays were performed to analyze the effect of this extract on oral tumor cell proliferation, survival and migration respectively. Western blot analysis was also performed to assess the effect of the extract on the expression of the proteins involved in various signaling pathways that stimulate tumor growth and progression in OSCC. In addition, LC-MS analysis was done to detect the important compounds present in the extract. **Results:** This is the first report showing the effect of Persicaria odorata on oral cancer. The present findings showed that methanol extract of Persicaria odorata has high potential in oral cancer prevention and treatment. The extract inhibited the proliferation, survival and migration of oral cancer cells in a dose dependent manner. Moreover, several proteins involved in tumorigenesis were found to be downregulated. Conclusions: The methanolic extract of Persicaria odorata exhibited potent anti-cancerous properties against OSCC. However, further investigations are required to validate these findings in vivo conditions. (DOI:10.9777/rr.2018.1137)

The proposed new methodology focused on reduced cost of production with enhaced yield of medicinally important *Ganoderma lucidum* mushroom.

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Ganoderma lucidum (king of herb) mushroom belongs to Polyporaceae (Ganodermataceae) of Aphyllophorales is well known for its application as medicinal products. It possess important properties of anti- inflammation, anti-carcinogen, stress reducer, anti-prostate and breast cancer agent, and anti-oxidant with immune-modulatory effects which makes it potential King of Herbs. Its cultivation/production under aseptic condition at laboratory scale as well as outdoor scale in fields and Forest makes it available for commercial purposes as medicinal proprietary product. However, its effective cost production and yearly production yield needs improvement and development in methodology and techniques focusing to enhance yearly production with affordable cost production. The current study highlights and suggest a new technique for laboratory and field cultivation using combination of wheat straw, tea leaves and wheat bran for the preparation of inoculum for spawn culture and

calcium carbonate and calcium sulphate was also used as a supplement. The hard wood Poplar (Populus deltoids), Mango (Mangifera indica) used as billets/log for cultivation of Ganoderma lucidum and Vermicompost was used as substrate for cultivation of G. lucidum The fruiting body of G. lucidum with supplemented vermicompost produced different physical texture as margin of fruiting body with deep red and brown color, increased thickness in fruiting body, and 30 percent more yield than previous studies. The billets of Poplar and Mango cultivated with vermicompost modification provided the best yield of mushroom among the substrate/colonization which took 1 week, 30 days and 45 days for the mycelium growth, primordial formation and harvesting, respectively. In total, 8 billets of Poplar using vermicompost produced 350 gm of dried G. lucidum in 120 days. Thus for an individual grower, cultivation using modified method is more profitable. (DOI:10.9777/rr.2018.1138)

Efficacy of *Origanum majorana* L. essential oil as novel plant based food preservative against aflatoxin B1 and lipid peroxidation

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Background: Food commodities contaminated with molds and mycotoxins are the severe problem. Several synthetic preservatives have been used to overcome the problem of food loss; however their indiscriminate use can cause adverse effect to environment and to human. Thus there is need to develop some safer plant based preservatives having no adverse effect on human and environment. Among plant products, essential oils of higher plants are gaining the attention to the scientific community due to their volatile and biodegradable nature. Thus, the recommendation of plant essential oils would be a novel approach to combat the problem associated with molds and mycotoxins. **Aim:** To explores the preservative potency of Origanum majorana L. essential oil (OMEO) and to analyze the probable mode of antifungal, antiaflatoxigenic and antioxidant activity **Methods:** GC-MS analysis to characterize activity of OMEO was assessed in terms of minimum aflatoxin inhibitory concentration (MAIC) which was found to be 1.5 µl/ml. Total phenolic content of OMEO was found to be 3.31 µg/mg. OMEO also exhibited considerable antioxidant activity, which was assayed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) having IC50 value equal 15.45 µL/mL. Strong antifungal, antiaflatoxigenic, and antioxidant potency of OMEO make it suitable forrecommendation as plant-based preservative during storage of food commodities. **Conclusion:** Present study recommend OMEO as a safe plant based preservative againstfungal and aflatoxin B1 contamination and oxidative deterioration of food commodities. (DOI:10.9777/rr.2018.1139)

Immobilization of β-galactosidase onto functionalized CNT-MoS2 nanoparticles using response surface methodology: Characterization, kinetics and its application

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Background: β-galactosidase is extensively utilized by dairy industry to improve sweetness, solubility,

flavor and digestibility of dairy products. Enzymatic hydrolysis of lactose by β-galactosidase is now becoming popular technology to produce lactose reduced milk & related dairy product for lactose intolerance people. On the other hand, by utilizing the trans-galactosylation property of β-galactosidase, synthesis of GOS (galacto-oligosaccharide synthesis), which are non digestible sugar, can act as good source of prebiotics, contributing to colon's health, by promoting the growth of beneficial bacteria. The immense importance of β-galactosidase for lactose intolerant people and dairy industry direct us to study this biocatalyst. **Aim:** To boost the stability and reusability of purified β-galactosidase by immobilization onto the suitable nanoparticles. **Methods:** This biocatalyst was immobilized onto the CNT-MoS2composite nanoparticles using gluteraldhyde as a cross linker and optimization was done by using Response Surface Methodology i.e. Box Behnken experimental design. **Results:** An overall 94.8% of immobilization efficiency was achieved. In order to determine the accuracy and significance of the quadratic model, ANOVA was performed. Immobilized enzyme showed shift in optimum pH, optimum temperature, thermal inactivation etc. on other hand, immobilized enzyme showed excellent reusability with the retention of 78% of activity after 10 uses. **Discussion:** CNT-MoS2 provides an excellent platform for immobilization of the given biocatalyst, which result in an overall of 94.8% of immobilization efficiency. The stability and reusability of the enzyme has been increased upto 60 days with retention of activity upto 78% after 10 uses. **Conclusion:** In this study, we propose the superiority of immobilized nanobiocatalyst over soluble enzyme, which would be beneficial for the dairy and food industry. (DOI:10.9777/rr.2018.1140)

Effect of polyamines (spermidine and putrescine) on growth, photosynthesis and antioxidant system in *Brassica juncea* treated with manganese.

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Polyamines (PAs) are well-established growth regulators playing key roles in stress management in various crop plants. In the present study, mitigative roles of spermidine (spd) and putrescine (put) were assessed in manganese stressed Brassica juncea plants. Spermidine (spd, 1.0 mM) or putrescine (put, 1.0mM) were applied to the foliage of Brassica juncea at 35 days after sowing (DAS) grown under manganese stress (30 or 150 mg kg^{-1} soil). High manganese stress diminished growth, chlorophyll content and photosynthetic attributes at 45 DAS whereas it enhanced electrolyte leakage, proline, and various antioxidant enzymes in the leaves of Brassica juncea plants. On the other hand treatment of polyamines (Spd and Put) under stress and stress-free conditions significantly increased the aforesaid growth traits and biochemical parameters and further accelerated the antioxidant enzymes and proline content, which were already enhanced by the high manganese stress. It is concluded that treatment of polyamines (through foliage) significantly increased the growth traits, photosynthetic efficiency and various biochemical attributes under high manganese stress and stress-free conditions. (DOI:10.9777/rr.2018.1141)

Identification of natural products as acetylcholinesterase inhibitor for treatment of Alzheimer disease through *in silico* approach

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Alzheimer's disease (AD) is a multifaceted disease involving various contributing factor for its progression. An estimate suggests that about 47 million people live with dementia worldwide, and more than 131 million by 2050 will suffer from one of the forms of dementia. The amyloid plaque and neuro-fibrillary tangles of hyper phosphorylated tau are the classical hallmark of the disease. AD affects memory, learning and behavior in a time dependent manner leading to vegetative state. The brain cholinergic system is damaged in AD leading to cognitive deficits. Acetylcholinesterase (AChE) plays a key role in controlling acetylcholine metabolism. The present work aims at in silico identification of some natural products as AChE inhibitors using shape similarity search. Shape based screening was performed using donepezil as scaffold for screening AnalytiCon Discovery NP library to identify compounds similar to Donepezil with similarity cutoff of 0.5. The ligands were filtered (Lipinski, CMC-50 & BBB filters), clustered and docked using Autodock 4.2. The compounds obtained were screened for ADME properties through preADMET server to identify virtual hits. Out of 4549 compounds identified 93 compounds were found to be CNS active. The docking study and ADMET properties identified some flavonoids, chalcone and heterocyclic bearing compounds to be active against AChE. The present work has identified some natural products as AChE inhibitors which will be further tested in various biological models. (DOI:10.9777/rr.2018.1142)

Photosafety mechanism of hesperidin against UV-R induced cell damage in keratinocytes

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Background: Prolonged ultraviolet radiation (UV-R) exposure causes skin disorders like erythema, edema, photoaging and photocarcinogenesis. Therefore its need to understand this problem and protect skin disorders for human welfare. Hesperidin (HES) belongs to flavonoids obtained from grapefruit and many other citrus fruits. It has been reported to exert a wide range of pharmacological activity. **Aim:** To understand the role of HES in skin cell photoprotection and explore the possible photosafety mechanism. **Methods:** UV/Vis and LC/MS-MS spectrometry were used for measurement of HES stability. Human keratinocytes cell line (HaCaT) irradiated under UV-A (7.92 J/cm2) and UV-B (3.24 J/cm2) with HES and alone. Photosafety was assessed by cell viability (MTT & NRU) assay followed by estimation of intracellular ROS (DCF assay), DNA damage (Comet assay), and flow-cytometric analysis for cell cycle and mRNA expression through RT-PCR methods. **Results:** HES was found photostable, i.e. there was no degradation observed in irradiated samples. The cell viability was observed 47% and 43%, irradiated by UV-A and UV-B, respectively. However, restoration of

cell viability was recorded at 5 µg/ml HES 98% and 95% under UV-A and UV-B exposure, respectively. HES significantly suppressed the ROS generation in UV-R treated cells. UV-R irradiated indicating extensive DNA damage (22% in UV-A and 25% in UV-B). However, this damage was reduced (5% at 5µg/ml) in the cells pretreated with HES. The flow-cytometric analysis demonstrates that a shift in G0/G1 (sub G1) phase was observed in UV-A (13.73%) and UV-B (22.31%) cells population. Conversely, pretreated with HES has shown no such shift in sub-G1 population in cell cycle. In RT-PCR analysis, it saw that HES prevent UV-R mediated Bcl-2/Bax, Cyto-c and p21 mRNA expression in HaCaT cells. **Discussion:** The degradation data showed that HES was stable under UV-R exposure. The cell viability and ROS scavenging analysis, confirm a significant protecation against UV-R. Reduction of DNA damage indicated that HES act as anti photogenotoxicity. It was also proved through decline of sub G1. The transcriptional evidence showed that this compound can attenuates the apoptotic marker genes. **Conclusion:** Our findings demonstrate that HES could be a promising way to reduce the adverse biological interactions with UV-R exposure. Therefore, we suggest the applicability of HES as a natural photosafety agent for human use. (DOI:10.9777/rr.2018.1143)

Production, optimization, characterization and kinetics of a partially purified laccase from Pleurotuscitrinopileatus and its application in swift bioremediation of azo dyes

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Background: In the present investigation the efficiency of laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) from Pleurotuscitrinopileatus was assessed for the decolorization of azo dyes. **Aim:** Enzyme production, characterization and kinetics of a partially purified laccase from Pleurotuscitrinopileatus were determined for its application in bioremediation of azo dyes. Methods & **Results:** Laccase has been partially purified by using 80% ammonium sulphate solution. Total activity, total protein, specific activity and purification fold for partially purified laccase were found to be 40.38U, 293.33mg/100ml, 0.91U/mg and 2.84, respectively. The pH and temperature optima of laccase were 5.0 and 50ºC, respectively, while the enzyme was most stable at pH 4.0 and temperature 30ºC when exposed for one hour. The Km of the partially purified laccase for substrates guaiacol, DMP (2,6-dimethoxyphenol) and syringaldazine (3,5-dimethoxy-4-hydroxybenzaldehyde azine) were 60, 95 and 26, respectively. This laccase has been tested for the use in the bioremediation of azo dyes in the absence of mediator molecules. Two dyes namely congo red and bromophenol blue were tested. **Discussion:** It was observed that laccase enzyme was very effective in the decolorization of these two dyes. More than 80% decolorization was observed within half an hour even in the absence of mediatorand their lower Km value indicates that efficiency of the enzyme is very high. The results were promising due to quicker decolorization in the absence of meditors showing that it can be used as a valuable biocatalyst for quick bioremediation of azo dyes. **Conclusion:** The enzymatic properties of laccase from P. citrinopileatus should be considered for a potential environmental (biodegradation and

bioremediation) or industrial applications. (DOI:10.9777/rr.2018.1144)

Photo induced, green synthesis of gold nanoparticles using aqueous extract of halophilic, unicellular green alga Dunaliella salina and itsinvitro anticancer activity on breast cancer cell line MCF-7

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Synthesis of gold nanoparticles, using biological entities via green route is an area of interest because of their potential application in the nanomedicine. In the current study, we have developed photo induced, low cost, ecofriendly method for synthesis of gold nanoparticle using aqueous extract of Dunaliellasalina (AED) which act as reducing as well as capping agent. The synthesis process were optimized as sunlight exposure, AED inoculums dose and HAuCl4 concentration. The synthesis of AuNPs was monitored using UV-Vis spectroscopy which exhibit SPR band at 535 nm after 55 min of bright sunlight exposure. Size and morphology of the biosynthesized AuNP were confirmed by TEM analysis, which confirmed the presence of spherical shape AuNPs with average size of 22.4 nm. SAED and XRD analyses confirmed the crystalline nature of AuNPs. FTIR analyses revealed the involvement of various functional groups present in AED in the synthesis of AuNPs. AED synthesized AuNPs and known anticancer drug Cisplatin were screened for their In vitroanticancer activity using MTT assay,calcein AM/PI assay, andAnnexin/PI on breast cancer cell line (MCF-7) and normal breast epithelial cell line (MCF-10A). AuNPs showed dose dependent anticancer activity and was not detrimental to normal cell line. (DOI:10.9777/rr.2018.1145)

Studies on establishment and differentiation of human pluripotent stem cells into β-cells

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Diabetes is a metabolic disorder characterized by progressively loss or dysfunction of pancreatic β-cells leading to insufficient insulin production and altered blood glucose level. A rocketing prevalence of diabetes worldwide has become a burning issue in biomedical science. According to International Diabetes Federation (IDF) ATLAS 8th edition, there are approximately 425 million diabetic patient worldwide in 2017 and the estimated diabetic patient number would be 629 million by 2045. The Diabetes capital of World, India homes for more than 70 million diabetes patients. Recent advances in biomedical research offered the islet β-cell replacement therapy as a potential treatment. However, lack of donors, need of large number of cells, immune rejection etc. have prevented it from being used in therapy. Stem cells harbour immense potential to differentiate into all cell types of human body so the researchers have explored the differentiation potential of stem cells in vitro for generating the

insulin producing β-cells. However, majority of the protocols of β-cells differentiation from pluripotent stem cells (ESCs and iPSCs) are associated with low efficiency and heterogeneity. Hence, the existing protocols of β-cell differentiation are not good enough to meet the required standard of the GM-grade β-cells. Keeping this in view, the present study aims to establish an efficient protocol of β-cell differentiation from human induced pluripotent stem cells (hiPSCs) using the various cocktail of molecules and very specific culture conditions. Inhibition of TGF-β (ALK 4, 5 and 7) using SB431542, and the BMP4 signaling pathway at a very specific stage will be used to generate NKX6.1 /NGN3 Endocrine Progenitors. Furthermore, growth arrest specific protein 6 (GAS6), an agonist of the AXL receptor tyrosine kinase subfamily and R428 as a receptor AXL inhibitor will be used for the maturation of β-cells. This study has the potential to provide a substantial solution of various associated problems to diabetes therapeutics. (DOI:10.9777/rr.2018.1146)

Analysis of extra domain and Switch I interactions in prokaryotic GTPases involved in ribosome assembly

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Introduction: GTPases comprise the largest class of essential ribosome assembly factors in bacteria. The conformational changes of switchI and switch II regions are associated a GDP-bound "off" state and a GTP bound "on" state. Based on sequence and structural similarity Ribosome associated GTPases are classified into TrmE Era-EngA-YihA-Septin-like super family which contains four universally conserved families, namely, MnmE (TrmE) Era, Der (EngA), and YihA. In contrast to the small GTPases from the ras- superfamily, the GTPases involved in ribosome assembly contain at least one extra domain in addition to the GTPase domain which has signatures to bind RNA. Moreover, these GTPases do not require Guanine Exchange Factors s to promote release of bound GDP and binding of GTP. **Aim:** We aim to study the switch I region of bacterial ribosome associated GTPases and its interaction with extra domain to better understand the GTP binding and exchange mechanism **Methods:** X-ray crystal structures of ribosome associated structures were retrieved from RCSB protein databank. The unresolved switch I regions for modelled using modbase and modeller tool. Energy minimization and simulation study was performed using GROMACS software tool. Analysis of trajectory of switch I was performed using VMD. UCSF chimera was used for visualization, superposition and analysis of the structures. Results and **Discussion:** GTPases involved in ribosome assembly contain at least one domain in addition to the GTPase domain. Structure analysis of Ribo-GTPases revealed that switch I regions are wide open in GTP or GDP unbound form. Molecular modelling and simulation study showed that the residue of switch I of G-domain interacts with the extra domains. We find the insertions in switch I region also facilitates its interaction with the extra-domain. Also, this residue of switch I showed correlated mutations with the extra domains suggesting that they are functionally related. (DOI:10.9777/rr.2018.1147)

Association of GSTM1 and GSTT1 gene (++/ --) genotype with risk of Non-Small Cell Lung Carcinoma in North Indian Population

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Background: The genes coding for separate isoforms of both the human glutathione S-transferase class mu and class theta enzymes (GSTM1and GSTT1) are polymorphic with a variable ethnic distribution. These enzymes detoxify reactive epoxides, including carcinogens produced by tobacco smoke. Because of this, the null polymorphism in the GSTM1 and GSTT1 gene has been studied widely as a possible source of inherited susceptibility and also possibly contributes to an increased risk of smoking-related lung cancer. **Aim:** To evaluate the association between GSTM1 and GSTT1 present/absentgenotypewith demographical clinico-pathological and risk correlation in non-small cell lung carcinoma (NSCLC). **Methods:** A total of 216 lung cancer patients and 100 controls were enrolled in a case-control study. The GSTM1 and GSTT1 were analyzed using PCR. Risk of lung cancer was estimated as odds ratio at 95% confidence interval using unconditional logistic regression models adjusting for age, sex, histology, stage of cancer, and tobacco use, chemotherapy and symptom index. **Results & Discussion:** 216 patients were included in the study, F/M ratio was 0.22 and the mean age was 59 years. Patients showed various histological subtype - 69% adenocarcinoma, 22% SCLC, 0.91% LCC, 2.31% mixed type carcinoma and rest data was not differentiated. 58% were smokers while 42% were non-smokers. Out of 216 NSCLC patients only 1.3% were in the stage Ia-IIb, 14.35%- III b and 39.35% in stage IV while in 44 % cases stages were not known. The therapy given to patients were; G+C 40%, P+C 7%, only G,P 6 % and E 2 % while 45% had no chemotherapy. The symptom indices were appetite loss 30.56%, weight loss 25%, chest pain 28.70%, breathlessness 53.24%, changes in voice 40.74%, back pain 36.11% and pain during swallowing 63.89%. Among the 166 cases of NSCLC patients, 30.34% were GSTT1 null and 69.66% were GSTT1 positive genotypes whereas in control subjects 8.7% were GSTT1 null and 91.3% were GSTT1 positive genotypes. 25% were GSTM1 null and 75% were GSTM1 positive genotypes while in control subjects 97.67% were GSTM1 positive and 2.33 % were null genotypes. Conclusions: The GSTM1 and GSTT1 null genotype is a risk factor for lung cancer. (DOI:10.9777/rr.2018.1148)

Enhancement of antimicrobial activity, stability and to investigate the mode of action of microencapsulated *Gaultheria procumbens* essential oil.

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Background: Aspergillus flavus is one of the cosmopolitan moulds, causing significant deterioration of food grains and their shelved products. Gaultheria procumbens L. commonly known as wintergreen oil is an aromatic plant of family Ericaceae. Its application as an insecticidal, antimicrobial, antileishmanial, antioxidant and antidiabetic agent has already been reported in traditional medicine system. The present study was undertaken to investigate the efficacy of chitosan-

cinnamic acid based microencapsulated Gaultheria procumbens L. essential oil (GPEO) against Aspergillus flavus (MZ-01), aflatoxin B1 secretion, mode of action and stability. **Aim:** Enhancement of antimicrobial activity, stability and to investigate the mode of action of Microencapsulated Gaultheria procumbens essential oil. **Methods:** Chemical characterization of GPEO was done through (GC-MS). **Results:** During chemical characterization (GC- MS), methyl salicylate (96.25%) was identified as the major component of GPEO. Microencapsulated GPEO exhibited strong antifungal and aflatoxin B1 suppressor activity than the uncapsulated GPEO and completely inhibited growth and toxin production at 1.25 µL/mL. The mode of action of microencapsulated GPEO was elucidated targeting ergosterol content in the cell membrane, the release of cellular ion contents and morphological alteration in A. flavus. **Discussion:** The current existing limitations of plant based preservatives such as low water solubility, strong organoleptic characteristics (flavour and aroma), low stability, etc. could be addressed by the modern advanced technologies such as microencapsulation. On the other hand it also results in enhancement of antimicrobial activity. **Conclusion:** The results demonstrate the potential of chitosan-based encapsulating material for the improvement of the antimicrobial efficacy as well as stability of GPEO. (DOI:10.9777/rr.2018.1149)

Force-induced rupture of double-stranded DNA in the absence and presence of covalently bonded antitumour drugs: Insight from molecular dynamics simulation

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DNA intra-strand crosslinks (ICLs) agents are widely used in the treatment of cancer. They may form a link between the same strands (intra-strand) or complimentary strands (inter-strand), and thereby increase the stability of DNA, which forbids the processes like replication and transcription. As a result, cell death occurs. We study the enhanced stability of a double stranded DNA in the presence of ICLs and compare our findings with results obtained in the absence of these links. Using atomistic simulation with explicit solvent, we apply a force along the helix direction and perpendicular to the helix direction and measure the rupture force and the unzipping force of DNA- ICLs, respectively. It is shown here that the rupture and the unzipping forces increase significantly in the presence of these links. The precise locations of ICLs in DNA affect the mechanism involved in the stabilization of dsDNA. Our result gives a atomistic idea of the process of DNA rupture and unzip in the presence and absence of antitumour drug. More computational studies like this would enhance our understanding about the molecular forces that govern the binding of important antitumour/anticancer compounds to DNA. Such information may be used to design alternative drugs that can stall replication and transcription processes and useful in designing better chemotherapeutic drugs in future. (DOI:10.9777/rr.2018.1150)

Novel molecular architecture through grafting to control drug release for cancer treatment using polymeric patch

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Background: Chemotherapy an effective treatment for cancer, drugs or chemotherapeutic agents used efficaciously kill the cancerous cells and also normal cells causing some adverse effects like nausea, vomiting, anaemia, hair loss just after administration of drug within an hour or days. Also most of the anti cancerous drugs are hydrophobic and poorly soluble in water there by reducing bioavailability to the system. Thus a suitable control drug delivery vehicle is required for their sustained and site specific delivery. **Aim:** Developing polymer based control drug delivery system for cancer. **Methods:** Polyurethane grafting on alpha cyclodextrin was done by using PTMG and HMDI based prepolymer followed by chain extension with CD. Polymers of varying degree of substitution and chain length of PU were synthesized to maintain hydrophobic and hydrophilic balance. Grafting was proven by various spectroscopic techniques. **Results:** The grafted copolymers exhibited enhanced thermal as well as mechanical properties as compared to pure polymers. Most striking feature that sustained drug release was attained in grafted systems against burst release in pure polymers and native drug. The developed copolymers were biocompatible as Hela cells grew well on polymeric films. In vitro cytotoxicity of drug loaded systems revealed better efficacy in cell killing and almost 78% killing was observed. In-vivo studies on melanoma model treated with drug loaded patch showed efficient tumor suppression as compared to control where tumor grew at faster rate. **Discussion:** These grafted copolymers showed sustained drug release which is due to grafting of PU chains making it hydrophobic systems against burst release in pure CD. It is the wrapping of PU which affects the drug release profiles. Biocompatible nature over HeLa cells further made them suitable biomaterial. In vivo results suggested that this inhibition of tumor by drug loaded copolymers was predominantly due to sustained release of drug for longer duration of time thud killing cells gradually. Conclusions: PU grafted CD copolymers were exhibited sustained drug release, were biocompatible and efficient tumor suppression was observed in In-vivo studies and thus can be a promising drug delivery vehicle against melanoma tumor. (DOI:10.9777/rr.2018.1151)

Role of black tea in prevention of Skin carcinogenesis

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Background: High Inorganic Arsenic (iAs) content in groundwater in West Bengal is a potential health hazard, resulting in many diseases including cancer of the skin. Arsenic exposure leads to DNA damage via generation of Reactive Oxygen Species (ROS), which, if not repaired, leads to initiation and finally progression of cancer. Epithelial to Mesenchymal Transition (EMT) plays a vital role in skin carcinogenesis. Modulation of these events by non-toxic phytochemicals may pave a way to prevent development of skin lesions, hence cancer. **Aim:** Prevention of skin carcinogenesis by black tea in Swiss Albino mice. **Methods:** Skin carcinoma was developed by topical application of DMBA along with iAs as a co carcinogen with Croton oil as a promoter. Histology using haematoxylin and eosin was performed to confirm the development of carcinoma and its different stages. ROS was estimated by a spectrofluorimeter and DNA

damage was measured by Single Cell Gel Electrophoresis (SCGE). Western Blotting was employed to study the expression of several markers, including those involved in EMT. **Results:** Treatment with DMBA and Arsenic led to the development of topical lesions (neoplasia), which was validated by histological examination. Extensive DNA damage was observed in the carcinogen-treated mice, which was effectively reduced by administration of black tea. EMT markers have been found to be aberrantly expressed in the carcinogen treated mice, as evident from Western Blot bands. The expression level of these proteins was modulated by black tea. **Discussion:** DMBA and arsenic lead to DNA damage due to ROS generation, repair of which is hindered owing to presence of iAs. Black tea effectively regulates the deviant expressions of proteins implicated in EMT. All these events cumulatively can aid in prevention of skin carcinogenesis in Swiss albino mice. **Conclusion:** Black tea plays a pivotal role in prevention of skin carcinoma. (DOI:10.9777/rr.2018.1152)

Nuclear Magnetic Resonance (NMR) analysis of a glucoside (Lusoside) isolated from Camel Milk

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NMR spectroscopy has a wide range of appications including the identification and structural studies of complex biomolecules like glucose , sucrose and milk oligosachharides etc. 1D H-NMR, 1D 13CNMR, 2D COSY, 2D TOCSY, 2D HMQC and 2D HMBC techniques were used to completely elucidate the structure of the biomolecules. The results obtained from the spectral data were systematically combined to elucidate the structure of glucoside (Lusoside). Full characterisation of Lusoside was achieved by assigning 1H and 13C signals, starting from the known to unknown signals. (DOI:10.9777/rr.2018.1153)

Knowledge, attitude, practice and drug utilization pattern in patients of epilepsy in North India

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Objective: To study knowledge, attitude, practice and drug utilization pattern in patients of epilepsy in North India. Materials and **Methods:** 50 patients who attended the neurology OPD and ward in SSH, BHU were included in the study. A questionare of 25 questions was administered to the patients in English or Hindi whichever the patient was proficient. The responses were recorded in yes, no or don't know. Drug utilization pattern was analyzed from the case sheets of the patients. **Results:** Most of the patients were of young age i.e<30 years (30%). Literacy rate was low with 72% of patients studied upto only school level. Patients belonging to Hindu religion (94%) were considerably more than Muslims. Good knowledge was seen as 94% patients had heard of epilepsy and 86% thought that it was treatable by modern drugs. Poor knowledge about cause was seen as 24% believed it to have a contagious spread. Positive attitude was seen as 72% thought that these patients can study, 84% felt that PWEs can do job and 92% felt that they can marry. However negative attitude was seen in few domains as 58% felt that they were discriminated by their schoolmates and 40% by their teachers and 52% wanted to hide the fact their daughter had epilepsy before marriage. Good practices

were seen as 74% knew that putting keys in a pt having convulsions was not helpful. The most common drugs used were Levetiracetam(50%), Valproate (40%), Clobazam (28%), Oxcarbazepine (16%), Carbamazepine (12%), Phenytoin (12%). 96% of the patients were controlled with antiepileptic drugs. 32% were controlled on single drig and 28% on 2 drugs. **Conclusion:** Analysis of Indian data revealed regional differences in KAP which could be attributed to factors, like literacy, awareness about epilepsy, and practice of different systems of medicine. There is a need to create awareness about epilepsy on a nation-wide basis to dispel the misconceptions and stigma through effective and robust programs with the aim to lessen the disease burden. The study also showed that most of the patients were controlled either on single or multiple drugs without the need of epilepsy surgery. (DOI:10.9777/rr.2018.1154)

Biofabrication and characterization of gold nanoparticles using endophytic *Fusarium* sp. and their applications

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Background: Low toxicity and cost effectiveness are the key features of bio synthesized nanomaterials. Endophytic fungi are relatively less explored microbes which according to studies could be efficient synthesizers of stable and monodispersed nanoparticles with noble shapes. Here we report the mycosynthesis of gold nanoparticles (AuNPs) by an endophytic Fusarium sp. designated as ACQR8 which has been isolated from the root tissues of Cissus quadrangularis, a well known medicinal plant. **Aim:** Green synthesis of gold nanoparticles from endophytic fungus Fusarium sp. and their applications. **Methods:** Gold nanoparticles were fabricated using cell free supernatant of endophytic Fusarium sp. The synthesis was confirmed by spectrophotometric technique. The physical conditions for biofabrication like temperature and pH were optimized. The characterization was done by TEM-EDX, FTIR and XRD techniques. The AuNPs were conjugated with commercial antibiotics like ciprofloxacin and tetracycline and the antibacterial efficacy was evaluated. **Results:** Temperature at 65°C and pH at 5 were best suited variables for the rapid biofabrication of AuNPs. The Surface Plasmon resonance of AuNPs was observed at 536 nm. The AuNPs were more or less spherical in shape and their size ranged from 13 nm to 28 nm with an average size of 21 nm with crystalline nature. The ciprofloxacin and tetracycline successfully coated the nanoparticles and showed an increase in growth inhibitory activity against bacterial pathogens. **Discussion:** The cell free supernatant of endophytic Fusarium sp. can efficiently fabricate monodispersed AuNPs. The best temperature and pH range for synthesis was in accordance to some previous reports. Tetracycline and ciprofloxacin coated AuNPs were proved to be more efficient in antibacterial activity as compared to antibiotics alone. **Conclusion:** The surface properties of AuNPs can be successfully exploited for interaction with antibiotics for enhancing their antimicrobial effects. (DOI:10.9777/rr.2018.1155)

To determine the distribution of anemia in people of gurugram, Haryana

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Background: Anemia is a condition, not a disease in which the number of RBCs (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a person to person and their age, gender, environment (altitude), smoking behavior, and different stages of pregnancy. Iron deficiency is thought to be the most common cause of anaemia globally, but other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival, can all cause anaemia. The prevalence of anaemia is an important health indicator and when it is used with other measurements of iron status the haemoglobin concentration can provide information about the severity of anemic conditions. **Aim:** To determine the distribution of of anemia in people of Gurugram, Haryana. **Material & Methods:** One hundred and sixty six cases were included in four months study period. The associations of Hemoglobin with anemia of different method were determined. **Results:** Out of 166 of participants, 85 (51.2%) were females and 81 (48.8%) were males. The study showed the association of anaemia with sex. 48 (28.9%) individuals were hypochromic of which 33 (19.9%) were females and 15 (9.0%) were males. Association of anaemic disease with age was also observed. Participants with less than 10 years had more hypochromia 14 (8.4%) and those with age of 30-40 had lowest level with P value 0.001. Highest number of participants showed RBC 4.5-5.5 million/mm^3 (24 individuals - 14.5%) with P value 0.000. **Discussion:** The increased incidence of anemia among females can be correlated with childbirth, menstrual cycle compounded with poor diet and lack of intake of iron supplements. India tops the list of anemic countries in the world. **Conclusion:** All the samples after processing revealed different types of anemia. There is the need of certain iron supplements in their diet to reduce the different types of anemia. Further analysis is required for detailed classification of anemia. (DOI:10.9777/rr.2018.1156)

Phytochemical screening and *in-vitro* antioxidant activity of *Terminalia belerica* fruit ethyl acetate fraction

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Background: The free radicals constitute a group of reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide, and super oxide anion. Overload of free radicals play a major role in the development of degenerative and chronic ailments. In current era, herbal products are measured to be the symbols of safety in comparison to the synthetic products that are regarded to be hazardous to human life and environment. Hence search of herbal products, their chemical characterization and antioxidant assessment bear significance for reducing ROS mediated diseases. **Aim:** The present study was designed to investigate the phytochemical composition and antioxidant potential of T. belerica fruit ethyl acetate fraction using various in vitro assays. **Methods:** Phytochemical screening was done by chemical methods. The in vitro antioxidant efficacy of ethyl acetate extract was

determined by DPPH free radical scavenging assay, reducing power assay, phosphomolybdate assay, hydroxyl radical scavenging activity assay and FRAP assay. Additionally Quantitative estimation of total phenol and flavonoid were also carried out. **Results:** Chemical analysis showed presence of major phytochemicals viz., phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides in the extract. Quantitative measurement of the sample showed higher content of phenolics and flavonoids. All the in vitro assays exhibited considerable antioxidant and free radical scavenging ability in the test extract. **Discussion:** Presence of appreciable antioxidant activity in the sample was directly correlated with phenolic and flavonoid contents. Hence T. belerica fruit phytoconstituents could be utilized for developing antioxidant drug of natural origin. **Conclusion:** T. belerica fruits possess considerable antioxidant and radical scavenging activities. (DOI:10.9777/rr.2018.1157)

Formulation and evaluation of nanoformulation for brain delivery with low toxicity

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Background: The blood–brain barrier (BBB) prevents the entry of drugs into the brain; it is a challenge to treat central nervous system disorders pharmacologically. Many anticancer, anti-HIV and other CNS drugs achieve very low bioavailability in brain. The development of nanotechnology provides potential to overcome this problem **Aim:** Formulation and evaluation of nanoformulation for brain delivery with low toxicity. **Methods:** The lactoferrin (Lf) conjugated poly (ethyleneglycol)–poly (lactide) nanoparticle (Lf–NP) was formulated to facilitate the transport of the nanoparticles across the blood brain barrier (BBB) by receptor-mediated transcytosis via the Lf receptor present on cerebral endothelial cells. Lf was thiolated and conjugated to the distal maleimide functions surrounding on the pegylated nanoparticles to form the Lf–NP. The conjugation was confirmed by Fourier transform infrared (FTIR) spectroscopy and electrospray ionisation (ESI) mass spectroscopy (ESI-MS). TEM observation and ELISA analysis confirmed the existence of active Lf on the surface of Lf-NP Results and **Conclusion:** The significant in vitro and in vivo results suggest that Lf–NP is a promising brain drug delivery system with low toxicity. (DOI:10.9777/rr.2018.1158)

Bioactivity of actinomycetes isolated from a pristine cave in garhwal Himalaya

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Background: A substantial amount of effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years. The rate of discovery of novel compounds from terrestrial actinomycetes has decreased significantly. Considering the alarming rate of increased drug resistance in pathogens and limitations in the isolation of effective bioactive components from terrestrial actinomycetes, it is paramount to characterize the new groups of actinomycetes from pristine habitats as a source of novel and potential bioactive secondary metabolites. **Aim:** The present work has been designed to screen

and characterize the bioactive metabolites of actinomycetes isolated from an unexplored cave in Garhwal Himalaya. The recovered isolates were screened for their antimicrobial potential against commonly encountered gram positive and gram negative pathogens. **Methods:** Actinomycetes inhabiting the study area were isolated from collected samples by culturing on selective media. Recovered isolates were identified on the basis of microscopic and cultural characteristics. Actinomycetes isolates were screened for their antibacterial spectrum via primary screening. The test bacteria used were Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, and Streptococcus pneumoniae. Isolates showing positive results in primary screening were subjected to secondary screening. **Results:** In the present study, a total of 19 actinomycetes were recovered. 15% of the actinomycetes showed inhibition against all the three gram positive test pathogens. Only 1% of the isolates were able to inhibit Pseudomonas aeruginosa. None of the actinomycetes inhibited E. coli. **Discussion:** The actinomycetes recovered in the present work have shown variable activity between gram positive and gram negative bacterial strains. The results clearly demonstrate that from the tested pathogens gram positive were highly susceptible to the screened actinomycetes as compared to the gram negative pathogens. The secondary screening results were significant. **Conclusion:** The discovery of bioactive compounds of actinomycetes is inimitable and unexcelled in clinical significance. The present work gave us an idea about the antibacterial capabilities of the actinomycetes recovered from the pristine cave. However, further work is required in order to indentify the bioactive compounds responsible for the bioactivity of these actinomycetes. (DOI:10.9777/rr.2018.1159)

Recognition of F-, CN-, and Fe2+ ions by a benzimidazolylterpyridene

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Background: The design of sensors capable of recognizing and sensing both cations and anions is one of the most challenging fields because of the important role they play in biological, industrial and environmental processes. The sensitive and selective detection of ferrous, fluoride and cyanide ions is important because of their crucial biological roles. **Aim:** Development of ligands (4'-[4-benzimidazol)- phenyl]-[2,2':6',2"]terpyridine) for the construction of suitable multichannel sensors that are capable of recognizing cations and anions. **Methods:** Synthesized compound was analyzed using CHN analysis, IR, 1HNMR, 13CNMR, ESIMS, absorption, emission spectroscopy, XRD. **Results:** A new sensor BIT- terpyridine, was synthesized, where the terpyridine moiety has been utilized as the cation binding site and the imidazole motif as the anion binding site. The receptor can act as colorimetric sensor for Fe2+ and F-/CN- ions in solution. The binding properties have been confirmed by absorption, emission and 1H NMR spectroscopic techniques. **Discussion:** The binding site for the Fe2+ ion in the system has been unambiguously established by single-crystal X-ray diffraction study of the Fe (II) complex of the receptor. Anion sensing studies indicate that in the presence of excess F-/CN- deprotonation of the imidazole N–H proton of the receptor occurs with the development of a bright

yellow color. **Conclusion:** The terpyridylimidazole ligand behaves as a triple-channel sensor for both Fe2+ and F-/CN- ions in solution. It is also interesting to note that, as the emission color of the receptor was finely tuned from violet to blue to green to finally yellow, the receptor can act as a suitable solvatochromic probe. (DOI:10.9777/rr.2018.1160)

Serum peptidome for cancer detection

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The low molecular weight region of the serum peptidome contains protein fragments derived from 2 sources (a) high-abundance endogenous circulating proteins and (b) cell and tissue proteins. While some researchers have dismissed the serum peptidome as biological trash, recent work using mass spectrometry based (MS-based) profiling has indicated that the peptidome may reflects biological events and contain diagnostic biomarkers.In this issue of the JCI, villanueva et al. report on MS-based peptide profiling of serum sample from patients with advanced prostate, bladder or breast cancer as well as from healthy controls. Surprisingly, the peptides identified as cancer-type-specific markers proved to products of enzymatic breakdown generated after patient blood collection. The impact of these results on cancer biomarker discovery efforts is significant because it is widely believed that proteolysis occurring ex vivo should be suppressed because it destroys endogenous biomarkers. Villanueva et al. now suggest that this suppression may in fact be preventing biomarker generation (DOI:10.9777/rr.2018.1161)

Vitamin A, β-carotene and vitamin E levels in cows with metritis

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Background: Metritis, an inflammation of the uterus, is caused by bacterial infection, and usually is seen following calving. It occurs most commonly after calvings complicated by dystocia, retained fetal membranes, twins or stillbirths. The antioxidant defense system of periparturient dairy cows may be overloaded with reactive oxygen species, predisposing animals to immune-system-related diseases, such as retained placenta, metritis, and mastitis. **Aim:** The aim of this study was to evaluate serum antioxidant vitamin levels in cows with postpartum metritis. **Methods:** Vitamin A, beta carotene and vitamin E levels were measured in blood samples collected from cows with metritis and health control group cows with HPLC. Microbiological analyses for E. coli screening were performed on bloody agar and salmonella shigella agar. Cows were fed with routine content (hay, silage, clover, turnip paste, pulp etc) was used in the ration. In addition, concentrated feed (protein ratio 21%) was added during the experiment. The blood samples were collected and analyzed from 7 days to 60 days after birth. **Results:** As a result, blood vitamin A, vitamin E and beta-carotene levels in the metritis group were found statistically lower than the control group at $p \leq 0.01$. **Discussion:** The prepartum supplementation of multiparous cows with β-carotene and vitamin E might be associated with a lower incidence of retained placenta and metritis that occurs after delivery. (DOI:10.9777/rr.2018.1162)

Statistical optimization of reaction condition of L-Methioninase from *Bacillus subtilis*

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Background: L- Methioninase (methionine γ-lyase) has important biotechnological application because of its hydrolytic property to catalyze L-methionine to α- ketobutyrate, methaneethiol and ammonia. Nessler's method was found to be simple and economically easy to estimate L-methioninase activity for released product ammonia. Response surface methodology used as an effective statistical tool to optimize conditions of enzyme and preferred the conventional method of "one variable at one time" because of accounting interactive effects of the variables with screening and prediction of large experimental domain. Process variables like temperature, pH, and substrate volume and incubation period are considered as significant parameters for assay method of L-methioninase. **Aim:** The objective of this study was to optimize reaction condition of L-Methioninase secreted from Bacillus subtilis using Response Surface Methodology. **Methods:** Bacillus subtilis was grown in nutrient media of pH 7 at 35C̊ and L-- methioninase was produced by adding 1% inoculum into nutrient media supplemented with 0.25% L- Methionine and incubated at 35C̊ , for 12-18 hrs in orbital shaker at 120rpm. The fermented broth was centrifuged at 10000 rpm for 40 min and pellets were resuspended in 0.05 M potassium phosphate buffer pH7 followed by sonication. Sonicated sample were used for the enzyme activity .One unit of L- methioninase activity was defined as enzyme required for production of 1 µmoles of ammonia per minute per ml of enzyme solution at standard conditions. The optimization strategy of RSM (MINITAB version15) was applied for different assay parameters (pH- 7 to 11.5, temperature-23˚C to 51˚C, substrate volume -0.2 to 0.8ml, incubation period-10 to 50min) to estimate optimum activity of L– Methioninase. The experimental data was analyzed on basis of multiple regressions and ANOVA. Second order polynomial model was designed by estimating predicted activity of L-methioninase using following quadratic equation:$y = \beta + \Sigma \beta X + \Sigma \beta X^2 + \Sigma \beta XX$ Results and **Discussion:** The value of coefficient of determination (R^2) as 0.9436 showed good fitness of RSM model for assay parameters. Multiple regression analysis and ANOVA showed optimum assay conditions of pH of reaction mixture, reaction time, incubation temperature, and substrate volume as 8.5, 30 minutes, 35˚C and 0.5ml respectively. The predicted activity of L-methioninase was estimated as 17.4 Unit at these optimized reaction parameters. The activity of L-Methioninase was enhanced by 0.6 fold after optimization of reaction conditions. Conclusion RSM has been employed as efficient statistical tool to enhance activity of L-methioninase from Bacillus subtilis in comparison to classical methods. (DOI:10.9777/rr.2018.1163)

Purification and characterization of Trypsin Inhibitor from *Garcinia cambogia*

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Background: Protease inhibitors are molecules which inhibit the activity of proteases. Trypsin inhibitors are serine protease inhibitors which reduce the biological activity of trypsin. Increased activity of trypsin is involved in the progression of many diseases. Isolation of Trypsin inhibitors may

help to reduce the activity of trypsin and thus may prevent the diseases caused by the excess activity of trypsin. **Aim:** Isolation and and Characterization of Trypsin Inhibitor (TI) from Garcinia cambogia. **Methods:** The seeds of Garcinia cambogia were collected from different villages of Calicut District, Kerala. The seeds were dried under shade and ground. The seed flours were defatted and extracted using 50mM Sodium Phosphate buffer with pH 7.6. Trypsin inhibitory activity was assayed on each step of purification according to the procedures described by M. L Kakade et al., (1974).TI was separated using Ammonium Sulfate precipitation and dialysis. Purification of TI was done by Ion exchange chromatography using DEAE-cellulose column and size exclusion chromatography with Sephadex G75. Activity staining was done according to the protocol followed by Felicioliet al. (1997). Temperature stability of the purified TI was analysed. Kinetic analysis was done and the inhibition constant KI was determined by Dixon plot at two different concentrations of BApNA (0.001M and 0.0005M). Protein content was determined using the method described by Lowry et al. **Results:** The Garcinia cambogia seed showed significant trypsin inhibitory activity when isolated using 50mM Sodium Phosphate buffer at pH 7.6. The homogenous band of activity staining indicates the presence and purity of trypsin inhibitor. Temperature stability study showed that trypsin inhibitory activity is highest at 37^0C and was stable up to 100^0C. The Dixon plot indicated that the inhibitor is competitive in nature. **Discussion:** Trypsin inhibitors from dry seeds of Garcinia cambogia were isolated, purified and characterised. **Conclusion:** Garcinia cambogia seeds showed significant trypsin inhibitory activity. (DOI:10.9777/rr.2018.1164)

Generation and directed differentiation of transgene free porcine (*Susscrofa*) induced pluripotent stem cell towards cardiomyocytes

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The World Health Organization has estimated that about 20 million peoples were died from cardiovascular disease (CVD) in 2015, which constitute about 30 percent of total death occurred in the world at that time. It has been estimated that the value of death rate by CVD alone may increases up to more than 75% of the total death in the world by 2030. Since human heart lacks a regenerative pathway unlike lower vertebrate hence the treatment of human heart diseases by regenerative medicine is remain a challenge for medical science. Therefore, the research has to be focused on cell transplantation. Porcine is known for its physiological and anatomical similarities with humans; hence it is the best model to treat human diseases. Pluripotent stem cells (PSCs) generated from porcine i.e., piPSCs will provide important clinical insights for cardiac cell therapy as the cardiovascular system of porcine is very similar to humans. We attempted to generate bona fide porcine pluripotent stem cell by using non-integrating strategy. In brief, target cells were transfected daily for day 17 by using various reprogramming factors. Noticeable morphological changes appear at day 6 of the transfection, where the cluster of proliferating cells begins to emerge. Morphological changes were more evident by day 15 and 18.iPSCs like colonies started to emerge by day 15. On the basis of morphology and stringent

pluripotent markers subset of 14 -16 clones were identified, mechanically picked and passaged further in standard embryonic stem cell culture conditions. (DOI:10.9777/rr.2018.1165)

The defect in energy equilibrium and family history augment osteoarthritis associated with matrix metalloproteinase: A study on Indian patients

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Background: Worldwide Osteoarthritis (OA) is highly prevalent disease. It is leading cause of disability and can negatively impact people's physical and mental well beings. A disturbance in Energy equilibrium is among the most common risk factor of OA. Matrix metalloproteinases (MMPs) are a potential marker for arthritis reported by some groups globally. MMP-9 is a gelatinases family member secreted extracellularly which can cleave extracellular matrix, and MMP-17 has been implicated in the activation of ADAMTS4, one of the key Aggrecanase that can cleave aggrecan protein, which is the major cause of Osteoarthritis. **Aim:** The aim of this study is to establish the association of serum MMP-9 and MMP-17 level with cartilage degradation. **Methods:** NIH criteria were used to categorize the patients for Body Mass Index (BMI). Human MMP-9 and MMP-17 were assessed with the help of sandwich ELISA assay kit. The association of Matrix Metalloproteinase and Clinico-pathological characteristics of the disease was assessed. Statistical analysis was performed by using SPSS-16.0 software. **Results:** The mean age of the study group was found 67.43±5.47 and 64.42±4.81 for control and cases respectively. Males are more affected than females. BMI, MMP-17 and MMP-9 were compared for study group. MMP-9 and Elevated BMI was found to be significant with controls and cases respectively, while MMP-17 was not found to be significant with study group. Family history of OA and Obesity was found to be significant with study group. Discussion & **Conclusion:** The results of above study imparted that disturbance in energy equilibrium and Genetic inheritance of OA may be a putative risk factor of Osteoarthritis. (DOI:10.9777/rr.2018.1166)

Impacts of diurnal variation ofultraviolet radiation and photosynthetically active radiation on phycocyanin of the cyanobacterium *Spirulina platensis*

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Background: Phycocyanin (PC) is one of the components of phycobiliproteins of cyanobacteria which constitute up to 60 % of the soluble proteins of the cell. PC is typically composed of α and β monomers with molecular masses in the range of 12-20 and 15-20 kDa, respectively. Properties of PC such as oxygen radical scavenging, anti-inflammatory, neuroprotective, hepatoprotective and anti-cancerous increases its importance in biomedical sciences. **Aim:** Impacts of diurnal variation of ultraviolet and photosynthetically active radiation on PC of the cyanobacterium

Spirulina platensis. **Methods:** The culture of cyanobacterium was irradiated in Petri dishes with white cool fluorescent, UV-B and UV-Aradiations. The Petri dishes were covered with different cut-off (395 nm, 320 nm and 295 nm) filter foils. Subsequently, equal amount of samples were removed after continuous exposure upto 48h at constant temperature of 25±2°Cand spectrophotometrically analyzed the photostability of PC and photosynthetic pigments. **Results:** The carotenoids, protein and activity of antioxidative enzymes (SOD, CAT and APX) increases significantly as ultraviolet radiation (UVR) exposure increases and the Chl. a content as well as absorbance and fluorescence values of PC decreases as duration of UVR exposure increases. While no significant changes was observed in PAR irradiated samples. **Discussion:** The effects of PAR, PAR+UV-A and PAR+UV- A+UV-B irradiations showed variable synthesis and destruction in PC due to uncoupling and photobleaching of its subunits. However, PAR had less deleterious effects in comparison to PAR+UV-A and PAR+UV-A+UV-B on PC. Conclusions: Overall, it may be concluded that PC content in the cyanobacterium Spirulina platensis was more stable in PAR as compared to PAR+UV-A and PAR+UV- A+UV-B irradiations. The stable PC of Spirulina platensis may be used in many fields such as pharmaceutical, biotechnological and biomedical industries. (DOI:10.9777/rr.2018.1167)

Mass transfer optimization for the antibiotic daptomycin in a stirred tank bioreactor

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Background: Daptomycin, is the first lipopeptide antibiotic to be approved by US-FDA, it shows strong activity against several multi-drug resistant Gram-positive pathogens. Deptomycin is a secondary metabolite produced by the actinomycetes Streptomyces roseosporus in the stationery phase of growth cycle. S. Roseosporus is an aerobic microorganism and O2 acts as a growth limiting factor. Its filamentous morphology increases the air mass transfer limitation when produced industrially in a bioreactor. **Aim:** To increase the volumetric oxygen mass transfer coefficient, KLa value for maximising the Daptomycin production. **Methods:** Experiment is carried out in a 3 L bioreactor using S. roseosporus strain grown on MGYP media and further sub-cultured in YEPD broth. Decanoic acid is added after 48 hrs of inoculation as a precursor. The growth and substrate utilization curve is plotted. KLa is measured using dynamic gassing out technique. **Results:** A typical microbial growth curve is obtained with lag, exponential, stationery and decline phase. Maximum cell biomass obtained is 18.8 g/l and Substrate concentration decreases from 60 to 2.6 g/l. sudden decrease in substrate concentration is observed on 6th day. Maximum and minimum KLa value obtained is 38.56 hr-1 and 12.32 hr-1 respectively. **Discussion:** The rheology of the media changes and shows Non- Newtonian behaviour as the batch continues. The rheology affects the KLa value and it decreases. Increasing agitation rate increases the KLa value. **Conclusion:** The S. roseosporus is a filamentous microorganism and require high amount of air for proper growth. The filamentous growth changes the rheology of the growth medium which results in decreased KLa value. Agitation rate can be increased within a range only. For the optimum growth non- conventional

bioreactors are required to be used. (DOI:10.9777/rr.2018.1168)

Evaluation of *in-vitro* extractability of calcium in *Elucine coracona* (Finger millet) with respect to processing, blending and supplementation

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Finger millet (Eleusine coracana) is a rich source of calcium and can be incorporated in diet by post menopausal women to alleviate the calcium deficiency related problems. In this study the finger millet was processed by soaking, germination and roasting for incorporation into a nutraceutical supplement Extractability of calcium was determined by complexometric titration using EDTA. There was a prominent increase in the in vitro extractability of calcium in case of germinated and roasted millets. Phytic acid was reduced by 70% during the overall processing. The effect of blending Finger millet with Horse gram (Macrotyloma uniflorum) was also carried out and the results showed an increased calcium extractability and content. Supplementation with vitamin C in the form of dry Amla (Phyllanthus emblica) powder also showed an increased mineral extractability. This combination of processing, blending and supplementation can be used in developing diet supplements for enhancing calcium intake in post menopausal women. (DOI:10.9777/rr.2018.1169)

Biopolymeric nano anti-diabetic drug

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Background: The present study focus on the design, synthesis, characterization and drug delivery application of core shell nanoparticles by making use of natural sources such as zein, pectin and flavonoids. **Aim:** Aim of the study was to encapsulate Mimosa pudica extract into zein-pectin core-shell nanoparticle and evaluate its hypoglycemic activity. **Methods:** The extract was prepared by soxhlet extraction from Mimosa pudica using ethyl acetate and its anti diabetic activity was studied.The extract was encapsulated into zein-pectin core-shell nanoparticle by ultrasonication method and was characterized by different techniques such as SEM, FT-IR, and UV-Visible spectroscopy. The extract encapsulated zein- pectin nanoparticles (EZPN) were then subjected to in-vivo studies. **Results:** The ethyl acetate extract of Mimosa pudica exhibited α-amylase and α-glucosidase inhibitory action as 523±0.7071 and 320.97±0.55 respectively. The SEM results showed that the EZPN's formed are amorphous, spherical with a size ranging from150-200 nm diameter. The UV-Visible and FT-IR characterization confirmed the incorporation of all the components in EZPN's. Non toxic nature of the extract and the EZPN's were confirmed. In vivo anti-diabetic activity study showed that lower doses of EZPN's at 200 mg/Kg. B.Wt. lowered plasma glucose concentration (124.5 ± 19.33); ALT and AST (110.33 ± 6.8; 230.33 ± 19.98) significantly. The liver glycogen (0.92 ± 0.02) level; hexokinase (251.43 ± 8.42) and glycogen phosphorylase (193.32 ± 16.59) activity was increased in lower doses of EZPN's. **Discussion:** The study indicates that when compared with control EZPN's exhibit significant anti-diabetic activity at a dose of

200mg/kg body weight than higher dose of EZPN's and different doses of extract. It was found that EZPN's are nontoxic and exerts promising effect in lowering hyperglycemia. **Conclusion:** It can be concluded that the EZPN's at lower dose of 200mg/kg body weight exerts promising effect in lowering hyperglycemia. (DOI:10.9777/rr.2018.1170)

A novel strategy for anti-hyperglycemic and anti-oxidative stress effect of *Vicia faba* seed extract in *Saccharomyces cerevisiae* 3187

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Background: Diabetes mellitus is a group of metabolic diseases that having features such as high levels of glucose in blood (hyperglycemia) and due to lack in production or action of insulin produced by the pancreas inside the body. Oxidative cellular damage is due to free radicals that contribute to the development of diabetes mellitus. **Aim:** The aim of this investigation was to examine the anti- hyperglycaemic potential and anti-oxidative stress effect of Vicia faba seed extract in yeast cells. **Methods:** Vicia faba seed extracts were exploited for their effects on glucose adsorption capacity, in-vitro glucose diffusion, in vitro amylolysis kinetics and glucose transport inside the yeast cells. Any nanoscopic differences in the cell surface morphology of the yeasts Saccharomyces cerevisiae were studied by using an atomic force microscopy (AFM). **Results:** The hypoglycaemic effect exhibited by the extracts of seed is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple in vitro model of yeast cells. Oxidative stress results in decrease in the mean cell volumes of S. cerevisiae and raise in cell roughness as they were exposed to increasing H2O2 concentrations for 1 h but if we treated with extract with H2O2 and it will cased reverse effect. **Discussion:** Hypoglycemic and anti-oxidative stress effect may due to synergistic effect of all the constituents present in seed extract or acting separately. **Conclusion:** In view of the adverse effects associated with the synthetic drugs and as natural resources are safer, cheaper and much effective so, conventional antidiabetic plants can be explored. Vicia-faba seed extract can be act as lead compound for drug discovery. (DOI:10.9777/rr.2018.1171)

Purification and characterization of *de novo* synthesized, Antioxidant β-amylase involved in raw starch degradation in fenugreek seeds

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Background: β-Amylase (E.C.3.2.1.2) hydrolyzes the α-1,4-glycosidic linkages in starch and related polysaccharides, removing maltose units from the non-reducing ends of the chain. After hydrolysis β-anomeric form of maltose is formed. The enzyme does not require cofactors for catalysis and play important role in seed germination and starch degradation. **Aim:** Purification and Characterization of De Novo Synthesized β-amylase from germinated Fenugreek Seeds. **Methods:** β-Amylase was purified by acetone fractionation (45-60%) followed by chromatography on carboxymethylcellulose and finally glycogen precipitation. Homogeneity and molecular weight were determined by using SDS-PAGE, size exclusion chromatography and western blotting techniques. De Novo synthesis and

antioxidant property of the enzyme were confirmed by using translational inhibitors and DPPH (1,1diphenyl-2-icryl hydrazyl) radical scavenging methods. **Results and Discussion:** The enzyme was purified 231 fold with specific activity of 782.01 units/mg. The molecular mass of the enzyme was determined to be 54 kDa. The optimum pH and temperature for the enzyme were 5.3 and 57 ºC, respectively. The enzyme had Km value of 16.2 mg/ml and Vmax of 833.33 µmole/min/mg. The enzyme hydrolyzed starch, glycogen, amylose and amylopectin but did not hydrolyzepullulan that showed inability of the enzyme to hydrolyzeα-1,6- glycosidic linkage. Inability to hydrolysis starch azure showed endo-amylase nature of the enzyme. The enzyme showed 20% activity with raw starch with respect to soluble starch (100%) i.e., the enzyme was capable of hydrolyze raw starch. The enzyme exhibited strong antioxidant DPPH radical scavenging activity with IC50 value of 300 µM and 99 µM for ascorbic acid and purified β-amylase, respectively. **Conclusion:** β-Amylase can synthesize De Novo, with potential to degrade raw starch and scavenging activity. Understanding the co-occurrence between amino acids composition and antioxidant activity could lead to the development of new class of effective antioxidant, which can serve as better alternative in many food applications. (DOI:10.9777/rr.2018.1172)

Development of novel polyherbal wound dressing material using chitosan polyvinyl alcohol composite

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Development of efficient wound dressing material is one of the most promising requirements in current medical applications. The current study delineates the preparation and evaluation of poly-herbal composite bilayer membrane as a wound dressing material. A novel bilayer composite of polyvinyl alcohol (PVA) and chitosan was fabricated using solution cast technique with an outer chitosan layer and inner PVA layer to meet the desired biological functionality of bilayered membrane. Azadirachta indica extract was loaded on chitosan film and Ficus benghalensis and Aloe Vera extract were loaded on PVA film layer. Furthermore, the developed bilayered membrane was characterized by various physicochemical and controlled release properties. Developed poly-herbal loaded membrane shows good tensile strength, water retaining capacity and optimal swelling property. Also, the membrane shows a high degree of hemocompatibility with sustained drug release activity. Drug release study from the membrane shows sustained release of active ingredient form the bilayer membrane. It is proposed that the developed herbal bilayer membrane exhibits immense potential to be used as wound dressing materials. (DOI:10.9777/rr.2018.1173)

Analysis of ground water during summer season nearby industrial area of district- Balrampur

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Present study deals with assessment for groundwater quality at different sites in district Balrampur. The samples were collected to analyze the physic-chemistry and suitability of water for

irrigation and domestic purposes. Selected ground water parameters were pH, dissolved oxygen (DO), TDS, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Acidity, Total Alkalinity, Free Carbon dioxide (free CO) as well as Total Hardness (Ca^{2+}/Mg^{2+}). To analyse the water quality in different areas named as 1, 2 & 3 were compared. Comparison showed the pH level was slightly acidic (6.7 •0.17) at site-1 & 2. Low values of DO (3.7 0.43) at site-3, high values of BOD (>3mg/L) at all four sites especially at site-2 (5.10 0.52), high value of alkalinity at all three sites and high values of Free CO2 (>2) at sites 1 & 3. Each parameter was compared with the standard desirable limit prescribed by WHO. For the statistical analysis, values of mean, standard deviations and correlation co-efficient (r) were calculated. The Karl Pearson Correlation matrix has approved the influence of temperature on cod, alkalinity and COD- alkalinity with significantly positive correlation. The study showed that groundwater quality is not so good for irrigational and drinking purposes and it should be well treated before use. (DOI:10.9777/rr.2018.1174)

A study of H-bond network originating from P-loop of NTPases

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Background: P-loop nucleotide binding and hydrolyzing proteins (NTPases) are involved in diverse functions ranging from signal tranduction, translation, DNA repair, membrane transport etc. A common structural element, Walker A motif (GXXXXGKS/T) or P-loop with variant (XXXX) and invariant G, GKS/T residue positions, plays an important role in imparting molecular function. P-loop has a typical arched shaped topology that accommodates and positions the phosphate moiety of the nucleotide for the hydrolysis to take place. **Aim:** We aim to study the H-bond interactions in and around P-loop motif of the diverse set of P-loop NTPases to reveal the structural features impacting the stability and topology and functions of P-loop. **Methods:** X-ray crystal structure data of P-loop NTPases were retrieved from RCSB Protein Databank. Identification, visualization and analysis of H-bond interactions of P-loop were performed using computational approaches. Results and **Discussion:** H-bond analyses of P-loop motif for 79 diverse and non-redundant NTPase X-ray structures were performed. We find that number of H-bond interactions originating from P-loop residues were diverse ranging from 5 to 14. 40% of H-bond was contributed by P-loop and residue located at least 10 residues apart. The H-bond interactions between P- loop residues and adjacent residues (within 10 residues) were 34%. The H-bond interactions between conserved invariant residues: 8%, between variant residue positions: 9% and between Invariant and variant positions were 9%. The H-bond variation indicates two groups of P-loop: rigid and flexible. A set of local and distant residues form a microenvironment around P-loop which provides stability and topology. A perturbation in local H-bond network and flattening in arched shape of P-of loop in mutant NTPases suggest its role in accommodating the nucleotides in the binding pocket. (DOI:10.9777/rr.2018.1175)

Heat induced expression of heat shock proteins in *Setaria cervi* and their cross reactivity with filarial infected human sera

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Background: Lymphatic filariasis is a mosquito born parasitic disease which causes a major health problem in the tropical and subtropical region. The available antifilarial drugs eliminate only larval stage but ineffective against adult worm therefore there is urgent need to cure this disease. HSPs are one of the most important proteins which maintain cellular homeostasis of parasite. **Aim:** To study the role of filarial Heat Shock Protein as a potential diagnostic marker during early infection of disease. Methodology: Bovine filarial parasite S. cervi was exposed to heat stress at 42°C for 1 hour and 2 hour respectively. Crude cytosolic extract was resolved on 10% SDS-PAGE. The bands obtained were excised by in trypsin digestion which was further identified by MALDI mass sequencing. Antigenic cross reactivity was assessed by using different category of filarial infected human sera through western blot. ELISA was performed using ScHSP70 and filarial infected human sera. **Results:** SDS-PAGE analysis showed over expression of proteins of molecular weight ≈ 90kDa, 70kDa, 60kDa, 27kDa, 18kDa and 12.5 kDa in the treated parasite. Sequences obtained by MALDI mass sequencing of these proteins were identified as HSP 70 and 18. Western blot analysis of filarial infected human sera revealed antigenic cross reactivity with whole IgG antibody against chronic and microfilariae infected human sera. Antigenic cross reactivity of scHSP70 was found higher in microfilarae infected human sera than chronic infected human sera by ELISA. **Discussion:** Present studied showed that heat stress induced the expression of heat shock proteins .These filarial HSPs are immunogenic in nature and showed antigenic cross reactivity with whole IgG antibody against human filarial sera like chronic, microfilariae. ScHSP 70 showed higher antigenic cross reactivity with microfilarial infected human sera. **Conclusion:** Therefore, this study suggests that filarial HSPs as an early diagnostic marker for lymphatic filariasis. (DOI:10.9777/rr.2018.1176)

Hydrogen peroxide improves growth traits, photosynthetic efficiency and biochemical attributes in tomato plants grown under copper stress

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H_2O_2 functions as a signaling molecule and modulates range of biochemical and physiological responses in plants. Therefore, an experiment was conducted to study the mitigative role of H_2O_2 in copper stressed tomato plants. H_2O_2 (0.1 or 0.5 mM) was applied to the foliage of tomato (Lycopersicon esculentum Mill.) plants at 30 days after transplantation (DAT) under copper stress (10 or 100 mg kg^{-1}soil). High copper stress (100 mg kg^{-1} soil) induced a significant reduction in growth traits, chlorophyll content and rate of photosynthesis at 40 days after transplantation. Activities of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) and leaf proline content also increased substantially with increasing copper stress. On the other hand, treatment of H_2O_2 under stress and stress- free conditions significantly increased the aforesaid growth traits and biochemical parameters. Moreover, H_2O_2 further accelerated the antioxidative enzymes and proline content, which

were already enhanced by the high copper stress. It is concluded that treatment of H2O2 (through foliage) significantly improved the growth traits, photosynthetic efficiency and various biochemical attributes under cu stress and stress-free conditions through modulation of antioxidant system. (DOI:10.9777/rr.2018.1177)

Kinetics of soluble and immobilized Urease from chickpea *(Cicerarietinum)*

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Introduction: Urease has a long and distinguished history. Being the first enzyme to be obtained in crystalline from Jac bean by Sumner in 1926. **Aim:** To perform the kinetics study of soluble and immobilized Urease from chick pea. **Methods:** Urease is isolated from cicer aritenium and it purified by Acetone fractionation and pH Fractionation subsequently. Inhibition studies done by (Cu^{2+}, Ag^{+}, Cd^{++}, Ni^{2+}) metal ion and, immobilization of Urease done by calcium alginate beads. Results & **Discussion:** Urease has been purified from cicer arietinum. pH fractionation result in 5 fold purification with 93.97%. The specific activity of purified enzyme (681.81units/ml) was much higher than partially purified (136.36 units/ml) and crude (36.36 units/ml) respectively. Protein content of purified enzyme was found to be 0.05 mg/ml. The activity of the immobilized enzyme was 204 units/beads, and the % immobilization was 60%and total units were 15. The Km and Vmax value of soluble enzyme found to be 8.9 and 0.4 extinction respectively. Immobilized enzyme found to be stable at 70° C also were soluble enzyme has optimum temperature of 60°C. The Q value of Immobilized enzyme was found to be 2 and activation energy 11.42 Kcal/mole. The Optimum pH of immobilized enzyme was 8.0. Inhibition of urease by metal ion (Cu^{2+}, Ag^{+}, Cd^{++}, $Ni^{2+)}$ was studied and found that Cu^{2+}, Ag^{+}, Cd^{++} act as noncompetitive inhibitor and Ni^{2+} act as a competitive inhibitor . The inhibition consent (Ki) urease using CuSo4, AgNo3, Cd(CHCOO)2, Nicl2, were 0.08 X10-^{5}M, 0.07 X 0^{-6}M, 0.05x 10^{-7}M, 1.7 X 10^{-4} M respectively. **Conclusion:** Kinetics Studies of soluble and immobilized urease will contribute towards the understanding of the structure and mechanism of Urease action in a much better way. (DOI:10.9777/rr.2018.1178)

Physical and functional characterization of extracellular vesicles derived from human platelets

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Background: Blood plasma contains very small size vesicles (<1000 nm), known as extracellular vesicles (EVs). They originate from a variety of cells including WBCs, endothelial cells but majority of them originate from platelets. Platelets release EVs upon activation by calcium ionophore (A23187). Platelet- derived EVs (PEVs) have active conformer of αIIbβ3 integrin on their surface and can bind with fibrinogen either in solution or immobilized on a matrix. **Aim:** Here we studied different population of PEVs based on their size and surface characteristics and interaction of integrin αIIbβ3 expressed on PEVs with fibrinogen. **Methods:** Platelets were isolated by differential centrifugation from fresh human blood, activated by calcium ionophore (1μM), followed by centrifugation to

pellet platelets. Supernatant containing PEVs was collected for quantitation by nanoparticle tracking analysis (NTA). Extent of fibrinogen (Fluorescent) binding with integrin αIIbβ3 expressed on PEVs was analysed by flow cytometry. Functional relevance of PEVs was assessed by observing their effect on platelet aggregation. **Results:** Platelets shed EVs while getting adhered on fibrinogen immobilized on a matrix suggesting the integrin αIIbβ3-mediated generation of EVs. Arg-Gly-Asp-Ser (RGDS, ligand of αIIbβ3) significantly inhibited release of EVs from platelets, principally generation of larger sizes (300-500 nm). PEVs interact with fibrinogen in solution and inhibited platelet aggregation in a count-dependent manner. **Discussion:** Our findings suggest that EVs population released from platelets have diverse properties. RGDS peptide markedly inhibited release of larger size EVs from platelets. Conclusions: This study is based on characterization of PEVs released from platelets. PEVs express active conformer of integrin αIIbβ3 on the outer membrane surface, which has strong affinity for fibrinogen revealing their potential role in homeostasis and thrombosis. (DOI:10.9777/rr.2018.1179)

Level of Immunoglobulin E (IgE) and eosinophils in allergic diseases

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Background: Total IgE (antibody) may be used for screening and detecting allergic diseases. A total IgE test may be ordered when a person has periodic or persistent allergic symptoms. Eosinophils are a variety of White Blood Cells (WBC) and one of the cellular components of immune system. Aim and Objectives: To study the Association of IgE and eosinophils in urticaria and allergic rhinitis (AR). Material and **Methods:** Patients of urticaria and AR was selected from the Out Patient Department (OPD) of U.P.U.M.S saifai Etawah. IgE were estimated by semi automated ELISA. WBC, differential leucocyte count (DLC; %eosinophil,) absolute eosinophil count (AEC) was done by Fully Automated Haematology Analyzer. Exclusion criteria: Any chronic systemic disease Study: Cross-Sectional study **Results:** We took total 125 patients in which total Urticaria patients were 86 and we found increased level of IgE in 81.3% patients while increased AEC found only in 16.2% patients. In Allergic Rhinitis we had 39 patients and we found increased level of IgE in 82.05% patients and increased AEC in 51.2% patients. % of eosinophils in blood was more high in Allergic Rhinitis 7.53% than Urticaria 4.13%. **Discussion:** We found in our study that total IgE was increased in both condition (Urticaria & Allergic Rhinitis) but AEC was increased only in AR, because the no. of patients of AR is 39 that's why a larger study group is required for making the conclusion. The % eosiophil in blood was more high in AR, so it is most likely that eosinophil count have a strong relationship with AR. Difference found among these 2 diseases may be because of their mechanism of immune reaction. **Conclusion:** Total IgE increased in both the allergic condition but eosinophil count is increased only in allergic rhinitis. (DOI:10.9777/rr.2018.1180)

Analysis of antioxidant properties of different extracts of *Calotropis procera* and different anti-diabetic drugs

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Background: Cellular damages, inactivation of enzymes are often caused by imbalances in the levels of free radicals and decreased levels of antioxidant enzymes. Many plants are used for the treatment of diabetes mellitus. Active hypoglycemic agents have been isolated from plants like Allium sativum, Gymnema sylvestre, Citrullus colocynthis, Trigonella foenum greacum, Momordica charantia and Ficus bengalensis etc and their mechanism of action have been studied widely. However the antioxidant properties of Calotropis procera in diabetes are still unrevealed. **Aim:** The present study deals with extraction and evaluation of various phytochemical constituents in leaf, root and stem of C. procera and comparison of their anti-oxidant properties with antidiabetic drugs. **Methods:** C. procera extracts were prepared as pet ether extracts (PEE), hydroethanolic extracts (HEE), and aqueous extracts (AQE). Phytochemical screening of extracts for total flavonoids, phenols, and tannins was performed. Qualitative High Performance Liquid Chromatography (HPLC) profiling was performed for chemical compound analysis in the extracts of Calotropis procera. The antioxidant properties of extracts were evaluated and compared with anti-diabetic drugs (insulin, metformin and pioglitazone).The antioxidant properties were evaluated against the standard antioxidants like ascorbic acid. Results and **Discussion:** It was found that significant levels ($p<0.001$) of antioxidants are present in the stem and leaf of Calotropis procera. Level of phenols was significantly higher in PEE and HEE of leaves of plant ($p<0.001$). PEE of leaves had significantly increased DPPH scavenging and superoxide radical scavenging activity respectively ($p<0.001$). **Conclusion:** Calotropis procera leaf has optimum antioxidant potential and can effectively remove free radicals, henceforth this plant can be used in future as a valuable source of pharmacologically active antioxidants to fight against diabetes mellitus. (DOI:10.9777/rr.2018.1181)

Role of curcumin in Arf6-cytohesin1 signaling axis–mediated thromboxane induced activation of phospholipase D in pulmonary smooth muscle cells.

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Background: Phospholipase D (PLD) catalyzes hydrolysis of phosphatidylcholine to produce phosphatidic acid (PA) and that plays a pivotal role in agonists induced increase in NADPH oxidase derived $O^{\cdot -}$ production in human pulmonary artery smooth muscle cells (HPASMCs). Curcumin is a well known antioxidant compound; however, its role in PLD activation is unknown. **Aim:** We seek to determine the role of curcumin in inhibiting conversion of ArfGTPfrom ArfGDP involving cytohesin-1, which results in the attenuation of U46619 induced activation of PLD in HPASMCs. **Methods:** We determined PLD activity, western blot, molecular docking and other assays by standard methods. Results and **Discussion:** Treatment of HPASMCs with U46619 stimulated PLD activity in the cell membrane, which was inhibited upon pretreatment with SQ29548 (Tp receptor antagonist), FIPI (PLD inhibitor), secinH3 (inhibitor of cytohesins) and curcumin. Upon treatment of the cells with U46619, Arf-6 and cytohesin-1 were translocated and associated in the cell membrane, which were not inhibited upon

pretreatment with curcumin. Cytohesin-1 appeared to be necessary for in vitro binding of GTPγS with Arf-6; however, addition of curcumin inhibited binding of GTPγS with Arf-6 even in presence of cytohesin-1. Our computational study suggests that curcumin predominantly inhibited Arf6GDPto Arf6GTP conversion, which appeared to be an important mechanism by which curcumin inhibits U46619 induced increase in PLD activity in PASMCs. **Conclusion:** This study suggests that U46619 caused stimulation of PLD activity occurs with the involvement of Arf6-cytohesin1 signaling axis. Curcumin inhibits U46619 induced increase in PLD activity. Curcumin also inhibits in vitro binding of GTPγS with Arf-6 even in presence of cytohesin-1. Our computational study revealed formation of cytohesin1-curcumin-Arf6GDP inactive complex, which appears to be the underlying mechanism for curcumin-mediated inhibition of U46619 induced increase in PLD activity in the PASMCs.(DOI:10.9777/rr.2018.1182)

Cumulative effects of photosynthetically active radiation, ultraviolet radiation and salt stress on reactive oxygen species generation and antioxidative enzymes activity in cyanobacteria inhabiting diverse habitats

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Background: Salinization is one of the major causes of soil degradation. Ultraviolet (UV) radiation, coming along with sunlight, impairs the photosynthetic apparatus of photoautotrophs. Cyanobacteria are considerably tolerant to salt and UV stress, therefore, have been used in reclaiming saline soils. Salt and UV, leads to oxidative damage through generation of reactive oxygen species (ROS), which are highly reactive and cause oxidative damage to biomolecules. To counteract this antioxidative enzymes are synthesized such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). **Aim:** We studied integrative effects of PAR, UV and salt stress on ROS generation and antioxidative enzymatic activities in Scytonema geitleri HKAR-12 and Nostoc sp. strain HKAR-2 isolated from roof top and hot spring respectively. **Methods:** Cyanobacteria were exposed to PAR, UV and NaCl (50, 100, 200mM) for different time intervals (12, 24, 36, 48, 60, and 72 h). ROS was estimated using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and enzymatic activities were determined following standard protocols. **Results:** PAR, UV and salt resulted in manifolds increase in ROS in both the cyanobacteria with maximum increase observed at 48h of UV, PAR and 200mM of salt treatment. All studied antioxidative enzymes showed maximum induction (3-3.5 folds) in 100 mM salt concentration on 36-48h of UV and PAR exposure followed by gradual decrease. However, in 200mM NaCl, activities declined after 24h of exposure. **Discussion:** PAR, UV and salt significantly induced ROS in both the cyanobacteria with more induction in Nostoc sp. strain HKAR-2. To counteract the ROS, significant induction of antioxidative enzymes was observed in both the cyanobacteria. However, induction of antioxidative enzymes was more in Nostoc sp. strain HKAR-2. **Conclusion:** Both the cyanobacterial species tolerated UV and salt stress but Scytonema geitleri HKAR-12 was found to be more tolerant. Hence, these cyanobacterial strains can be employed for

reclamation of saline and sodic soils. (DOI:10.9777/rr.2018.1183)

Isolation, identification and bio-potential characterization of endophytic fungi isolated from *Saraca asoca*

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Background: The endophytic fungi are comparatively less studied group of microbes producing surfeit of compounds having the biomedical and agricultural importance. Residing inside their host, they are known to increase the defense of host plant by producing secondary metabolites active against all kinds of microbial pathogens and additionally, there mycelia synthesizes nanoparticle of different metal for medical use. **Aim:** Isolation identification and bio-potential characterization of endophytic fungi isolated from saraca asoca. **Methods:** Isolation, and identification of endophytic fungi were done through available microscopic techniques and screening of their myco-synthesis of silver and gold nano-particle, antibacterial and antioxidant activities were performed using the disc diffusion and DPPH assay respectively. **Results:** A total of 36 endophytic fungal isolates representing 22 different morphotypes were recovered from Saraca asoca. All morphotypes, (100%) were synthesizes gold nano-particle at 95^{0}C for 10 minutes, Interesting was that 13.63% were synthesizes silver nano-particle at 95^{0}C for 10 minutes. Out of all morphotypes, 45.45% were active against more than two or more human bacterial pathogens with inhibition zones between 7.0-20.0 mm, while 13.63% showed human fungal pathogen against candida albicans with inhibition zone 5-10mm.While 50% display anti-oxidant activity.Though only 4.54% morphotypes displayed positive cellulase activity, 68.18% showed the protease activity, while 27.27% exibited amylase activity and 4.54 were showed the lipase activity **Discussion:** The endophytes was effective against both gram positive and gram negative bacteria however, the equal grade of activity was noticed against gram nigative bacteria as well as gram positive ones. **Conclusion:** The preliminary results of this experiment trigger the interest in bio-potential of the crude metabolite and mycelia obtained from culture which will further be purified and characterized. (DOI:10.9777/rr.2018.1184)

A biochemical and proteomic analysis reveals survival mechanisms under drought stress in horsegram

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Background: Drought stress is one of the most devastating environmental stresses severely affecting crop growth and yield. To withstand drought stress and sustain the agricultural productivity there is need to identify natural stress tolerant crop resources. Horse gram (Macrotyloma uniflorum) is a highly drought tolerant yet underexploited legume, commonly known as 'kulthi'. The U.S. National Academy of Sciences in 1978 identified horse gram as potential food source for the future. Insurmountable drought tolerance and pest resistance together make it agriculturally an attractive crop. It is called poor man's pulse as it sustains millions of people. Horse gram has many future implications in neutraceuticals, functional foods and

therapeutics.**Aim:** To decipher the biochemical, antioxidant enzymatic and proteomic mechanisms in horse gram in response to drought stress. **Methods:** Leaf and root tissues of horse gram were analyzed for biochemical parameters like relative water content, proline, etc. and antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) etc. under control and drought stress conditions. For proteomic analysis, three protocols i.e. Trichloroacetic Acid (TCA)-acetone, phenol and multi-detergent were compared for extraction of proteins. **Results:** Proline, phenols, GST, POD and SOD showed elevated levels of activity under drought stress. The phenol-acetone method gave the best quality and quantity of protein from horse gram. The proteome of horse gram reveals that some hypothetical and known proteins to be up regulated under drought stress conditions. Discussions: Horse gram utilizes osmolytes and antioxidant enzymes as defense mechanism against drought stress. Developed protocol for protein analysis could be applicable not only to important leguminous but also non-leguminous crops. Conclusions: The identified targets through biochemical and proteomic analysis reveal their critical role in drought tolerance and can be explored for imparting stress tolerance in various crop plants. (DOI:10.9777/rr.2018.1185)

One step green synthesis of gold nanoparticles using turnip seed extract for subsequent biomolecule immobilization

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Background: Nano biotechnology is a rapidly growing field of science that lead to the possibility of exploiting the structures and is dedicated to the study of nanoparticles, nanostructures, nanodevices, and nanoanalytics. Synthesis of metal nanoparticles and nanomaterial has attracted much more attention these days due to their physical, optical and chemical properties. Nowadays, gold nanoparticles (AuNPs) have attracted much more attention towards itself due to its application in the field of nanomedicines, biomolecule immobilization and therapeutics. Beside the conventional methods, bio-reduction potential of plants, are being explored. These green synthesized AuNPs are easy to synthesize, size controllable and are easily characterizable due to its red colouration, monodispersity, easy functionalization, biocompatibility and non-toxicity. **Aim:** Synthesis of gold nanoparticles using green methodology so can be utilized as an effective support for bio- molecule immobilization. **Methods:** Gold nanoparticles were synthesized by using turnip seed extract. Absorption and crystallinity of AuNPs were measured by UV- Vis spectrophotometer and X-ray diffraction (XRD) analysis. Morphology and shape of nanoparticles were examined by Transmission electron microscope (TEM). SEM, FTIR and EDX analysis were also done for its characterization. **Results:** UV-Vis spectrophotometer showed the maximum absorption peak (λmax) at 525nm with visual color change to pinkish red due to surface plasmon resonance. XRD and EDX revealed the crystalline nature and elemental composition of AuNPs. TEM revealed spherical shape and size of the nanoparticles ranging from 5-50nm. FTIR showed the presence of amine and other stabilizing functional groups. **Discussion:** Turnip seed extract act as reducing and stabilizing agent. FTIR of extract as well as AuNPs have revealed presence of different functional groups such as amines,

alcohols, ketones and aldehydes involved in reduction and stability of nanoparticles. **Conclusion:** An ecofriendly method for gold nanoparticle synthesis using plant extract has been proposed. Biomolecules present on the surface of AuNPs and its size and shape will be helpful as a support for biomolecule immobilization. (DOI:10.9777/rr.2018.1186)

FTO gene polymorphism and it's association with type 2 diabetes mellitus in north Indian populations

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Aim: The present study investigated the association of variant-rs9940128 (in intron 1) of the fat mass and obesity-associated (FTO) gene with type 2 diabetes mellitus (T2DM) in North Indian Population. **Methods:** Unrelated study subjects (n-110 normal glucose-tolerant [NGT] controls and 110 cases [T2DM]) were randomly selected. Genotyping was done by the polymerase chain reaction restriction fragment Length polymorphism method and samples were sequenced to validate the genotypes obtained. **Results:** The polymorphism rs9940128 A/G of the FTO gene was found to be highly significant and thus was associated with T2DM. Conclusions: Among North Indians, the rs9940128 A/G variant of the FTO gene was associated with T2DM. (DOI:10.9777/rr.2018.1187)

Fluorescein-based probes for the detection of hypochlorite anions: synthesis, characterization and bio-activity

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Background: The hypochlorite anion plays vital role in biological system. It is produced by the peroxidation of chloride ions catalyzed by the enzyme myeloperoxidase in activated leukocytes and act as a critical microbicidal agent in natural defense. However, its abnormal levels cause many inflammation- related diseases, including cardiovascular diseases, lung injury, rheumatoid arthritis, and cancer. So the detection of hypochlorite anion is of great importance. **Aim:** Development of fluorescein based probes for endogenous detection of hypochlorite anions in living cells. **Methods:** Synthesized compounds (FTNA & FTMA) were analyzed using CHN analysis, IR, 1HNMR, 13CNMR, ESIMS, absorption, emission spectroscopy and XRD. Their cytotoxicity assay (MTT) was also carried out. **Results:** Two novel probes (FTNA & FTMA) based on the fluorescein hydrazone are synthesized and well characterized using spectroscopic techniques and x-ray crystallography. These probes are found sensitive for recognition of hypochlorite anions among various anions with fast response, high sensitivity and excellent selectivity at physiological pH. Furthermore, the fluorescence signals of probes were also studied endogenously in living cells on the addition of different inhibitors. **Discussion:** Results shows that the sensing mechanism follow hydrolytic pathway which results in the fluorescence enhancement. FTNA only visualized the endogenous hypochlorite. MTT assay clearly shows that both the drugs FTMA and FTNA are cytotoxic at 24h and 48h against MDA MB 435s cell line with significant IC50 values. Further, the treated cells showedmorphological changes as compared to untreated cells. Plasma

membrane blebbing was seen suggesting that cells were undergoing apoptosis. **Conclusion:** The synthesized probes show fast response, high sensitivity and excellent selectivity for hypochlorite anion at physiological pH Furthermore, these excellent attributes enable us to demonstrate, the endogenously produced hypochlorite in living cells and are active against MDA MB 435s cell line at micromolar concentration. (DOI:10.9777/rr.2018.1188)

Evaluation of Enzyme-linked immune-electro transfer blot (EITB) assay for the diagnosis of human neurocysticercosis

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Background: Neurocysticercosis (NCC), caused by larva of Taenia solium, is the most common parasitic infection of central nervous system (CNS). Imaging tools always may not useful for diagnosis since sometimes it cannot differentiate NCC from other lesions and some NCC lesions may be missed. Moreover, these techniques are expensive and not readily available in developing countries. So we developed cyst fluid antigen based EITB for diagnosis of NCC. **Aim:** To evaluate the efficacy of EITB for diagnosis of human neurocysticercosis. **Methods:** Total 80 MRI confirmed symptomatic NCC patients and 150 healthy individuals were included. Sera were collected from all symptomatic and healthy individuals and EITB was performed with cyst fluid antigens for diagnosis of neurocysticercosis. **Results:** On EITB, several bands of < 50 were commonly recognized by antibodies from sera of NCC patients. The 15kDa was the most common band identified on immunoblot with sensitivity 92% and specificity 90.67%. Bands of 17kDa, 47kDa, 23kDa, and 25kDa along with 15kDa had sensitivity 92%, 95%, 95%, 93% respectively and specificity 90.67%. Presence of these bands along with 15 kDa probably is the best diagnostic marker for NCC. **Discussion:** Lentil lectin-specific T. solium glycoproteins based EITB has been widely accepted for sero-diagnosis of NCC. Sera are considered positive for cysticercosis when one or more reactive bands of < 50 kDa are identified on immunoblot. However, the process of antigen preparation is laborious and complex. Moreover, this test needs to be validated in highly T. solium endemic populations. **Conclusion:** This is the first study to evaluate the diagnostic efficacy of cyst fluid based EITB. We report that the 15 kDa reactive antigenic band with other bands on cyst fluid based EITB is highly sensitive and specific for the diagnosis of NCC. (DOI:10.9777/rr.2018.1189)

Pharmacogenomics of aspirin resistance in ischemic stroke: Focus on UGT gene

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Background: Stroke is the leading cause of neurological disability and death worldwide. Aspirin, an antiplatelet agent is considered as a golden standard for the treatment as well as prevention of secondary stroke. Interindividual variation in response to aspirin, which gets reflected in the form poor response and adverse drug reactions has led to the concept of "Aspirin Resistance". Genetic variants have been suggested

to influence the drug response. **Aim:** To screen all the variants of UGT gene (encodes aspirin metabolizing enzymes) and correlate with ischemic stroke. **Methods:** Two hundred ischemic stroke patients and equal number of age and sex matched controls were included in study. Patients were categorized as responders and non-responders based upon their outcome using mRS. The DNA was isolated using phenol chloroform method. Variation in UGT gene was evaluated by GSA (Global screening assay) and validated by PCR-RFLP. **Results:** UGT1A6*2 Thr181Ala variant was found to be significantly associated with aspirin resistance in ischemic stroke patients. **Discussion:** On account of genetic variation, UGT might influence aspirin resistance since the outcome of patients on aspirin treatment was poor. Several variants of UGT have already been reported to influence the drug response. However, association of UGT variants in response to aspirin in Malwa region of Punjab has not been evaluate where stroke is highly prevalent. **Conclusion:** Information regarding gene variants influencing drug response may aid clinicians in choosing an appropriate treatment strategy for ischemic stroke patients. (DOI:10.9777/rr.2018.1190

Expression profiles of immuno-modulators during the wound healing phase of lizard tail regeneration

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Background: Epimorphosisis observed in vertebrate classes. However, of all the different species capable to regenerate, members of Gekkonidae selectively re-grows only tail amongst their appendages. Induced autotomy of tail, leads to a regulated inflammatory phase prior to theepithelial covering of wound. The multi-layered wound epithelium is a major organizer that orchestrates the subsequent events of regeneration. This work is an attempt to understand the role of major inflammatory mediators in fashioning regeneration. **Aim:** The study analyzes the impact of various inflammatory mediators on epimorphic regeneration of tail in a reptilian model Hemidactylusflaviviridis by visualizing the temporal expression status of COX (Cyclooxygenase), iNOS (Nitric oxide synthase), Interleukin 6 and Interleukin 1- β. **Methods:** The wound healing phase from resting (0 days post-amputation - 0 dpa) till wound epithelium (4 dpa) was uniformly divided and on each day, using appropriate analytical tools the temporal protein and gene expression profilesof IL-6 and IL-1β, along with activity of COX and iNOS enzymes were studied. **Results:** The major regulatory enzymes of inflammation namely COX and iNOS, showed opposite trend of expression and activity, where levels of the former raised conspicuously while that of latter decreased up to 3dpa and hiked suddenly at 4dpa stage. Further, IL-6 and IL-1β significantly decreased temporally, suggesting the negative correlation among regenerative and inflammatory processes. **Discussion:** Literature study emphasizes the role of inflammation for onset of wound healing but its prolonged stay might adversely affect the tissue replacement. The molecular markers of inflammation, when checked, depict its reduced level during the reconstructive process, further supporting the above notion. Hence, it could be construed that the events occurring during inflammatory phase play a seminal role in the successful realization of regeneration. **Conclusion:** This study is an attempt

to sketch the impact of inflammation on regeneration.The mechanism of regeneration will be explicitly portrayed only if the correct information of the inflammatory front is incorporated. (DOI:10.9777/rr.2018.1191)

Identification of some natural products as β-catenin/TCF4 interaction inhibitor from ZINC database: A combination of ligand based qualitative 3-D pharmacophore modeling, drug likeness filter, and structure based molecular docking, and in silico ADMET analysis

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Background: Dysregulation of β-catenin/TCF4 interaction in Wnt/β-catenin signaling plays a critical role in gastrointestinal cancers. Hence, inhibition of β-catenin/TCF4 interaction could be a crucial target in designing of drugs against gastrointestinal cancers. Bioactive natural products with therapeutic potential are abundantly available in nature and some of them are beyond exploration by conventional methods. Therefore, computational approaches become a versatile tool for exploration of drug candidates in drug discovery setup. **Aim:** Identification of some natural products as β-catenin/TCF4 interaction inhibitor. **Methods:** Ligand-based qualitative 3-D pharmacophore models were developed based on a set of 32 β- catenin/TCF4 interaction inhibitors using PharmaGist webserver to understand the essential structural features of a β-catenin/TCF4 interaction inhibitor. The best pharmacophore model was selected and virtual screening was performed through ZINCPharmer webserver (ZINC Natural Products, last updated 09/23/14), considering a root-mean-square deviation of 0.1, to obtain 100 novel virtual hits. The top 100 hits were further filtered using DruLiTo software to identify compounds with drug-like properties. Thirty five compounds were subsequently docked on β-catenin (PDB id – 1JPW) using Autodock suite 4.2. Finally, ADMET analysis was performed using PreADMET webserver, only for nine compounds. Results and **Discussion:** The best pharmacophore model with a score of 41.243 and features including 4 spatial, 2 aromatic, 1 donor, and 1 acceptor was chosen. Thirty five compounds with drug-like properties were subjected to molecular docking and nine compounds with docking score less than −7.0 kcal/mole and predicted inhibitory constant (Ki) < 8 μM were identified. All the identified compounds showed interaction with active site residues (Lys435, Arg469, Lys508, Arg515, and Glu571) on β-catenin, which interacts with TCF4. Five compounds were found suitable based on predicted in silico human intestinal absorption, Caco2 cell permeability, skin permeability, blood brain barrier penetration, plasma protein binding, cytochromes P450 metabolism, P-glycoprotein inhibition, hERG inhibition, and carcingogenicity. **Conclusion:** Five natural products identified require further evaluation in in vitro and in vivo experiments to facilitate discovery of selective β-catenin/TCF4 interaction inhibitors. (DOI:10.9777/rr.2018.1192)

Categorization of molecular frames after adenylate cyclase activation in a background of MKK3/6 stabilization during SAC+BER treatment-an *in vitro* study

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Background: Cancer cells often have delicate regulation of apoptosis resulting in sustenance of survivability and establishment of tumorigenic growth coupled with resistance to anticancer drugs. In this context alternate strategies are considered to improve therapeutics. Report advocated induction of apoptosis after S-allyl-cysteine (SAC) and berberine (BER) individual treatment in HepG2 cells. However the role of proinflammatory cytokine in setting in the programmede cell death is not considered elsewhere after SAC or BER treatment. **Aim:** Intrinsic regulation of cell survival pathways and its role in alteration of HepG2 cell death in association with change in cell proliferation after SAC+BER treatment. Moreover bagatelle of molecular waves associated with mechanistic regulation of programmed cell death after drug treatment was analyzed. **Methods:** WST assay was performed for dose responsive analysis. Cell cycle regulatory factors were estimated by immunoblot while NFκβ and E2F localization were analysed by electrophoretic mobility shift assay. Status of tAIF distributory molecules was estimated by immunocytochemistry. Mitochondrial membrane potential and lysosomal integrity analysis was performed by flow cytometry. **Results:** MKK3/MKK6 mediated p53 stabilization in parallel to adenylate cyclase-PKA-PP2A dependent reduction in nuclear localization of NFκβ potentially induced lysosomal disintegration resulting alteration in scramblase-APLT activities and generation of programmed cell death. DNA fragmentation by nuclear tAIF and Rb-E2F interaction associated cessation of cell proliferation further potentiate the effective reduction in cell survivability. Discussions: Bid truncation in treated group effectively modulated tAIF distribution that inturn synergistically generated'eat me' signal corresponding to classical pathway over cell surface. Necrostatin pre-treatment effectively reduced annexinV-FITC fluorescence accentuating a significant role of necroptosis in the protentiation of apoptosis after SAC+BER treatment. Conclusions: Adenylate cyclase activation dependent molecular interactions indicated the role of AIF truncation in the reduction of cancer cell survivability and may be targeted in future alignment of therapeutics against liver carcinoma. (DOI:10.9777/rr.2018.1193)

Investigation of the Anticancer Potential of Karambel on Oral Squamous cell carcinoma

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Background: Despite the extensive research carried out in the field of cancer therapeutics, it still remains one of the most life threatening diseases globally. Oral cancer is the sixth most common

malignancy in the world and approximately 1, 28,000 people are dying due to this disease every year. Surgery, chemotherapy and radiotherapy, still remain the corner stone for the treatment of oral cancer; however, these approaches are not very effective and are linked with chemoresistance and multiple adverse side effects. Thus, herbal medicines are extremely vital in this regard on account of their minimal side effects for prevention and treatment of the disease. Karambel or Dillenia indica (DI) is one such herbal medicine, which is not commercially cultivated, but is found in wild in the Terai & Dooars region and Katha Reserve Forest in the North-East region of India. It is a traditional medicine used for the prevention and treatment of different diseases. Objective: To investigate the anticancer effects of Karambel in oral cancer. Materials and Methods: To determine the antiproliferative and cytotoxic effect of Karambel on oral cancer cells MTT assay, cell cycle arrest, PI-FACS, PI apoptosis, Annexin-V/PI apoptosis assay, live and dead assay, colony forming assay, DPPH assay and wound healing assays were performed. To determine the mechanism of action of the DI extract, the oral cancer cells were treated with the DI extract and the expression of the proteins involved in cancer cell proliferation, inflammation, apoptosis, survival, invasion and metastasis were analyzed through western blot method. Results: The methanolic extract of DI displays anti-proliferative and cytotoxic effect in oral cancer cells in a dose dependent manner. The extract also showed high potential in inducing cell cycle arrest at the G1 phase and inhibiting metastasis of oral cancer cells by in vitro methods. It also inhibited apoptosis and exerted antioxidant potential with increased free radical scavenging activity. Apart from these the protein expression analysis showed that the extract significantly inhibited the expression of the proteins involved in cell proliferation, inflammation, survival, apoptosis, invasion and metastasis. Conclusion: The effect of Karambel or DI extract correlate with its traditional use as an anticancer agent, thus making it an interesting source for further investigation. (DOI:10.9777/rr.2018.1194)

Antioxidant attributes of edible oyster mushroom

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Background: Recently, studies in various types of free radicals have gained attention around the globe as it causes oxidative stress, which may lead to aging and numerous diseases. Antioxidants play an important role in maintaining human health by performing the action of scavenging free radicals. Oyster mushrooms have become attractive as functional foods around the globe because of their nutritional and medicinal attributes. **Aim:** To elucidate the biochemical content and antioxidant activity of oyster mushroom. **Methods:** Biochemical analysis was carried out using standard method, while DPPH radical scavenging, metal ion chelating assay, phosphomolybdate assay, reducing power assay were used to evaluvate the invitro antioxidant activity of oyster mushroom. **Results:** The result obtained revealed the presence of flavonoids, terpenoids, saponins, anthraquinone in the methanolic and aqueous extract. Total phenol and flavonoid contents were found in the range of 19.78-23.59µg propyl gallate equivalent/mg and 23.27-26.44µg quercetin equivalent/mg in aqueous and methanolic extracts, respectively. Comparatively, lower activities were observed for aqueous and methanolic extracts in DPPH radical

scavenging and reducing power assays. Appreciable metal ion chelating activity was demonstrated by aqueous (57-86%) and methanolic (36-71%) extracts in the concentration range 100-400µg/ml, that result dose dependent response. Total antioxidant capacity for both the extracts was found in the range 83-96µg PGE/ml at test concentration. **Discussion:** In the present work fruiting bodies of P. sajor-caju has shown presence of number of bioactive compounds. These compounds are known for their medicinal attributes including antioxidant properties. **Conclusion:** The bioconstituents present in P. sajor-caju extracts have shown promising antioxidant activity viz., metal ion chelating activity and total antioxidant capacity in the present work. Hence results validate the use of edible mushroom P. sajor-caju as medicinal food. (DOI:10.9777/rr.2018.1195)

A meta-analysis of triple-negative breast cancer in India

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Background: Breast cancer is the most prevalent cancer in women worldwide. Among the different breast cancer subtypes, triple-negative breast cancer (TNBC), which is more prevalent among younger women, presents the most aggressive form. Numerous clnicopathological studies carried out in the world strongly supports the utterly poor prognoses and high recurrence rate of TNBC. **Aim:** There is significant variation in prevalence rates of TNBC reported by various studies worldwide. We performed a literature- based meta-analysis of these studies. **Methods:** We searched the databases of Google and PubMed for studies that reported on the prevalence of TNBC in India as well as other part of the world. Results and **Discussion:** Data were obtained from 36 studies that involved 13 studies from India (7002 patients with breast cancer) and 23 studies from other parts of the world (111768 patients with breast cancer). Our analysis revealed that the percentage of TNBC ranges from 6.7-27.9% in different countries and India tops amongst all of them, followed by Indonesia, Algeria and Pakistan. Most of the other countries (Netherland, Italy, London, Germany) have TNBC incidence below the mean level (i.e. 15%). The high incidence of TNBC in Indian population is associated with several risk factors, which primarily include lifestyle, obesity, family history, and BRCA1 mutations. The treatment of TNBC is greatly hampered due to lack of targeted therapies and hence, it demands earnest attention towards extensive research for prevention and the development of treatment modalities with high efficacy. **Conclusion:** Our report strongly suggests that India ranks amongst the top in the world in the incidence and prevalence of TNBC. This finding has significant clinical relevance as it may contribute to poor outcomes in patients with TNBC in India. However, extensive researches are needed to understand the determinants of TNBC. (DOI:10.9777/rr.2018.1196)

Identification of chitinolytic isolate and production, purification of extracellular chitinase

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Background: Chitinase enzymes belong to glycosyl hydrolases family which degrade chitin. Chitinase have applications in asthma, inflammation induced from chitin and allergen, osteoarthritis and biocontrol **Aim:** Identification of chitinolytic isolate and production, purification of extracellular chitinase. **Methods:** A chitinolytic isolate was characterized and further identified by using Sanger DNA sequencing method and phylogenetic analysis. Chitinase enzyme production using this isolate was carried in a medium containing nutrient agar media mixed with 10% colloidal chitin. Enzyme purification was done using ammonium sulphate precipitation at 50% saturation and further salts and low molecular constituents were removed using dialysis membrane with 20 KD cut off. **Results:** The Isolate was identified as Cellulosimicrobium aquatile. The activity of supernatant was 10 mU/ml and after purification it was observed 116 mU/ml. After purification, the purification fold increased 1.719 times. Activity of Chitinase enzyme was measured in terms of µmole of N-Acetyl-d-glucosamine per ml. **Discussion:** Till date more than 250 bacteria has been reported as chitinase producing bacteria. Cellulosimicrobium aquatile have been first time included in this regime. **Conclusion:** Cellulosimicrobium aquatile have chitinolytic potential and chitinase produced by it may be used for biocontrol and treatment of disease. (DOI:10.9777/rr.2018.1197)

Cell free mitochondrial DNA in serum and milk associated with bovine mastitis: A pilot study

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Background: Mastitis is inflammation of mammary gland affecting domestic animals. Fragments of the mitochondrial genome released from dying cells are considered surrogate markers of mitochondrial injury. **Aim:** The study aimed to assess the association of increased cell free mitochondrial DNA (mtDNA) content in serum and milk of bovine mastitis. **Methods:** Milk and serum samples were collected from 20 sub-clinical mastitis and 10 normal animals from dairy farm in Punjab. Mastitis was confirmed by California mastitis test and bacterial isolation. Oxidative stress, nitric oxide and inflammatory cytokines were also estimated. Quantitative real time polymerase chain reaction was conducted in samples from both mastitis and healthy animals targeting the mtDNA genes cytochrome b. **Results:** Mastitis animals showed higher oxidative stress markers and nitric oxide along with higher level of inflammatory cytokines. Cell free mtDNA was significantly higher in serum and milk of mastitis animals comparing to that of healthy control. **Discussion:** Bovine mastitis is inflammation of the mammary gland and associated with increased oxidative stress. Mastitis animals showed higher oxidative and nitrosative stress along with higher level of inflammatory cytokines. This may be due to production of reactive oxygen species produced by mitochondria and inflammatory cells to directly act as antibacterial, and also signal to drive the production of inflammatory cytokines. The higher cell free relative mtDNA content in mastitis animals indicates injury to the mammary epithelial cells

and thereby releasing the mtDNA in the milk and blood. This mtDNA may be a marker of exposure to reactive oxygen species and/or oxidative stress in bovine mastitis. **Conclusion:** In summary, this is the first report about association of the cell free mtDNA in bovine mastitis with increased cell free relative mitochondrial DNA content in mastitis animals. This mtDNA quantification may be used as a screening as well as biomarkers for diagnosis of mastitis. (DOI:10.9777/rr.2018.1198)

Anti-inflammatory activity of methanolic extract of the bark of *Prunus cornuta*.

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Background: Prunus cornuta, belongs to the family Rosaceae, is a deciduous medium sized tree with grey brown to brown bark, leaves are oblong to lance-shaped, 8-15 cm long, long pointed with finely toothed margin and small white flowers are borne in long drooping clusters. P. cornuta found in the Himalayas at altitude of 2500-3500 meters. **Aim:** To ascertain the anti-inflammatory activity of the methanolic extract of the bark of Prunus cornuta. **Methods:** In-vitro and In-vivo studies were employed to examined anti-inflammatory activity by following methods like the human red blood cell (HRBC) membrane stabilization method, inhibition of albumin denaturation, heat induced hemolysis and carrageenan induced method. **Results:** The results of this study demonstrate that the crude methanolic extract of bark of P. cornuta possesses significant anti-inflammatory activity and have potential as leads for the discovery of anti-inflammatory phytocompounds. **Conclusion:** The present study revealed that P.cornuta has broad spectrum of anti-inflammatory activity and potential source of anti-inflammatory agents that could be useful for infectious disease. (DOI:10.9777/rr.2018.119

Mitigation of heavy metal stress, using primed seeds - A review

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Bioaccumulation and bio-magnification of heavy metals (HMs) in the environment are increasing day by day, creating unpleasant situation for all living organisms; plants are the great sufferers due to their sessile nature. In plant system HMs at toxic levels react with several vital cellular bio-molecules such as nucleus, proteins and DNA, result in the production of reactive oxygen species (ROS), may cause morphological, physiological, metabolic, and molecular aberrations. In response to HMs, plants are equipped with a number of mechanisms to impede the HM toxicity. The key elements of these mechanisms are the formation of phytochelatins (PCs), metallothioneins (MTs), ligand-metal complex, non-enzymatic compounds like proline and soluble alcohols etc. But these mechanisms are not sufficient to mitigate the heavy metal stress. Therefore, other alternate option regarding the removing the effects of HMs is seed priming treatment, using a number of inorganic chemicals and PGRs such as nitric oxide, nitrates, selenium (Se), salts of Ca and Mg, salicylic acid (SA) and brassinosteroids (BR) respectively. Seed priming has potential to erase phytotoxic effects of HMs by strengthening the plant's antioxidant system and

improving seedling establishment and vigour during crop growth. Considering all the facts, this review explores the mitigation of HMs stress by using primed seeds. (DOI:10.9777/rr.2018.1200)

3,5-dihydroxy-4',7-dimethoxyflavone: Isolation and characterization from *Alpinia nigra* and *in-silico* studies for its drug development strategy

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Alpinia nigra (Zingiberaceae) is widely distributed in Northeast India and well known for its various medicinal purposes such as anti-helminthic, anti-inflammatory and anti-cancer properties. It has been widely investigated and reported to possess a wide variety of secondary metabolites belonging to different classes such as terpenoids, alkaloids and flavonoids. Flavonoid is a small molecular weight secondary metabolite and generally follows all criteria of pharmaceutical to use as a drug. In the present investigation, 3,5-dihydroxy-4',7-dimethoxyflavone, was isolated from ethyl acetate extract of A. nigra leaves for the first time. The structure of compound was elucidated using various spectroscopic approaches such as FTIR, NMR and XRD and was further confirmed and validated from previously reported FTIR and NMR data. Prospective of in silico based physiochemical characterization of the compound as drug development was found to have good pharmaceutical qualities as a drug because it follows 100% Lipinski rule five, Lipinski rule 3, Veber, Ghose, BBB, CMC 50 like rule. WLOGP value of this compound is 2.89, thereby; it would not be effluated from CNS by the p-glycoprotein. According to its pharmacokinetics, the compound is expected to have high gastrointestinal absorption, work as CYP1A2, CYP2C9, CYP2D6, CYP3A4 inhibitor and bioavailability score is 55%. Moreover, the compound is water soluble (XLOGP3 = 2.55) with molecular weight of 314.29 g/mol and three rotatable bonds representing the compound to be excellent drug lead-like properties. (DOI:10.9777/rr.2018.1201)

Autophagic vacuolization, multicellular spheroid formation and release of cytochrome C induced by Curcumin

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Background: Oral cancer is the sixth most common cancer worldwide and is one of the leading causes of death in developing countries. Curcumin is a compound that exerts anti-proliferative and apoptotic effects via multiple molecular targets. Recent studies have demonstrated that cytochrome C plays an important role in cell death. **Aim:** The present study was aimed to explore the pathway involved in the apoptotic activity of curcumin in oral cancer cells. **Methods:** We employed oral cancer KB cell to assess cytotoxicity assay, clonogenicity assay, morphology analysis with inverted phase contrast microscopy and the release of pro-apoptotic factor (Cytochrome C) from mitochondria into the cytosol after curcumin treatment. **Results:** The IC50 of curcumin was close to 80 µM in 48 H treatment which dramatically decreased the number of viable KB cell colonies. Curcumin inhibited the growth of the KB cells, induced autophagic cell death and form multicellular spheroid in a time and dose-dependent manner. In addition, curcumin

treatment resulted in the release of cytochrome C from mitochondria into the cytosol, which plays an important role in cell apoptosis. Discussion & **Conclusion:** Our data for the first time suggest that curcumin induces autophagic vacuolization, multicellular spheroid formation and release of cytochrome C in KB cell. These findings provide a novel insight into the facts underlying the anticancer property of curcumin. (DOI:10.9777/rr.2018.1202)

2.45 GHz microwave irradiation induced mitochondrial dysfunction in hippocampus of mouse brain, with ameliorative effect of donepezil treatment

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Background: 2.45 GHz microwave (MW) frequency is widely used in wireless communication. Exposure-related effects of MW on neurological functions have been addressed in voluminous findings. The deleterious effects of MW involve many aspects of neuronal functions i.e. ionic concentration, receptors, synaptic proteins including energy homeostasis in the brain. **Aim:** Present study elucidates the effect of 2.45 GHz MW on mitochondrial functions of hippocampus (HP) in mice brain and to test the therapeutic potential of donepezil against MW induced effects. **Methods:** Mice were irradiated to 2.45GHz (averaged SAR-0.023 W/Kg) for thirty days two hours daily (10.00-12.00 IST). Donepezil was administered (5 mg/Kg, p. o.) simultaneously with MW radiation. HP was dissected out from mice brain after cervical dislocation. Mitochondria were isolated and processed for estimation of citrate synthase (CS), succinate dehydrogenase (SDH) & cytochrome c oxidase (COX) activity; mitochondrial calcium, membrane potential (MMP) and ROS level. **Results:** MW irradiation did not affect CS and SDH activity; donepezil treatment maintained basal CS activity and exhibited a significant increase of SDH activity. COX activity was declined in MW exposed group and donepezil treatment restored to basal level. Mitochondrial calcium and ROS was increased significantly in MW exposed group. An inverse decrease in MMP was also noted, while Donepezil treatment showed basal level of calcium, MMP and reduced ROS level. **Discussion:** MW exposure affects neuronal enzymes, ionic imbalance, ROS generation and induced apoptosis. Our results indicated that 2.45 GHz MW exposure caused mitochondrial dysfunction in HP. Donepezil exerted a protective effect on mitochondrial function of HP by limiting ROS generation and enzymatic alterations. **Conclusion:** 2.45 GHz MW irradiation induces mitochondrial dysfunction in HP of mice brain and donepezil has an improving effect on mitochondrial dysfunction. (DOI:10.9777/rr.2018.1203)

Evaluation of serum total and ionized calcium in CKD patients

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Background: Chronic kidney disease (CKD) is emerging to be an important chronic disease globally, mostly due to increase in the incidence of Diabetes Mellitus (DM) and hypertension. Abnormalities in calcium, phosphorus, calcitriol

and parathyroid hormone (PTH) are associated with CKD. Patients of CKD have much higher chances of vascular and soft tissue calcification if calcium is over-supplemented. **Aim:** The aim of this study was to evaluate serum ionized and total calcium in CKD patients and to predict if ionized calcium estimation is a better predictor of calcium levels in the serum. Materials and **Methods:** For this study, we evaluated 60 newly diagnosed patients of CKD who were not on any calcium supplement. Levels of creatinine, albumin, phosphorus, calcitriol, PTH, total calcium and ionized calcium were analyzed. Statistical analysis was done between ionized calcium with various parameters using Pearson two tailed correlation coefficient for association. **Results:** We found strong association of ionized calcium with creatinine, total calcium and phosphorus while no significant correlation was observed with albumin, albumin corrected calcium, PTH and calcitriol levels. Ionized calcium proved to be a better predictor of serum calcium level than total calcium level and albumin corrected calcium. **Conclusion:** Ionized calcium is physiological/functional component of serum calcium. We propose that ionized calcium should be estimated routinely instead of total calcium as it will help in proper supplementation of calcium and prevent metastatic calcification of various organs which occurs due to over-supplementation of calcium based on hypocalcemia predicted by serum total calcium level and albumin corrected calcium estimation. (DOI:10.9777/rr.2018.1204)

Analysis of OVATE Family Protein gene family from early land plants unravels evolutionary course and history

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Early land plants particularly the mosses, liverworts, hornworts and lycophytes forms the connecting link between the aquatic and higher plants. Several morphological and adaptive features evolved either from pre-existing genetic elements, or de-novo that allowed successful colonization of terrestrial ecosystem. It is therefore imperative to elucidate the course of molecular evolution acting upon the genes, influencing their structure and function that resulted in genotypic and phenotypic diversification. Multi- gene families are descended from a common ancestor through segmental or whole genome duplications followed by processes such as sub-functionalization, neo-functionalization, retention of redundant function, or pseudogenization that contribute to their diverse functions. The present study was performed on a multigene family, Ovate Family Proteins (OFPs), to understand the course of evolution on gene structure, functional attributes, and species-specific diversification in seedless plants. OFPs are plant specific transcriptional repressors and are acknowledged for their roles in important growth and developmental processes in angiosperms; though knowledge about their role in development and adaptation in early land plants is almost negligible. To address these lacunae, comprehensive in-silico analyses were carried out using the whole genome sequence and transcriptome data of four early land plants i.e. Marchantia, Physcomitrella, Selaginella and Sphagnum. Our analysis revealed the presence of 4, 19, 6 and 3 OFP members in Marchantia, Physcomitrella, Selaginella and Sphagnum, respectively. Cross-genera comparative analysis

revealed a drastic change in the structure and characteristics in OFPs suggesting functional diversification during the evolutionary process. This study attempts to unravel the role/s of OFPs in early land plants which may provide the basis for targeted research on the developmental and evolutionary attributes. (DOI:10.9777/rr.2018.1205)

In-silico targeting of sterol C-24 reductase in *Leishmania donovani* - A novel approach for treatment

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Background: Due to insufficient availability of antileishmanial chemotherapeutics it is an urgent need to search for new low cost molecules which have better efficacy and with low toxicity. We have employed in silico analysis for identifying new antileishmanial drug for possible use in therapeutics **Aim:** The sterol helps in the structural stability and survival of the Leishmania parasite. The enzymes are unique and novel in Leishmania. Many of them are not well characterized and their crystal structures are yet to be determined. We aimed to apply structure based drug designing (SBDD) approaches to target this enzyme. **Methods:** Homology modeling of Leishmania donovani Sterol C-24 reductase was performed (LdS24reductase) using Phyre2 server. Ramachandran plot was generated by Procheck. The active site residues of the target protein were examined by the docking of NADP to the target. Following preparation of protein and 2052 ligands (NCI diversity dataset), AutoDock Vina was applied for in-silico virtual screening. Molecular dynamic simulation of top five ligand-protein complexes was performed to understand the dynamics and behavior of the system. **Results:** Phyre2 server uses 4QUV (sterol reductase from Methylomicrobium alcaliphilum) as template. Ramachandran plot showed 90.4 % residues in favored, 9.2% in allowed and only 0.3 % in disallowed region. Docking of NADP elaborates the binding pocket and active site residues. We found approximately ten ligands which showed binding affinity above -10 kcal/mol. We explored top five ligands for its analysis using Gromacs. **Discussion:** SBDD approach against LdS24reductase enzyme suggests top ten ligands with high binding affinity. Ligplot analysis of the respective docked ligand indicates the binding pattern of the ligands including the noncovalent interaction. These results suggest that top five ligand might possess antileishmanial property. **Conclusion:** Structure Based Drug Designing approach (SBDD) may offer better strategy for development of new chemotherapeutic regime against leishmaniasis. (DOI:10.9777/rr.2018.1206)

Effect of inhibition of prolyl oligopeptidase on proteome of filarial parasite *Setaria cervi*

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Background: Prolyl oligopeptidase (POP) plays important role in the maturation and degradation of neuropeptides/peptide hormones and is a regulator of inositol (1,4,5) P3 signaling in mammals. In filarial parasite S. cervi, POP inhibition lead to calcium induced mitochondrial mediated apoptosis via stimulation of IP3 signaling. **Aim:** To

study the effect of inhibition of prolyl oligopeptidase on proteome expression in filarial parasite S. cervi. Methodology: The adult female S. cervi were exposed to Z-Pro-prolinal (ZPP), a specific POP inhibitor for 8 h. To study the proteome expression the crude somatic extract of the parasite was subjected to 2-dimensional gel electrophoresis. Further, to study the expression of phosphoproteins, the gel was subjected to phosphoprotein imaging. The altered proteins were identified using MALDI MS/MS. **Results:** A total of 109 and 112 protein spots were observed in 2D electrogram of control and ZPP treated parasites respectively. The protein spot analysis using PD-QUEST software showed 56 up-regulated and 32 down-regulated protein spots in ZPP treated parasites. The major proteins identified were found to play important role in diverse biological functions like calcium signaling, energy metabolism, cell cycle regulation, stress response and structural proteins. The phosphoprotein imaging revealed upregulation of G protein signaling proteins. **Discussion:** Inhibition of POP upregulated the calcium dependent proteins such as calreticulin and calpain-6 and phosphoproteins such as Rho dependent GTPase activating protein and regulator of G protein signaling protein. Therefore, the proteome analysis confirms that POP is indirectly involved in IP3 signaling mediated calcium release from the ER lumen in filarial parasites. **Conclusion:** Inhibition of POP significantly altered the proteome expression of S. cervi which suggests that POP play important role in the survival of the filarial parasites. (DOI:10.9777/rr.2018.1207)

Designing and identification of novel leads as anticancer

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Background: Quinones still comprise one of the largest classes of antitumor agents for beating cancer. They are naturally occurring and their cytotoxic potential make up for an attention. Conjugation reactions with bionucleophiles and Quinone redox cycling are the general mechanisms contributing to quinone toxicity. It has proved to be a good ligand for the topoisomerase II receptor which is the basic enzyme for restoring native DNA topology. Topoisomerases are ubiquitous enzymes required for untangling DNA after processes such as replication, transcriptions and other processes that distort DNA topology. **Aim:** To investigate the structure- activity relationships (SAR) and molecular docking of 2-chloro-3-amino-1, 4- naphthoquinone derivative for novel leads anticancer therapy. **Methods:** Several members of 2-chloro-3- amino-1, 4-naphthoquinone series were selected and performed quantitative structure- activity relationships (QSAR) study using VLife MDS software. Molecular docking study was performed using Schrodinger maestro 15. The study utilized crystal structure of Topo II alpha (PDB ID: 1ZXM) and doxorubicin as the standard. **Results:** The quantitative structure- activity relationships (3D-QSAR) and molecular docking of 2-chloro-3-amino-1, 4-naphthoquinone derivative provides an insight on structural requirement of Topo II alpha inhibitors for optimal inhibitory activity. The result showed mostly hydrophobic forces plays an important role in the binding of these inhibitors to the Topo II alpha. Among various compounds, AAME1 showed highest glide score (-11.5) compared to standard drug (-8.06). **Conclusion:**

We have successfully performed docking studies of a manually designed library of 2-chloro- 3-amino-1, 4-naphthoquinone derivatives for the designing of novel Topo II alpha inhibitors. Docking study indicated that presence of appropriate substituent resulted in compounds which will bind deep into the active site and make favorable interaction with the key residue of active site. The results of the present study may provide a new parameter for further design of novel 2-chloro-3-amino-1, 4-naphthoquinone as potent Topo II alpha inhibitors in the treatment of cancer. (DOI:10.9777/rr.2018.1208)

Phytochemical screening, HPTLC screening and in-vitro antioxidant activity of *Centella asiatica* extracts

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Background: There is currently immense interest in natural antioxidants and their role in human health and nutrition. However, traditionally used medicinal plants await such screening. **Aim:** The study aimed to identify the phytochemicals present in different solvent extracts of Centella asiatica (i.e. PECA- Petroleum ether extract of C. asiatica, CCA- Chloroform extract of C. asiatica, EACA- Ethyl acetate extract of C. asiatica, ECA- Ethanolic extract of C. asiatica, HACA- Hydro-alcoholic extract of C. asiatica) and evaluate the respective in-vitro antioxidant potentials. **Methods:** The phytochemical screening of extracts was done with standardized procedures and the antioxidant potential of different solvent extracts of Centella asiatica was assessed by its free radical scavenging activity 2, 2-diphenyl -1- picrylhydrazyl (DPPH) as well as hydrogen peroxide scavenging assay respectively for reducing capability. **Results:** In all different solvent extracts of C. asiatica revealed excellent free radical scavenging activity as revealed by 2-2-diphenyl-1-picryl-hydrazyl (DPPH) assay with EC50 values for ECA=128.752±1.85 µg/ml, HACA=274.884±1.21 µg/ml and hydrogen peroxide assay against the standard (Butylated hydroxytoluene) BHT, with the EC50 values ECA=429.69±0.92 µg/ml HACA=458.08±0.58 µg/ml while rest solvent extracts shown very less antioxidant activity. **Conclusion:** The present study indicates that the Centella asiatica extracts have good antioxidant activity which can be used in stress and anxiety and also a good source to be used as natural drugs. (DOI:10.9777/rr.2018.1209)

Screening of Anti-Inflammatory compounds from marine macro algae

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Marine seaweeds are increasingly viewed as potential sources of bioactive compounds with enormous pharmaceutical, biomedical and neutraceutical importance to reduce risk factors of diet-related chronic diseases. In an attempt to identify the anti-inflammatory compounds; Seaweeds were collected from Mandapam and Rameshwaram in Tamil Nadu. Initially we targeted on eicosanoids compounds from marine macro algae (HYPNEA MUCIFORMIS). However we found difficulties that these compounds are very difficult to isolate in stable form. Then we concentrated on the targeted crude extraction of terpenoids with

chloroform. Crude extracts was fractional by means of column chromatography using silica gel 60- 120 mesh size. Standardization studies have been conducted with high purity silica gel. Different mobile phase has been used for standardization and concluded that Chloroform: Methanol was working well. The following ratio was used Chloroform: Methanol (30:0 to 30:0.6). Anti-inflammatory activity of fractions from column chromatography were checked using lipooxygenase (LO enzyme studies, LPS activated murine macrophage cell line (RAW 264.7) and cytotoxicity assay in HeLa cells. Anti- inflammatory results showed that significant activity were noticed in all samples compared with the positive control (LPS). The fractions gave high cell viability as compared with crude extracts. This revealed that pure compounds have more activity than crude. Since crude may contain some inhibitory compounds. More active molecules was identified from fraction no. 33 of SARGASSUM TENERRIMUM. This compound was identified as E-15-Heptadecenal, Sesquiterpene aldehyde by using 1H-NMR ans 13C- NMR spectra. This compound could be used as a drug for anti-inflammatory activity. (DOI:10.9777/rr.2018.1210)

Studies on human Mesenchymal Stem Cell (MSCs) derived exosomes

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Exosomes (40-150 nm) are extracellular vesicles (EVs) that originate from multivesicular bodies of the endocytic pathway fusing with the plasma membrane. They are found in all body fluids, and are produced by most of the cell types but the rate of production is high in MSCs as well as in cancer cell. Exosomes, are enriched in signaling components, including lipid raft domains, bioactive lipids, transmembrane and cytosolic proteins, lectins, and various RNA species, including both mRNA and miRNAs. The exosomes in tumor progression and metastasis serve as a therapeutic agent by conferring several advantages as they can reprogram tumor behavior. Considering, MSCs-derived exosomes as a novel, very effective tool in regenerative and in immune-modulatory therapies, we have evolved purification strategies and technologies to obtain a pure population of exosomes with a reasonable yield. MSCs from different sources like human adipose tissue and Wharton's jelly were used for the isolation of exosomes. The exosomes- containing conditioned medium from cultured cells is used as the starting material for exosomes purification. Exosomes were isolated and purified by using commercially available isolation kit. Now, we will attempt to elucidate the role of exosomes in cancer. (DOI:10.9777/rr.2018.1211)

FOXO3A gene associated with aging: A pilot study of North Indian population

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Background: Aging is characterized by the general decline in body functions and the increased susceptibility to age-related pathologies. The fork head box O (Fox O) transcription factor family is a key player in an evolutionary conserved pathway. It promotes longevity downstream of insulin and insulin- like growth factor receptors in a variety of organisms, but the mechanisms by which FOXO extend lifespan remains elusive. Aim: To study the

genetic assessment and FOXO3A genetic variants in healthy study groups. **Methods:** Identify the genetic variants by PCR and Sequencing. This study includes 31 participants N=14, 07 and 10 as healthy aged, diseased (negative control) and healthy young (positive control) respectively. **Results:** The mean age for study group were found to be 85.93 ± 9.25, 25.1 ± 3.9 and 61.86 ± 9.26 for healthy aged, healthy young and diseased respectively. A total 40 Genetic variants were discovered in the region of FOXO3A. At nucleotide position 108567572 Del G, 108567577 Ins G were found to be significantly associated with diseased patients. 108567311 (Del T) mutation were mostly observed with the younger age and disease population but not up to the significant. **Discussion:** In this study, we analyzed 40 genetic variants out of which eight rs550848748 rs940385121 rs972234040 rs17532174 rs2253310 were previously reported across the FOXO3A to detect association with healthy aging. All SNPs presented on intronic region but one rs4946936 present on 3'utr but none of them is non synonymous. Conclusions: This study shows genetic variants the G nucleotide at 108567572 and 108567577 may be a molecular marker for healthy aged population. (DOI:10.9777/rr.2018.1212)

Comparative study of lipid profile in regular and non –regular haemodialysis in Chronic Renal Failure (CRF) patients

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Background: Chronic renal failure patients with CKD stage - 5, their Glomerular filtration rate have 15 ml/min or less they need to kidney transplant or dialysis to survive. Studies show that CRF patients are very prone to cardiovascular disease. During dialysis elevates of lipid profile (VLDL-C, LDL-C, triglycerides and total cholesterol) so regular dialysis has become the atherogenic major factor in mortality and morbidity. **Aim:** The present study was done to know whatever haemodialysis affect on lipid profile in CRF patients whose were taking dialysis regular and irregular. The nephrologist had prescribed all patients 2 dialyses at least in a week. **Methods:** The study was divided into two groups both are suffering chronic renal failure with CKD-5 stage last 4-month group first 18 patients undergoes haemodialysis regular time interval and at least two times in a week. Group second patients have same condition but some reason they were unable to take haemodialysis but at least once in a week. We collected serum sample after overnight fasting 3 times the regular interval of 30 days from, MLB medical college Jhansi .and analyzed Total cholesterol, VLDL, LDL, HDL, Triglycerides, by the automated machine. CRF Patients were near free from another disease. In Statistical analysis comparison between two groups was done by students t-test in Graph Pad Prism 7 software. **Results:** The age groups were 20 to 60 year olds and 80% patients were male. HDL-C, Triglycerides, VLDL-C level was found elevated in regular haemodialysis taking patients compare to irregular Haemodialysis. There was no significant change ($p>0.05$) find out cholesterol, LDC-C level in between group first and group second. There was no significant difference ($p>0.05$) observed between male and female CRF patients regular and irregular taking dialysis. **Discussion:** Chronic renal failure disease (CRF) is a significant health problem, the prevalence of which is increasing all over the world. The main cause of death in this patient population is cardiovascular

disease (CVD) related mortality. CRF is associated with premature atherosclerosis and increased the incidence of cardiovascular morbidity and mortality .the present study observed that CRF patients long-term regular dialysis effect lipid profile level. We also find out that the lipid profile level between female and male equally affected by dyslipidemia. **Conclusion:** it is concluded that number of dialysis increase the level of HDL-C, Triglycerides, VLDL-C regular CRF patients and they were no effect of cholesterol, LDC-C level. Dialysis patients need to cure of dyslipidemia. In irregular patients hypertriglyceridemia treatment is necessary that will improve the quality of life of CRF patients. (DOI:10.9777/rr.2018.1213)

Thermodynamic and conformation aspects of the interaction between antibody binding protein and IgG

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Background: Antibodies (IgGs) through protein-protein interactions play a vital role in stimulating cell signaling and providing stronger immune system in humans and proA (protein A) and proG (proteinG) are known to invade cells defense system through the mechanism of strong binding to IgGs. **Aim:** To gain an insight into the thermodynamic and conformational aspects of the interaction of antibody binding proteins with IgG. **Methods:** We had investigated the variable binding modes of proA/proG and their conformational states upon binding with IgG using solution based techniques such as CD, ITC and fluorescence. **Results:** In ITC, proA and proG binds with 1:3 and 1:1 stoichiometry to IgG respectively. Binding studies suggested that proA and proG undergo conformation rearrangement at the binding interface of IgG. CD and fluorescence results revealed that IgG does not undergo any significant conformational change. **Discussion:** In ITC, the multiple binding ratio of proA: IgG indicated the presence of conformational flexibility in proA, confirmed by CD results. By contrast, proG binds with 1:1 stoichiometry to IgG, which also involves key structural rearrangement within the binding interface of IgG-proG complex, confirmed by fluorescence KI quenching study. The binding competition experiments established that proA and proG cannot bind IgG concurrently. It is implicit from CD and fluorescence results that proA and proG dictate the phenomenon of IgG recognition. **Conclusion:** This study reports comprehensive energetic and conformational aspects of the interaction of proA/proG with IgG. These results showed the existence of structural flexibility in proA and proG that may be essential in order to bind to various types of IgGs which may allow these bacterial proteins to have functional diversity adding extra advantages to the microorganisms in their survival. These results enhanced our knowledge of mechanism adopted by proA and proG for binding with IgG. (DOI:10.9777/rr.2018.1214)

Active involvement of defense mechanism to mitigate adverse effects of UV-B radiation in *Withania somnifera* L. Dunal, an important medicinal plant

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Background: Withania somnifera, major producer of withanolides, is a constituent of over 200 medicinal formulations for the treatment of various disorders including cancer. Various abiotic factors have been found to modulate the active metabolite composition in W. somnifera. However effect of UV-B radiation on W. somnifera is in infancy stage and in order to develop high metabolite producing variety, it is desirable to get information regarding plant's response under different environmental conditions. **Aim:** The present study aims to find out the plant's responses to UV-B treatment **Methods:** W. somnifera plants were UV-B treated for 1, 2, 3, 4 and 5 hours. The damage to the plant was accessed through NBT staining to the plants and 14 genes from three different pathways were analyzed through Real time PCR. Further biochemical assays were performed to attest the RT-PCR data. **Results:** After 3 hour of UV-B treatment, signs of leaf damage appeared as indicated by NBT staining of leaves. Genes from DNA repair pathway (ruvB1 and ruvB2) were upregulated under UV-B treatment. In addition DAD1 and Bax inhibitor genes were also upregulated. A total of six genes from withanolide biosynthetic pathway and 4 genes from flavonoid pathway also showed variable overexpression under UV-B irradiation as compared to control. Biochemical assays for quantification of total flavonoids and total phenolics resulted in higher accumulation of these metabolites in UV-B treated samples as compared to control. **Discussion:** Initial UV- B treatment to W. somnifera resulted in upregulation of DNA-repair genes and anti-apoptotic genes which suggested active involvement of defense mechanism in plant to mitigate the adverse effects of UV-B radiation. Up to 3 hours of exposure, protective metabolites such as phenolics and flavonoids increased which suggest their role in UV-B protection in W. somnifera. **Conclusion:** Initial UV-B exposure caused quick activation of the defense system in W. somnifera and increased production of protective metabolites. (DOI:10.9777/rr.2018.1215)

Canaga odorata (Lam.) Hook. f. & Thoms essential oil as a plant based preservative in food system

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Industrial relevance: Based on the findings of the present study the Canaga odorata (COEO) essential oil can be recommended to agri-food industries for developing plant based preservative having anti-fungal, anti-aflatoxigenic and antioxidant potential. Aim of the study: To explore the in vitro and in situ preservative potential of COEO and to analyze the probable mode of anti-fungal and anti-aflatoxigenic action. **Methods:** Mycoflora analysis of some stored oilseeds and selection of the most aflatoxigenic Aspergillus flavus isolate as test fungus. GC-MS analysis to characterize COEO. Fungi toxicity of oil against the test fungus and some storage fungi. Anti-aflatoxigenic potential of COEO. Antioxidant activity by DPPH and ABTS free radical assay. Mode of antifungal action and effect of EO on cellular methylglyoxal (MG). In situ anti-fungal and anti-aflatoxigenic effect (by HPLC) of the COEO. Phytotoxicity assay of COEO. Results and **Discussion:** 692 mold isolates were obtained during mycoflora analysis and A. flavus AF-M-K5 was identified as test fungus. GC-MS analysis depicted linalool (19.99%) and benzyl acetate (18.25%) as major components. The minimum

inhibitory concentration, aflatoxin inhibitory concentration and fungicidal concentration of COEO against AF-M-K5 were 2.0, 1.5 and 5.0 µL/mL respectively. COEO showed broad spectrum fungitoxicity against storage fungi. The IC values for DPPH and $ABTS^{+}$ assay were 1.02 µL/mL and 0.92 µL/mL respectively. COEO reduced ergosterol content and enhanced leakage of Ca^{2+}, K^{+}, and Mg^{2+} ions emphasizing plasma membrane as antifungal site. Reduction in MG content by COEO suggested mode of reduction in AFB1 biosynthesis. COEO protected stored mustard seeds from A. flavus (75%), storage molds (73.02%) and aflatoxin B1 production during storage and caused no toxicity in seed viability. **Conclusion:** Present study recommend COEO as a safe plant based preservative against fungal and aflatoxin B1 contamination and oxidative deterioration in stored oilseeds. (DOI:10.9777/rr.2018.1216)

Automated medical bandage for empowerment of differently abled person/Multi drug delivery system designed for dose dependent drug release

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Background: Dating back to time since medication has started, it has always been challenging for physically disable patients to care on their own. An attendant is required for timely medication and dosage requirement. Especially in case of poor and lower middle class family, where members have to move out for daily earning. **Aim:** Automated bandage provides a controlled technique of drug delivery for various disease and wounds. **Methods:** An electric conducting bandage made of cotton thread covered with conductive substance for the coated in hydrogel loaded with concerned medications or electro active hydrogel made up of bacterial cellulose and conducting polymers. The bandage has a battery of suitable voltage and a microcontroller controlled b a digital synchronized custom made device or smartphone. The device would trigger the microcontroller and regulate the amount of current passing into the wire thereby releasing drug. **Results:** Regulation of automated bandage is entirely switch based system controlled by the will of patient. The drug reaches to the destination through a channel. As prescribed by doctor, medicine can be applied ecto or sub-dermally. This helps patient to be self-dependent even if they are differently abled or alone at home or even old aged. **Discussion:** the medication can be pain killer, antibiotic, tissue regenerating growth factor or any drug that is to be released in sub-dermal layers in case of chronic skin diseases, wounds etc. this method could be very beneficial for differently able people or partially paralyzed ones. **Conclusion:** Automated bandage gives a new dimension to the medicare world. It can be designed with bio-degradable fibres generating eco-friendly equipment with cost effective value. Empowerment of differently abled/old patients with proper medication will be advancement in science and technology. Future prospect can be injecting medicine to the blood level which will help to combat various diseases. (DOI:10.9777/rr.2018.1217)

Salvianolic acid B ameliorates the pathological changes associated with lipopolysaccharide induced acute lung injury in mice

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Background: Acute lung injury (ALI) is an acute inflammatory disease characterized by excess production of inflammatory factors in lung tissue. Salvianolic acid B (SVA-B), an active component isolated from Chinese herb Salviae miltiorrhizae, is shown to possess a wide range of biological activities like anti-inflammatory, anti-oxidant and anti-carcinogenic effects. **Aim:** This study aimed to investigate the effect of salvianolic acid B on lipopolysaccharide (LPS)-induced acute lung injury in mice. **Methods:** Adult albino mice were divided into 5 groups with six animals each and were named according to challenge/treatment/dose: SHAM/control group (saline alone), the LPS group challenged with LPS (Escherichia coli Serotype: 055:B5, Sigma, USA, 100 µg/kg), and the SVA-B group pretreated with SVA-B (@1, 5 and 15 mg/kg orally) 7 days before LPS challenge. Bronchoalveolar lavage fluid (BALF) samples and lung tissues were collected 6 h after LPS administration. Histopathological and biochemical (MPO, oxidative stress markers) parameters and levels of inflammatory cytokines (TNF-α and IL-6) levels in BALF were monitored. **Results:** SVA-B significantly attenuated LPS-induced LPS induced pulmonary oedema and microvascular permeability by decreasing the lung wet/dry weight ratio and lung tissue injury. SVA-B decreased LPS induced inflammatory cells recruitment and inflammatory cytokines production. SVA-B decreased LPS induced histopathological changes in the lungs and reduced LPS induced oxidative stress. **Discussion:** Acute lung injury is characterized by an acute inflammatory process in the airspaces and lung parenchyma. In the present study, pretreatment with SVA-B attenuated LPS- induced inflammatory changes such pulmonary hyperpermeability and edema, cytokines secretion, neutrophil cell influx and MPO activity in the lung. Additionally, SVA-B pretreatment resulted in a decline oxidative stress. **Conclusion:** The study indicated that SVA-B ameliorates LPS-induced acute lung injury in mice largely through suppression of inflammation, oxidative stress and histopathological changes in lungs and thus may have a therapeutic potential in the prevention of the disease. (DOI:10.9777/rr.2018.1218)

PPAR: A novel target for the treatment of type 2 diabetes.

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Background: Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors involved in regulation of lipid metabolism, lipoprotein synthesis in liver. These are activated by thiazolidinediones results in increase insulin sensitivity hence can be used in treatment of type 2 diabetes. **Aim:** Relevance of PPAR in current therapy of diabetes mellitus disorder. **Introduction:** The peroxisome proliferator- activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPAR plays a vital role in cellular differentiation, development, and metabolism of carbohydrates, lipids and proteins. These contain various functional domains viz. N-terminal region, DNA-binding domain, flexible hinge region, ligand binding domain and C-terminal region. Three types of PPARs have been identified. PPAR-α, expressed in liver, kidney, adipose tissue and heart. PPAR-β expressed in brain and adipose tissue. PPAR-γ expressed in adipocytes, placenta and macrophages. PPAR-α

agonists are pharmacologically related to fibrates and activate PPAR-α for lipid metabolism. PPAR-γ is activated by thiazolidinediones, treating metabolic syndrome by lowering blood glucose level. PPAR-β agonism changes the metabolic preference from glucose to lipids. Dual PPAR agonist (Glitazars) bind to both PPAR-α and PPAR-γ receptor, these include Saroglitazar, Aleglitazar, etc. **Conclusion:** PPAR agonists may have therapeutic usefulness in metabolic syndrome by increasing fatty acid consumption in skeletal muscles and adipose tissue hence can be used as a novel target for the treatment of type 2 diabetes. (DOI:10.9777/rr.2018.1219)

Carbon ion radiation in combination with PNKP inhibitor radiosensitizes cultured lung cancer cells

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Background: High LET (linear energy transfer) carbon ion radiation has significant biological advantages for treatment of cancer as compared to low LET gamma radiation. There are ongoing efforts to incorporate molecularly targeted agents (radiomodulator) as part of combined radiochemotherapy to enhance its efficiency as well as cost effectiveness. Polynucleotide kinase/phosphatase (PNKP) plays an important role in the repair of DNA double strands breaks. Inhibition of PNKP may regulate cellular radiosensitivity, making it an attractive therapeutic target. **Aim:** To investigate the effect of carbon ion radiation in combination with PNKP inhibition on cultured lung cancer A-549 cells. **Methods:** Carbon ion radiation having the energy of 62MeV with corresponding entrance LET 290KeV/μM was used to irradiate the cells. Cells were irradiated with dose of 2Gy and 4Gy with the combination of 30 μM A12B4C3 (PNKP inhibitor). Cell viability was measured by MTT assay and trypan blue exclusion assay. To assess the DNA damage Comet assay and DAPI staining was performed. Caspase-3 activity was measured to evaluate apoptosis. **Results:** High LET carbon ion radiation with A12B4C3 enhances the cell death as compared to only irradiation group. Similarly, comet formation was enhanced when carbon ion irradiation was combined with A12B4C3. DAPI staining also revealed that there was significant DNA damage in combined treatment as compared to only irradiated group. Colorimetric caspase 3 assay signify that A12B4C3 in combination with carbon ion radiation enhances the cell death through apoptosis. **Discussion:** These results indicate that A12B4C3 as PNKP inhibitor has potent radiosensitization activity when combined with carbon ion radiation in A-549 cell line. Further studies are required to confirm the role of A12B4C3 in DNA repair pathway in human lung cancer cell line A549. **Conclusion:** A12B4C3 an inhibitor of PNKP activity, may be used as radiomodulator to suppress recurrence of cancer on the exposure of carbon ion radiation. (DOI:10.9777/rr.2018.1220)

Detection of Reactive Oxygen Species (ROS) in cyanobacteria using fluorescence microscopy and fluorescence spectroscopy

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Background: Reactive oxygen species (ROS) are cell signaling molecules, synthesized inside the cells as a response to routine metabolic processes. In ultraviolet radiation (UVR), ROS concentration increases several folds in the cells that become toxic for the cell survival. 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) is a non-fluorescent, cell-permeable dye which is hydrolyzed intracellularly into its polar, but non-fluorescent form of DCFH by the action of cellular esterases. Oxidation of DCFH by the action of intracellular ROS and other peroxides turns the molecule into its highly fluorescent form, i.e., 2',7'-dichlorofluorescein (DCF), which can be detected by various fluorescent methods. **Aim:** Detection of Reactive Oxygen Species (ROS) in Cyanobacteria by using fluorescence microscopy and fluorescence spectroscopy. **Methods:** The UVR irradiated cyanobacterial samples were incubated with 2',7'- dichlorodihydrofluorescein diacetate (DCFH-DA). Cells were visualized under a fluorescence microscope equipped with the following filter set: UV: (DAPI) EX 340 nm EM 488 nm, blue: (FITC) EX 495 nm EM 510 nm and green: (PI 550) EX 550 nm EM 650 nm. The fluorescence intensities of the samples were measured by a fluorescence spectrophotometer with an excitation wavelength of 485 nm and an emission band between 500 and 600 nm. **Results:** The image obtained from fluorescence microscopy shows ROS generation in all studied cyanobacteria samples. The Green/Red ratio shows higher levels of ROS in UVR treated samples in comparison to control samples. The results obtained from microscopic analysis were further supported by data obtained from fluorescence spectroscopy. **Discussion:** UVR depended generation of ROS has been reported to affect the lipids, nucleic acids as well as photosynthetic machinery. The highest level of ROS generation in the UV-B region of UVR was observed. The use of a fluorescent oxidant-sensing probe DCFH-DA provides an easy way of detecting ROS in cyanobacteria. **Conclusion:** This method provides reliable, simple, rapid and cost effective means for detection of ROS in cyanobacteria. (DOI:10.9777/rr.2018.1221)

Piracetam ameliorates LPS-induced neurotoxicity in rats

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Background and Aim: The present study was performed to investigate the effect of piracetam on neuroinflammation induced by lipopolysaccharide (LPS) and resulting changes in cognitive behavior. **Methods:** Neuroinflammation was induced by a single dose of LPS solution infused into each of the lateral cerebral ventricles in concentrations of 1 µg/µl, at a rate of 1 µl/min over a 5-min period, with a 5-min waiting period between the two infusions. Piracetam in doses of 50, 100, and 200 mg/kg i.p. was administered 30 min before LPS infusion and continued for 9 days. On ninth day, the behavioral test for memory and anxiety was done followed by blood collection and microdissection of the hippocampus (HIP) and prefrontal cortex brain regions. Results and **Discussion:** Piracetam attenuated the LPS-induced decrease in coping strategy to novel environment indicating anxiolytic activity. It also reversed the LPS- induced changes in the known arm and novel

arm entries in the Y-maze test indicating amelioration of spatial memory impairment. Further, piracetam moderated LPS-induced decrease in the mitochondrial complex enzyme activities (I, II, IV, and V) and mitochondrial membrane potential. It ameliorated changes in hippocampal lipid peroxidation and nitrite levels including the activity of superoxide dismutase. Piracetam region specifically ameliorated LPS-induced increase in the level of IL-6 in HIP indicating anti- neuroinflammatory effect. Further, piracetam reduced HIP Aβ (1–40) and increased blood Aβ level suggesting efflux of Aβ from HIP to blood. Conclusions: The present study indicates preclinical evidence for the use of piracetam in the treatment of neuroinflammatory disorders. (DOI:10.9777/rr.2018.1222)

Pharmacologically imperative, antifungal bio-organic molecules discovery: An approach to extract and develop natural antifungal from novel mangrove Actinobacteria

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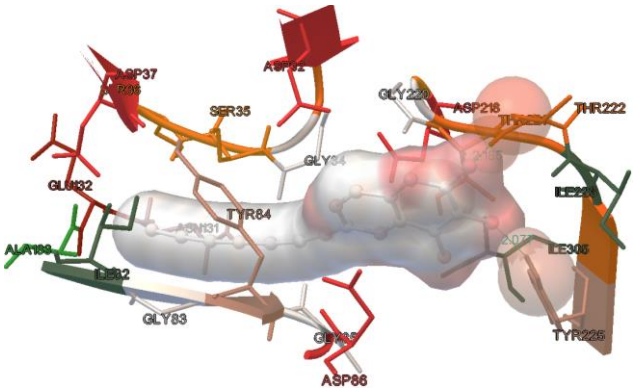
Background and Aim: The focus of the present study was to discover naturally available medicinal products from mangrove actinobacteria. Most commonly, Streptomyces were isolated and identified along with Actinomycetes, Nocardia, and Micromonospora. **Methods:** Indented to focus on the discovery of Streptomyces novel therapeutics, it was solely isolated, characterized through 16S rRNA (VITGAP241). High-throughput approach was done to screen most potent antifungal bio-organic molecules. Structurally identified and screened compounds were purified through column chromatography technique and confirmed via ^{1}H, ^{13}C NMR, FTIR and GCMS. Compound purity was determined using HPLC. **Results:** An in vitro competitive ELISA based protease inhibition assay was carried out followed by an antifungal activity (Zone of inhibition 18mm) assessment using agar well diffusion method. To get more insight into the binding mode of most potent compounds to secreted aspartic protease 1 (PDB ID: 3FV3) which confers virulence in C. albicans. Molecular mechanistic values that were obtained from molecular docking studies reveal the possible drug candidacy ability of screened compounds (Binding energy range -8.17 to -40.41 kcal/mol). The necessary inhibitory constant values were found only in nano molar (8-156 nM). Discussion and **Conclusion:** There was a resemblance of identical results were found between in vitro and in silico evaluations. The anticipated druggability properties of potentially identified compounds were uncovered by establishing a SAR along with ADMET, PK/PD extrapolations. Future prospects: Drugs can be formulated using nanofibers using polymers to check its encapsulation efficacy and in vitro enzyme release (DOI:10.9777/rr.2018.1223)



Computational discovery and experimental verification of a drug indicated for overactive bladder for the reversal of memory and cognitive deficits in rat model neurodegeneration.

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Background: Today, the urgency to discover effective interventions for cognition and memory impairment is greater than ever. Discovery of new indications for already approved drugs can advantage to the patients by getting rapid access to novel treatment choices and also to the developer by cost-effective solutions to expensive drug development process. Aim: Drug repurposing for memory and cognitive deficits: in silico, in vitro, and in vivo investigation of Drug Bank database for acetylcholinesterase enzyme (AchE) inhibitors Methods: In this study, we developed an integrative computational framework based on virtual screening workflow methods to screen 4199 drugs (FDA approved drugs and drugs approved outside United States) with acetylcholinesterase (AchE) as the enzyme structure. Interestingly, six hits were identified by the virtual screening process were further investigated using detailed molecular dynamics simulation to determine the ability of the hits to interact with the target in a stable manner. Results: Based on this in silico screening protocol, a drug indicated for overactive bladder, identified as a potential compound that could target acetylcholinesterase (AchE) enzyme. For the first time, we found that our drug inhibits AChE enzyme at sub-micromolar concentrations in vitro and also showed a significant reversal of memory and cognitive deficits in in vivo rat model of neurodegeneration. Discussion: We evaluated and compared the effects of intragastrically-administered our drug with donepezil, a marketed AchE inhibitor, in cognitive and behavioral assays including the novel object recognition test, Y maze and Morris water maze test. We founded that our drug can restore memory loss and cognitive dysfunction to a similar extent as donepezil. Conclusion: These findings suggest that our drug may become a viable treatment option for memory and cognitive deficits with a good safety profile in humans. (DOI:10.9777/rr.2018.1224)

Elucidation of the interactive effects of alcohol and restraint stress on mice hippocampus: Effect of melatonin

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Background: Stressful events and alcohol abuse are the cumbersome situations which can synergistically predispose the negative effects on the brain. Oxidative stress generated by chronic immobilization and alcohol consumption cause severe neurotoxicity in the hippocampus region that ultimately leads to cognitive dysfunction Aim: To elucidate the effect of melatonin on hippocampus of stressed mice, alcohol drinking mice and combination of both. Methods: Male Swiss albino mice were given alcohol (ALC) (15% v/v) or restraint stress (RS) or both (RS for 6 h per day) up to 28 days. We found increased ALC consumption in the ALC + RS group as compared to the ALC group. Morris water maze (MWM) test and novel object recognition test (NORT) revealed the spatial and recognition memory impairment in RS and ALC + RS group. Results: ALC + RS group showed more profound oxidative stress and augmentation of pro-inflammatory cytokine (IL-1b) as compared to RS or ALC group alone. Melatonin

(20 mg/kg, p.o) treatment for 14 days significantly prevented the raised oxidative stress, release of IL-1b, GSH depletion and augmentation of AChE activity in the hippocampus. Moreover, semi-quantitative reverse transcriptase PCR results showed that combined exposure of ALC and RS leads to over-activation of NF-kB transduction inflammatory pathway and down-regulation of the Nrf2/HO-1 axis which was significantly ameliorated by the melatonin treatment. Discussion & **Conclusion:** In conclusion, our results indicated that ALC + RS exerted the deleterious effects on the hippocampus which were alleviated by the melatonin treatment. (DOI:10.9777/rr.2018.1225)

Green synthesis of zinc oxide nanoparticles using *Rubia cordifolia* root extract against different bacterial pathogens

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Background: Wide application of nanoparticles stimulates the need for synthesizing them but, the conventional methods are usually hazardous and energy consuming. **Aim:** This lead to focus on "greensynthesis" of nanoparticles which seems to be easy efficient and ecofriendly approach. In this study the green synthesis of zinc oxide nanoparticles was carried out using root extract of Rubia Cordifolia a reducing agent and their antimicrobial activity against various bacterial pathogens. Zinc oxide nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity. **Methods:** Zinc oxide nanoparticles were synthesized using zinc nitrate and NaOH by precipitation method. Results and **Discussion:** Microbiological tests were performed using varying concentrations of green ZnO NPs with size 14.18 nm. Green synthesized NPs showed antibacterial activity against Micrococcus luteus, Staphylococcus aureus, Escherichia coli. Zinc Oxide nanoparticles were characterized by XRD, SEM, EDX and TEM. **Conclusion:** The green synthesis of Zinc Oxide nanoparticles appears to be cost effective, eco-friendly and easy as compare to other methods for the nanoparticles synthesis and its antibacterial activity. (DOI:10.9777/rr.2018.1226)

Effect of valproic acid on the gene expression of Base Excision Repair (BER) enzymes in *Leishmania donovani*

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Background: Leishmaniasis is a vector borne protozoan disease of major concern in poor countries. Due to lack of effective preventive and prophylactic agents, control of Leishmaniasis relies primarily on chemotherapy. New formulations like amphotericin B, its lipid formulations, and miltefosine have shown great efficacy against leishmaniasis but the emergence of resistant strains limit their usefulness and development of new antileishmanial drugs is urgently needed. Survival of Leishmania donovani in highly oxidative environment inside the host macrophage indicates it has an efficient DNA repair pathway. Studies on cancerous cells suggest that histone/protein deacetylases (HDAC) have significant roles in the regulation of DNA repair enzymes. Also, HDAC inhibitors (HDACi) induce hyperacetylation of

histones and some non-histone proteins leading to chromatin remodelling and transcriptional activation. **Aim:** To assess the effect of valproic acid (VA) on the level of gene expression of Base Excision Repair (BER) enzymes in L. donovani **Methods:** HDAC assay, Insilco Analysis, Cell viability assays and RT-PCR **Results:** Through assays we demonstrated that Leishmanial HDACs are biologically active. Localization studies through microscopy indicated that class I HDACs are nuclear whereas class II HDACs are predominantly present in the cytoplasm. Out of HDACi included in this study, TSA, apicidin, sodium butyrate and VA, VA could reduce the viability of L. donovani more than others. IC50 of VA for L. donovani was 40mM. Surprisingly, at lower concentration (5-10mM), VA increases the cell proliferation (up to 10-15%) but at higher concentration (>10mM) cell viability was gradually reduced. Furthermore, treatment of cells with 5mM VA leads to upregulation of BER transcripts namely polymerase β, UNG and ligase-IIIα up to 7, 4 and 2 folds, respectively. Discussions: We presume that activation of DNA repair enzymes is due to increased histone acetylation. **Conclusion:** VA has varying effects at different concentrations on L. Donovani and further studies are needed to understand its mechanism of action as well as explore its therapeutic potential against leishmaniasis. (DOI:10.9777/rr.2018.1227)

Evaluation of antifouling potential of extract of seaweeds against bio-film forming bacteria on titanium

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Background: Environmental problems caused by tin and copper based antifouling compounds have highlighted the need to develop new environment friendly antifouling coating. **Aim:** To screen the antifouling potential of extracts of seaweeds against some marine and other fouling bacteria. **Methods:** Seaweeds such as (Sargassum wightii and Stoechospermum marginatum were collected, washed, air dried andfinely powdered samples were subjected to solvent extraction by cold steep method. The crude extract was eluted with different polar to nonpolar solvents and subjected to FT-IR, GC-MS and TLC analysis. The bio-film forming bacteria, Micrococcus luteus (MTCC4821), Flavobacterium aquatile (MTCC7307), Pseudomonas aeruginosa (MTCC7453), and Bacillus flexus (MTCC 2422), were isolated from the cooling pipes (Titanium) of Fast Breeder Test Reactor at Kalpakkam, Atomic power station. The ethyl acetate fraction of seaweeds was incorporated into the epoxy sol gel coating solution on titanium coupons using Spin Coating method and the coating was evaluated by Electrochemical Impedance Spectroscopy. For in vitro bio-film adhesion analysis, epoxy coated Ti coupons were exposed in the respective bacterial culture medium for six hours. For post exposure analysis, total Viable Count was done followed by Epifluorescence microscopy and Live/Dead staining. **Results:** Out of eight, the ethyl acetate fraction of S. wightii was effective against both gram positive and gram negative bacteria at MIC value of 0.75 and 0.375 mg/ml for Pseudomonas aeruginosa and Micrococcus luteus respectively. Total Viable Count showed one order reduction in both gram positive and negative bacterial load on bio-film. Epifluorescence microscopic analysis of Acridine orange and Live/Dead staining showed significantly less bio-film adhesion on ethyl acetate

fraction impregnated Ti- coupons in comparison to control and anodized coupon. **Conclusion:** The anti-fouling property of S. wightii could be due to the presence of fatty acids, considering its rich diversity of secondary metabolites, so this seaweed could be used as a Green inhibitor against fouling bacteria. (DOI:10.9777/rr.2018.1228)

Identification, sequence analysis and expression of BURP domain containing Protein under Boron toxicity in rice plants

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Background: Boron toxicity is a nutritional disorder affecting crop production in many parts of the world. This study explored the expression of BURP domain containing protein associated with B tolerance in rice (Oryza sativa L.). The BURP-domain proteins consist of (i) an N-terminal hydrophobic domain, (ii) a short (conserved) segment, (iiii) an optional segment of repeat units, and (iv) the C-terminal BURP domain. The majority of members of BURP domain containing proteins are induced by various stress factors such as drought, salt, cold, abscisic acid and boron toxicity. **Aim:** The exact role of BURP-domain containing proteins in rice plants, still remains to be elucidated especially under boron toxicity. Rice is one of the most important crops and a model plant for monocot species. However, only two BURP protein- encoding genes, OsRAFTIN1, and RA8, have been reported. Even though the rice genome has been sequenced, a genome-wide analysis of the BURP family in this species has not been done. Towards a long term goal of revealing the functions of all members in this family, transcript expression levels of this family will be investigated in rice seedlings under Boron toxicity. **Methods:** To identify the conserved motifs in BURP proteins, online MEME (Multiple EM for Motif Elicitation) will be employed to analyze the protein sequences of BURP members from different rice plant species. The SMART search program will be used to confirm the BURP domain and to obtain the protein sequences of the BURP domain, and the protein sequences of BURP domain will be in turn used to generate an alignment. Results and **Discussion:** The presence of the conserved BURP domain in diverse plant proteins suggests an important and fundamental functional role for this domain. It is possible that the BURP domain represents a general motif for localization of proteins within the cell wall matrix. The other structural domains associated with the BURP domain may specify other target sites for intermolecular interactions. (DOI:10.9777/rr.2018.1229)

Hairy root cultures: a potent approach for the production of high-value Secondary metabolites

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Aim: Hairy root, a plant disease which is a novel source for the production of valuable secondary metabolites due to its rapid growth without any phytohormone, numerous branching, and genetic and biochemical stability. Hairy roots are similar to wild type roots with regard to the differentiated morphology and biosynthetic machinery and also accumulate similar secondary metabolites.Hairy root cultures had been also tried as an

experimental system to elucidate the secondary metabolite pathways. Methods & **Results:** Agrobacterium rhizogenes, a soil bacterium, mediates the hairy root production by transferring a portion(T-DNA) of root inducing plasmid into explants of target plants.T-DNA contains oncogenes (i.e. rol A, rol B, rol C and rol D)which also gets incorporated into host nuclear genome and induces the formation of hairy roots. Discussion and **Conclusion:** Since last three decades, a large number of plants including medicinal plants had been successfully transformed with Agrobacterium rhizogenes for the production of valuable secondary metabolites (used as pharmaceuticals, pigments and flavors) through the development of in vitro hairy roots. This review main focuses on the principle and advantages of using hairy root cultures to produce secondary metabolites and its pathway elucidation studies in various plant species. (DOI:10.9777/rr.2018.1230)

Significance of DHA in memory development of infants

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Background: Docosahexaenoic acid (DHA) is the major lipid in the human brain. It is omega -3 polyunsaturated fatty acid (PUFA's) vital for normal brain function. DHA comprises 50% weight of the neuronal plasma. **Aim:** Significance of DHA in memory development of infants. **Introduction:** Docosahexaenoic acid (DHA) concentrations are high during gestation and first three years of infant life. Animal studies confirm DHA enhanced advanced cognitive function and keen visual data processing prowess. Modern day diet significantly lacks DHA and natural sources are limited, predominantly maternal post-partum milk. Improvement in memory sharpness in adults and impacts on productivity at later stages of life is recognized from concept of advanced cognition being uniquely human. **Conclusion:** Learning and behavioral relationship of healthy school children with DHA is affirmed by myelination of brain frontal lobes through enriched tissue content that includes high concentration of the fatty acid. Also, health benefits of DHA on diseases such as hypertension, depression and onset of diabetes mellitus at later stages of life were also positively enforced. (DOI:10.9777/rr.2018.1231)

Antioxidative and hypolipidemic effect of *Pueraria tuberosa* in rats with high fat diet induced nonalcoholic fatty liver disease

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Background: Non alcoholic fatty liver disease (NAFLD) is chronic liver diseases ensuing from excessive fat accumulation in liver. Excess hepatic fat accumulation results from excess triglycerides (TG) delivery from diet to liver and from excess carbohydrate conversion to TG. The pathogenesis of NAFLD is multiplex and closely entangled with insulin resistance and obesity. **Aim:** The aim of this work is to evaluate the antioxidant and hypolipidemic effect of previously reported antidiabetic role of aqueous extract of Pueraria tuberosa in rats with high fat diet induced non alcoholic fatty liver disease. **Methods:** Non alcoholic fatty liver disease was induced in rats with high fat diet (HFD) for 16 week and grouped into HFD control, PT 50mg/100g BW+HFD, PT

100mg/100g BW+HFD, finofibrate 100mg/kg BW + HFD. Triglycerides, cholesterol, glucose, SGOT, SGPT level was assessed in blood plasma. SOD, Catalase and LPO enzyme activity was assessed in blood sample. **Results:** Aqueous extract of PT shows the antioxidant properties by decreasing the lipid peroxidation and increasing the superoxide dismutase and catalase enzyme activity. It also shows the hypolipidemic effect on NAFLD rats by decreasing the TG and cholesterol level. It also shows the important effect on the prevention of progression of liver damage by decreasing the liver function test enzyme SGOT and SGPT enzyme activity. **Discussion:** In this experimental study we analyses the therapeutic effect of PT in case of NAFLD on biochemical level. **Conclusion:** All the results demonstrated that PT can exhibit therapeutic effect on non-alcoholic fatty liver disease by improving antioxidant enzyme activity, decreasing TG, cholesterol level and decreasing liver function test enzyme SGOT and SGPT level. (DOI:10.9777/rr.2018.1232)

Association of oxidative stress with hypertension in elderly subjects

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Introduction: Oxidative stress is associated with hypertension (HTN), and important risk factor for cardiovascular events. Many processes involved in the pathophysiology of HTN, vascular damage, due to oxidative stress is principally important. This study evaluated the association between oxidative stress and HTN. **Methods:** Seventy five patients with hypertension and 75 age matched control were enrolled in the study. Total oxidant status (TOS) and total antioxidant status (TAS) were determined by novel automatic colorimetric methods. **Results:** Mean value of TOS were significantly higher in HTN than in normal control subjects (33.70 ± 8.26 vs. 30.70 ± 8.58 µmol H2O2 Equivalent/L, P = 0.03). Mean of TAS were markedly lower in HTN compared to normal control subjects (1.55 ± 0.95 vs. 2.04 ± 1.63 mM Trolox equivalent/l, P = 0.025). **Conclusion:** HTN showed markedly higher oxidative stress measured by novel automatic colorimetric methods, compared to healthy. Taken together, our findings suggest the involvement of oxidative stress in HTN. (DOI:10.9777/rr.2018.1233)

An algal biopolymer sodium alginate induced antioxidant defense responses in *Solanum lycopersicum* Linn.

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Background: Control of plant diseases using biopolymer is gaining momentum in recent years because of its ecofriendly nature. Hence, in this study an algal biopolymer sodium alginate was tested for its potentiality of inducing resistance factors in tomato plant. **Aim:** To study the potency of sodium alginate in inducing antioxidant defence responses of Solanum lycopersicum Linn. **Methods:** The tomato leaves were sprayed with different concentrations (0.2%, 0.4%, and 0.6%) of sodium alginate. Experimental leaves were assayed for H2O2, Catalase (CAT), Superoxide dismutase (SOD), Ascorbate peroxidase (APX) and Guaiacol peroxidase (GPX) enzyme activity by standard

protocols. **Results:** Sodium alginate (0.4%) treatment recorded an increase in H2O2 content on day 2 which further increased by 40% on day 6. An increased CAT activity was evident in tomato leaves treated with 0.2% biopolymer on day 2. A maximum SOD activity was recorded on day 4 in 0.4% and APX was also recorded on day 8 in 0.4% sodium alginate treatments. An increased GPX activity was evident in 0.4% and 0.6% biopolymer treated leaves on day 4 and 6. Both a qualitative and quantitative increase in SOD and GPX were evident on day 4 and 6 by sodium alginate in tomato. **Discussion:** A significant increase in the levels of SOD by sodium alginate pre- treatment suggests its role in neutralizing the superoxide. The levels of SOD activity also to correlated with levels of H2O2. Further, higher level of H2O2 observed appear to correlate with higher levels of SOD and CAT. An increase in POX activity could play a protective role against ROS. The induction of isoenzymes of APX and GPX in tomato may also contribute to survival of the tomato during pathogen infection. **Conclusion:** Based on the above results we concluded that sodium alginate could be a potent elicitor of antioxidant defense in tomato. (DOI:10.9777/rr.2018.1234)

Santalin A as a potential phytochemicals of *Pterocarpous santalinus* (Lal chandan) for modulation of mTOR receptor in diabetic nephropathy: *In-silico* assessment by molecular docking

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Background: Diabetic nephropathy (DN) is a primary cause of end-stage renal disease globally. Activation of mTOR and reduced autophagy has been identified as one of the pathogenic pathways. The present available drugs have been successful in inhibiting the mTOR signaling, but show low oral bioavailability and suboptimal solubility. Rapamycin is a selective inhibitor of mTOR, shown significant protection against DN. However, its continuous use is associated with side effects. Thus, search of novel drugs are on great demand. In present study in silico approaches has been adopted to identify potential compounds with optimal oral bioavailability and better solubility properties, with no toxic effect. Materials and **Methods:** The receptor protein mTOR with PDB ID: 3FAP was retrieved from RCSB protein data bank. Total 20 compounds were selected from the list of 113, obtained from LC-MS of Lalchandan. Further in silicomolecular docking calculation was done by using YASARA software. Druglikeness and molecular property of best docked compounds was checked by using Lipinski rule of five and AdmetSAR server. **Results:** Five compounds showed interaction with mTOR protein. Out of these Santalin A showed best interaction with binding energy 11.453[kcal/mol] and dissociation constant 4025.6841[pM]. Further, the Lipinski druglikeness and admetSAR results also showed that Santalin A has good optimal oral bioavailability, non-carcinogenic and no toxic effect, suggesting all drug like properties as compared to standard drug Rapamycin. **Conclusion:** Santalin A, can be taken up as a potential lead compound for biological testing for developing a new drug for diabetic nephropathy, acting via mTOR signaling inhibition pathway. (DOI:10.9777/rr.2018.1235)

Antiproliferative effect of isoquinoline alkaloid against N, N-dimethyl hydrazine induced colon cancer in albino wistar rat

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Background: In this study, we investigated the in vivo anti-proliferative action of isoquinoline alkaloid (M1) in dimethyl hydrazine (DMH) induced colorectal carcinoma (CRC) using albino Wistar rats. Our study provided the evidence that M1 can potentiate comparable anti-proliferative properties towards colon cancer treatment. **Aim:** The present study is aimed to elucidate the anticancer potential of isoquinoline alkaloid (6, 7-dimethoxy-1, 2, 3, 4-tetrahydro-isoquinoline-3-carboxylic acid (M1)) obtained from Mucunapuriens seeds. **Methods:** All animals were randomly divided into 5 groups of 6 animals each (n=30), M1 was administered to DMH induced CRC rats at 10 and 25 mg/kg doses for 28 days. Various physiological, oxidative parameters, histopathology, were performed to evaluate the anti-CRC potential of M1.The docking studies of M1 were performed with CRC molecular targets, namely COX-2 (PDB: 4COX), IL-2 (PDB: 1M47) and IL-6 (PDB: 1IL6) using AUTODOCK 1.5.4. **Results:** Protective action of M1 was observed with histological and different biochemical tests in DMH-induced colon cancer. The measurement of serum AST, ALT, LDH and CK was also carried out from the similar experiment. All the enzyme levels were increased almost two folds ($p<0.01$) in DMH treatment group as compared to control but treatment with test compound restored the levels to normalcy. M1 treatment (25mg/kg) attenuated the levels of both IL-2 and IL-6 with pronounced effect on IL-6.As per docking studies, M1 showedhighest interaction energy with IL-6 as compared to IL-2 and COX-2. M1 showed moderateinteraction with IL-2 and COX-2. **Discussion:** M1 therapy revealed ameliorative effect as restored the levels of GSH, SOD and CAT to normalcy and enhancing the anti-oxidative physiological processes promoting its protective action. Further histopathology and SEM analysis were performed to document the morphological changes associated to DMH administration and drug treatment. Both the analyses revealed less tumoral vacuoles formation in M1 and 5-FU groups, demonstrating the protective action of the compounds in cancerous condition. **Conclusion:** Our study collectively suggest that M1 might be active against colon carcinoma by reducing inflammatory parameters, oxidative stress parameters and varied effect on various enzyme levels. (DOI:10.9777/rr.2018.1236)

A novel role of mast cells in erythrophagocytosis during chronic inflammatory and autoimmune diseases

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Anemia is a normally associated with various autoimmune and chronic inflammatory diseases. Mast cells play a vital role to initiate inflammatory cascade. Previous study with human patients with mastocytosis has shown its correlation with anemia in 50% of cases. We attempted to reveal the interaction of mast cells with erythrocytes in the present study in vitro. Mast cell interaction with opsonized and oxidatively damaged erythrocytes which mimics erythrocytes during autoimmune and chronic inflammatory diseases respectively

was analyzed with RBL-2H3, P815 and bone marrow derived mast cells. Murine erythrocytes were either opsonized with anti mouse RBC sera or treated with tert-butyl hydroperoxidase (t-BHP), to induce oxidative damage. Normal, opsonized or oxidatively damaged erythrocytes were labeled with carboxyfluorescein succinimidyl ester (CFSE) to track erythrophagocytosis. Uptake of fluorescently labeled erythrocytes was analyzed by flowcytometry as well as confocal fluorescence microscopy. Oxidatively damaged and opsonized erythrocytes were readily taken up by mast cells whereas normal erythrocytes were not. We show, for the first time, direct erythrophagocytosis of oxidatively damaged erythrocytes by mast cells in resting as well as in activated state in vitro. This suggests the active involvement of mast cells in erythrocyte clearance under oxidative stress during chronic inflammatory conditions. Mast cells express some of the receptors in common with macrophages which are involved in erythrophagocytosis. We found VCAM-1 and TIM3 receptors on RBL mast cells which may be the possible receptors involved in mast cell-erythrocyte interaction. To further explore the preferred mechanism of erythrophagocytosis by mast cells, various inhibitors were used and uptake was analyzed by flowcytometry. Partial inhibition of phagocytosis by individual inhibitors provided a clue that this process may be controlled by several pathways. The mechanisms revealed in the present study might be helpful to circumvent the adverse autoimmune or chronic inflammation related anemic disorders. (DOI:10.9777/rr.2018.1237)

Population heterogeneity: A new way to understand mycobacterial virulence

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The cell to cell variation in protein levels in a clonal cell population is termed as expression noise. The concept of expression noise is largely overlooked in understanding mycobacterial pathogenesis and bulkof research considers a basic assumption that members in a clonal cell population are phenotypically identical. We believed distinct roles for the expressing cells and cells defected for expression of a protein (that could have potential role in virulence) when expressed in a noisy fashion in wild type population. We identified two overlapping expression noises for proteins coded by two distant but related genetic loci namely TlyA and EspG in wild type Mycobacterium marinum. TlyA protein is shown to possess various properties that could contribute to mycobacterial virulence including its inflammatory nature. To explore the distinct roles for cells that are co-operating for TlyA expression and cells defected for the same, we attempted to generate a population rich in TlyA defectors by selecting wild type population over capreomycin. The selected population was analysed for its virulence potential in mice model. Our initial observations suggest that CAP selection alters the virulence potential in a way that selected population conditionally retains its virulence ability. (DOI:10.9777/rr.2018.1238)

Stability indicating method development and validation of abiraterone in human plasma by using LC-MS/MS

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Aim: A selective, sensitive, liquid chromatography mass spectrometry/ mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of abiraterone in human plasma using abiraterone D4 as an internal standard. **Methods:** Analyte and internal standard were extracted from human plasma by Liquid-liquid extraction using a mixture of diethyl ether and dichloromethane (70:30, v/v). The chromatography was performed on WATERS XEVO TQS by using Hypersil gold (50mm x 4.6 mm) column. The processed samples were analyzed by LC-MS/MS technique in the multiple reaction monitoring mode using the respective [M+H]+ ions, m/z 350.29 to 156.12 for abiroterone and m/z 354.28 to 160.17 for the abiraterone D4. **Results:** The peaks were obtained at retention time of analyte and internal standard same at 1.7±0.3 min This analytical method is valid for the determination of abiraterone over a range of 0.2540 ng/ml to 305.2780 ng/ml. Signal from the detector were captured in a computer and processed using MassLynx SCN 4.1 v software. **Conclusion:** This method was found suitable to analyze human plasma samples for the application in pharmacokinetic and BA/BE studies. (DOI:10.9777/rr.2018.1239)

In-Silico distributional study of photolyases genes in cyanobacteria

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Background: Cyclobutane pyrimidine dimers (CPD) are the major DNA photoproducts induced by ultraviolet (UV) radiation. Photoreactivation is an evolutionary ancient mechanism present in cyanobacteria which utilizes photolyase (PL) for repairing UV-induced damaged DNA in light dependent manner. Photolyases are monomeric proteins of 50-60 kDa with stoichiometric amounts of two non- covalent chromophore/cofactors. **Aim:** Study the distribution of various types of PLs in cyanobacteria. **Methods:** In present study 85 fully sequenced cyanobacteria were used and amino acids sequences coding PLs were retrieved from NCBI database. Multiple-sequence alignments were performed with the Clustal X and results were visualized using Bio-edit program. Evolutionary analyses were conducted using MEGA 7 program. **Results:** Six different types of PLs are present in cyanobacteria. Out of six, five PLs showed similarity to prokaryotic PLs, and one PL was similar to the PLs found in eukaryotes. Different strains of Prochlorococcus marinus, e.g., MIT 9313, CCMP1375 and MIT 9303, and Atelocyanobacterium thalassa were found to lack PLs. The CPD PLs are evenly distributed in all studied cyanobacterial groups, and hence, constitute the most abundant PLs. Cry_DASH PLs which repair CPD in single stranded DNA is completely absent in the order Nostocales, whereas it is unevenly distributed in other orders. In the order Synechococcales, distribution of PLs was found to be habitat dependent. All PLs are highly conserved at their light sensing and catalytic domain. **Discussion:** The presences of various types of PLs in cyanobacteria could offer survival advantage against lethal UVR. The distribution of PLs in cyanobacteria could be characteristics of habitat and intensity of UVR received by them. **Conclusion:** This study provides knowledge of

various types of photolyases present in cyanobacteria. Further studies are needed to find out the possible reasons for presence of PLs sequences having more resemblance with higher organisms and their distribution trends in cyanobacteria. (DOI:10.9777/rr.2018.1240)

In-silico characterization of the diversity of the extra cytoplasmic function (ECF) sigma-factors of *Mycobacterium Sp*.

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Background: Despite concerted global efforts to eradicate Mycobacterium tuberculosis (MTB), it remains one of the deadliest pathogens and major cause of human death. Recently, Global Tuberculosis Report, 2017, suggested that ~10 million TB incidences were reported in 2016 and nearly half million cases of MDR-TB remained untreated. Despite different approaches employed for tuberculosis control viz; antibiotic-sensitivity assay, vaccine-development, molecular diagnostics etc; transcriptional regulation of this pathogen remains most promising which depends primarily upon its habitat mainly controlled by the Extra Cytoplasmic Function (ECFs) sigma-factors.Here, we carried out detailed in silico analysis for different sigma-factors of 334 Mycobacterium Species to elucidate their molecular diversity. **Aim:** In silico characterization of the ECF sigma-factors of Mycobacterium Sp. **Methods:** The 10 ECF sigmaalong with SigA, SigB, SigF homologs of MTB were retrieved using NCBI-BLAST and multiple-sequence alignment using Clustalx2.1 was generated. Subsequently, phylogenetic tree was created using Neighbour Joining method with MEGA 7.0.26 software. Logos were generated using WebLogo. Motif analysis. Homology-modeling and superimposition were carried out using Motif-Scan, Modeller9v2 tool and Chimera packages, respectively. **Results:** The phylogenetic analysis demonstrated four different groups of sigma factors viz; GroupI, II, III and IV. On analysis of the ECF-class, highly conserved motifs binding to regions 2 and 4 were revealed. The core conserved motifs identified were; AEDXXQE, WLXXV and LXXLYYEQR (in region 2.4 and 4.2, respectively). Besides, several specific insertions/deletions between regions 2 and 4 as well as the N-terminus and C-terminus regions were important for diversity in the function of the ECF- class. Furthermore, structural superimposition of the ECF-sigmas suggested that SigG, SigI, SigJ belong to one group; SigH, SigE, SigM shared another group while SigL, SigD, SigC and SigK shared still another group. **Discussion:** The motif analysis revealed that there are insertions/deletions of amino acids in ECF- sigma that lead to their altered recognition at distinct promoters in response to different abiotic stresses. The in silico classification of different ECF-sigmas into subgroups closely resembled with their similar in vivo functions in Mycobacterium. **Conclusion:** This analysis will lead to better understanding of the diversity as well as transcriptional-regulation mediated by the ECF-sigma which is mainly responsible for the persistence of this pathogen. Modulation of the function of one/more of these potent transcriptional- regulators might lead to better control of tuberculosis in near future. (DOI:10.9777/rr.2018.1241)

A scientometric study of research output of herbal drug development in India for cancer treatment.

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Background: Cancer is a disease which is the second most leading cause of death. It is characterized by uncontrolled cell division, lack of apoptosis, self sufficiency for repair and growth, and spreading and invading in nature. Millions of dollar spent every year on the research for developing anticancer drugs, most of the clinical drugs are herbal in origin. Hence, the pharmaceutical companies prefer to research on traditionally used herbals to reduce time and money. Several academic as well as research institutions are also working on the same way which result the publication of numerous research articles annually. In this study, an attempt was taken to evaluate the research and its output in India. **Methods:** In this study, scientometric data were retrieved from Pubmed database on 31.12.2016 using seven years filter to analyze the authors, their institutions, geographical region, journal and type of cancer for which the research performed. All data were analyzed scientometrically which is a mathematical and statistical tool to measure the scientific productivity in terms of individual, institutional, regional and/or geographical contribution to a subject of a field of interest. **Results:** The present study shows that the publications vary with time, Asian Pacific Journal of Cancer Prevention published the maximum literature, Maximum works were related togeneral mechanism of cancer and amongst specific cancer, breast cancer was the top most cancer studied in india, Uttar Pradesh had maximum contribution in India, Indian institutions namely Annamalai University, Karnataka, Karunya University, Tamil Nadu, Central Drug Research Institute, Lucknow and University of Kalyani, Kolkata had maximum contributing Indian institution in herbal drug research for cancer. **Conclusion:** This study analyzed the herbal research output in India for the development of anticancer drugs using an interdisciplinary approach and revealed that Indian researchers preferred to work jointly with interdisciplinary and publish their research work in open access journals. The contribution of Annamalai University in Indian institution, Karnataka among Indian state was highest. (DOI:10.9777/rr.2018.1242)

Covalent immobilization of *β*-amylase onto functionalized molybdenum sulfide nanosheets, its kinetics and stability studies: A gateway to boost enzyme application

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Background: Enzymes, being highly effective and selective in catalysis, find widespread use in food, pharmaceuticals and other industrial fields owing to its mild and green reaction.β-Amylase finds application in food and pharmaceutical industries.The drawback of soluble enzyme could be overcome by immobilization onto some suitable matrices which without altering the properties of enzyme much, are as effective in catalysis as the free counter-part.MoS2 nanosheets have superior properties over other nano-materials, making it a suitable candidate for enzyme immobilization. **Aim:** β-Amylase nanobiocatalyst was prepared by immobilizing the enzyme from peanut onto functionalized MoS2

nanosheets, prepared by hydrothermal method.Comparative study of the two catalysts was performed. **Methods:** Na2MoO4.2H2O (0.25 g) and L-cysteine (0.50 g) were dissolved in 25 mL each of MQ and stirred for 10 min at 40 °C, respectively. Thereafter, both the solutions were mixed and stirred for 10 min at 40 °C. During the process, HCl (0.1 M) was added to the solution to maintain its pH approximately at 5. The black color powder was taken out, washed with MQ water and ethanol thrice and dried in open air at 60 °C for 12 h. To a preparation of 1 mg/mL of MoS2 in buffers, different concentration of glutaraldehyde and enzymes were added for immobilization to occur, according to the Response Surface Methodology experiment. Results and Discussions: By employing Response Surface Methodology, approximately 92% immobilization efficiency was achieved.Thermo-stability; pH stability, reusability and storage stability of immobilized β-amylase were superior with respect to the soluble enzyme.Immobilized enzyme retained 80% residual activity, after 10 cycles and 83% residual activity upon storage over a period of 50 days. **Conclusion:** The betterment of enzyme upon immobilization in terms of pH, temperature and stability confers wider range of application for maltose production and accordingly, suitable for food and pharmaceuticals industries. (DOI:10.9777/rr.2018.1243)

Ayurvedic plants having xanthine oxidase inhibitor: A status update

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Background: Xanthine oxidase (XO) present in normal tissues in the form of dehydrogenase enzyme which oxidizes hypoxanthine to xanthine and finally to uric acid. The over activity of xanthine oxidase causes hyperuricemia, nephropathy and gout. XO is also a source of reactive oxygen species in cardiovascular system and causing oxidative damage in the myocardium. In clinical practices allopurinol commonly used as XO inhibitor for gout treatment. However, it induces serious side effects such as renal failure, impaired liver function and hypersensitivity reactions. Hence, there is a need to look for new xanthine oxidase inhibitors. Certain class of secondary metabolites presents in medicinal plants e.g.

flavonoids, polyphenolic and sterol compounds have been reported to possess XO inhibition. These findings have opened the possibility of phytochemical exploration of Ayurvedic plants for possible XO inhibitors for the management of gout. **Aim:** The aim of the study to explore the XOI potential of ayurvedic plants. **Methods:** The XOI potential of plants extract was tested following standard protocol spectrophotometically using allopurinol as positive control. The absorption increments were monitored every 60 seconds for 10 min at 290 nm indicating the formation of uric acid. **Results:** Various extracts of plants of vata samanam category and others were assayed in-vitro for XOI activity, whereas ethyl acetate extract of Z. amatum and hexane extract of S. suaveolens shows more than 50% inhibition at 100µg/mL. Alcoholic extract of S. diphyllum found as less XO inhibitory activity. The IC50 value of allopurinol used, as the standard was 7.54 µM. **Conclusion:** In

conclusion, this study indicates XOI potential of ayurvedic plants, may be useful for the treatment of hyperuricaemia and gout, which correlates with the ethno- botanical data on the use of these plants in Indian folklore. Detail data would be presented in the conference. (DOI:10.9777/rr.2018.1244)

Identification of stress responsive phosphoglycerate kinase in bovine filarial parasite *Setaria cervi*

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Background: Lymphatic filariasis is an infectious tropical parasitic disease, caused by tissue dwelling nematodes e.g. Wuchereria bancrofti, Brugia malayi and Brugia timori. A potent macro/microfilaricidal drug is urgently needed to combat the disease. To develop new drugs it is essential to look for specific targets in the filarial worms that can be exploited. Phosphoglycerate kinase (PGK) seems to be interesting potential drug target due to its importance in energy metabolism of parasites ATP synthesis. PGK (EC 2.7.2.3) produces ATP after catalyzing 1, 3-bisphosphoglycerate to 3-phosphoglycerate using magnesium as a cofactor. It is also a nuclear mediator in DNA replication and repair. PGK has been explored in helminthes such as Fasciola hepatica as vaccine candidate. **Aim:** Identification of stress responsive Phosphoglycerate kinase in bovine filarial parasite Setaria cervi. In this study we have measured enzyme activity of PGK in different life stages of S. cervi and biochemical analysis of PGK; in the control, antifilarials as well as clorsulon (CLN) treated parasites. **Methods:** The worms were exposed to ABZ, DEC+ABZ of 200μM and CLN of 10, 20 μM concentration in the KRB maintenance medium at 37ºC, and 5% CO for 7h. The motility and viability of the parasites was recorded at various time intervals. Different oxidative stress parameters were measured in the parasites after 7h. Results and **Discussion:** A significant amount of enzyme activity was detected in the somatic extract of different developmental stages of the parasite. Among different stages, excretory secretory product of microfilariae showed a higher level of PGK activity in adult male than female. We observed that albendazole and clorsulon showed marked decrease in the motility and viability of the adult parasite after 7h. The activities of antioxidant enzymes increased generation of reactive oxygen species, lipid peroxidation and protein carbonyls. In this case also did not show significant alterations in case of DEC+ABZ. Thus, it can be concluded from our studies that ABZ and CLN alone could be a potential macrofilaricidal. **Conclusion:** Phosphoglycerate kinase activity has been detected in S. cervi a bovine filarial parasite. S. cervi life stages showed difference in PGK activity suggesting that the enzyme is developmentally regulated. The inhibition of ScPGK by antifilarial drug and clorsulon may lead to decrease in energy by less production of ATP. Thus exhibiting the important role played by filarial PGK in the survival of these parasites. (DOI:10.9777/rr.2018.1245)

Interaction of bioactive component of *Pueraria tuberosa* with PKC-α and NF-kB in reference to diabetic nephropathy: *In-vivo* & *in-slico* analysis by molecular docking

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Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal failure worldwide. Protein kinase C and NF-kB are important factors involved in its pathogenesis. Earlier we have reported the nephroprotective potential of Pueraria tuberosa (PTY-2r) through different pathways. **Aim:** The aim of this study, to explore the role of aqueous extract of Pueraria tuberosa i.e. PTY2r and its bioactive component on PKC-α and NF-kB in kidney of DN rats. **Methods:** Streptozotocin (STZ, 55mg/kg body Weight, i.p) induced rats were divided randomly into 2 groups, i.e. DN control, and DN+PTY-2r. The effect of extract on PKC-α and NF-κB was investigated through immunohistochemistry. The bioactive components of PTY-2r were analyzed via LC-MS/MS based on their M/Z value and retention time. Through molecular docking study the interaction of different component with PKC-α and NF-κB were analyzed. **Results:** The in-vivo study showed that PTY-2r significantly suppressed the the expression of PKC-α and NF-κB in glomerulus region of kidney of DN rats. The molecular analysis showed that five compounds namely Tuberostan, Robinin, Tuberosin, Hydroxytuberosin and Puererone showed interaction for both PKC-α and NF-κB. Out of these Tuberostan showed best interaction with binding energy 11.21[kcal/mol] and dissociation constant 6066.8193[pM] for PKC-α. For NF-κB, Robinin showed best interaction with binding energy 9.368 [kcal/mol] and dissociation constant 135882.9375 [pM]. **Discussion:** Activation of PKC-α is considered as an important signaling molecule in diabetic complications. Activated PKC-α in kidney induces NF-κB activation. NF-kB is a known transcription factor for expression of IL-6, TNF-α and iNOS, thus, it's up-regulation is linked to inflammation induction in the kidney of DN patients. PTY-2r significantly suppressed the expression of PKC-α & NF-κB, thus prevent the inflammatory signaling pathway. Molecular docking supported this study. **Conclusion:** This study concluded that different bioactive components of PTY-2r are involved in prevention of progression of diabetic nephropathy. (DOI:10.9777/rr.2018.1246)

Expression of chemokine receptor in Helicobacter pylori associated gastric cancer patients

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Background: The overwhelming evidences have supported that chemokine-chemokine receptor systems can regulate transformation, growth, neovascularization and metastasis of tumor cells. The abnormal expression of CXCR5 could be observed in many tumors and was associated with tumor progression. However, the expression or function of chemokine receptor CXCR5 in H. pylori (Helicobacter pylori) induced gastric cancer (GC) has not been investigated. **Aim:** To detect the expression of CXCR5 in GC patient in presence/ absence of H. pylori infection and it relationship with cagPAI (cag Pathogenicity Island) genes. **Methods:** Cancer tissues and corresponding normal tissues from 80 GC patients were included in this study. H. pylori infection was diagnosed by PCR and rapid urease test. The presence of cagPAI genes (cagA, cagE, cagM and cagT) were analyzed by PCR. The expression of CXCR5 in gastric cancer

tissues and adjacent normal tissues was evaluated by real time PCR. **Results:** Of 80 GC patients included in the study, 43 (53.75%) were H. pylori positive. Out of 43 patients infected with H. pylori, 27 patients had all four genes (cagA, cagE, cagM and cagT). The expression of CXCR5 was significantly up- regulated ($P < 0.05$) in cancer tissues compared to normal tissues irrespective of H. pylori infection. The expression of CXCR5 was significantly higher in presence of all four cagPAI genes positive H. pylori strains compared to partially deleted ones. **Discussion:** Significantly up-regulated expressions of CXCR5 in H. pylori positive than H. pylori negative samples from normal and cancerous sites indicate that CXCR5 is involved in the oncogenesis and progression of H. pylori induced gastric cancer. **Conclusion:** Current findings emphasized that significantly up-regulation of CXCR5 is associated with cagpAI genes in development of GC. This study may have significant promise for the advancement of cancer therapy, either in terms of improving diagnosis or predicting prognosis. (DOI:10.9777/rr.2018.1247)

Cell division regulatory protein DivIVA interacts with both genome segregation and divisome components of *Deinococcus radiodurans*

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'Min' system is known to play a critical role in spatial regulation of cell division at least in rod shaped bacteria. In these bacteria, DivIVA or MinE regulates the localization of MinC / MinD complex, which in turns blocks FtsZ nucleation at the poles and thus the plane of cell division gets fixed at mid cell position. Deinococcusradiodurans, a radio-resistant bacterium possesses 'Min' system comprised of Min C, Min D, Div IVA and truncated Min E. We showed that DivIVA of D. radiodurans forms dimer and interacts with genome segregation components like ParA2, ParA3, ParA4, ParB1, ParB3 and ParB4 as well as core cell division proteins. Molecular basis of DivIVA interaction to two macromolecular complexes was investigated. The multiple sequence alignment and conserved domain analysis was carried out with the full-length sequence of DivIVA. Interestingly, it has been observed that only N-terminal of this DivIVA is conserved across its homologs, and is 26 % identical with full length of DivIVA of Bacillus subtilis. So, DivIVA was divided into N-terminal, C terminal and middle domains. All these variants were cloned and expressed, and the interactions of different domains of DivIVA with its segrosome and divisome partners were monitored. Using bacterial two-hybrid system and co-immunoprecipitation, we concluded that N-terminal of the protein is involved in dimerization. Interestingly, N-terminal domain showed physical interaction with genome segregation components like ParA1, ParA2, ParB2 and ParB3, whereas middle- domain interacts with Min C of this bacterium. On the other hand C-terminal does not interact to either self or to other proteins monitored in this study. DivIVA-RFP expressing in Escherichia coli showed foci formation at the poles. Results suggesting the regulatory role of DivIVA in cell division and genome segregation will be presented. (DOI:10.9777/rr.2018.1248)

Comparative study of solid-state fermentation and submerged fermentation for protease inhibitor production from *Agaricus bisporus*

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Background: Edible mushrooms have not been studied thoroughly for the protease inhibitor production. Agaricus bisporus, an edible mushroom is a source of various biomolecules which have nutritional as well as therapeutic value. **Aim:** This research work involves the comparison of the production of an inhibitor of serine protease enzyme from Agaricus bisporus through solid-state fermentation (SSF) and submerged fermentation (SmF). **Methods:** The mycelia were grown on different types of agro-waste raw materials, through SSF, in roux bottles. The substrates used were wheat bran, rice bran, and chickpea bran (10% each) and supplemented with cyano-bacterial biomass (2%) and 50% moisture content. The mycelia were extracted after ten days of incubation in the BOD incubator at 260 C.For SmF, shake flask cultures of A. bisporus were incubated at 260 C at 180 and 100 rpm for the first four days and the next five days, respectively. The crude extracts obtained from both the fermentation methods were assayed for the activity of the protease inhibitor produced. The biomass produced was calculated for SSF and SmF through glucosamine assay and biomass dry weight determination, respectively. **Results:** Wheat bran substrate gave higher protease inhibitor activity in SSF. The protease inhibitor activity was increased in SSF compared to SmF by 43%. **Discussion:** SSF proved to be a better method in comparison to SmF, owing to the anchorage provided to the fungal mycelia by solid state substrate. This helped in enhanced growth of the mycelia and production of the protease inhibitor. **Conclusion:** This is the first study on A. bisporus for protease inhibitor production. The scale-up of the protease inhibitor production from A. bisporus by SSF using wheat bran as the substrate will prove economical and environment-friendly as wheat bran is a low cost and ubiquitously available raw material. (DOI:10.9777/rr.2018.1249)

Recognition driven biological studies using a new hydrazone derivative

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Background: Imbalances in the intake of metal ions such as Al^{3+} and Cu^{2+} is the root cause of several hematological and neurological disorders like Parkinson's, Alzheimer's, and Wilson's disease, while fluoride ions overexposure is associated with many serious gastric, kidney disorders and skeletal fluorosis, etc. Thus, development of a multiple ion chemosensor can be highly beneficial for the detection of these metal ions in vivo. In the present study we discuss a chemosensor H2L which exhibits good cell membrane permeability and senses $Al^{3+,}$ Cu^{2+} and F- ions in cell lines. **Aim:** To synthesize and characterize a hydrazone derivative H2L for the detection of metal ions and then extend this recognition driven cell imaging studies. **Methods:** H2L was synthesized and characterized using elemental analyses, spectroscopic studies (FT-IR, 1H, 13C NMR, ESI-MS, UV/vis, fluorescence) and X-ray single crystal analysis. The interaction of H2L with Cu^{2+} and Al^{3+} ions and their sensing mechanisms were investigated in detail by spectroscopic studies. **Results:** The synthesized and well characterized chemosensor H2L responded to Al^{3+} and Cu2+ ions via fluorescence turn-on and turn-off

pathways. The turn-on ensemble of H2L-Al^{3+} turned off by the sequential addition of F- ions. **Discussion:** The newly synthesized hydrazine derivative H2L has been characterized by spectroscopic techniques, crystallizes in triclinic system with P1 spacegroup as authenticated by its single crystal X-ray crystallography. H2L responds to Al^{3+} and Cu^{2+} ions via different routes in mixed aqueous solution over other competitive metal ions. The addition of Al^{3+} ion to H2L displayed drastic enhancement of the emission intensity while the addition of Cu^{2+} ions to H2L caused quenching of the emission. This recognition driven process has been exploited to understand the biological application of H2L using cell imaging in the absence and presence of metal ions. On the other hand, H2L-Al^{3+} ensemble exhibited decrease of fluorescence intensity on addition of F- ions. Thus F- ions could revert the sensing of Al^{3+} ions resulting in emission of parent chemosensor due to coordination ability of Al^{3+} to F- ion. **Conclusion:** We have developed a new chemosensor H2L based on a hydrazone derivative which detects Al^{3+}, Cu^{2+} and F - ions. This phenomenon has been driven to understand their behavior through cell imaging. (DOI:10.9777/rr.2018.1250)

Inhibitory role of silver nanoparticles against important bacterial pathogen of rice (*Oryza sativa*)

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Rice (Oryza sativa) is the main staple food in Indian subcontinent. Rice productivity declined by fifteen percent due to the infestations of multiple diseases. Among these, bacterial diseases like Xanthomonas oryzae causes bacterial blight (BB) is one of the most fatal disease in rice growing countries. Broad spectrum antibiotics were applied to control the out-break of this disease, but still it remained unsolved. Nanotechnology has ushered in a new era of disease therapeutics with the development and application of different nanoparticles, where silver nanoparticle captures attention. In the current experiment, silver nanoparticle was synthesized from rice leaves. Fresh leaves of rice were collected from the experimental field of ICAR-IIAB, Ranchi, and leaf extract was prepared by filtration and was mixed with 1mM silver nitrate (AgNO3) solution in different ratios (0.5%, 1.0%, 2.0% and 5.0%) at room temperature for overnight, till the colour of the mixture changes to yellow-brown confirming the formation of silver nanoparticle. UV-Visible spectrophotometric analysis showed surface plasmonicresonance (SPR) at the prescribed range of silver nanoparticle. During characterization, silver nanoparticles showed 128nm size as analyzed by particle size analyzer along with a zeta value of -36.6mV whichexhibits strong stability. Synthesized silver nanoparticle also showed FTIR and XRD value at prescribed range. This rice leave synthesized silver nanoparticle exhibited anti-bacterial activity when tested against rice pathogen, Xanthomonas oryzae, cultured and evaluated through standard microbiological methods.Anti-bacterial potency of silver nanoparticle were also maintained when applied silver nanoparticle product from tannin, isolated from natural gum based materials. Process optimization and bulk formulation is on progress

before field applications. Field trial on silver nanoparticle application against this disease will be started soon. (DOI:10.9777/rr.2018.1251)

Microbiota pattern in colorectal cancer

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Background: The human colon harbours as many as 15,000 – 36,000 bacterial species that constitute the intestinal microbiome. A dis-balance of this intestinal microbiota community acts as a source of infection leading to acute/chronic inflammation, and gastrointestinal cancers including colorectal cancer (CRC). The fact that CRC prevalence is increasing in India at alarming rate and literature reveales that gut microbiome can promote carcinogenesis, study of microbiota associated with CRC is intriguing. **Aim:** To identify the compositional differences of microbiota in patients diagnosed with Colorectal cancer (CRC), Inflammatory Bowel Disease (IBD) and healthy individuals **Methods:**1.Species identification using 16s rDNA sequencing:- Total DNA isolated from the flash-frozen tissue samples from (1) CRC tumor site, (2) adjacent normal tissue,(3 IBD and (4) IBS using the manufacturer protocol (Qiagen All-prep kit). DNA-seq libraries were constructed, barcoded, and pooled for sequencing, using Ion Reporter TM Software. 2. Quantification the relative abundance:- Toxic bacterial gene expression analysis was done using SYBR Green based RT-q PCR. The reaction was normalized with universal bacteria controls. The fold change was calculated based on the obtained Ct values. **Results:** There is a significant difference found in sequences among different bacterial species of Bacteroidestes and fermicutes phyla in CRC samples.Toxic bacterial genes like Enteropathogenic e. coli (EPEC), Adherent invasive E. coli (AIEC), Enterotoxigenic Bacteroides fragilis (ETBF), Fusobacterium nucleatum (fadA) showed differences in their relative abundance in CRC samples. Discussion and **Conclusion:** This study evaluated the abundance of Fermicutes in relation to colorectal adenomas compared to controls. The human gut microbiota has been shown to have a dynamic and observable impact on the human host. Current results have shown that interactions between the host and the bacteria colonizing the gut can contribute to carcinogenesis. Thus, there is a need for a mechanistic understanding of the role of the gut microbiome in tumor development as well as its progression. (DOI:10.9777/rr.2018.1252)

Oxidatively modified lipids during metabolic disorder induce alteration in eNOS via Erk mediated pathway leading to endothelial cell dysfunction

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Background: Endothelial nitric oxide synthase (eNOS) plays an important role in maintaining vascular endothelial cell (EC) function and alteration in its expression has been shown to cause endothelial dysfunction (ED) and development of atherosclerosis. Extra cellular regulated kinase (Erk), an upstream regulator has been identified as a critical player in maintaining ECs homeostasis. However, Erk-mediated regulation of eNOS in ECs in the presence of atherogenic stimuli such as modified lipids has not been well explored. **Aim:** We have investigated the role of Erk-mediated regulation of eNOS in ECs in the presence of oxidatively modified lipids. **Methods:** Primary Human Umbilical Vein

Endothelial Cells (HUVECs) were used as in vitro ECs model in all the experiments. MTT and Trypan blue dye exclusion assays were performed to assess cell viability. Gene silencing was done by siRNA transfection to determine the role of Erk in regulating its downstream proteins. Western blotting and qPCR were performed to detect expression and regulation of different ED associated markers. Results and **Discussion:** We observed that oxidatively modified lipids altered ECs viability and downregulated the expression of eNOS and Erk in HUVECs. Expression of different ED markers such as Intercellular Adhesion Molecule-1 (ICAM-1) and monocyte adhesion on ECs increased and expression of von willebrand factor (vWF) decreased significantly in the presense of modified lipids. Further, silencing of Erk using siRNA caused appreciable decrease in eNOS and ECs viability which indicate that decrease in eNOS under oxidative stress condition is partly mediated by Erk. **Conclusion:** The present study indicates that Erk is important in maintaining ECs homeostasis and modified lipids cause ED by downregulating eNOS through Erk mediated pathway. (DOI:10.9777/rr.2018.1253)

Phtyochemical Screening and HPTLC fingerprinting of Panchavalkala (A Polyherbal Ayurvedic Formulation) for diabetes Management among North Indian Population.

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Background: Ayurveda holistic approach for the treatment of disease and based on the property of drugs i.e. rasa, guna, virya, vipaka, and doshkarma. Panchavalkala was one of the Ayurvedic formulation which is a combination of five astringent drugs named: Nyagrodha (Ficus bengalenesis Linn.), Udumbara (Ficus glomerata Roxb.), Ashvatha (Ficus religiosa Linn.), Parisha (Thespesia populanea Soland ex correa) and Plaksha (Ficus lacor Buch-Ham.) and evaluated clinically for diabetes. Panchvalkala had properties like anti-inflammatory, antiseptic, antidiabetic, antioxidant, immune-modulatory, antibacterial, antimicrobial wound healing and astringent properties. The present study aims to examine the phytochemical and HPTLC profiling of this antidiabetic polyherbal Ayurvedic formulation i.e. Panchavalkala for standardization. **Methods:** The kwath of the formulation (Panchvalkala) was prepared as per standard protocol described in Ayurvedic Pharmacopoiea of India and followed by Preliminary phytochemical screening and HPTLC studies using CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner and WinCats-4 software. **Results:** The phytochemical screening of the Panchvalkala kwath showed the presence of alkaloids, phenol, triterpenoid, flavonoids, tannins, saponins and carbohydrate. The HPTLC fingerprinting analysis revealed distinct band pattern which will help in proper identification and standardization of the formulation. **Conclusion:** The results scientifically validate the use of Panchvalkala kwath for diabetes management in the traditional medicine and its HPTLC fingerprinting along with phytochemical profiling can be used for its identification and standardization. (DOI:10.9777/rr.2018.1254)

Study of rapamycin production in a non-conventional bioreactor

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Background: The microbial production of an antifungal antibiotic rapamycin using Streptomyces hygroscopicus has been investigated by many workers owing to its low productivity and large number of applications which includes immunosuppressive action for renal transplants. Air lift reactor (ALR) is a well studied system with lower shearing rate and lesser power consumption. **Aim:** Present work is aimed at employing an internal loop airlift reactor with H/D ratio of 10 for production of rapamycin. **Methods:** Rapamycin production was carried out using optimized media. Production of rapamycin was carried out using Streptomyces hygroscopicus at pH 6.0, 28C and different aeration rates. Analysis of rapamycin was done using HPLC. **Results:** Rapamycin production was carried out using statistically optimized media. It has been found that due to low shearing rate, specific growth rate is enhanced with higher biomass production. Air flow rate has been correlated to volumetric oxygen transfer coefficient (kLa) and found that 1.5 vvm of airflow rate correspond to the kLa which is obtained at 1 vvm in stirred tank bioreactor with comparable production. **Discussion:** This study finds its application in investigating the kinetic characteristics of rapamycin production in airlift reactor with perspective of scale up. Airlift reactor has economic advantage over conventional stirred tank reactor and has simple design and flow patterns. **Conclusion:** Production was carried out for 120 hours and production was evaluated at different flow rates it was found that maximum production was obtained at 1.5 vvm. (DOI:10.9777/rr.2018.1255)

The study of correlation of Micronutrient in type-2 diabetes mellitus and it's associated chronic complications

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Objective: To clarify the role of zinc, copper, chromium, magnesium and selenium in patients with type 2 diabetes mellitus and it's associated chronic complication (DM). **Methods:** Total 150 patients with type 2 DM were enrolled in this study, Together with 75 case of type 2 DM and 75 control healthy subjects, matched for age and sex, who served as the control group. Overnight fasting serum levels of glucose (FBS),PPBS, Glycated hemoglobin A1c (HbA1c) and lipid profile were estimated in all the subjects. Copper, chromium, magnesium, zinc, and selenium were estimated in all subjects. **Results:** Statistically significant differences between group 1(with and without complications), and group 2 (control). The control group were found significant differences in magnesium, selenium, zinc and Copper with case but not in chromium level. FBS showed high significant negative correlations with magnesium and selenium and significant positive correlation with copper. The HbA1c showed significant negative correlation with magnesium and selenium, and positive correlation with copper. Zinc, magnesium and selenium showed significant decrease in both the groups than the control group. Magnesium showed significant decrease in diabetes mellitus than control group. Conclusions: Trace elements could have a role as cofactors in the pathogenesis and complications of type 2 DM. Trace element supplementation might have utility in the treatment of this complex disorder. (DOI:10.9777/rr.2018.1256)

Screening of nickel tolerant endophytic bacteria from plants growing in nickel contaminated soil

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Background: Environmental contamination of heavy metal is a serious concern due to its detrimental effect on biological system. Eukaryotic systems are exposed to nickel through air, water and soil. Nickel results in the formation of oxidative stress by generating Reactive Oxygen Species (ROS), which has detrimental effect on the metabolic reactions within the cell. Several plants have the potential to bio- accumulate metals from soil and water due to the presence of endophytic bacteria. These endophytic bacteria play an important role in absorption of metals and enable the plant to survive in heavy metal contaminated areas. **Aim:** The present work focuses on isolation, characterization and bio accumulation capacity of endophytic bacteria from Vigna radiata (Green gram) in the artificially induced nickel contaminated soil. **Methods:** The isolation of bacteria was performed on Nutrient broth substituted with Nickel chloride salt (50ppm). The morphological, biochemical, antibiotics susceptibility studies of isolates were performed. The isolates were then subjected to heavy metal analysis for bioaccumulation capacity. The textile industry releases nickel along with the toxic dye effluent thus the ability of the isolate to degrade dye was also determined. Results and **Discussion:** Total six endophytic bacterial strains from non-chelated and chelated soils were isolated. The isolates showed nickel tolerance in range of 200-300 ppm. The strains SNi-3 and CSNi-3 showed dye degradation capability against textile dyes such as Aniline, Azure-B, Coomassie Brilliant Blue, Methylene Blue, and Rose Bengal. SNi2 was resistant to streptomycin. **Conclusion:** The isolated strains can be used in bioremediation of heavy metals and textile dye. (DOI:10.9777/rr.2018.1257)

A quantitative estimation of serum calcium, magnesium, zinc, apolipoprotein A, apolipoprotein B and lipids in patients with type 2 diabetes mellitus.

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Background: Metabolism of several minerals has been reported to be altered in diabetes mellitus which might have specific roles in the pathogenesis and progress of this disease.Impaired lipid metabolism has been implicated in cardiovascular complications in diabetic patients. **Aim:** The study was designed to quantitatively estimate serum calcium, magnesium, zinc, apolipoprotein A, apolipoprotein B and lipids in patients with type 2 diabetes mellitus. **Methods:** Study was conducted at Dr. R.P.Govt Medical College, Kangra at Tanda. 100 patients of type 2 diabetes mellitus and 100 healthy controls were taken after obtaining informed consent. Fasting glucose, HbA1c, calcium, magnesium, zinc, apolipoprotein A, apolipoprotein Band lipids were estimated using appropriate kits. **Results:** There was a statistically significant difference in the mean values of calcium, magnesium, zinc, apolipoprotein A, apolipoprotein B, total cholesterol, triglycerides, HDL and LDL in type 2 diabetics and controls. **Discussion:** Lower concentrations of serum

calcium, magnesium and zinc in diabetics as compared to controls suggests that impaired metabolism of these minerals may have a contributory role in the progression of diabetes mellitus and later development of complications. The assays for apolipoprotein A and apolipoprotein B should be included in the standard lipid profile, for better risk prediction in diabetics. **Conclusion:** The poor glycemic control and the association with type 2 diabetes mellitus strongly suggest that serum calcium, magnesium, zinc, apolipoprotein A, apolipoprotein B and dyslipidemia estimation should be a part of the screening panel in the risk detection. Supplementation of these minerals may be beneficial for delaying the disease progression and associated complications. (DOI:10.9777/rr.2018.1258)

Differential histamine receptor expression and signaling cause heterogeneity in tumor cell response to mast cell mediators

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Background: Mast cells (MCs), specialized secretory cells, are important component of tumor microenvironment. Several studies have shown that mast cells either promote or suppress tumor growth and development where as in few cases they are reported to be inert. The role of mast cells in tumor is still controversial. Most of the role of mast cells depends on the release of pro-inflammatory mediators upon activation. However, these functions of MCs in cancer have been poorly understood. **Aim:** To investigate the direct and indirect effect (their exocytosed mediators) of mast cells on the growth of leukemia (L1210), Lymphoma (YAC-1, EL-4) and mastocytoma cell line (P815) invitro. **Methods:** Cancer cells (YAC-1, P815, L1210, and EL4) and Mast cells (RBL-2H3) were used in the investigation. Pyrimidine, Rentidine, and JNJ777120 (Histamine receptors antagonist) were used. FACs, MTT, qRT-PCR and Western Blotting were used in the study. **Results:** We observed that mast cell mediators reduced the cell viability and proliferation in YAC-1 cell line. In contrast, in EL-4 cell line, they induced a weak but significant increase in growth. We found that the differential response of these cells could be due to the histamine receptors expression (H1, H2, H3 and H4) in all tumor cell lines. Treatment of histamine receptor antagonist, Pyrimidine (H1R-antagonist) enhanced the proliferation whereas Rentidine (H2R- antagonist) inhibited the cell growth through apoptosis and cell cycle arrest at Go-G1 phase. We further demonstrated that mast cell mediators down regulated the expression of Survivin, Cox-2, and SNAP-23 in YAC-1 whereas opposite effect was seen in EL4. **Discussion:** Overall observations suggest that activated mast cell mediators showed functional heterogeneity such as anti-proliferative effect, growth tolerance and enhanced growth in YAC1, p815 and L1210, and EL4 cell lines respectively. **Conclusion:** This suggests that mast cell mediators, along with histamine receptors could be a new therapeutic target for the treatment of leukemia and Lymphoma. (DOI:10.9777/rr.2018.1259)

High prevalence of OXA-232 in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) represents the most serious concerns since they are susceptible to very few antibiotics.OXA-48-type carbapenem-hydrolyzing class -D β- lactamases are widely distributed amongEnterobacteriaceae. These enzymes show high-level hydrolytic activity against penicillinsand low-level hydrolysis towards carbapenems. The true prevalence of OXA-48 type β-lactamases is relatively unknown due to the varying level of carbapenemase activity and difficult to detect with phenotypic methods. **Aim:** To detect the prevalence of OXA-48 and its variants in clinical isolates of E. coli and K. pneumoniae. **Methods:** A total of 500 non-repeatEnterobacteriaceae isolated fromvarious clinical specimens were includedin the study. Total DNA from phenotypic resistant clinical isolates were extracted by boiling method. PCR was performed for detection OXA-48 type, other carbapenemases and aminoglycoside resistance gene. PFGE was performed for OXA positive isolates to detect the clonal relationship among OXA producers. **Results:** Of 500 isolates, 202 (40.4%) were E. coli and 298 (59.6%) were K. pneumoniae. OXA-48 type, i.e. OXA-232 were detected in 116 (38.9%) of E. coli and 83 (41.1%) K. pneumoniae isolates. NDM co-occurred among 98 (84.4%) E. coli and 72 (86.7%) K. pneumoniae OXA producing isolates. All OXA producers were also found to harbor other carbapenemases, ESBLs, and aminoglycosides. PFGE analysis showed that OXA producer isolates belonged to different pulsotypes. **Discussion:** Rapid increase in CPE has emerged as threat for infection control globally. Among CPE E. coli and K. pneumoniae account for significant portion of nosocomial infection. The true prevalence of OXA type β-lactamases has always remain debatable due to low hydrolysis of carbapenem, however the present study gives a fair idea about true prevalence of OXA type β-lactamases. **Conclusion:** The study shows that OXA-232 variant is major OXA-48 type β-lactamase in our hospital. The OXA-232 producer belonged to different clonal groups. (DOI:10.9777/rr.2018.1260)

Identification of dysregulated micro RNAs in Obstructive Sleep Apnea (OSA)

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Background: Obstructive Sleep Apnea (OSA) is one of the most common disorders and is growing on a global scale as a major health concern. It is characterized by repetitive episodes of respiratory disturbance accompanied by oxygen depletion and sleep disruption.Literature has established a strong association between microRNAs and obesity, a major risk factor for obstructive sleep apnea but has not been studied in OSA. Therefore, our hypothesis states that some miRNAs such as miR-21 and miR-29 which are differentially regulated in obesity may play a role in OSA. **Aim:** The aim of this study is to investigate the expression of miR-21 and miR-29 in obese and non-obese OSA patients compared with healthy volunteers. **Methods:** 28 subjects (14 diseased and 14 healthy) were selected for this study and subjected to total RNA isolation from Peripheral Blood Mononuclear Cells (PBMCs) using Trizol. miR-21 and miR-29 expression analysis was done

using SYBR Green based RT-q PCR. The reaction was normalized with RNU6 and non-template controls. The fold change in miR-21 and miR-29 was calculated based on the obtained Ct values. **Results:** The expression of miR-21 was found to be up-regulated significantly by 3-fold in obese OSA patients and by 1.4-fold in non-obese OSA patients compared with healthy volunteers. Similarly, miR-29 was up-regulated in obese OSA patients by 2-fold. **Discussion:** This study shows that miR-21 and miR-29 are differentially regulated in OSA patients than the healthy volunteers. Higher miRNA expression in obese subjects than non-obese subjects elucidates that obesity directly influences the pathogenesis of OSA. **Conclusion:** OSA which has been categorized as a heterogeneous disorder with multiple pathophysiological risks is still under detailed study. Our study concludes that miRNA expression profile differs significantly in obese and non-obese OSA subjects than healthy volunteers and elucidates the involvement of miRNAs as potential biomarkers of OSA. (DOI:10.9777/rr.2018.1261)

Neuroinflammation: a bull's eye for neuroprotection in Parkinson's disease?

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Background: Parkinson disease (PD) is a neurodegenerative disorder characterized by dopaminergic neurons affected by inflammatory processes. Neuroinflammatory processes play a significant role in the pathogenesis of PD. Epidemiologic, animal, human, and therapeutic studies all support the presence of a neuroinflammatory cascade in disease. **Aim:** Is neuroinflammation promoted PD pathogenesis or progression? **Methods:** The literature is thoroughly reviewed to understand the neuropathologic features of PD. Pathology of PD at molecular level are summarized from cell biological and animal studies. Results and **Discussion:** Post-mortem analyses of human PD patients and experimental animal studies reveal that the increment in pro-inflammatory factor levels and activation of glial cells are the common features of the PD brain. Pathology of PD get worsen on chronic release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 by activated astrocytes and microglia leading to DA neuron degeneration in the substantia nigra pars compacta (SNpc). As disease progresses, neighbouring glial cells are engaged by inflammatory secretions resulting in a vicious cycle of autocrine and paracrine amplification of inflammation perpetuating tissue injury. Such pathogenic processes contribute to neurodegeneration in PD. Previous studies have shown that peripheral immune system also acts as a key player in the pathogenesis or progression of PD. Infiltration and accumulation of immune cells from the periphery are detected in and around the affected brain regions of PD patients. **Conclusion:** A better understanding of the role of neuroinflammation in PD will provide new insights into the pathological processes involved in its progression and can help us to establish effective therapeutic strategies. (DOI:10.9777/rr.2018.1262)

Synthesis, Spectroscopic in *vitro* cytotoxicity and DNA damage studies of Cu (I) complexes with N, N'-disubstituted thiocarbamide ligands

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Structural analysis of three new substituted thiocarbamide ligands N- (naphthyl)-N'-(isobutoxycarbonyl) thiocarbamide (H L^1), N-(4-methoxyphenyl)-N'-(isobutoxycarbonyl) thiocarbamide (H L^2)& N-(2-methoxy-4-nitrophenyl)-N'-(isobutoxycarbonyl) thiocarbamide (H L^3) and their copper (I) complexes [(H L^1) CuCl] (1) , [(H L^2) CuCl] (2) and [(H L^3)2CuCl] (3) was performed using various spectroscopic techniques (FT-IR, ^{1}H and ^{13}C NMR, UV-Visible),TG analysis and single crystal X-ray studies of (H L^1) and [(H L^1) CuCl] (1). The copper (I) complexes possess trigonal planar geometry coordinating through two thione sulfur atoms from two ligand molecules and one chloride ion. Two intramolecular hydrogen bonding interactions present between (-N1H) and carbonyl oxygen, (-N2H) and coordinated chlorine stabilize the TBP structure of the complexes. Determination of in vitro cytotoxicity of ligands and their complexes using five human carcinoma cell lines 2008, C13*(cervical carcinoma), A2780, A2780/CP and IGROV-1(ovarian carcinoma) revealed that copper (I) complexes were more potent inhibitors than the ligands against all the cell lines. The most effective were complex 2 and 3. The comet assay test of complexes 2 and 3 against 2008, C13* and IGROV-1 cell lines indicated significant damage to the DNA structure. (DOI:10.9777/rr.2018.1263)

HPV - Also a cause for Head and Neck Squamous Cell Carcinoma

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Head and Neck Squamous Cell Carcinoma malignant disease is associated with human papillomavirus (HPV) 16 infection. Human papillomavirus (HPV) is a virus often infecting humans. It is often present on skin or mucous membranes. This diverse DNA virus is often linked to many various benign and malignant neoplastic lesions. Over 40 types of HPV are transmitted through sexual contact and infect the anogenital region which might be secondly transmitted to the oral mucous. The biology of HPV- positive oropharyngeal cancer is distinct with P53 degradation, retinoblastoma RB pathway inactivation, and P16 upregulation. By contrast, tobacco-related oropharyngeal cancer is characterised by TP53 mutation and downregulation of CDKN2A (encoding P16). The best method to detect virus in tumour is controversial, and both in-situ hybridisation and PCR are commonly used; P16 immunohistochemistry could serve as a potential surrogate marker. HPV-positive oropharyngeal cancer seems to be more responsive to chemotherapy and radiation than HPV-negative disease. Many patients with oropharyngeal cancer have no common risk factors, and recent epidemiologic and molecular studies have identified high- risk types of human papillomavirus (HPV), especially HPV- 16, as the potential etiologic agents. (DOI:10.9777/rr.2018.1264)

Ocular pathologies associated with xenobiotic stress from pesticide exposure

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Background: WHO records reveal multiple health hazards and fatalities from the widespread use of pesticides in many parts of the world due to their description as farmers' friends, primarily as a result of occupational exposure. Developing countries primarily dependent on agriculture for their economies are especially reliant on these chemicals and the consequential repercussions on public health have become increasingly evident with the rise of diseases like Parkinsons' and Alzheimer's. **Aim:** Despite the significant volume of data available on the detrimental impact of pesticides, there is a lacuna of knowledge where the effect of pesticide exposure on the eye is concerned. The current study aims to shed light on this neglected area of xenobiotic insult and reveal a hitherto undiscovered link between a miscellany of ocular pathologies such as keratectasia, opacification, neovascularisation and chronic pesticide exposure. **Methods:** The consequences of chronic pesticide exposure on the eye, with special focus on the ocular surface were primarily explored by mimicking the on-field scenario in murine model. Cytological, histological and flowcytometric parameters were taken into consideration to determine the risk to corneal tissue. **Results:** Visual acuity of the subjects was determined and it was observed that chronic exposure to pesticides leads to heightened ocular morbidity wherein there were visible pathophysiological changes to the ocular surface. Further, it was discovered that those with chronic exposure to these xenobiotics reported epiphora (increased lacrimation) and ocular irritation. **Discussion:** A detrimental impact on the corneal layers and an amplified expression of inflammation markers such as TNF-α, VCAM-1 and ICAM-1 was consequently observed. **Conclusion:** It was found that along with ocular toxicity, chronic exposure to pesticides significantly increased the risk of disorders like keratectasia and corneal neovascularisation which negatively impact vision and if left untreated, may lead to blindness. (DOI:10.9777/rr.2018.1265)

TDZ-induced plant regeneration in *Brassica oleracea* L. var. *botrytis*: effect of antioxidative enzyme activity and genetic stability in regenerated plantlets

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The effects of various combinations of plant growth regulators on regeneration potential from seedling-derived leaf tissues of Brassica oleracea L. var. botrytis were evaluated. Callus was induced from 2- wk-old leaf explants. The explants were incubated on Gamborg's (MSB5) medium. The maximum frequency of callus induction (85.56%) was recorded on MSB5 medium supplemented with 9.1 µM thidiazuron (TDZ) and 0.5 µM α-naphthalene acetic acid (NAA). Optimum shoot induction (54.44%) was obtained on MSB5 medium supplemented with 4.5 µM TDZ and 0.5 µM NAA. The maximum number of shoots per explant (5.33) was recorded on MSB5 medium with 4.5 µM TDZ and 0.5µM NAA, whereas the maximum shoot length (4.86 cm) was recorded for shoots cultured on MSB5 medium supplemented with 4.5 µM TDZ and 5.7 µM gibberellic acid (GA3). However, optimum root induction (71.11%)

occurred on half-strength Murashige and Skoog basal medium supplemented with 4.9 µM indole-3 butyric acid (IBA). Studies on the antioxidant activity of superoxide dismutase, ascorbate peroxidase, and peroxidasein seedlings, callus, regenerated shoots, and regenerated plantlets cultured on 4.5 µM TDZ and 0.5 µM NAA medium revealed the roles of these key antioxidative enzymes in callus induction and regeneration. The genetic stability of the regenerated plantlets was assessed using inter simple sequence repeat primers. The monomorphic amplification products confirmed true-to-type in vitro regenerated plants. This in vitro regeneration method can be useful in the large-scale production of genetically uniform plants, for genetic transformation, and conservation of elite germplasm of plant species. (DOI:10.9777/rr.2018.1266)

Evaluation of 5-Demethylnobiletin against lead induced toxicity in *Caenorhabditis elegans*

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Background: Toxic amount of transition elements including lead (Pb), arsenic (As), mercury (Hg) and cadmium (Cd) penetrated to the environment by human activities and has been reported to persuade cognitive and behavioural deficits. In comparison to other heavy metals, lead is the most deleterious element, associated with neurodegenerative, mutagenic, teratogenic and carcinogenic effects. Currently, lack of complete remediation therapy against lead induced stress is the major concern related with organisms. In recent years, the identification and characterization of medicinal plants to combat Pb induced toxicity has gained increasing scientific interest. **Aim:** In this study, we investigated the toxicological behaviour of lead and explore the potential protective effects of a polymethoxyflavones compound, 5-Demethylnobiletin, which is isolated from Gardenia lucida, against sub-chronic administration of lead using Caenorahabditis elegans as a model. **Methods:** C. elegans treated or untreated with 5-Demethylnobiletin and exposed with Pb were investigated for behavioural assays, stress resistance assays, lifespan assay and gene reporter assay. For further mechanistic evaluation quantitative RT-PCR was performed. Results and **Discussion:** 5-Demethylnobiletin exerted excellent antioxidant activity, reduced intracellular oxygen species and enhanced lifespan against Pb induced lethality. 5- Demethylnobiletin showed upregulated expression of GST-4 and SOD-3 against Pb induced oxidative damage, using the transgenic strain CL2166 and CF1553 and also elevated levels of synaptic acetylcholine and dopamine in wild-type worms. In addition, 5-Demethylnobiletin significantly reduced lipid levels and α-synuclein aggregation in the transgenic strain NL5901. Moreover, 5-Demethylnobiletin up-regulated mRNA expression of mtl-1, jnk-1 and pink-1 genes in wildtype N2 exposed with Pb. **Conclusion:** The results from this study suggest that the protective and reinstating effects of 5-Demethylnobiletin against lead toxicity might be regulated via by upregulation of mtl-1, jnk-1 and pink-1 genes in C. elegans. Therefore, 5-Demethylnobiletin may be useful as therapeutic agent or supplements to treat or slow lead induced pathologies. (DOI:10.9777/rr.2018.1267)

Assessment of antioxidant profile of *Trichodesma indicum* from shivalik hills

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Free radical induced oxidative stress causes several patho-physiological conditions in plants. Antioxidants decrease oxidative stress thus are significant for human health. Various plants of Shivalik hills from Western Uttar Pradesh have been tested for its antioxidant potential and free radical scavenging capacity. Trichodesma indicum was assessed for its flavonoid and other phenolic contents, enzymatic and non-enzymatic antioxidants and proved to be very useful. It was evident from the study that plant is rich source of antioxidants and proved to have radical scavenging activity. Higher proline and ascorbate contents were observed in all accessions. Catalase, peroxidase, superoxide dismutase and IAA oxidase activity were assayed and presence of enzymes was reported in high quantity in all accessions. Stress tolerance potential as analyzed using lipid peroxidation tests and low MDA levels was reported in all samples studied proving high antioxidant potential in Trichodesma indicum. (DOI:10.9777/rr.2018.1268)

Relation of vitamin D with breast cancer; an experimental approach

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Vitamin D and calcium are metabolically interrelated and highly correlated dietary factors. From the experimental studies it has been observed that anticarcinogenic effects arises due to their participation in regulating cell proliferation, differentiation, and apoptosis in normal and malignant breast cells. There may be involvement of vitamin D and calcium in the development of breast cancer. Specifically, for inverse associations between vitamin D, calcium intakes and breast cancer there is some epidemiologic evidence; Serum, plasma, and/or blood levels of vitamin D metabolites have been inversely associated with breast cancer risk; high sunlight exposure, most probably reflecting vitamin D synthesis in the skin, has been associated with a reduced risk of breast cancer; breast density has been inversely related to vitamin D and calcium intakes; calcium has been associated with a reduced risk of benign proliferative epithelial disorders of the breast; certain polymorphisms of the vitamin D receptor might modify breast cancer susceptibility. Low intake of vitamin D causes low synthesis of 25(OH)D in liver so kidney also produce low level of 1,25(OH)2 D. Due to insufficient amount of 1,25(OH)2 D unable to maintain the calcium concentration in metabolism which causes low differentiation and high proliferation of cell that leads to breast cancer. (DOI:10.9777/rr.2018.1269)

Fractal analysis of colony margins as an aid for screening oligotrophic freshwater yeast cultures for bioclarification of turbid polluted water resources in the iron ore mining region of goa

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Background: Yeast is known to produce characteristic cell wall polysaccharides useful in many cell- cell and cell to inorganic particle interaction phenomena. Sediment binding properties of oligotrophic freshwater yeasts are underexplored. In Iron ore mining areas of Goa,

water resources are polluted due to high turbidity due to mineral colloids. For bioclarification of the turbidity there is felt need to identify a promising strain of freshwater yeast. Puchkov (2016) has provided examples of using image analysis in the studies of both themacroscopic and the microscopic microbiological objects obtained by various imaging techniques. Fractal analysis has been found useful in characterizing microbial colonies. **Aim:** Methods As a part of ongoing work to access the feasibility of employing interesting strains of ascomycetous and basidiomycetous yeasts isolated from local freshwater ecosystem for bioclarification of turbid polluted water resources in Goa's Iron ore mining area a need was felt for a reliable procedure to screen and select potentially useful strains. We tested the hypothesis that fractal dimension of colony margins of yeast could serve as an aid to screen and select strains capable of bioclarification of turbidity. **Results:** Different fractal dimensions for 16 yeast strains were obtained. These were positively or negatively co-related with bioclarification ability of the strains producing clear small or large flocs, leading to identification of 2-4 superior strains showing better sediment bioclarification potential. **Discussion:** Fractal analysis of yeast colony margin was proved to be an useful aid to characterize freshwater yeast strains and This image analysis based technique holds excellent potential for rapid screening of a large number of yeast strains required in multifarious biotechnological applications. **Conclusion:** Utilize the fractal dimensions for establishing a clear positive or negative correlation with their bioclarification potential. (DOI:10.9777/rr.2018.1270)

Digital library of Indian medicinal plants & their metabolites

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Background: The plants have potential to synthesize a wide variety of chemical compounds that can be used to perform important biological functions. But, only a few amounts of the world's biodiversity have been tested for biological activity. Thus, the discovery of known/new bioactive compounds from previously unknown sources is a vital part of the discovery process that saves time and resources. **Aim:** To develop a bio-informatics tool for new drug discovery & exploration of Indian medicinal plants chemical diversity. **Methods:** A universal reversed phase UPLC gradient program was developed for generation of UPLC/HPLC-MS profile (LC-MS fingerprint). The separation of wide range of plants metabolite were carried out on Waters BEH C-18 150 x 2.1 mm, 1.7µ column and developed MS/MS fingerprints of non- target plant metabolites. The HPLC, LC-MS and MS/MS fingerprints were utilized to develop the Digital Library of Indian Medicinal Plants & their Metabolites **Results:** A Web-based digital (LC-MS/MS) library of non-targeted plant secondary metabolites with suitable software search algorithm was developed for exploration of Indian medicinal plants chemical diversity. **Discussion:** On the basis of above facts CSIR- CDRI, Lucknow developed a digital library of Indian Medicinal Plants & their metabolites contain, 216 medicinal plant species from 73 families having 529 chemical/Mass spectrum fingerprints. It has targeted applications (1-4) for medicinal plants chemistry. 1. Identification and authentication of plant species based on their Mass Spectrum/HPLC/LC-MS Fingerprints. 2. Discovery of known bioactive compounds from previously unknown sources (inverse search). 3.

Distribution of known compounds in different biological source (Plants/herbs). 4. Identification of known/unknown derivatives of bioactive compounds in different biological source (Plants/herbs) using LC-MS/MS data. **Conclusion:** The developed digital library of Indian medicinal plants and their metabolites provide mass spectrometry based methodologies to explore the chemistry of plants. (DOI:10.9777/rr.2018.1271)

Vascular parkinsonism clinicoradiological profile

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Objective: To study the clinical and radiological features of vascular parkinsonism (VP). **Methods:** Cross-sectional study where 15 patients with VP underwent motor and cognitive evaluation and brain MRI. **Results:** Patients with VP were, 93% male and 7% female with average age of 67.6 yrs. with a range from 35 years to 85 years; all had vascular risk factors - arterial hypertension (62.55), T2DM in 25%, CAD and CKD in 12.5% each, smoking in 50%. Around 62.5% had past stroke, 25% had TIAs. They presented with an insidious onset of parkinsonism (87.5%) and acute onset in 12.5% and a rapidly progressive clinical course. Predominant lower body Parkinsonism (50%), hemiparkinsonism in 37.5%, postural instability (75%), freezing of gait (25%) and falls in 50%, urinary incontinence and pyramidal signs were more common in patients with VP. Movement Disorders Society's Unified PD Rating Scale (MDS-UPDRS) scores were higher in patients with VP with less responsiveness to levodopa. They had greater cognitive impairment and 80% fulfilled diagnostic criteria for probable vascular dementia. Most patients with VP had brain MRI changes: multiple lacunar infarcts (66.7%) or extensive white matter disease (26.7%). Conclusions: VP can be clinically diagnosed by parkinsonism at an older age, characterised by lower body predominance, urinary incontinence, pyramidal signs, postural instability with freezing of gait and falls, and dementia with less responsiveness to levodopa. (DOI:10.9777/rr.2018.1272)

Pueraria tuberosa enhance insulin secretion from pancreas through DPP-IV inhibition and incretin hormones regulated β cells survival signalling pathway.

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Objective: The main objectives of this research were to explore the effect of polar fraction of tubers of Pueraria tuberosa (PT) on incretin hormones regulated pancreatic β cells survival and DPP-IV activity. **Methods:** Chronic diabetes was induced with STZ (65mg/kg bw) in rats for 60 days and grouped into diabetic control and PT. Expression of genes was assessed by PCR, IHC, and ELISA. Morphological analysis of tissue was observed using H & E stain. The components of PT were analyzed via HPLC-MS based on their chemical formula, molecular mass, and retention time. In silico molecular docking approach has been used to see the interaction of active phytochemicals of PT on the basis of their binding energy [kcal/mol] and dissociation constant [pM] using YASARA software. Interactive visualization was done using Discovery studio 3.0. **Results:** In comparison to diabetic controls, PT administered

rats showed decreased DPP-IV activity in the intestine, leading to enhanced basal plasma insulin concentration. Through molecular docking, we found Puerarone and Robinin to be the most potential phytochemicals of PT for DPP-IV inhibition. Binding energy (kcal/mol) and dissociation constant (pM) of Robinin with DPP- IV protein were found to be 7.543 and 2,957,383.75, respectively. For Puerarone, it was 7.376 and 3,920,309, respectively. In comparison to diabetic control, the size and number of islet cells along with the plasma level of GLP-1, GIP, and pancreatic expressions of GLP-1R, GIP-R, Bcl2, and insulin were enhanced significantly after PT treatment. Through in silico molecular docking, tuberostan showed the best interaction for GLP-1R with binding energy at 8.15kcal/mol and dissociation constant at 1061624.125 pM. Puererone showed the best interaction for GIP-R with binding energy at 8.31kcal/mol and dissociation constant at 810381 pM. Conclusions: Pueraria tuberosa and its active phytochemicals plays potential role in DPPIV inhibition and also acts as incretin receptors agonist and protects against STZ-induced diabetes by down regulating β cells apoptosis. (DOI:10.9777/rr.2018.1273)

Targeting triple negative breast cancer by moringin

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Background: The triple negative breast cancer (TNBC) is a highly aggressive tumor. Although some chemotherapeutic agents have been developed, these agents produce several side effects and are highly expensive. Thus, safe and cost-effective agents are required. **Aim:** The main focus of this study was to examine the potential of moringin against triple negative breast cancer cells. We also delineated the underlying mechanism by which moringin exhibits anti-cancer activities against TNBC. **Methods:** We used triple negative MDAMB-231 cell line during the study. Moringin containing benzyl-isothiocyanate, is produced by myrosinase-catalyzed hydrolysis of glucomoringin (GMG).The MTT and clonogenic assays were used for the cytotoxicity; DAPI staining for apoptosis and wound healing assay for cell migration. Results and **Discussion:** Moringin inhibited the viability and proliferation of MDAMB-231 cells in a dose- and time-dependent manner. At concentrations as low as 25 μM, moringin inhibited the proliferation of cancer cells after 24 hours of treatment.The long-term colony formation by cancer cells that mimics in vivo situation, was also significantly inhibited by moringin. DAPI staining suggested that the moringin induced apoptosis in breast cancer cells. Moringin also induced ROS generation in MDAMB-231 cells. As revealed by wound healing assay, moringin inhibited the migration of cells. **Conclusion:** Moringin exhibit anti-cancer activities against triple-negative breast cancer cells. The generation of ROS may contribute to its anti-cancer activities. Studies are in progress to substantiate these observations using multiple TNBC cells lines and to elucidate the in-depth molecular mechanism. (DOI:10.9777/rr.2018.1274)

Evaluation of mycophenolic acid in batch submerged fermentation by using *Penicillium brevicompactum*

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Mycophenolic acid (MPA) is an antibiotic and an immunosuppressant drug that is produced by the microfungus Pencillium brevicompactum as an extracellular secondary metabolite, when grown in submerged fermentation condition. Numerous screening processes were carried out to identify the suitable media composition and the optimal pH and temperature requirements for the batch production. Shake flask batch cultures were incubated at 28 °C for 10 days at 200 rpm and production of MPA was evaluated. A 10 days batch fermentation process was carried out in a 3L stirred tank bioreactor with an initial pH of 5.5 and an incubation temperature of 28°C, with an agitation rate of 200 rpm. The DO was maintained at 40%. Glycine and Methionine were used as precursors. A detailed study showed that the lag phase of the culture lasted till 72 hours, followed by the log phase till 144 hours. MPA was produced between 144-240 hours. The maximum specific growth rate was 0.068 h^{-1}. The maximum concentration of MPA and the rate of production were found to be 1.38g/L, and 6.5 $mg/L^{-1}h^{-1}$ respectively. This kinetic study will further applied for comparing the results of different modes of fermentation processes and scale up of MPA. (DOI:10.9777/rr.2018.1275)

Fungal decolorization of azo dye by extracellular enzymes of *Pleurotus ostreatus* and *P. citrinopileatus*

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Background: Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paperprinting. They are generally recalcitrant to biodegradation due to their xenobiotic nature. However microorganisms, being highly versatile, have developed enzyme systems for thedecolorization and mineralization of azo dyes under certain environmental conditions. **Aim:** The present work focuses on the decolorization of azo dye by Pleurotus species i.e., P. ostreatus and P. citrinopileatus. Synthetic dyes used in present work were coomassiee brilliant blue (CBB), bromocresol green (BG) and Methyl red (MR). The production of lignolytic enzymes i.e., laccase, MnP were also analysed since these enzymes play role in decolorization and degradation. **Methods:** Methodology contains culture and maintenance of different pleurotus species. Enzyme assay of laccase and MnP was carried out following the medthod as described in Dhaliwal et al. (1991) and Paszczynski et al. (1985). Decolourization of azo dye done by their visible spectra were recorded with a UV-visible (Elico- SL191) spectrophotometer at λmax= 595nm, 515nm and 523nm (CBB, BG and methyl red respectively). **Results:** It was observed that maximum lignolytic enzyme activity was achieved by Pleurotus ostreatus in comparison to P. citrinopileatus. Further, investigation was done with crude enzymes obtained from culture of Pleurotusostreatus were tested for decolorization of azo dyes. After 4 hour of treatment of coomassiee brilliant blue, bromocresol green and Methyl red with crude enzyme, decolourization was recorded as 67.04%.72.90%, and 67% respectively. **Conclusion:** The present investigation suggest that white rot fungi Pleurotus ostreatus can be used in bioremediation of dye

contaminated ecosystem. (DOI:10.9777/rr.2018.1276)

Combination of ivermectin and albendazole induces oxidative stress associated apoptosis in adult filarial parasites: Biochemical and proteomic approach

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Background: Lymphatic Filariasis is one of the most prevalent tropical diseases. Available antifilarial drugs effectively eliminate larval stages of the parasite but are ineffective against the adult worms. **Aim:** Present study aims to explore the underlying mechanism of action of effective drug regimens against adult filarial parasites. **Methods:** In the present study, S. cervi adult filarial parasites were exposed to ivermectin (50µM), albendazole (200 µM) alone as well as in combination ivermectin+albendazole (50 +200 µM) for 8h under ex vivo conditions. To elucidate the combined mechanism of action, biochemical and proteome studies were performed. **Results:** The motility and viability of parasites was found to be significantly reduced in a concentration dependent manner in the iver+alb exposed parasites. Level of oxidative stress markers ROS, protein carbonyl, lipid peroxidation and NADPH oxidase activity was found to be significantly increased. Significant reduction in cytochrome c oxidase and increase in calpain and caspase- 3 activity was observed in iver+alb treated parasites. Proteome profile of cytosol revealed 13 upregulated and 16 downregulated spots. These differentially expressed spots were identified as stress responsive, energy metabolic and antioxidant enzymes in iver+alb treated parasites. **Discussion:** The present study revealed that combination iver and alb (50+200 µM) was effective in killing adult parasites. Combined effect of drugs lead to the generation of oxidative stress by elevating ROS, protein carbonyl and lipid peroxidation levels. This could alter the mitochondrial membrane permeability leading to release of cytochrome c. Reduced activity of cytochrome c oxidase and increase in the calpain and caspase-3 activity suggested the initiation of apoptosis. The proteome profile of cytosol revealed that antioxidant enzymes and proteins play crucial role in the survival of adult filarial parasites. **Conclusion:** Iver+Alb (50+200 µM) could be used as potential drug regimen against lymphatic filariasis. (DOI:10.9777/rr.2018.1277)

Biochemical characterisation and relative enzymatic activity of wtGNE and mutants (D207V, V603L, R193C, V727M, R308C)

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Background: UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE) is a bifunctional enzyme (N- terminal epimerase and C-terminal Kinase domain) that catalyzes the rate limiting step in sialic acid biosynthesis. Homozygous missense mutations in either epimerase or kinase domain of GNE are involved in the development of a neuromuscular disorder GNE myopathy, characterized by defect in proximal and distal skeletal muscles sparing the quadriceps. **Aim:** The pathomechanism of this disease is poorly understood. The complete GNE protein structure is not known. Individual domain studies limit drug binding affinity to enzyme active sites when both domains interact and fold in

quaternary structure. Thus it is of prime importance to determine crystal structure of full length GNE protein and study substrate binding parameters. A major limitation for obtaining crystal structure of full length GNE protein is large scale expression of recombinant GNE in functionally active form, we aim to clone, express and purify GNE in pET30a from C41pLysS. Both wild type and mutant GNE were expressed from E. coli followed by functional activity determination using epimerase and kinase assays. **Methods:** Full length GNE and various pathologically relevant GNE mutants were subclonedinto pET30a vector and expressed in E. coli C41 (PLysS). The protein was purified from soluble fraction using Ni-NTA affinity chromatography and confirmed by immunoblotting using specific antibody. The functionality of overexpressed protein was established by specific epimerase assay using Morgan-Elson Methods. Both wildtype GNE and mutant GNE proteins (epimerase mutants (D207V, R193C, R308C) & GNE kinase mutants (V603L, V727M) were analysed for enzymatic activity. Results & **Discussion:** Full length GNE was expressed as soluble protein in C41 (pLysS), with 1mM IPTG induction at 160C/overnight. The conditions were optimized for wild type &mutant protein. The activity of crude cell lysates was determined by Morgan Elson method. Wild type GNE showed approx. 40% epimerase activity while D207V, R193C, R308C epimerase mutants showed no activity. Also the kinase mutant V727M showed no epimerase activity indicating that mutation in one domain affected the activity of other domain. Interestingly V603L kinase mutant showed 10% epimerase activity suggesting each mutation may be unique in affecting GNE activity. Thus our study will provide insights into mutation analysis of GNE and offer a platform for development of better therapeutic molecules. (DOI:10.9777/rr.2018.1278)

Stage specific proteome analysis in filarial parasite Setariacervi after exposure of albendazole

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Background: Present antifilarial drugs strategies only eliminate the larval stages of filarial parasites. Therefore, there is an urgent need of drugs which are macrofilaricidals. Albendazole (Alb), which has also been reported as a potent microfilaricide, binds to tubulin inhibiting polymerization and subsequent formation of microtubules. The WHO has recommended the use of DEC+Alb combination therapy form as a drug administration programme. However, the exact mechanism of their action is still unknown. Proteome studies contribute markedly to our understanding of parasite biology, host-parasite interactions, and mechanisms of drug action. **Aim:** The proteome analysis of different stages of bovine filarial parasites in control and drug treated parasites. **Methods:** An equal number of motile adult female, uterine microfilariae and excretory product of S. cerviparasites were incubated in KRB maintenance medium containing 200 µM of albendazole for 6h at 37°C, 5% CO2.Further to study the expression of altered proteins were identified using MALDI MS/MS.. **Results:** The total number of spot identified in adult female was 153, in uterine microfilariae 80 and 74 were observed in Excretory Secretory product. In drug treated parasites, we identified the total number of 278 proteins, thus decreasing the overall number of proteins identified in different life stages as compare to control. **Discussion:** In this study most

proteins showing decreased expression in drug treated parasites. Through MALDI-MS analysis calcium binding proteins like calponin and chaperons like protein disulphide isomerase, Hsp60, Hsp70 were upregulated. Some of the downregulated proteins are major sperm protein and glycolytic proteins. **Conclusion:** This approach provides provisional evidence for relative protein abundance and the presence or absence of a particular protein in any given stage. (DOI:10.9777/rr.2018.1279)

Association of Vitamin C and Selenium with nodular thyroid disease

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Background: Antioxidants have long been known to play a crucial role in thyroid physiology. This is due to the oxidative nature of the gland which produces large amounts of free radicals from iodine and tyrosine residues as well as hydrogen peroxide during thyroid hormone synthesis. Thus the maintenance of a proper oxidant-antioxidant balance becomes more so important in case of thyroid diseases in order to avoid oxidative stress. Vitamin C is a very important non-enzymatic antioxidant in our body which acts as a free radical scavenger. Selenium acts as a constituent of antioxidant enzymes and also is a part of the thioredoxin reductase system. Thus the role of selenium is twofold in normal functioning of the thyroid. **Aim:** The purpose of the current study is to evaluate the levels of nutritional antioxidants vitamin C and trace element Selenium in patients with benign and malignant thyroid nodules and try to associate them with disease status. Materials and **Methods:** 40 cases with thyroid nodules (31 benign and 9 malignant) and 16 controls were included in the study. Vitamin C was estimated from plasma by spectrophotometric method and Selenium was estimated from whole blood by spectroscopic method. **Results:** Vitamin C levels were found to be lower in both benign and malignant cases compared to healthy controls. Selenium levels were lower in malignant cases compared to healthy controls. **Conclusion:** Low values of both vitamin C and Selenium indicate the presence of increased oxidative stress and an impaired nutritional antioxidant defence which may have a possible role in the development of the disease. (DOI:10.9777/rr.2018.1280)

Myristica fragrans Houtt. seed essential oil as potent botanical preservative against post- harvest molds and aflatoxin contamination in rice samples

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Background: Rice is an important cereal food all over the world. Across the globe, rice contamination due to moulds and their associated mycotoxins especially aflatoxin B1 (AFB1) is a major problem in the storage condition. Different synthetic fungicides are currently being used but they cause adverse effect on consumer health. Therefore, food industries are looking towards some safer alternatives of natural origin. **Aim:** The aim of the present study was to evaluate antioxidant, antifungal, antiaflatoxigenic activity and mode of action of chemically characterized Myristica fragrans Houtt. essential oil (MFEO). **Methods:** Chemical characterization of MFEO was done through GC-MS analysis. Efficacy of MFEO

against most toxigenic strain of A. flavus was determined in the terms of minimum inhibitory concentration (MIC) and minimum aflatoxin inhibitory concentration (MAIC). Probable mode of antifungal action was determined by assessment of ergosterol content in plasma membrane and cellular ion leakages. Antioxidant activity of MFEO was calculated through DPPH$^{\cdot}$ and ABTS$^{\cdot}$+ assay. The phytotoxicity assay on rice seeds was also done by seed germination test. Results and **Discussion:** Analysis of stored rice samples revealed the presence of 15 fungal species and Aspergillus was recorded as dominant genus with 6 species. AF LHP R14 was identified as highest AFB1 producer. Chemical characterization of MFEO resulted in elemicin (27.08%) and myristicine (21.09%) as major components. The MIC and MAIC of MFEO were 2.75 and 1.50µL/mL, respectively. Dose dependent reduction of ergosterol content and increase in cellular ion leakages confirmed plasma membrane as probable target site of antifungal action. The IC50 value of MFEO through DPPH$^{\cdot}$ and ABTS$^{\cdot}$+ assay were 3.74 and 0.24 µL/mL respectively. Phytotoxicity assay showed 100% seed germination. **Conclusion:** The tested MFEO may be recommended as food preservative in view of their biological activity against food borne toxic moulds, AFB1 secretion, free radical generation and nonphytotoxic nature. (DOI:10.9777/rr.2018.1281)

Antioxidant and cholinesterase inhibitory activity of phenolic rich extracts from *Bombax ceiba* L.flowers

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Cognition impairment is the most recurrent form of dementia in aged people indicated by permanent neuronal loss and atypical behaviour. Disparities in cholinergic pathway have been reported as major cause of cognition impairment, where deficiency of acetylcholine occurs due to hydrolysis of acetylcholine by acetylcholinesterase. Besides acetylcholinesterase, butyrylcholinesterase also inactivate acetylcholine as well as butyrylcholine, which is considered as a new target in drug discovery for neurodegenerative diseases. Bombax ceiba (Bombacaceae) commonly known as semal or silk cotton tree is also known as red cotton tree or Indian kapok tree. Bombax ceiba is an imperative plant of tropical and subtropical region which have been mentioned in the traditional systems of medicine such as Ayurveda, Siddha and Unani due to its various pharmacological activity. In the present investigation we have investigated the choline esterase and antioxidant activity of B. ceiba extracts by biochemical assay. Besides these activity evaluations, preliminary phytochemical testing and quantification of total phenolic and flavonoids have also been performed by HPTLC method. Finding of present study indicated that B. ceiba extract are rich in polyphenolic contents (19.10 ± 0.74 QcE and 28.01 ± 1.28 GaE). Results of antioxidative potential evaluation suggested that these extracts have high free radical scavenging potential (65.49±2.49%) and are also able to reduce iron like radicals (93.78 FSE). Beside antioxidant potential B. ceiba extracts also inhibited the choline esterases effectively (IC50 31.22 ±1.42 µg/ml). Current investigation on

hexane and ethanolic extracts from B. ceiba flowers indicated that phenolic rich extracts could be used in development of effective plant based cholinesterase inhibitors. (DOI:10.9777/rr.2018.1282)

Repurposing *Putranjiva roxburghii* for possible cytotoxic lead

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Background: Putranjiva (Putranjiva roxburghii Wall, Syn-Drypetes roxburghii family- Euphorbiaceae) native of India and widely grown all over Asia, particularly in Nepal, Sri Lanka, Bangladesh, Thailand, China, and Myanmar. In traditional systems of Indian medicine i.e. Ayurveda and Siddha, it is used for azoospermia, diuretic, ophthalmopathy, catarrh and constipation. Many attempts were made to re- purpose its medicinal use by in-vitro and in-vivo biological studies. However, its chemoprevention potential is still unexplored. **Aim:** The objective of the present study is to isolate and characterize the chemical constituents from different parts of Putranjiva and evaluate its cytotoxic potential. **Methods:** We have extracted the powdered material of twigs, seed, and stem bark with alcohol by cold percolation and partitioned into various fractions using organic solvents of varying polarity. In addition to phytochemical investigation, HPLC fingerprint of each fraction was developed to correlate the chemoprevention action. The chemo-prevention potential of extracts/fraction was evaluated on six cancer cell lines A549 (Human lung carcinoma cell line), PC-3 (human prostate cancer cell lines), K562 (Lymph leukemia type), NCI-H520 (lung carcinoma), A-431(epidermoid carcinoma) and HEK-293(human embryonic kidney cells). **Results:** In-vitro studies on cancer cell lines showed that butanol extract of stem bark has shown most chemoprevention action in human prostate carcinoma (PC3 Cell lines, IC50=10µg/ml). While hexane fraction of twigs and stem bark IC50 have moderate activity (PC3 cell lines, IC50 = 32.61µg/ml and 46.61µg/ml, respectively). Additionally, methanol extract of seed and ethyl acetate fraction of bark were also found to be effective against lung carcinoma (NCI-H520 cell lines, IC50= 40µg/ml and 17.35µg/ml, respectively). Phytochemical studies showed that hexane fraction of twigs contain mainly triterpenes along with some fatty acids and esters. Most active butanol fraction of twigs has resulted into a major compound of phenolic group. **Conclusion:** Results indicate the possible chemoprevention potential of Putranjiva. However, further detailed studies are required to establish mode of action and confirm present preliminary results. Chemical fingerprint analysis further confirmed that plant has secondary metabolites of diverse class which may serve to repurpose its therapeutic use. The controversial folklore claim has already imposed many questions over its use. The detail will be presented in the conference. (DOI:10.9777/rr.2018.1283)

Distribution and structural study of glutaredoxins in cyanobacteria

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Background: Glutaredoxins (GRXs) are oxidoreductase group of enzyme which helps in

maintaining cellular redox status of animal and plant systems. Additionally, GRXs are also involved in iron homeostasis. Cyanobacteria are photosynthetic Gram-negative, oxygen-evolving photosynthetic microorganisms which are potentially important for bioenergy and valuable chemicals production. These organisms are subjected to fluctuating light which could alter the redox status and their growth. **Aim:** To study distribution and structural diversity of GRXs in cyanobacteria. **Methods:** Sequences of cyanobacterial GRXs were retrieved from NCBI database and subjected to phylogenetic analysis in MEGA 7.0. Multiple sequence alignment was performed in Clustal W. Structures were generated using homology modeling approach using Modeller 1.18. Structures were visualized and analyzed in UCSF Chimera 1.11.2. **Results:** Phylogenetic analysis revealed four classes of GRXs in cyanobacteria. Class I and II GRXs are distantly related to each other while class V and VI GRXs are diverged group having maximum amino acid substitution rate. Each modeled structure possess thioredoxin fold containing 3 layers of α/β/α mixed with 4 β-sheet strands in 4312 order; strand 3 is antiparallel to rest of β-strands. Protein core is made by β-strand. Class I has great variability in their active site and glutathione binding site. Comparatively rest of the classes has higher conservation in their active sites. Active sites are comprised of neutral amino acids in general which are hydrophilic in nature. Active sites of the protein present in surface region are made by α helices and loops. **Discussion:** GRXs are divided into different classes based on their active site motif sequence. Among them Class I and II are common in all organisms whereas Class V and VI are restricted to some species of Cyanobacteria. Conclusions: The catalytic site and glutathione binding residues in GRXs, other than Class I, are highly conserved in cyanobacteria. (DOI:10.9777/rr.2018.1284)

Identification of selective MMP-9 inhibitors through multiple e-Pharmacophore, ligand- based pharmacophore, molecular docking and density functional theory approaches

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Background: Matrix metalloproteinase-9 (MMP-9) is a significant target for the development of drugs for the treatment of arthritis, CNS disorder, and cancer metastasis. The critical adverse effect of MMP-9 inhibitors may be due to the presence of hydroxamate as zinc-binding group (ZBG), that can chelate the zinc ion and also other divalent cations. **Aim:** To identify highly specific, less toxic, non-ZBG inhibitors of MMP-9, by e-pharmacophore and ligand-based (3D-QSAR) approaches which would be promising to develop drugs for the treatment of various disorder. **Methods:** The experimentally known MMP-9 inhibitors were used to grow up the ligand-based three pharmacophore models, and the X-ray crystallographic structures of MMP-9 with different inhibitors were utilized to develop five energy-optimized structure-based (e-pharmacophore) models utilizing Schrodinger suite. All developed pharmacophores were validated and applied to screen the Zinc database. Pharmacophore matched compounds were subjected to molecular docking to retrieve hits with novel scaffolds. The Induced fit docking (IFD) analysis provided significant information about the driving of

inhibitor to approve a suitable bioactive conformational position in the active site of protein. Density functional theory (DFT) was utilized to explore electronic features of hits. **Results:** The e-pharmacophore and 3D-QSAR predictions retrieved total 24 hits with diverse scaffold, good ADME properties and without ZBG as MMPs inhibitor. Out of these 24 hits, ninehits have selective MMP-9 binding affinity than other MMPs (MMP-2, MMP-3). The IFD and DFT studies also supported the binding affinity and interaction of reported hits. **Discussion:** This study illustrates that the combined pharmacophore approach is advantageous to identify diverse hits which have better binding affinity to the active site of the enzyme. **Conclusion:** Structure- and ligand-based methods were used to perform the virtual screening (VS) of database compounds to obtain potent and selective MMP-9 inhibtors. (DOI:10.9777/rr.2018.1285)

Genomic association of ENPP1 gene with diabetic CKD patients of eastern Uttar Pradesh, India

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Background: ENPP1 is a significant candidate gene for diabetic chronic kidney disease. **Aim:** The present study tries to investigate the association of the ENPP1 (K121Q) polymorphism with Diabetic CKD Patients in eastern Uttar Pradesh population. **Methods:** 162 Diabetic CKD and 155 healthy control individuals participated in this hospital based study. K121Q polymorphism was determined by using PCR-RFLP. **Results:** The mean age of study groups were 57.70±9.8 and 55.2±10.6 for cases and controls respectively. It was observed that KK & KQ genotype frequency of ENPP1 gene is 60.5% and 36.4% respectively with Diabetic CKD patients in the present study. The allelic frequencies of Diabetic CKD group (K 78.7%, Q 21.3%) deviate from control group (K 84.19%, Q 15.81%).Genotype KQ was more frequent in patients than controls, shows that Q allele may be associated in Diabetic CKD patient and may have more risk for population of eastern Uttar Pradesh. Beside this, KQ genotype has significantly higher risk of GFR decrement. No association found between Diabetic CKDand KK genotype [P=0.950, OR=1.082, 95%, CI =0.090–13.008]. **Conclusion:** This finding suggests that K121Q polymorphism of ENPP1 gene is associated with Diabetic CKD disease in eastern Uttar Pradesh population but not up to the statistically significance. (DOI:10.9777/rr.2018.1286)

Myelodysplastic syndrome related hematopathology and aberrant JAK-STAT signalling axis

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Hematological disorders like myelodysplastic syndrome(MDS) arises due to clonally dysregulated hematopoiesis forming dysplastic cells which lacks proper maturity and immune functional capacity. The reason behind this dysregulation can be abridged to the abnormalities in various signalling pathways controlling proliferation, differentiation, maturation and apoptosis of bone marrow cells. In the present

study we have focussed in the JAK-STAT signalling, which is one of the very important pathways, playing crucial role in haematopoiesis and studies related to myelodysplastic syndrome (MDS) has not been totally explored.MDS was mimicked in a mouse model by administration of a potent carcinogen N-ethyl- N-nitrosourea (ENU). The control and MDS groups of animals were subjected to a variety of tests, including cell morphology study in peripheral blood and bone marrow, cytochemistry and histochemistry of bone marrow smears, karyotyping and flowcytometric expression analysis of the phosphorylated forms of proteins like JAK1, STAT3 and STAT5 (denoted as pJAK1, pSTAT3 and pSTAT5) and the phenotypic expression of proteins like CD45 and CD71. The immune functional capacity of bone marrow derived cells was assessed by the following Cell Mediated Immunity (CMI) parameters like MTT, NBT as well as the innate immunity study. Focussing on the morphology of the blood and bone marrow cells, dysplastic nature was evidently discernible in diseased groups when compared to control. The expression of Common leucocyte antigen CD45 was less in comparison to the expression of transferrin receptor CD71 which was increased in MDS mouse model. JAK1 showed an upregulated expression followed by STAT5. Therefore, it can be concluded that downregulation of CD45 may have helped in the upregulation of JAK- STAT signaling and CD71 expression. This aberrant signaling may be among one of the activated signaling axes that lead to affected hematopoietic lineages in preleukaemic condition i.e. myelodysplastic syndrome. (DOI:10.9777/rr.2018.1287)

Biochemistry of Staphylococcus biofilms and development of antibiofilm strategies

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Background: Staphylococcus biofilms, particularly methicillin-resistant one (MRSA) are the major reason behind persistent hospital acquired infections. These biofilm residing cells are less susceptible to antimicrobial treatments, by both active and passive mechanism. MRSA infections are surface protein dominant, whereas other S. aureus infection are shown to be rich in polysaccharide content. **Aim:** Aim of the study was to establish how the understanding of the biochemistry of the biofilm matrix could be useful in developing an antibiofilm treatment regime using different S. aureus strains. **Methods:** 96 well plate crystal violet biofilm assay, biochemical characterization of EPS, Confocal Laser Scanning microscopy, bioinformatics. **Results:** Biochemical characterization and treatment of proteinase K along with the antibiotics on bap (biofilm-associated-protein) -positive and bap-negative S. aureusstrains showed that such treatment regime was successful, wherein the biofilm was dominated by the presence of proteins. A detailed in silico characterization of Bap proteins and other homologous proteins revealed the presence of a heptapeptide in hydrophobic core of protein and indicated to be responsible for the protein-protein interactions. The synthetic hepta-peptide was tested if the masking effect on surface proteins could inhibit the S. aureus biofilm development and act as an 'antibiofilm-peptide'. Results clearly showed that the heptapeptide was able to inhibit early adhesion as well as biofilm development in the S. aureus biofilms. **Discussion:** It was demonstrated that how Ca^{2+}can inhibit the biofilm development in Bap-dependent S. aureus biofilms.

Of late, we showed that a surface protein playsa very critical role, especially in the MRSAand can be a potential target to treat biofilm mediated infections. **Conclusion:** This study shows that how fundamental understanding of biochemistry of biofilm matrix may assist in developing various methods to deal with biofilm-mediated infections and sensitization of S. aureus biofilms for antibiotic treatment, where Bap-like surface proteins play a major role. (DOI:10.9777/rr.2018.1288)

Development and evaluation of bio-flexy films using a novel biopolymer from Ananas cosmosus loaded with nanosized topiramate

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Background: Topiramate, anticonvulsant drug possesses t1/2:7-9 hours (low); protein binding: 96%; water solubility: 22mg/L enhances acts as selective GABA reuptake inhibitor. Side effects include abdominal pain, pharyngitis, suicidal thoughts and sudden unexpected death. **Aim:** The aim of Research work was to formulate nanosized bio-flexy films using a novelbiopolymer isolated from Ananas cosmosus fruit pulp containing Topiramate as a model drug. The soft palate drug delivery helps bypass first-pass metabolism in the liver and pre-systemic elimination in the gastrointestinal tract gets avoided Ananas cosmosus biopolymer was used to prepare bio-flexy films because of its biodegradability, biocompatibility, non-toxic, non-irritant in nature and non-reactive on soft palatal surface. Physicochemical characterization of biopolymer displayed inbuilt properties of filmability, mucoadhesivity. **Methods:** Bio-flexy films were prepared by solvent casting technique. Drug to polymer ratio was chosen at five levels for Ananas cosmosus FOO1-FOO5 containing varying ratios of biopolymer from 1%-10% and 1% of nanosized Topiramate and compared with Sodium carboxyl methyl cellulose standard films. Bio-flexy films were evaluated for thickness, surface pH, weight uniformity, folding endurance, in-vitro release and stability studies. **Results:** The percentage yield of Ananas cosmosus biopolymer was found to be 0.972±0.008%. Thickness of formulated bio-flexy films was ranging from 0.030 mm±0.005 to 0.042 mm±0.003, Folding Endurance: 100-160, Surface pH: 7.01±0.02 to 7.01±0.01, Weight Uniformity: 0.078±0.05 to 0.083±0.04, Drug Content Uniformity: 68.9%±0.55 to 72.94%±0.52 (drug was uniformly dispersed in bio-flexy films), Swelling Percentage: 62%±0.4 to 74%±0.2 (drug released through bio-flexy films by swelling followed by erosion), Tensile Strength: 78.58 gm. ±1.2 to 81.22 gm±1.0 (bio-flexy films can withstand any undue pressure or strain), Percentage Moisture Uptake (PTU): 2.0%±0.11 to 2.4%±0.10, **Discussion:** The biopolymers showed excellent filmability, mucoadhesive and mucoretentiveproperties. The functional groups present in the bio-polymer were comparable to the groups present in the mucoadhesive polymers. Drug to polymer ratio was chosen at five levels for Ananas cosmosus; FOO1 (1:1), FOO2 (1:3), FOO3 (1:5), FOO4 (1:6), FOO5 (1:10), and five levels for Sodium carboxyl methyl cellulose FSO1 (1:1), FSO2 (1:3), FSO3 (1:5), mFSO4 (1:6), FSO5 (1:10). Formulations possessed adequate thickness, strength, durability, in range of physiological pH, in-vitro release thus suitable for soft palatal formulation. Formulations FOO1 (containing Topiramate: Ananas cosmosus biopolymer (1:5)) was found to be best formulation. **Conclusion:** Based on all above mentioned evaluation parameters, Formulation FOO1 (containing Topiramate: Ananas cosmosus

biopolymer (1:5)) was selected as Best Film as in-vitro release study results revealed prolonged duration of period, R2=0.9557, Peppas korsmeyer as best fit model, follows Fickian Diffusion (Higuchi Matrix) release mechanism using BITS Software 1.12.Stability study revealed stable bio-flexy films with no significant change in physical appearance and stable pH. Prepared formulations of Topiramate loaded bio-flexy films are suitable for soft palatal delivery. (DOI:10.9777/rr.2018.1289)

Inflammatory molecular tinkering responsible for melanoma cell metastasis- an *in vivo* study

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Background: Metastasis, the release, journey and colonization of cancer cell from a primary site to far away require epithelial-mesenchymal transition (EMT) that allows cancer cells to dedifferentiate, earns enhanced migratory and invasive capabilities. Inflammatory cytokines like TGF-, which are produced and released form tumor-infiltrating immune cells, are potent inducers of EMT.This inturn indicates a possible link between inflammation and invasion to metastasis. **Aim:** Present study was conducted to enlighten molecular signaling associated with TGFβ signaling mediated EMT in cancer cells during malignancy. Further analysis was performed to evaluate underlying molecular pattern of interaction present behind inflammatory cytokine mediated modulation in tumor cells which make them invasive in nature. **Methods:** B16/F10, a perpetual mice melanoma, were subcutaneously injected to 4 weeks aged (10-12gm body weight) swiss albino mice at right thigh region. Exposed specimens are sacrificed after four weeks of exposure. Primary and secondary colonized tumours were collected, identified and assayed for histological and immunological parametrics to determine activity of LIMK2 and its association with filament stabilization. NWASP-Arp2/3-NEDD complex formation and cofilin interaction to filament stabilization were estimated through co-immunoprecipitation. FACS analysis carried out with MCR1 protein for determination of secondary colony formation. **Results:** Presence of MCR1 protein, a potent marker for B16/F10 cell line, in secondary colony confirmed establishment of metastatic tumor. TGF1- TGFR1 mediated P190RhoGAP dependent co-localization of FAK-Src activated ROCK-LIMK2 associated interaction of cofilin with NWASP-Arp2/3-NEDD complex and modulated isotypic switching of cadherin at cell surface. **Discussion:** Correlation and interaction of inflammatory cytokine with metastasis and following secondary tumor formation were perceived. Therefore inflammatory intermediates may be considered as marker to assess the progression of metastasis in in vivo. Conclusions: Mechanistic interaction in P190RhoGAP-FAK-Src axis played a pivotal role in inflammatory cytokine guided metastatic progression and may be characterized in future therapeutics against metastatic progression in cancer biology. (DOI:10.9777/rr.2018.1290)

Heat treatment and soluble proteinaceous factors may influence swarms and morphology of Gold nano and microparticles produced using Rhizobium sp. cultures from root nodules of Mimosa pudica in a simple, novel slide based system

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Background: There is a lot of interest in autonomous movement and collective behavior of synthetic nanomaterials which have important applications in nanomedicine, nanobiotechnology and nanosensors. Present work using viable cultures and cell free extracts of Rhizobium sp. to produce and analyze swarms of monodisperse Au Nanoparticles (Au NPs) and polydisperse Au microparticles (Au MPs) was inspired by previously reported work on chemically triggered swarming of commercially available Au MPs. **Aim:** We aimed at developing a simple, glass slide based technique for rapid production, microscopic visualization, morphological analysis, study of swarming behavior and monitoring of effect of heat on Au NPs and Au MPs forms and assemblages using cell based and cell free microbial system such as Rhizobium sp. to test the hypothesis that unidentified proteinaceous factors could be involved in producing simple monodisperse and complex polydisperse geometric forms of Au NPs and Au MPs. **Methods:** We used wild type Rhizobium cell suspensions prepared from surface sterilized root nodules of Mimosa pudica. Pure Rhizobium strains were isolated using Congo Red Yeast extract mannitol agar medium. Cell free extracts from wild type and pure cultures were prepared by warming cell suspensions for 15, 30, and 45 sec durations and using membrane filters. Cultures and extracts were separately mixed with HAuCl4 in equal proportions in each treatment on slides and monitored microscopically. For studying effect of heat slides were warmed on a Spirit Lamp for 15, 30, 45 seconds. Photomicrography was done with digital microscope. **Results:** Mixed, dense swarms of monodisperse Au NPs were obtained with polydisperse Au MPs using wild type and pure cultures as well as cell free extracts. Heat treatment yielded interesting forms and complex assemblages exhibiting fractal properties. **Discussion:** Specific Rhizobium sp. cell bound heat responsive proteins may trigger monodisperse Au NPs swarms and simple and complex assemblages. Some of these assemblages such as open and closed rings, interlocked rings are unique. **Conclusion:** Using our simple slide based technique a lot of scope exists for applying a combination of Microbial cell free extracts and timed heat treatment to obtain defined monodisperse GNP swarms and study postulated heat responsive protein mediated genesis and architecture of biotechnologically useful forms of Au NPs and polydisperse Au MPs. (DOI:10.9777/rr.2018.1291)

Deregulation of Wnt signaling pathway in aplastic anemia mouse model

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Background: Hematopoiesis is the process by which all types of mature blood cells are produced in the adult bone marrow (BM) from hematopoietic stem/progenitor cells (HSPCs). Any catastrophe in the BM causes alteration of steady-state hematopoiesis leading to evolution of

hematopoietic disorders such as Aplastic anemia (AA). AA is a paradigm of acquired BM failure, which is manifested as peripheral blood (PB) pancytopenia, hypocellular BM and prevalence of adipocytes and impaired hematopoiesis. As a chronic effect of protracted pesticide toxicity, AA development is very common among Asian population, and is associated with defect in HSPCs. **Aim:** To investigate the role of Wnt signaling pathway, a crucial regulator of adult hematopoiesis, in the context of AA in a novel mouse model induced by chronic pesticide exposure. **Methods:** Inbred adult Swiss albino mice were exposed to an aqueous mixture of pesticides containing cypermethrin, chlorpyriphos and hexaconazole and development of AA was confirmed by PB and BM analysis. For Wnt signaling study we used flow cytometry i. to evaluate expression level of Wnt3a, Wnt5a, β-catenin, WIF1 (Wnt inhibitory factor), TERT (telomerase reverse transcriptase) and NFAT (nuclear factor of activated T-cells) and ii. to measure intracellular calcium level (Ca^{2+}) in AA BM. **Results:** Results showed significant down-regulation of canonical Wnt3a/β- catenin/TERT and non-canonical Wnt5a/Ca^{2+}/NFAT signaling pathway in AA BM, which was due to expressional decline in Wnt ligands such as Wnt3a and Wnt5a and simultaneous increase in Wnt antagonist WIF1 expression in BM post pesticide exposure. **Discussion:** Down-regulation of canonical Wnt3a pathway impaired proliferation and survival of BM cells and on the other hand, down-regulation of non-canonical Wnt5a pathway altered hemato-reconstitution ability of BM cells under stress condition. **Conclusion:** Deregulation of Wnt signaling pathway in BM plays a crucial role behind the pathogenesis of pesticide induced AA. (DOI:10.9777/rr.2018.1292)

Cucurbitacin B & withanone combination (CucWi-N) for cancer treatment

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Environmental insults including industrial pollution, heavy metals, smoke and radiations have been identified as significant factors contributing to carcinogenesis and its promotion to aggressive stages including metastasis. A wide range of synthetic molecules are in use as chemotherapeutic molecules. However, most if not all, have adverse effects. Hence, development of new preventive and therapeutic natural compounds has been prioritized. Cucurbitacin B and Withanone have been shown to possess promising anticancer potential in our earlier studies. Currently, we developed a combination of Cucurbitacin B and Withanone (CucWi-N), and analyzed its anticancer potential using non-small-cell lung cancer cells. In in silico study, Cucurbitacin B showed capacity to abrogate mortalin-p53 interaction (necessary for cancer cell proliferation). In combination with Withanone, it had synergistic affinity to engage hnRNP-K protein (necessary for cancer cell migration). In in vitro analysis, we found that the combination significantly and dose-dependently caused changes at cellular and protein level indicating (i)

induction of replicative senescence, (ii) sensitization of cancer cells to environmental stressors, and (iii) inhibition of stemness and aggressiveness of the cancer cells. Tumor assays in Balb/c nude mice with CucWi-N showed suppression of tumor growth and lung metastasis. We propose that CucWi-N is a potential natural anticancer drug that warrants further mechanistic and clinical studies. (DOI:10.9777/rr.2018.1293)

Exposure to electromagnetic radiation 2450 MHz induces cholinergic toxicity in experimental rats

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People in modern society are exposed to an ever increasing number of 3G smart phones and Wi-Fi irradiate electromagnetic radiation and some studies have demonstrated that these radiations can precipitate several neurobehavioural disorders. Duration and frequency of exposure of EMR is critical to alter acetylcholine. Therefore, the present study explores to different frequencies of EMR exposure on hippocampus cholinergic neurotransmitter as well as cognitive behavior in rats. Animals were divided into four groups (i) Control (ii) EMR-900 MHz (iii) EMR-1800MHz (iv) EMR-2450MHz. Experimental rats were exposed to EMR (900, 1800 and 2450 MHz) everyday for one hr for 28 consecutive days. The cognitive deficits in terms of novel arm entries in Y-maze paradigm was evaluated at D-1, 7, 14, 21, 28 respectively and neurotransmitter evaluated at the completion of EMR exposure. The results revealed that EMR-2450MHz exhibited significant decrease in novel arm entries. EMR-2450 MHz caused decrease in the level of acetylcholine and increased acetylcholinesterase activity in hippocampus. There was no any significant difference between EMR-900, 1800 and control. Therefore, exposure of EMR-2450 in rats caused significant cognitive deficit which can be linked to cholinergic toxicity. (DOI:10.9777/rr.2018.1294)

The study of protein complex in repression of the tumour causing *dMyc* in *Drosophila melanogaster* ovary cells through biochemical approach.

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The ovarian germline stem cells (GSCs) of Drosophila females are located at the anterior tip of germarium. The well known homolog of the human c-myc oncogenein Drosophila ovary germline, diminutive (dMyc) is self renewal factor and stimulate high metabolic rates, including increased protein synthesis and cell growth. dMyc is highly expressed in self renewing Germline stem cells but downregulated in further differentiating 16-cell cysts and cells cystoblasts (CB). The other factors as Bam and Brat are translational regulators that favours differentiation are expressed in differentiating cells CBs. Mei-P26 a TRIM-NHL tumor suppressor, Argonaute 1 microRNA binding protein and Bgcn is found to be expressed and equally distributed in self renewing GSCs as well differentiatng stem cells CBs. dMyc transcripts were tightly downregulated in the cystoblast differentiation up to the 16-cell cyst stage. The mechanism of the downregulated dMyc mRNAs from CBs to 16 cell cyst stage is not known. Previously, it was shown that Mei-P26, Bgcn and Ago1 contribute regulation of dMyc mRNA

through unknown mechanism. Here, we show that a multiple protein complex of Mei-P26 with differentiation factors Bam, Bgcn, Brat, and Ago 1 in differentiation undergoing cells represses a reporter bearing dMyc mRNA in Drosophila Schneider's cells. These findings highlight the importance of a multiple protein complex in cystoblast in repression of dMyc, a stem cell maintenance factor that causes tumorigenesis upon its overexpression. (DOI:10.9777/rr.2018.1295)

Need for genetic studies in couples with recurrent abortion

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Background: Recurrent abortion or recurrent pregnancy loss is defined as two or more consecutive pregnancy failures before 20th week of gestation and it is estimated to affect ~1% of couple trying to conceive. The incidence of recurrent abortion is 1 in 300 pregnancies worldwide. Although various factors such as genetic disorders, uterine malformations, endocrine factors, hematological, autoimmune diseases are associated, the exact cause of recurrent abortion remains unknown in about 50% of cases. **Aim:** To identify chromosomal abnormalities if any, in couples with recurrent abortion and subsequent genetic counseling. **Methods:** 27 couples with recurrent pregnancy loss as study subjects and 10 age-matched healthy control couples with atleast one child were enrolled in the study. Peripheral blood lymphocytes were collected, cultured and treated with colchicine to arrest growth at metaphase stage and karyotype was analyzed. The results of cytogenetic analysis were correlated with various demographic, clinical and lifestyle characters of couples. **Results:** Among 27 couples with recurrent abortion, 4 females and 2 males had chromosomal abnormalities of which 5 were autosomal and 1 sex chromosomal abnormality. Chromosomal changes were invariably seen in couples having more than 5 abortions. Study also showed a positive correlation between chromosomal changes and age of couples, history of infection and obesity. **Conclusion:** With the help of karyotypic studies, many chromosomal variations are identified in this pilot study warranting an elaborate chromosomal evaluation in all recurrent abortions. Moreover, awareness about the importance of genetic testing and counseling in couples can reduce the incidence of recurrent abortion and foetal morbidity and mortality. (DOI:10.9777/rr.2018.1296)

Silicon induced antioxidant defense responses in *Solanum lycopersicum* against leaf spot disease caused by *Septoria lycopersici*

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Background: Silicon has been recognized as an "agronomically essential element" all over the world. It is probably the only element which is able to enhance the resistance to multiple stresses. Hence, in this study Silicon was tested for its potential to induce resistance factors in tomato plant. **Aim:** To study potency of silicon in inducing antioxidant defense responses in Solanum lycopersicum Linn against leaf spot disease. **Methods:** The tomato leaves were pretreated with different concentrations (0.5, 1.0, 1.5 mM) of silicon and inoculated with Septoria lycopersici.

Experimental leaves were assayed for cell death, Catalase (CAT), Superoxide dismutase (SOD), Polyphenol oxidase (PPO), Ascorbate peroxidase (APX), Guaiacol peroxidase (GPX) activity and Proteomic changes by 2D PAGE. **Results:** Silicon at 1.0mM and 0.5mM concentration recorded increased GPX and CAT activity, whereas increased SOD activity was recorded in tomato leaves treated with 1.0mM silicon at 48 hrs. The 2D-PAGE recorded a total of 33 and 14 unique spots in control and silicon treated leaves respectively. Key proteins induced upon silicon treatment includes NBS-LRR, Serine/threonine protein kinase and β-amyrin oxidase. Cell death analysis revealed that silicon reduced cell death up to 60% and also disease symptoms. **Discussion:** Peroxidase (POX) activity of leaves of tomato treated with siliconpeaked at 72 hrs which could promote lignification and suberization. An increased activity of antioxidant defense enzymes such as CAT, SOD, PPO, APX and POX in tomato may contribute to survival during pathogen infection. Induction of 'R' gene products by silicon also evident in tomato leaves. The expression of NBS-LRR have been shown to induce by salicylic acid and wounding mediating defense signaling pathway. The presents study augments that induction of NBS-LRR and Protein kinase by Si treatment along with to elicitation of antioxidant defence in protects tomato from Septoria lycopersici infection. **Conclusion:** Silicon induces resistance in tomato by promoting antioxidant defense mechanism, activation of 'R' genes and protein kinases. (DOI:10.9777/rr.2018.1297)

Synthesis, Spectroscopic, X-ray crystal and *in vitro* cytotoxicity studies of some novel N, N'-disubstituted thiocarbamide derivatives

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The green synthesis of five N, N'-substituted thiocarbamides were performed by the reaction of pentoxycarbonyl chloroformate with naphthyl amine for H2L1, with 2-chloro-4-nitroaniline for H2L2, with 2-methoxy-4-nitroaniline for H2L3, with 3-nitroaniline for H2L4 and by the reaction of 2, 2, 2- trichloroethoxycarbonyl chloroformate with naphthyl amine for H2L5. These compounds were fully characterized by using various spectroscopic (FT-IR, 1H and 13C NMR) and single crystal X-ray studies of H2L1 and H2L5. The crystal structure of both the compounds is triclinic with space group P-1 and unit cell dimensions a = 7.139(5) Å, b =10.751(5) Å, c = 12.442(5) Å, α =109.738(5) ° β = 98.872(5) º, γ = 106.335(5) º Z = 2 for H2L1 and a = 8.115(5) Å, b = 8.899(5) Å, c = 12.238(5) Å, α = 75.345(5)º β = 87.734(5)º γ = 64.477(5)º , Z = 2 for H2L5 , respectively. An intramolecular hydrogen bonding interaction observed between N1-H and carbonyl human carcinoma cell lines 2008, C13*(cervical carcinoma), A2780, A2780/CP and IGROV- 1(ovarian carcinoma).The IC50 values of compounds H2L2 — H2L4 demonstrated them to be very promising anticancer agents. (DOI:10.9777/rr.2018.1298)

Adaptations to hypoxic-stress in stimulated platelets within thrombus microenvironment

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Background: Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor. The oxygen-sensitive αsubunit is stabilised in the presence hypoxia, hypoxia-mimetics and certain non-hypoxic stimuli, while the beta-subunit is constitutive. Low oxygen pathophysiological conditions (high altitude, air travel) are suggested to be pro-thrombotic states. However, the mechanistic link between hypoxic stress and platelet hyperactivity or thrombus formation remains to be established. **Aim:** Here we studied platelet response to hypoxic stress and adaptation of activated platelets to potential oxygen-deprivation within cell aggregates or thrombus niche. **Methods:** Platelets were isolated by differential centrifugation from fresh human blood. Total RNA extraction, reverse transcription and quantitative PCR were carried out for mRNA expression. Hypoxia simulation was induced using hypoxia chamber. Protein expression analysis was carried out by immunoblotting. Platelet-derived extracellular vesicles (PEVs) were quantitated by nanoparticle tracking analysis (NTA). Intracellular calcium was measured by fluorescence spectrophotometry using Fura-2 AM. **Results:** Here we have demonstrated that enucleate platelets express HIFα mRNA transcripts. HIFα protein levels increased following exposure to hypoxia. Inhibition of protein translation markedly attenuated hypoxia-induced HIFα expression, while inhibition of proteasome degradation markedly increased HIFα accumulation. Hypoxic-stress induced HIF stabilization was associated with shedding of PEVs and rise in intracellular calcium in human platelets. **Discussion:** Our findings suggest that pre-formed HIFα mRNA in enucleate platelets allows HIFα protein synthesis and stabilization in response to hypoxia. Accumulation of HIFα in hypoxic platelets possibly leads to PEVs shedding and increased calcium signaling which might underlie platelet hyperactivity in oxygen-compromised states. Conclusions: Elucidation of HIFα regulation in hypoxic platelets will shed light on platelet physiology at high altitude and points to a novel target for potential antithrombotic therapy. (DOI:10.9777/rr.2018.1299)

Role of thioredoxin and glutathione reductase redox system in bovine filarial parasite, *Setaria cervi*

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Background: The glutathione and thioredoxin are the major thiol dependent redox system playing crucial role in the maintenance of cellular redox homeostasis. But their importance in filarial parasites is not well explained. **Aim:** To elucidate the role of thioredoxin and glutathione reductase redox system in bovine filarial parasite, Setaria cervi. Methodology: In the present study, adult female Setaria cervi, bovine filarial parasites were incubated with DHBA and auranofin, the respective inhibitors of GR and TrxR at different concentrations for 8 h. The effect of inhibitors was observed on the motility and viability of parasite as well as on the various oxidative stress and apoptotic markers. Results and **Discussion:** The auranofin at 10 μM concentration significantly decreased the motility and viability and affected the survival of the filarial parasites leading to their death within 8h. However, at similar concentration DHBA showed no significant effect on the motility and viability of parasite. Auranofin suppressed the parasite's antioxidant system leading to increase in level of ROS. Further, the increase in the level of Ca^{2+} binding proteins in cytosol and PDI activity in

ER suggested that auranofin induced Ca^{2+} mediated oxidative stress leading to apoptosis in filarial parasites. The proteome analysis of auranofin treated parasitesshowed the marked alteration in the protein expression with 32 downregulated and 23 upregulated protein spots in comparison to the control. Through MALDI-MS analysis some of these altered spots were identified as the calcium binding proteins and the glycolytic enzymes. **Conclusion:** Overall this study suggests that TrxR is the major enzyme for maintaining the cellular redox homeostasis and survival parasites and could be used as potential drug target. (DOI:10.9777/rr.2018.1300)

In silico studies for potential natural inhibitors for isocitrate dehydrogenase type II of *Mycobacterium tuberculosis* (H37Rv)

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Background: Tuberculosis is a life threatening bacterial disease caused by Mycobacterium tuberculosis which has affected the population of almost all part of the world since time immemorial. Although remarkable discovery has been achieved in combating disease control and its propagation but due to emergence of resistant strain of Mycobacterium tuberculosis exhaustive search for the panacea to counter the bacteria is still on. **Aim:** In this study we had proposed a molecular docking studies of 35 natural compounds against proposed drug target Isocitrate Dehydrogenase type II(PDBID: 5kvu) of Mycobacterium tuberculosis (H37Rv). These compounds are well known for their many therapeutic effects and health benefits. Isocitrate Dehydrogenase is a crucial enzyme for Tricarboxylic Acid Cycle which in turn is an essential metabolic pathway for the persistence and survival of the Mycobacterium in active phase. Isocitrate Dehydrogenase type II got no similarity with Human Isocitrate Dehydrogenase thus making it a suitable candidate as a drug target. Material and **Methods:** PubChem database was used to check the efficacy of these different natural compounds. Docking studies were performed with the help of Autodock 4.0. NADP+ was used as a reference ligand to compare the docking results with natural compounds. Molecular dynamics study of the docked complex has been also performed to infer the deep insight of the various interactions and stability of the docked complex. **Results:** The docked complex consists of target receptor protein and most potent inhibitor ligand amentoflavone; which has scored height minimum binding energy among all natural compounds. **Discussion:** Natural compounds showed good binding energies with the receptor protein. **Conclusion:** Docking and Molecular dynamics study showed that Amentoflavone, a multibeneficial compound got the highest minimum binding energy and stability in molecular docking and molecular dynamics simulation studies respectively, and thus may act as a strong antitubercular compound. (DOI:10.9777/rr.2018.130

Correlative study of serum Hepcidin level and serum iron reserve parameters in preeclampsia and HELLPsyndrome

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Background: Pregnancy may lead to various complications such as gestational diabetes, proteinuria, hypertension, preeclampsia, eclampsia etc. Hypertensive disorders during pregnancy are a major cause of morbidity and mortality. In the

developing world, it leads to 10-15% maternal deaths (Mehta et al, 2015). Among all hypertensive disorders of pregnancy HELLP syndrome bears the worst prognosis with maternofetal complications 7-70% and maternal mortality rate 1-24% (Pokharel et al, 2008). Hepcidin was

first isolated from human urine and named on the basis of its site of synthesis (hep-) and its in-vitro antibacterial properties (-cidin). **Aim:** Correlative study of serum Hepcidin levels and serum Iron reserve parameters in preeclampsia and HELLP Syndrome **Methods:** In our study we have taken patients of both HELLP syndromes taking consideration that HELLP syndrome - in some cases a severe form of preeclampsia but in rest cases it is diagnosed without previous features of preeclampsia. A total forty patients of preeclampsia and HELLP syndrome are taken in our study with equal number of age matched healthy controls from Sir Sunderlal Hospital, BHU, Varanasi after ethical clearance from ethical committee of Institute of Medical Sciences, BHU. 5 ml of blood was taken for hepcidin estimation after proper consent from patients and controls. Serum was used for the estimation of hepcidin levels. The method of estimation was competitive sandwich ELISA. **Results:** After that we analyzed the data using statistics and found the mean and standard deviation for cases as 5.773 and 3.442 & for controls 3.560 and 2.46. Statistical analysis showed that the means are not from the same group with p value 0.00717. **Conclusion:** From the data we concluded that there is significant increase in hepcidin levels among HELLP syndrome and preeclampsia patients in comparison to controls and decrease in serum transferrin saturation levels. **Discussion:** Iron balance is necessary for normal physiology; however, iron disorder is associated with many types of diseases including hereditary hemochromatosis (HH), β-thalassemia, anemia of inflammation, and iron- refractory iron deficiency anemia (IRIDA). Previous studies by various authors revealed that iron disorder is due to the dysregulation on hepcidin–ferroportin (FPN) axis. Thus, correcting hepcidin–FPN axis would be potential therapeutic strategy for iron disorders. (DOI:10.9777/rr.2018.1302)

Licarin A, a lignan from *Myristica fragrans* induces G1 arrest and autophagy in non-small cell lung cancer cell line A549

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Lung cancer is one of the leading cancers worldwide with relatively poor prognosis. There is an increasing concern over the resistance developed by cancer cells to chemotherapy which is found to be related to some extent with defective apoptosis. This necessitates investigation of new drugs that targets other cell death mechanisms like autophagy thereby improving current therapeutic strategies. Licarin A (LCA), a lignan has been reported for its anti-inflammatory, antiparasitic, anti-mycobacterial and anticancer properties. In this study, LCA purified from Myristica fragrans seeds was used to investigate anti-proliferative effect and its ability to induce autophagy in A549. Data from MTT assay indicated that LCA inhibited proliferation of A549 cells in a dose and time dependent manner with

an IC50 of 26.85 ± 2.769μM at 24h. Cell cycle analysis by flow cytometry showed that LCA induced G1 arrest. In addition, increased p53 and decreased cyclin D1 protein levels were observed. LCA treated cells showed increased autophagic vacuoles when stained with acridine orange and monodansylcadaverine dye. Also, increased mRNA and protein expression of Beclin 1, LC3II were observed along with degradation of p62. These results confirmed activation of autophagy by LCA. To understand the role of autophagy in the cytotoxic activity of LCA, cells were pre-treated with chloroquine, a late autophagy inhibitor. Chloroquine pre- treatment caused a reduction in the cytotoxic effect and downregulated degradation of p62 indicating LCA induced autophagy played a pro-death role in A549 cells. Our findings provided evidence for LCA induced autophagic cell death in A549 cells and mayfind its future application in lung cancer therapy. (DOI:10.9777/rr.2018.1303)

A novel method to establish an *in vitro* model system from early chick embryo to study muscular dystrophy

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Background: Muscular dystrophy (MD) is an X-linked recessive disorder affecting most exclusively the males. Wherein, Duchene muscular dystrophy (DMD) is the most commonly occurring sub type of MD. Proteins like Dystrophin, sarcoglycan, laminin, etc. form a sarcoplasmic complex that is involved in development and maintenance of muscles. Any genetic alteration in proteins of this assembly causes MD condition. Cure of DMD is still not available. Current drugs address only symptoms. To develop an effective drug and to understand complete biology of the disease, efforts are made to develop in vitro models out of mice and human skeletal muscle cells. **Aim:** To develop an in vitro model for DMD studies from avian embryonic stem cells. **Methods:** The chick embryos were isolated with the help of filter ring method from the freshly laid eggs. Subsequently the pluripotent cells of the embryos were isolated from the embryos and plated without any enzymatic degradation. These were then further cultured to establish into muscle cell lines using serum and factors like FGF-2. These muscle cells were then targeted by disrupting their cytoskeletal protein by antibody anti-dystroglycan. Morphometric studies and RNA expression of these targeted muscle cells was analysed. **Results:** The morphological features of the targeted muscle cells showed resemblance to dystrophic cells. There were evident variations in the m- RNA expression. The culture also featured atropy of these cells. Conclusions: The dystrophy like cells developed in-vitro through antibody blockade of α-dystroglycan showing features of muscle dystrophy are easy and economical to obtain and can serve as a model to study certain initial aspects of the disease Muscle Dystropy and also can be used to study the effects of various pharmaceutical compounds. (DOI:10.9777/rr.2018.1304)

Fungal L-Asparaginase: production, characterization and its application in acrylamide mitigation.

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Background: Microbial L-Asparaginase (LA) is an enzyme that catalyzes hydrolysis of L-asparagine to aspartic acid and ammonia. Free asparagine has been found to be the main source of acrylamide in fried starch based foods. The Swedish studies conductedin 2002 revealed the occurrence of acrylamide in starch-based foods that processed at a temperature of 120 °C or above, leading to the introduction of L- Asparaginase into the food technology. Acrylamide thus formed is considered as a potential cause of cancer. It is considered to be a neurotoxin (causing peripheral neuropathy) after occupational exposure in humans. Therefore, for reducing the acrylamide levels in fried foods, one of the alternatives could be the use of L-Asparaginase as this enzyme hydrolyzes free asparagine into a non-precursor. **Aim:** The aim of this study is to investigate the acrylamide mitigation potential of L-Asparaginase produced from Aspergillus terreus sp. BV – C strain using Solid state fermentation. **Methods:** The following methods were used: Solid State Fermentation (SSF) for enzyme production, Ion-Exchange and Size Exclusion Chromatography for purification of the extracellular enzyme, Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis for determination of molecular weight of purified protein and High Performance Liquid Chromatography for detection of reduction in acrylamide levels in fried potato products. **Results:** The efficacy of the enzyme combined with physical treatment methods towards acrylamide content was found to be 93% as compared to untreated potato samples. **Discussion:** Pretreatments such as blanching, mono- and di-valent salt solutions combined with L-Asparaginase can reduce the acrylamide content drastically to 93% as compared to the commercial formulations which can reduce the acrylamide levels to 50%. **Conclusion:** L-Asparaginase from Aspergillus terreus sp. BV – C strain opens a new frame of reference to the commercial use of L-Asparaginase for acrylamide mitigation in fried food items. (DOI:10.9777/rr.2018.1305)

Antibacterial and antioxidant activity of an endophytic fungus, EHL2 isolated from *Euphorbia hirta*

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Background: The endophytic fungi are relatively less investigated group of microbes producing plethora of compounds having the biomedical and agricultural importance. Residing inside their host, they are known to strengthen the defense of host plant by producing secondary metabolites active against all kinds of microbial pathogens and additionally, these metabolites have great importance for humans and plants. **Aim:** Isolation and characterization of endophytic fungi from Euphorbia hirta for exploring their antibacterial and antioxidant activities. **Methods:** Isolation and identification of endophytic fungi were done through available microscopic techniquesand screening of their antibacterial and antioxidant activities were performed using thedisc diffusion and DPPH assay, respectively. **Results:** A total of 18 endophytic fungal isolates representing 14 different morphotypes were recovered from Euphorbia hirta. Out of all morphotypes, 42.85% were active against more than two or more bacterial human pathogens with inhibition zones between 6.0-19.0 mm, while only 7.14% showed the positive antioxidant activity. The culture EHL2

displayed the potential against broad range of bacterial pathogens showing impressive activity against Enterococcus faecalis (19mm zone of inhibition), Staphylococcus aureus (14mm) and Citrobacter frundii (11mm), respectively. **Discussion:** The endophyte EHL2 was effective against both gram positive and gram negative bacteria however, a higher grade of activity was noticed against gram positive bacteria as compared to gram negative ones. **Conclusion:** The preliminary results of this experiment trigger the interest in antimicrobial potential of the crude metabolite of culture EHL2 which will further be purified and characterized. (DOI:10.9777/rr.2018.1306)

Hentriacontane suppresses LPS–induced inflammation in RAW 264.7 cells and mice model

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Aim: Inflammation is the normal protective response of living tissue to injury being caused by physical stimuli, chemical stimuli or microbial toxins. However, prolonged inflammation leads to the pathogenesis of many diseases, such as arthritis, asthma, multiple sclerosis and many more. Inflammation is normally controlled and selflimited. The various drugs for inflammation, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs have serious side-effects. Hence an alternative to these drugs is natural drugs. Hentriacontane, a natural compound has been demonstrated to have various pharmacological effects including antitumor activity and antimicrobial activity. The aim of the present study is to depict the anti-inflammatory potential of hentriacontane both in-vivo and in-vitro. Material and **Methods:** Alterations in both pro-inflammatory (TNF-α, IL-6, MCP-1 and IL-1β) and anti- inflammatory (IL-10) by hentriacontane was studied both in RAW264.7 cells and in mice. Suppressive potential of hentriacontane on NO, PGE2, and LTB4 on LPS induced translocation of NF-κB in RAW264.7 cells was studied. Further investigations on effect of hentriacontane on phagocytic index, carrageenan induced paw oedema in mice and on organ weight were done. **Results:** Hentriacontane, at all the concentrations, 10μM, 5 μM and 1μM in-vitro and 5mg/kg, 2mg/kg and 1mg/kg in-vivo significantly reduced all the parameters of inflammation in the experiments under study. The highest concentration used in the two models presented the most significant results. Conclusions: The results indicate that hentriacontane is a potent suppressor of inflammatory cytokines and other mediators. Moreover it also has regulatory effect on NF-κB. Hence, Hentriacontane is a potential candidate for investigations to develop anti-inflammatory drug. (DOI:10.9777/rr.2018.1307).

Green synthesis of silver nanoparticles using cell extract of the cyanobacteriumFischerella sp. strain HKAR-13

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Background: Metallic nanoparticles have drawn a lot of attention due to their unique physical and chemical properties, which largely differ from their bulk properties. The modification of properties was observed due to size effects, modifying the catalytic, electronic, and optical properties of the nanoparticles. In the last few years, green synthesis based on bacteria, fungi, cyanobacteria, and plant extracts are extensively investigated due to their eco-friendly protocol and better morphological control. **Aim:** Green synthesis of silver nanoparticles by using cyanobacterial cell extract and its applications. **Methods:** The cyanobacterial biomass (8 g; wet weight) was taken and boiled for 30 min at 60 °C in the double distilled water (100 mL). This extract was filtered through Whatman filter paper No. 1 and silver nitrate was added to attain a final concentration of 1 mM and then kept under bright sunlight. **Results:** After addition of silver nitrate solution (1-5 mM) the original color of the cell extract changed from reddish blue to dark brown within 1 h, suggesting the synthesis of Ag-CNPs. Spectroscopic analysis of the reaction mixture showed a prominent peak at 430 nm. Analytical techniques such as X-ray diffraction, Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM) were used to elucidate the formation and characterization of Ag-CNPs. **Discussion:** The role of biomolecules such as proteins, enzymes, amino acids, carbohydrates, and vitamins present in the cell extract has been implicated in the reduction of Ag+ ions. Ag-CNPs have wide range of applications in areas such as health care, cosmetics, environmental health, biomedical sciences, chemical industries, space industries, drug-gene delivery and also show antibacterial, anticancerous and antifungal activities. Conclusions: Synthesized silver nanoparticles showed reduction of Ag(I) ions to Ag(0) which suggests that the cyanobacterial extract is acting as reducing agent.Various potential therapeuticuse of these nanoparticles were also studied. (DOI:10.9777/rr.2018.1308)

The cation-ions mediated modulation in the catalytic efficiency and structural stability of *Staphylococcus aureus* moonlighting protein enolase

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Background: The emergence of numerous antibiotic-resistant varieties of S. aureus has necessitated the discovery of effective key elements in S. Aureus, which can serve as drug targets. The strategies to collapse the invasive behavior of S.aureus can help in the identification of potential candidates for molecular drugs. **Aim:** To investigate the impact of cations on the structure and catalytic efficiency of S. aureus enolase. **Methods:** The enolase gene of S. aureus has been cloned, overexpressed, and purified as a hexa-histidine- tagged variant. Initially, the Enolase was kinetically characterized and the effect of monovalent and divalent cations in enolase catalysis and structure were studied. In line with this, the impact of pH on activation of enolase by ions were studied. The neurotoxin and anticaries mediated inhibition of enolase and role of selected cations in enolase inhibition were also investigated.

Results and Discussion: Enolase was capable of reversible conversion of Phosphoenolpyruvate (PEP) to 2-phospho-D-glycerate (2-PGA) with the K for PEP was found to be $0.231065\pm0.01393x10^{-3}$M and V was $0.6492\pm0.00065x10^{-4}Mmin^{-1}$. Enolase activity was inhibited by NaCl, even at low concentrations. But KCl, on the other hand, had a stimulatory effect upon the catalytic activity of enolase. The enzyme was strongly activated by Mg^{2+} and Zn^{2+}. The Mn^{2+} and Cu^{2+} have a slight activating effect upon the catalytic activity of enolase while Ni^{2+} and Ca^{2+} are very weak activators. A total loss of catalytic activity was observed in presence of Hg^{2+}. The pH 7.5 found to be optimum for activation of enolase by ions. The pH above or below the optima was found to be ineffective. Neurotoxins like acrylamide reversibly and 2,5-hexanedione irreversibly inhibited enolase. The divalent cations found to be effective in protecting enolase from inhibition only in reversible reaction.Mg^{2+}found to be playing a major role in structural stability and reduction of inhibitory impact. **Conclusion:** Mg^{2+} being natural cofactor binds both conformational and catalytic site of enolase considerably with more affinity in comparison with Zn^{2+} or Mn^{2+}.So, Mg^{2+} resulted in the formation of a more stable closed form of the enzyme which favors the substratebinding. Which induce the event of cooperative binding of catalytic cations to enolase. This event eventually leads to having a considerable amount of reaction. (DOI:10.9777/rr.2018.1309)

Essentiality of *Leishmania donovani* glutamine synthetase as determined by gene overexpression and knockout approaches

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Leishmaniasis is a group of vector-borne diseases, caused by more than 20 species of the protozoan belonging to genus Leishmania. Chemotherapy for visceral leishmaniasis is problematic as the available drugs are toxic, costly and shows drug resistance. Hence, there is a necessity to look out for the novel drug targets based on the knowledge of molecular mechanisms, employed by the parasite for its survival. Focusing on the problem, a novel target glutamine synthetase (GS) was identified from Leishmania donovani. GS catalyzes the synthesis of glutamine from glutamate and ammonia with hydrolysis of ATP. The product of this reaction glutamine, takes part in a wide variety of metabolic and biochemical processes that supports growth and proliferation of cells. GS exists as a single copy gene in parasite genome and the enzyme exists in both promastigote and amastigote form of the parasite. To study the functional role of glutamine synthetase in Leishmania donovani, gene overexpression and knockout strategies were employed. LdGS overexpressing parasites were generated by episomal expression of the enzyme. GS overexpression was confirmed by enzyme activity and western blot. Approximately two-fold increase in enzyme activity compared to wild-type was observed and densitometric analysis of western blot showed~1.9 fold increase in expression of protein. Functional studies revealed the role of this enzyme in growth and infection of the parasite. Single knockout (SKO) cell lines of LdGS were generated by targeted gene disruption. Confirmational study of gene disruption was done by polymerase chain reaction, enzyme activity, and western blot. Almost 35 percent decrease in

expression of protein and enzyme activity compared to wild-type was observed in SKO parasite. Attempts are being made to generate complementation cell lines and double knockout parasites for validation of GS as drug target. (DOI:10.9777/rr.2018.1310)

Identification of lymphatic filarial Bio-markers

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Lymphatic filariasis (LF) is a common vector borne disease caused by nematodes Wuchereria bancrofti, Brugia timori and Brugia malayi. LF affects over 856 million people in 52 countries worldwide. In the year 2000, World Health Organization has launched the Global Program to Eliminate Lymphatic Filariasis (GPELF), by the year 2020. However the major bottleneck in combating LF is the low efficiency and limited sensitivity of the available diagnostic tests. Still, the most popular diagnostic test in India is "night blood

film examination" which suffers from a major limitation that blood samples have to be collected during odd hours at night. Therefore a better option would be to develop a diagnostic method which could be performed during the day and could define patients at the risk of developing disease symptoms. In our study, we are trying to identify marker protein/s from infected serum samples by using gel electrophoresis. In proteomic studies, it was seen that serum protein is significantly higher in chronic LF patients as compared to normal subjects. Further, denaturing gel electrophoresis of infected patients' sera revealed novel protein bands of approximately 210 and 205 KDa which were not seen in normal population. The presence of these protein bands in infected serum could probably serve as a hallmark of Lf and may help in determining disease condition and its acuteness. (DOI:10.9777/rr.2018.1311)

Curcuminoids suppress NF-kB activation pathway in glioma and breast cancer cells

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Background: Curcuminoids are the yellow coloring phenolic compounds isolated from the golden spice turmeric. Although these agents have demonstrated potential against some cancer-types, it's potential against glioma and the underlying mechanism remains poorly understood. Aim: We examined the activities of curcuminoids in C6 glioma and breast cancer cells. We also examined if curcuminoids inhibit pro-inflammatory NF-κB activation pathway in glioma cells. Methods: The breast cancer cells (MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-453) and C6-glioma cell lines were used during the study. Curcuminoids containing curcumin, demethoxycurcumin and bisdemethoxycurcumin were obtained from Arjuna Natural Extracts Ltd Alwaye, Kerala. The other parameters used were: MTT and clonogenic assays for the cytotoxicity; AO/PI and DAPI staining for apoptosis; immunofluorescence assay for NF-κB activation; and wound healing assay for cell migration. Results and Discussion: A dose- and time-dependent decrease in the viability and proliferation of glioma cells and breast cancer cells was observed after the treatment of cells with curcuminoids. At concentrations as low as 25 μM, curcuminoids

inhibited the proliferation of cancer cells after 24 hours of treatment. The long-term colony formation by cancer cells that mimics in vivo situation was also significantly inhibited by curcuminoids. AO/PI staining suggested that the curcuminoids induced apoptosis in cancer cells. Hydrogen peroxide induced translocation of NF-κB-p65 from cytoplasm to nucleus that was significantly reduced by the pretreatment with curcuminoid. Curcuminoid also induced ROS generation in cancer cells. As revealed by wound healing assay, the curcuminoids inhibited the migration of cells. **Conclusion:** Curcuminoids exhibit anti- cancer activities against glioma and breast cancer cells. The generation of ROS and inhibition of NF-κB activation may contribute to the anti-cancer activities of curcuminoids. Studies are underway to delineate the in-depth molecular mechanism by which curcuminoids inhibit NF-κB activation and exhibit anti- cancer activities. (DOI:10.9777/rr.2018.1312)

Biosorption of heavy metals by *Pleurotus* species

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Background: Heavy metals concentration to the environment has been increasing continuously as a result of industrial activities and technological advances, posing a significant threat to the environment and public health because of their toxicity, accumulation in food chain and persistence in nature. The ability of the basidiomycetes to absorb and accumulate metals together with excellent mechanical properties of fungal mycelia provides an opportunity to utilize such candidates in selective sorption of industrial heavy metal ions from polluted water. **Aim:** The present work focuses on the, to select the suitable Pleurotus species for heavy metals removal. Second objective of my work is to evaluate the heavy metal removal in liquid media by Atomic Absorption Spectrophotometer and third is to evaluate the extracellular enzymes activity of Pleurotus species. **Methods:** Methodology contains culture and their maintenance of the two different species of pleurotus i.e. P. flabellatus & P. eryngii stock culture was maintained on PDA at 27±2°c. Laccase Activity (EC 1.10.3.2): Laccase activity was determined according to Dhaliwal et al. (1991). Atomic Absorption Spectrophotometer Analysis: Estimation of Cu, Mn, Ni & Zn in liquid filtrate of Pleurotus species after 5 days interval with the help of Atomic Absorption Spectrophotometer (AAS). **Results:** The mycelial growth rate of P. flabellatus was not inhibited up to 25ppm, whereas 10ppm in case of P. eryngii against zinc. Nickel inhibits the growth rate of both Pleurotus species. The maximum ligninolytic enzyme activity was achieved by P. eryngii. **Conclusion:** The present investigation suggests that Pleurotus can be used in bioremediation of heavy metals from contaminated ecosystem. (DOI:10.9777/rr.2018.1313)

Expression and localization of NANOS2 in spermatogonial germs cells during spermatogenesis in rat

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Nanos is an evolutionarily conserved family of RNA-binding proteins that are expressed specifically within the germ cells of both

invertebrate and vertebrates. The present study was designed to determine the expression of Nanos2 in rat spermatogonial germ cells (SGCs) during spermatogenesis. Nanos2 were localized immunohistochemically and immunofluorescence microscopy in paraffin embedded testicular sections whereas the expression of mRNA was done through semi-quantitative RT-PCR. Immunohistochemical studies demonstrated presence of NANOS2 immunoreactivity in SGCs as wells as in spermatogonial stem cells. Nanos2 was dominantly localized in the cytoplasm of SGCs although; staining intensity of NANOS2 signal varied depending upon the stage of the cycle of seminiferous epithelium. In addition, NANOS2 was also localized in the outer dense fibers of flagella of spermatids and in the head cap of late spermatids. The mRNA transcript of Nanos2 (129 bp) was detected in isolated spermatogonial stem cell population. These results suggest that NANOS2 might play an important role in germ cell as well as lineage differentiation during early embryonic development because of its stage or cell specific expression. (DOI:10.9777/rr.2018.1314)

In vitro anti-diabetic potential of *Pterocarpus marsupium* extract

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Background: Diabetes mellitus is a metabolic disorder characterized by abnormally high levels of blood glucose due to disorder in carbohydrate, fat, and protein metabolism and leads to the development of micro vascular complications. **Aim:** The aim of the study is to check in vitro anti-diabetic potential of aqueous and methanolic extract of heartwood of Pterocarpus marsupium plant by α-amylase and α- glucosidase enzyme Inhibition assay, and glucose uptake in yeast cells. **Methods:** Heartwood was subjected to sequential solvent extraction, total amount of phenols (mg GAE/gm of extract), flavonoids (mg Quercetin equivalent/gm of extract), and tannin (mg GAE/g of extract) content were analyzed by in vitro method like ABTS+ and DPPH scavenging activity, α-amylase andα -glucosidase inhibition assay, and glucose uptake in yeast cells. **Results:** Total phenols, flavonoids and tannins in methanolic extract of heartwood were 124.4 ± 5.6, 485.5.± 12.7 and 71.6 ± 6.2, mg/gm of extract respectively. In DPPH and ABTS+scavenging activity the IC50 valueswere28.7µg/ml, 11.4µg/ml, 13.5µg/ml and7.8µg/ml, 2.9µg/ml, 4.2µg/mlfor aqueous, methanolic extract of heartwood and standard ascorbic acid respectively. In α- amylase and α -glucosidase inhibition assay, IC50 value for standard Acarbose, aqueous and methanolic extract of heartwood were 44.09, 66.72, 48.20 and 45.17, 72.32, 48.12, respectively. The IC50 values for glucose uptake assay were found to be 106.72µg/ml, 101.99 µg/ml, and 98.50 µg/ml, for metformin, aqueous and methanolic extract of heartwood respectively. **Discussion:** In vitro anti-diabetic as well as antioxidant activity may be due to presence of high amount of polyphenols, flavonoids and tannins in methanol extract of plant heartwood which minimized the oxidative stress to some extent. Conclusions: Methanolic extract of heartwood of P. marsupium possessed both antioxidant and antidiabetic activity. It showed hypoglycemic effect by increasing glucose uptake in yeast cells and inhibiting α-amylase and α-glucosidase activity in vitro. (DOI:10.9777/rr.2018.1315)

Anti-hyperglycemic activity of napin-like protein of *Momordica charantia*

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Background: Diabetes mellitus is most common metabolic disorder which results in many secondary complications of different organs. Therefore, management of diabetes is necessary with control of blood glucose level on a daily basis. Bitter gourd (Momordica charantia) is known for its anti-diabetic properties and has been used in traditional medicine worldwide. We have identified a novel protein from M. charantia, which showed structural features of Napin-like proteins. Napin like proteins from different plants, have been reported to have an array of biological activity. However, anti-diabetic activity of napin- like protein has not been demonstrated. **Aim:** To evaluate anti-hyperglycemic activity of recombinant napin like protein (Acc. No. AJ488931) of M. charantia (rMcnapin). Materials and **Methods:** Purification of the rMcnapin expressed in Pichia pastoris was carried out. Evaluation of anti-hyperglycemic activity of rMcnapin was carried out in vitro by glucose uptake assay using HepG2 cells and in vivo using experimental streptozotocine-induced diabetic animals). **Results:** The rMcnapin enhanced glucose absorption in HepG2 cells with increased expression of Glut4. The rMcnapin significantly reduced blood glucose level in diabetic animals. **Discussion:** The present study indicates that the napin-like protein of M. charantia was able to enhance glucose uptake by increasing the expression of GLUT 4 and its translocation to the plasma membrane in cultured cells. In vivo studies show that the protein is able to reduce blood glucose level in diabetic rats and maintained normoglycemia in glucose tolerance test. The protein thus has anti-hyperglycemic activity **Conclusion:** In summary, this work led to the establishing a novel property of the napin-like protein anti-hyperglycemic activity. (DOI:10.9777/rr.2018.1316)

Role of nitric oxide and glucose in alleviation of salt stress inhibited photosynthesis in wheat (*Triticum aestivum* L.)

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The present study was conducted to assess the involvement of nitric oxide (NO) and glucose (Glu) in reversal of salt inhibited photosynthetic characteristics in wheat (Triticum aestivum L.). These experiments were conducted in the net house, department of botany Aligarh Muslim University, AMU, Aligarh. Salt stress is one of the major abiotic stresses, which are known to reduce productivity of various crop species and wheat is considered a most prominent crop in the world. Wheat cultivar (WH 711) was subjected to

100 mM NaCl treatment exhibited decreased photosynthetic and growth characteristics, nitrogen assimilation and cysteine metabolism. In contrast, proline content and antioxidants was found to be induced under salt stress. Supplementation of 50 µM Nitric oxide (SNP, sodium nitroprusside) and 6% glucose (Glu) to salt grown plants increased photosynthetic potential of plants through increase in nitrogen- and sulphur-assimilation capacity and proline accumulation. The interaction effect between NO and Glu was additive on photosynthetic responses. The results

of the study bears an important impact on the involvement of NO and Glu in alleviating salt stress effects and provide new ways to mitigate salt stress problems in crop plants. (DOI:10.9777/rr.2018.1317)

Molecular characterization of Alr2321 as a glyoxalase I enzyme from *Anabaena* sp. PCC 7120 and confers its role in abiotic stress.

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Abiotic stresses significantly enhance cellular reactive oxygen species (ROS) level which results in toxic methylglyoxal (MG) production. Glyoxalases (GlyI and II) ubiquitously found across organisms catalyze conversion of toxic methylglyoxal into nontoxic lactic acid whose properties and function are still unknown in cyanobacteria. Here, first attempt to characterize glyI from Anabaena sp. PCC7120 using in silico and wet lab approaches. Whole genome sequence analysis showed that Anabaena sp. PCC7120 contains cluster of ten genes posses glyI domain (PF00903) in which only three genes (alr2321, all1022, alr4469) caring conserved glyI motif (HEHE). In all these three genes, we have characterized alr2321 which showed maximum 65% similarity with Ni2+ dependent glyI of E. coli having conserved βαββββ motif. Complementation of alr2321 in E. coli gloA mutant in the presence of methylglyoxal suggested it is an active enzyme of glyI family. Kinetics analysis in the presence of different divalent metal and substrate (MG) revealed that Alr2321 showed maximum activity in the presence of Ni2+/Co2+, although it is inactive Zn2+-bound form. The glyI reaction product (S-lactoylglutathione) formation in the presence of Alr2321 was further confirmed by HPLC. The effect of various abiotic stresses showed that UV-B leads to the highest upregulation of alr2321 transcript followed by heavy metal and metalloids, UV, salinity, drought and heat (5.5 to 2 fold). Multistress response of glyI gene(alr 2321) indicates its future utility in developing transgenic cyanobacteria. (DOI:10.9777/rr.2018.1318)