Pakistan Journal of Biochemistry and Molecular Biology

Preface

Fourteenth Biennial Conference of Pakistan Society for Biochemistry and Molecular Biology (PSBMB) was held during December 9-12, 2018 at Dr. A.Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan. Theme of the conference was "MOLECULAR BIOSCIENCES: RESEARCH AND INNOVATIONS". Hundreds of scientists, post-doctoral fellows and graduate students from all over Pakistan and other countries attended this conference.

Here we present abstracts of poster presentations delivered during the conference. Editorial board is grateful to the organizing committee of PSBMB 2018 for providing abstracts of poster presentations for publication in PJBMB.

Editorial board

Pakistan Journal of Biochemistry and Molecular Biology

Poster Presentations of 14th Biennial Conference of PSBMB (December 2018)

ROLE OF INFLAMMATORY MOLECULES IN THE PROGRESSION OF CARDIOVASCULAR DISEASE

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Cardiovascular diseases (CVDs) are the world's leading cause of death with high incidence and prevalence. According to World Health Organization (WHO), approximately 17.7 million people died due to CVD globally. Among different types of CVDs, Coronary Artery Disease (CAD) and Ischemic Stroke (IS) are the most common one. Several risk factors have been reported to be involved in CAD and IS such as obesity, age, sex, family history, diabetes mellitus, hyperlipidemia, depression, hypertension, smoking and genetic variations. Inflammation also plays a vital role in the pathogenesis of these diseases. Various cytokines have been found to be involved in the inflammatory pathways of CAD and IS. Specifically the variations in interleukin (IL) genes have been related to these diseases. It has been reported that during the inflammatory process, interleukin (IL) genes act as anti-inflammatory molecules whereas some interleukin (IL) genes act as pro-inflammatory molecules. Genetic alterations in these genes may cause increased risk to the pathogenesis of CAD and IS. These genetic variations were found to be inconsistent with different ethnic groups in the world. The present study is designed to investigate the genetic alterations in interleukin (IL) genes, and their relationship with CAD and IS. To execute the proposed research, blood samples will be collected from CVD patients and healthy individuals. DNA will be extracted from the samples. Polymerase chain reaction (PCR) will be carried out for genotypic analyses. The results obtained will be confirmed by DNA

sequencing. The proposed study may provide an insight in the mechanisms involved in increasing susceptibility towards CAD and IS and may also be helpful in detecting the role of interleukin (IL) genes in development and progression of CVD.

PHYLOGENETIC RELATIONSHIPS OF SPECIES OF GENUS *ABUTILON* BASED ON CONSERVED REGIONS OF CHLOROPLAST DNA

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Genus *Abutilon* belongs to family Malvaceae. Nearly 200 recognized species of genus *Abutilon* are distributed in tropical and subtropical areas of the World. In Pakistan, 18 out of 200 species have been reported which are distributed in Sindh and Baluchistan. Members of genus *Abutilon* are perennial herbs, shrubs and small trees with the height ranges from 8 inches to 10 feet. They are recognized in all over the World for medicinal, economical and ornamental importance. Classification has been constructed on the basis of their vegetative and reproductive characteristics including floral, seed and fruit characters. These all characters are ambiguous in case of dry or damaged sample, as morphological characters are difficult to distinguish between various species. Urbanization is also cause hindrance to trace members of genus *Abutilon* from their reported geographical locations which results in reproducibility of results. There is a need to resolve its ambiguity and find out interspecific relationships using molecular data. In this study, chloroplast DNA regions will be used to reconstruct the phylogenetic tree. To achieve this goal samples will be collected from different areas of Pakistan, identification and confirmation will be performed by the help of taxonomist from center of plant conservation, University of Karachi, submitted to herbarium for accession number. Whole DNA will be extracted and amplification of desired sequence will be performed by polymerase chain reaction. After amplification of

desired sequence of cpDNA, amplicon will be sent for sequencing. Sequenced cpDNA sample will be analyzed and submitted to GenBank and phylogenetic analysis will be performed. This phylogenetic analysis will be helpful for placement of species of genus *Abutilon* in their correct position, assessment of botanical diversity and also reveals patterns and relationships.

AMELIORATION OF THE BLOOD GLUCOSE LEVELS AND NORMALIZATION OF BLOOD PRESSURE FOLLOWING 7-DAY HALF AN HOUR WALKING IN A TYPE 2 DIABETIC PATIENT

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World Health Organization's recently compiled data shows that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. The current prevalence of Type 2 diabetes mellitus in Pakistan is 11.7%. Type 2 diabetes (formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. It is much more common than type 1 accounting to around 90% of all diabetes cases worldwide and is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Liver and muscle cells inadequately respond to insulin thus the entry of glucose is restricted consequently resulting in Hyperglycemia. Family history and genes play a role in type II diabetes. Written informed consent was acquired from the patient. The prescription from the consultant included Diabold (4mg) before breakfast, Sitamet 1000mg at night Tramol plus whenever she felt fatigued. The prescription continued and was intervened by a half an hour walk daily at 7 pm for 7 days before the meals thus travelling a distance of 85 meter. A warm up of 15 min was also introduced before walking. Pre intervention fasting & random blood glucose levels were recorded and even at the end of the intervention also. Blood pressure and pulses were checked and recorded regularly before & after walking. The pre intervention

fasting glucose levels were attenuated together with the random blood glucose levels. Her blood pressure which was monitored daily, acquired normalization. The results are discussed in terms of modulated metabolism following post intervention in a type 2 Diabetic patient.

INFLUENCE OF GROWTH HORMONE SECRETEGOUGE RECEPTOR (*GHSR*) GENE POLYMORPHISM(S) ON CHICKEN GROWTH TRAITS AND CARCASS YEILD

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Growth enactment and carcass yield are commercially important phenotypic characters of chicken. The cumulative demand of the chicken meat urge producers to elevate consistent chicken growth, more meat within a short time. Growth hormone secretagogue receptor *(GHSR)* gene is responsible for the growth performance in chicken. GHSR binds with its ligand ghrelin, which is a peptide hormone produced by chicken proventriculus and stimulate the growth hormone release. *GHSR* gene is also well known for appetite stimulation and energy homeostasis regulation in birds. The current study aims to elucidate the association of Single Nucleotide Polymorphisms (SNPs) of a *GHSR* gene on growth traits and carcass yields in Ross, Cobb and Hubbard breeds. For this purpose, blood samples will be collected from chickens followed by DNA extraction using standard protocol. Targeted region of the gene will be amplified by Polymerase Chain Reaction (PCR). For this, primers will be designed using "Primer 3" software. Restriction Fragment Length Polymorphism (RFLP) will be performed for genotypic analyses. The results will be verified using DNA sequencing. This study might be beneficial in phylogenetic analyses moreover in the design of breeding programs. Furthermore, the research would be advantageous in the identification of potential biomarkers which are responsible for economically important phenotypic characters.

INVESTIGATION OF GENETIC BASIS OF BARDET BIEDL SYNDROME IN A PAKISTANI FAMILY

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Bardet-Biedl Syndrome (BBS), is a rare systemic autosomal recessive disorder that is clinically and genetically heterogeneous. It arises due to defects in BBS genes with the underlying cause being alterations in the gene sequences. The primary clinical features of BBS, that manifest in the first decade of life, include rod-cone dystrophy, polydactyly (congenital), obesity, cognitive impairment, reduced intelligence, renal dysfunction and genital malformation. To date, 21 genes have been identified to be involved in the BBS. These BBS-associated genes encode proteins involved in the regulation and maintenance of ciliary structures and mutations in these genes lead to defective cilia. Primary cilia play a key role in sensory perception and various signaling pathways. Three BBS-associated genes *BBS6, BBS10* and *BBS12* have been previously reported to encode for chaperonin-like proteins. These proteins play an important role in assembly of the BBSome, which is a multi-protein complex essential for mediating activity of ciliary trafficking. In accordance with the recent findings, half of the clinically-diagnosed BBS families showed variations in these three genes, explaining the importance of defects in chaperone proteins as pathogenic factors. Based on the evidences, current study aims to detect the variations present in *BBS6, BBS10* and *BBS12*. Genetic polymorphisms in *BBS6* (*rs1545* and *rs1547)*, *BBS10 (*c.1958-1967*del* and *rs1489342987)* and *BBS12* (*rs587777802* and *rs121918327)* will be screened through tetra-primers Amplified Refractory Mutation Systems Polymerase Chain Reaction (t-ARMS PCR), followed by direct DNA sequencing and genotyping to screen the genetic variations. Statistical analyses will be thereafter executed for interpretation of the acquired results, to reveal any possible associations of the variations with BBS phenotypes. In future, the results may help in the identification of genetic diagnostic biomarker(s) for clinical implications.

THERMOTOLERANCE INDUCTION IN AROMATIC AND NON-AROMATIC RICE USING FOLIAR PROLINE SPRAY APPROACH

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Foliar application is a shotgun approach to mitigate against the deleterious effects of abiotic stresses to enhance the growth and yield of plants. In this context, the present study is designed to evaluate the effects of foliar proline on thermotolerance of aromatic (super basmati) and non-aromatic (JP-5) rice in response to high temperature stress. The rice varieties were exposed to heat stress as well as exogenously applied proline (40mM concentration) at flowering and maturity stages at control (32±2°C), heat shock (45±2°C) and recovery (32±2°C) conditions. The stress induced damages were quantified through stress indicators including electrolyte conductivity (EC), proline accumulation, malondialdehyde (MDA) level, H_2O_2 production and total soluble protein. The results indicate a decrease in EC, MDA and H_2O_2 upon foliar application of proline while increased proline content was observed indicating the role of proline as an osmoprotectant to combat the damages due to high temperature stress. It can be concluded that exogenously applied proline can help combat stress induced damages due to high temperature stress which may play a role for enhanced stress tolerance in rice.

PRODUCTION, OPTIMIZATION AND KINETIC ANALYSIS OF XYLANASE FOR PREPARATION OF CROSSLINKED ENZYME AGGREGATES

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Xylanase is a hydrolytic enzyme which cleaves the β-1, 4 backbone of the complex plant cell wall polysaccharide known as xylan. Xylanase plays an important role in various industrial applications. Although, the industrial applications of xylanase are well known, but the use of this enzyme is restricted due to its poor stability, limited reusability, sensitivity to the environmental conditions and high production cost. Keeping all these drawbacks in consideration, there is a need to establish an alternate approach to provide stability to the free enzyme. Crosslinked enzyme aggregates (CLEA) technology is a fast and versatile method to produce immobilized enzymes by precipitation and crosslinking immobilization method. Carrier free enzyme aggregate is a carrier free (CLEA) immobilization which have number of industrial advantages as compared to carrier bound immobilization techniques. In this study, initially four most reported xylanase producing *Aspergillus* strains were screened. Among them, *Aspergillus flavus* KIBG-IB34 was selected due to its high production yield. Fermentation parameters were optimized to further enhance the microbial growth and enzyme titre. For partial purification, different precipitating agents (ammonium sulphate, isopropanol, butanol, ethanol, and methanol) were used. Thereafter, the kinetic parameters of enzyme were characterized as the preparation of CLEA based immobilized xylanase is mostly depends on immobilization parameters such as the nature of cross linker, precipitating agents, aggregation time and aggregation temperature. Immobilization efficiency and protein loading efficiency will be estimated. Furthermore, compare stability and recycling efficiency both crosslinked xylanase aggregates and free xylanase.

INTERVENTION INDUCED ALLEVIATION OF DEPRESSION, ENHANCED COPING SELF EFFICACY AND WAYS OF COPING IN A TYPE 2 DIABETIC PATIENT

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The co-occurrence of depression in Diabetes Mellitus type 2 is attributed to a variety of factors, including the psychological and psychosocial impact of the disease. Multifaceted interventions have facilitated in the management of type 2 Diabetes. A 30 min walk, for 5 days is regarded as a recommended intervention for its management. Diabetes mellitus type 2 is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin accompanied with depression. The Zung Self-Rating Depression Scale; 20-item self-reported questionnaire is widely used as a screening tool to evaluate psychological and somatic symptoms associated with depression. The responses were calculated in terms of index score. Coping Self efficacy scale is a combination of two vital concepts in chronic situation and challenges, *coping* and *self-efficacy* and about the general belief in oneself to solve problems and reach goals. The Ways of Coping Questionnaire is used to evaluate the coping processes. An informed consent was obtained from a type 2 diabetic patient, keeping in view the ethical grounds. Her regular medication was continued along with an 85-meter walk for 7 days. Her depression, coping self-efficacy and ways of coping were assessed before and after the intervention. One-week intervention ameliorated her depression and coping self-efficacy was improved. An improvement was evident in problem focused coping and tension reduction following post intervention. The results are discussed in terms of modulated metabolism following the intervention.

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IDENTIFICATION OF THE POTENTIAL TYPE 2 DIABETES SUSCEPTIBILITY GENETIC ELEMENTS IN SOUTH ASIAN POPULATIONS: A COMPUTATIONAL APPROACH

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Type 2 Diabetes (T2D) is one of the rapidly prevailing health risks in the different populations of the world. The lack of significant population-specific data about T2D demands the identification of latent genetic risk factors behind T2D in different ethnic groups that had not identified so far. In this study, we present an insilico pipeline for the identification of uncharacterized T2D-related genetic elements that could make the South Asian (SAS) populations susceptible to this disease. Two databases, Genome-Wide Association Studies (GWAS catalog) and Gene Expression Omnibus (GEO) were used initially to retrieve all T2D-related studies. The genes retrieved from these studies were annotated using the Database for Annotation, Visualization and Integrated Discovery DAVID. The variant analysis of selected genes was accomplished using wANNOVAR. The only population that was considered relevant in wANNOVAR was the SAS and all cogent variants reported in this population at MAF < 0.05 were selected. Finally, SNPnexus fetched the genetic variants which are purely related to T2D. This results in the identification of seven genes that could make the SAS populations susceptible to T2D. Furthermore, these genes are not previously described to associate with the risk of T2D in these populations. We, therefore, infer that this study would not only contribute towards knowledge-base but will also suggest relevant genetic factors for the development of genotyping arrays for targeted therapeutics and management of the T2D in the South Asians.

NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF LORAZEPAM: A DOSE RELATED STUDY

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Present study was designed to monitor the dose dependent effects of lorazepam; a benzodiazepine (CNS depressant). It is the primary drug of choice for treatment of anxiety and to produce calming effects. However, repeated administration of this lorazepam causes dependence, and this might be caused by increased dopaminergic neurotransmission. Besides dopamine, 5-hydroxy tryptamine (5-HT) has also been reported to have pivotal role in the pathophysiology as well as treatment of anxiety and addiction. Repeated administration of lorazepam might involve altered 5-HT metabolism as well. Present study was therefore designed to monitor dose-dependent effects of lorazepam and to select its optimum dose for further experiments and pharmacological interventions. Effects of lorazepam were monitored on food intake, growth rate, activities in familiar and novel environments, light dark box activity, forced swim test and metabolism of dopamine and 5-HT. oral administration of lorazepam was done at the doses of 0mg/kg, 2mg/kg, 4mg/kg and 6mg/kg. Behaviors parameters were monitored following single administration of lorazepam. Rats were decapitated and whole brain samples were collected and stored at -70°C until neurochemical analysis by HPLC-EC. Findings from the present study could be implicated to increased therapeutic utility of lorazepam and related benzodiazepines.

IN QUEST OF PREVALENCE, RISK FACTORS OF CEREBRAL PALSY IN PAKISTAN: THE JOURNEY CONTINUES IN QUETTA

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Cerebral palsy, a neurodegenerative disorder, refers to cerebral damage associated impaired motor functioning occurring in children in an early age thus exhibiting delayed milestones. The prevalence established world-wide, is still in its budding stages in Pakistan. This study is an attempt to consolidate the regional findings from Pakistan. The prevalence of Cerebral Palsy in Gujranwala hospital was 0.417% when surveyed by the authors in 2015-2016. Males being the most effected and spastic was the most common type. A study conducted at the Department of Pediatrics, Faisalabad depicted 75% Cerebral Palsy cases and the majority were spastic. Out of 102 cerebral palsy 90% were spastic and were dominated by males in Armed Forces Institution of Rehabilitative Medicine in Rawalpindi. A household survey in rural district of Sindh found cerebral palsy to be the most common disability. In Karachi's Squatter settlement 20 cases of Cerebral Palsy have been reported stating 80% males. In an OPD of Neurosurgery Lady Reading Hospital in Peshawar, 82 cases of CP have been reported and the greater percentage was occupied by males while district Swabi, KPK reported 1.22/1000 live birth as CP cases. A survey conducted in Quetta under the umbrella of the Department of Biochemistry, University of Karachi revealed only a single Complex for Special Education catering to Cerebral Palsy. According to the principal, 61.35% were suffering from Cerebral Palsy; however, 11 documented questionnaires could be obtained from parents of CP afflicted children as diagnosed by a physician revealed only 23.9% cases. The occurrence of more females with three sisters belonging to the same family was an eye opener to investigate into the possible risk factors such as consanguineous marriage, hypoxia, birth Asphyxia and environmental factors that have been also explored in previous researches making it prudent to explore underlying mechanisms.

ASSOCIATION OF *IRAK2* GENE VARIATION(S) WITH THE PROGRESSION OF RHEUMATOID ARTHRITIS

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Lymphocytes, with 2 trillion cells, constitute 1% of total normal human body weight. On antigen exposure, selective cells multiply in number to destroy antigens. However, in some disorders, the body loses its activity to recognize self and non-self-cellular components and tissues. According to CDC, the primary target site of RA is joints. Starting from hands, wrists, and knees it leads towards the joint tissue damage that cause chronic pain, shakiness and other deformities. Globally it affects 1% of the population and is considered as the second leading cause of disabilities. Although the definite cause for arthritis is still not clear, some genetic and environmental factors may contribute to the progression of disorder. Progression of RA initiates when macrophages appear at the site of infection, antigen degradation occur by the activation of T- helper cells, pro-inflammatory cytokines and proteases. Most common proinflammatory cytokines such as IL-1, and IL-23 are involved in the pathophysiology of RA which is affected by NF-κB mediated activation of cytokine gene expression. The current study is designed to investigate the association of single nucleotide polymorphism(s) of interleukin-1 receptor-associated kinase 2 (IRAK2) with the susceptibility of RA. Blood samples will be collected from the patients of RA after taking the informed consent. DNA will be extracted followed by gene amplification by PCR and confirmation of product will be done by gel electrophoresis. SNP identification will be confirmed by sequencing of target gene. To target associated proinflammatory cytokines levels, ELISA will be performed. Metabolic activity of cells will be determined through MTS assay. Data analyses will be done by bioinformatics and statistical tools. Identification and association of IRAK2 gene SNPs may improve our understanding of the molecular mechanism

involved in RA development and may also act as biomarkers in future for the early diagnosis of RA.

INVESTIGATING THE EFFECTS OF TRIFLUOPERAZINE ON ANDROGEN-INDEPENDENT PROSTATE CANCER CELL LINES, PC-3 AND DU-145

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With over 4,500 new cases reported annually in Pakistan, Prostate Cancer (PCa) is the fourth most prevalent cancer type and poses a major health safety issue. If diagnosed at the initial stages, androgen-dependent PCa may be cured with androgen deprivation therapy and local decisive interventions via surgery or radiation. However, once metastasized and transformed into androgen-insensitive type, PCa treatment remains elusive. Existing chemotherapy, for patients with this type of malignancy, is associated with severe adverse effects. Finding a drug that has anti-cancer attributes and associated with tolerable adverse effects remains the ultimate goal in cancer therapy. The current study is designed to investigate the additional actions of Trifluoperazine, an antipsychotic drug from the family Phenothiazine, on Dopamine D_2 receptors, Alpha 1A Adrenergic receptors and Calmodulin in PC-3 and DU-145 cell lines which are established targets of the selected drug and previously reported to be overexpressed in androgen-independent type of PCa. Gene and protein expression for the above-mentioned receptor types in PC-3 and DU-145 cells, before and after drug exposure in a dose-dependent manner, will be assessed through Quantitative Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-qPCR) amplification and Immunocytochemistry respectively. The effects of the selected drug on morphology and viability of PC-3 and DU-145 cells may be evidenced by growth inhibition and cytotoxicity assays, followed by flowcytometric analysis to distinguish between cell death due to apoptosis and necrosis. In future, the outcomes of this study may lead to elucidation of the targets of the tested drug on

androgen-independent PCa cell lines using integrated computational, proteomic and functional analyses. Hence, the selected drug may have clinical implications and serve as a potent substitute for conventional chemotherapeutics in PCa treatment.

OMICS APPROACHES TO DISCOVER PUTATIVE BIOMARKER IN OSCC PATIENTS FOR EARLY DIAGNOSIS

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Squamous cell carcinoma is cancer of mucosal lining contributing greater than 90 % of oral cavity cancer and ranked as eleventh most common cancer globally. Highest morbidity rate has been observed in Pakistan, India, and Bangladesh. Each year 500,000 new cases are diagnosed globally while 5-year survival rate is only 50%. Alcohol and tobacco are major risk factors of OSCC in developed world while smokeless tobacco products (gutka, naswar) are main risk factors in Asian countries. These risk factors affect several pathways by changing expression of several genes and gene products. The objective of current study was to identify differentially expressed genes through real time quantification at molecular level and study the comparative profiles of differentially expressed proteins using traditional proteomics approaches. For proteomics approach, serum samples from local population suffering from OSCC and recommended for surgery were collected. High abundant proteins were removed by using albumin/IgG removal kit. Protein estimation of depleted sample was carried out using BCA assay. Serum samples were resolved on 12% SDS-PAGE to visualize depletion pattern. For comparative proteomics analysis, 2-D gel electrophoresis was performed to study differential expression in diseased and control samples. For genomic approach, tissue samples of OSCC patients along with adjacent non-cancerous tissue removed during surgery were used to quantify gene expression using RT-qPCR. Our study is likely to provide information regarding specific biomarkers associated with OSCC. Furthermore, it is expected that the outcome of this research might be beneficial as a possible diagnostic and prognostic tool in future.

CONSTRUCTION OF FUSION PROTEIN FROM *MYCOBACTERIUM TUBERCULOSIS* TO PERK UP SENSITIVITY OF TB SERODIAGNOSIS

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is amongst the deadliest diseases worldwide, especially in endemic regions including Pakistan. Currently TB diagnosis profoundly depends upon antibody profiles of various *Mycobacterium tuberculosis* antigens. To improve sensitivity of diagnostic test, fusion proteins containing epitopic regions of multiple different antigenic proteins can be a fruitful approach. In this regard, fusion protein was made by joining antigenic regions of CFP21 to Rv1352. AgX was attached to fusion protein for soluble expression. Fusion Construct along with its native proteins were cloned and expressed in *E. coli*, purified through Ni+ affinity chromatography. Native proteins were injected in the rabbits for immune response generation. ELISA was performed to monitor the levels of antibodies in blood samples, drawn at regular intervals. Immunoreactivity was observed through western blotting and secondary structure prediction was done by CD spectroscopy. Serodiagnostic potential of fusion protein will be estimated through ELISA. Promising results will prove the fate of fusion protein for their usefulness in TB serodiagnosis.

PAMIDRONATE, AN ANTI-OSTEOPOROSIS DRUG AS CHEMOTHERAPEUTIC AGENT

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Breast cancer is one of the predominant cancers diagnosed among women throughout the world. In Asia, Pakistan has highest incidence rate of breast cancer (BC). The absence of early detection marker and poor diagnosis are leading cause of breast cancer and

due to which mortality rate is higher. Triple negative breast cancer (TNBC) is an aggressive type of BC because of poor prognosis and shows resistance against hormonal therapy and chemotherapy. In Pakistan around 17.28% population was diagnosed with TNBC. BC is usually detected by mammogram at stages I, II, or III. Therapeutic preferences are used from primary surgical process followed by adjuvant and radiotherapy. Various signaling pathways are found to be involved in the development of BC. Malfunctioning of the signaling cascade may cause aberration in normal cell growth that result in cancer development. Mutation in cellular signaling pathways including MAPK, P13K/AKT/mTOR, and MVA affect cell growth and proliferation. Mevalonate (MVA) is a complex metabolic pathway, essential for the generation of diverse cellular end products and cell growth cycle. MVA intermediates are used as therapeutic drug targets and induce cell apoptosis in various cancer. Bisphosphonates (BPs) are the MVA pathway inhibitors. BPs are FDA approved drug for the treatment of osteoporosis and it was approved as standard drug for prevention of bone metastasis in breast cancer. Current study focuses on investigating the role of Pamidronate (PAM), a member of BPs as anti-cancerous drug on BC cell line. Cytotoxic effect of PAM was evaluated on BC cell line. Genomic approach was also used to identify the changes in gene expression of BC cell line induced by PAM. Present study may provide help in understanding the role of PAM as an anticancer agent for the development of new therapeutic targets.

EFFECT OF PHYTO-MEDIATED SILVER NANOPARTICLES ON OXIDATIVE STRESS AND HISTOLOGY OF HEPATIC TISSUES IN RATS

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An eco-friendly, phyto-mediated synthesis of silver nanoparticles (AgNPs) is a fast-growing area of research in nanotechnology. Use of medicinal plants for the synthesis of silver nanoparticles have proven to be the good therapeutic agent for the treatment of

various diseases. Despite its vast area of synthesis and applicability in various consumer products, there is limited data available regarding their effects on human health and the environment. This study enlightens the phyto-mediated synthesis of silver nanoparticles in an eco-accommodating way using aqueous aloe vera leaves extract (AVLE) and evaluate its effects on oxidative stress and histology of hepatic tissues on a rat model. The AgNPs were synthesized under ambient conditions using AVLE. The synthesized nanoparticles were characterized by UV–visible spectroscopy and SEM; and evaluate its potential effects on biochemical parameters and histology of the liver in rats. 30 male albino Wistar rats were randomly divided into three groups (n=10): group I: received no treatment; group II: received aloe vera leaf extract (300 mg/kg b.w); and group III: received AgNPs (10 mg/kg b.w) via an intragastric tube for 14 days. Results showed the successful synthesis of phyto-mediated AgNPs with maximum absorption spectra at 400 nm, spherical morphology, and 20-24 nm average particle size. The liver biochemical indices (ALT, AST, and ALP) showed no significant difference, whereas a decrease in oxidative stress (MDA), improve levels of antioxidant enzymes (SOD, CAT, GSH) and no histological alteration have observed as compared with the control group. In conclusion, the phyto-mediated AgNPs suggesting its potential role in clinical therapeutics for the prevention of various diseases.

ROLE OF SONIC HEDGEHOG SIGNALING PATHWAY IN THE DEVELOPMENT OF HCC

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Liver cancer is the second highly prevalent cancer responsible for malignant associated mortality worldwide. Hepatocellular carcinoma (HCC), Cholangio carcinoma and Sarcoma are common liver cancer. Globally, HCC is the most common carcinoma and prevalent in Asia and sub-Saharan Africa. Yearly, 560,000 cases of liver cancer are reported and mortality rate is also high due to delay in diagnosis. Overall survival rate is only 3.5%. In Pakistan,

incident rate of HCC is 7.6/0.1 million and 2.8/0.1 million males and females respectively. Various risk factors for development of HCC are HCV, HBV, alcohol consumption and fatty liver diseases. Different methods for early stage treatment are liver transplantation, surgical resection and embolization but are useful for only 30-40% patients. In advanced stages, chemotherapy is the only available option. There is always a high need for the identification of new therapeutic targets. HCC is highly associated with activation of many oncogenes in cellular signaling cascades such as EGFR-Ras-MAPK pathway, c-MET signaling cascades, IGF signaling, PI3K/AKt/mTOR pathway, Wnt signaling pathway, beta catenin pathway and Sonic hedghoge (Shh) sigaling pathway. It is reported that during different malignancies Shh pathway becomes reactivated in differentiated liver tissues that leads to rapid cell growth and proliferation. Shh signaling cascade functionally active in growing embryo and is inactive in developed liver tissues. During development of HCC, different genes of Shh pathway are found to be up regulated. Differential gene expression analysis is an important approach that can be used to evaluate the involvement of Shh pathway genes in the development and progression of HCC in local population. Current study focuses on the gene expression analysis through RT-PCR to evaluate the role of Shh pathway in hepatocarcinogenesis and that may provide a possible therapeutic target for HCC.

EFFECTIVENESS OF DUPHASTON IN ERADICATION OF POLYCYSTIC OVARIAN SYNDROME: A CASE STUDY

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Duphaston, a synthetic progestogen, has similar actions to progesterone and is used to treat menstrual irregularities which is one of the main ailment of polycystic ovarian syndrome (PCOS). PCOS, also known as “STEIN LEVENTHAL SYNDROME” after the name of its discoverer occurs in the women of reproductive age. According to the American society of human reproduction and Embryology/American society for reproductive medicine (ESHRE/ASRM) consensus at Rotterdam in 2003 workshop the

diagnosis of PCOS is based on (1) chronic anovulation, (2) Polycystic Ovaries in ultrasonography and Clinical/Biochemical parameters for hyperandrogenism. Other signs and symptoms include acne, irregular menstrual cycles or amenorrhea, hirsutism, difficulty in getting pregnant, obesity and insulin resistance. Informed consent from a PCO diagnosed patient (aged 23) was obtained, keeping in view the ethical considerations. Duphoston along with Metformin (500 mg thrice a day) was prescribed by her physician as follows: The dose for Duphaston was 10 mg twice a day for 5 days before the menstrual cycle, then abstinence for 21 days. This was repeated for 6 months. The pre-data collection was followed by the post Duphaston data collection after 6 months. The pre-treatment findings exhibited elevated levels of LH while the FSH level was relatively low. Ultrasound reflected enlarged ovaries with multiple small follicles, approximately to 12 or more with a measurement of 2-9 mm thus showing an irregular ovarian outline. Post treatment findings exhibited normal levels of LH and FSH. Size and shape of ovary was normal (0.7mm), devoid of follicles thus acquiring a normal menstrual cycle. The results are discussed in terms of Duphaston and Metformin induced eradication of PCOS.

BIOPRESERVATIVE PROPERTIES OF ANTIBACTERIAL SUBSTANCE PRODUCING INDIGENOUS STRAIN OF BACILLUS SPECIES

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Many antimicrobial compounds have been described so far in account of their bio preservative abilities. In addition to vast variety of antimicrobial producing organisms, Bacillus genus has been reported to produce antimicrobial compounds which could be exploiting to control food spoilage bacteria and food borne pathogens. Although Bacillus strains are known to produce a vast array of antimicrobial compounds, some compounds remain to be identified. Therefore, this study was carried out for investigating biopreservative potential of an indigenous strain of Bacillus species to prevent food spoilage. The indigenous strain of Bacillus

species was an isolate of rhizosphere soil. This strain was identified by cultural characteristics; Gram's staining reaction, and spore staining procedure. Biochemical tests included motility test, citrated utilization test, catalase test, oxidase test and ampicillin resistance test. Antagonistic effects of this strain were determined against Bacillus and Staphylococcus species using spot test. Biopreservative effects were determined with tomato pulp. Results demonstrated that antibacterial substance produced from the indigenous strain of Bacillus species was active against both Bacillus and Staphylococcus species. Addition of culture of the indigenous strain of Bacillus species prevented the spoilage of tomato pulp in laboratory environment at room temperature. Our results are promising for use of the indigenous strain of Bacillus species as a biopreservative strain in food industry. However, further molecular characterization need to be done in future studies.

CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILING OF BACTERIA ISOLATED FROM DIFFERENT COSMETIC PRODUCTS

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Cosmetic products comprise essential minerals, growth factors, organic, inorganic compounds and humidity which provide suitable conditions for growth of microorganisms. Cosmetic products may be contaminated by three ways; (1) application of unsterile raw material as ingredients (2) in the course of production process (3) during use of cosmetics. Although the microbial standards of cosmetics have been progressively improved, the contamination has been frequently reported and even in some cases, has created serious problems for consumers. The aim of present study was to isolate and identify bacteria from Cosmetic Products and to assess the antibiotic susceptibility patterns of isolated bacteria. Cosmetics samples selected for investigation belonged to Mascara and Eye liner. Samples were collected from saloons as well as homes used by either single person or used in sharing with other family members. Identification of recovered bacterial strains was carried out on the

basis of cultural, morphological, biochemical methods. A total of 14 Mascara samples including 05 samples from saloon (shared), 04 samples from home based single users, and 05 from home based multiple users and 8 samples of eye liners which included 03 samples from saloon (shared), 02 samples from home based single users and 03 samples from home based multiple users were investigated. Among Mascara, 12 samples yielded bacterial growth (*n*=28) of both gram positive and gram negative bacteria while 02 were negative. While 04 eyeliner samples contained bacteria (*n*=13) and 04 sample were sterile. Overall 41 bacterial isolates, 28 from Mascara and 13 from eye liners were recovered and characterized. In conclusion, shared cosmetics samples were found contaminated with bacteria while majority of home based single users (non-shared) samples were sterile suggesting that sharing the cosmetic products may render them susceptible to bacterial contamination and could serve a source of bacterial dissemination leading to skin infections.

ANALYSES OF GENETIC VARIATIONS IN HYPOXIA-ASSOCIATED GENE CARBONIC ANHYDRASE 9 (*CA9*) IN SQUAMOUS CELL CARCINOMA OF LUNGS AND ESOPHAGUS

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Squamous Cell Carcinoma (SCC) originates from the epithelial cells, lining various organs including respiratory and digestive tracts. Ranked as the third and fourth most prevalent cancers in Pakistan, lung and esophageal SCC pose a serious threat to health. High energy and metabolic demands within the rapidly proliferating tumor cells may lead to hypoxia which causes pH imbalance in tumor microenvironment. Expression of key pH regulatory proteins in hypoxic tumors may play a prominent role in the survival and invasiveness of different types of malignant tumors. *CA9* gene encodes for a membrane-bound protein called Carbonic Anhydrase 9 that is overexpressed in tumor cells in response to hypoxia-mediated

pH shift. CA9 protein has been reported to play pivotal roles in maintenance of the intracellular pH and might help in metastasis of tumor cells through the acidification of extracellular pH. Genetic variations in *CA9* have been of research interest due to their prominent functions in cancer progression and invasion. The designed study is targeted to investigate the genetic variations in *CA9* and their associations with lung and esophageal SCC. After the approval for the use of human subjects from institutional ethical committees of the concerned institutes, blood samples from a total of 150 patients with lung and esophageal SCC and from the same number of age and sex matched controls will be collected. Genomic DNA will be extracted through standard phenol-chloroform method. Genetic variations will be assessed through tetra-primers Amplified Refractory Mutation System Polymerase Chain Reaction (t-ARMS PCR). Direct DNA sequencing will be used to validate the findings and their associations with SCC will be analyzed through statistical tools. The outcomes of this study may bring better insights for prognostic biomarkers in patients with SCC of lungs and esophagus.

EVALUATION OF HEALTH-RELATED QUALITY OF LIFE USING SF-36 IN A POLYCYSTIC OVARIAN SYNDROME DIAGNOSED AND CONVALESCENT FEMALE

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Health-related quality of life (HRQol) is a multidimensional concept used to describe physical, emotional, and social aspects of particular diseases or their treatment. Females afflicted with Polycystic ovarian syndrome may have negative modulations on the quality of life not only because of the hormonal disturbances but also because of adverse clinical complications including reproductive (menstrual irregularity and infertility), metabolic (insulin resistance, diabetes, and cardiovascular risk), and psychological disabilities (anxiety and depression). Although the patho-physiology with respect to polycystic ovarian syndrome in 15%–20% of women at reproductive age might be obscure but have elucidated as factors responsible for the reduction of life

quality. After obtaining the informed consent SF-36 tool was administered to the woman diagnosed with polycystic ovarian syndrome. This was re-administered after her treatment of 6 months when her patho-physiology was reinstated. The eight health domains were recorded, coded and scored according to established criteria. The scoring demonstrated that physical functioning, role limitation due to physical health, energy, emotional well-being, and general health were improvised after treatment, however, role limitation due to emotional problem were not modulated and pain experienced was ameliorated. The results are discussed in terms of the hormonal induced alleviation of depression and improvised health related quality of life.

ANTITUSSIVE EFFECT OF SOME MEDICINAL PLANTS ON SULPHUR DIOXIDE INDUCED COUGH IN ANIMAL MODEL

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Natural products have been used by human beings for treating different pathological conditions since the time immemorial. Numerous plants have been reported to have antitussive activity. The aim of our present study was to evaluate the ethanolic extract of *Arenaria serpyllifolia* (Carophyllaceae) whole plant, *Caesalpinia pulcherrima* (Caesalpiniaceae) aerial parts and *Fragaria nubicola* (Rosaceae) whole plant for antitussive activity at the doses of 200 and 400 mg/kg in healthy albino rats. Cough is a symptom and also a defensive reflex of removing the noxious, irritating substances and pathogens from larynx, trachea and bronchi. Cough is usually considered as the disease, related to lungs. A number of substances are responsible for stimulation of cough reflexes. Among most common are dust, histamine and bronchoconstriction.*Arenaria serpyllifolia* is a small annual herb commonly called Thyme leaved sandwort, found in uncultivated land, arid meadows and cliffs. *Caesalpinia pulcherrima* is an ornamental plant usually 3.7-4.3 m in height known as Peacock flower. *Fragaria nubicola* grows in wooded valleys, forest margins and meadows. It is commonly

known as wild strawberry. These plants have been used traditionally for cough suppression by different societies. In this study cough was induced by sulphur dioxide induction method. Animals were divided into eight groups of ten animals each and all the drugs were administered orally. Group I served as control group while group II served as standard. A dose-dependent inhibition of cough was observed for all extracts. At doses of 200 and 400 mg/kg all three extracts were proved highly significant compared to standard. So it proves and supports traditional use of plants for relieving cough.

MOLECULAR CHARACTERIZATION OF ANTIBIOTIC RESISTANCE AND VIRULENCE FACTORS GENES FROM GUT FLORA ISOLATES OF BROILERS

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The emergence of multidrug resistance poses serious global health and environmental crisis. Antibiotics are being used therapeutically for infection treatments and sub-therapeutically in agriculture, poultry and livestock. In poultry, these are being administered as antibiotic growth promoters (AGPs) prophylactically in feed for disease prevention, increased feed efficiency and weight gain. It is suggested that the intensive use of antibiotics in clinical settings and agriculture sector has led to the wide spread dissemination of antibiotic resistance (AMR) in microbes. Chicken gut harbors complex microbial communities having distinct role in host health and performance. The AGPs positively support poultry production and growth, they also exert selective pressure on the gut micro flora for the establishment of resistance genes. This resistance could be natural or acquired by the environment through horizontal gene transfer. These virulence factors are also thought to be acquired by commensal flora in association with resistance determinants. This may lead to the wide spread of resistant and virulent flora in the chicken gut and environment ultimately affecting human health. *Escherichia coli* is a human and avian opportunistic pathogen found

in chicken gut and is used as bio indicator for AMR monitoring of the environment. It is important to assess the risk of food animal-related AMR and virulence factors on public health. So, this study aims to establish antibiotic susceptibility profile of *E. coli* isolates from chickens that were previously grown on different diet groups, by disc diffusion assay. The detection of antibiotic resistance and virulence genes will be performed using multiplex PCR. To further investigate the acquired origin of resistance plasmid isolation and conjugation assay of resistant isolates will be performed. The findings of this study may provide critical information related to the dissemination of MDR *E. coli* strain of poultry origin.

MUTATIONS IN *KIF14*, ENCODING A MICROTUBULE MOTOR PROTEIN, IS A NOVEL CAUSE OF AUTOSOMAL RECESSIVE PRIMARY MICROCEPHALY

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Autosomal recessive primary microcephaly (MCPH) is a rare neurodevelopmental ailment characterized by a reduced brain volume and mild to severe level of intellectual disability. Cranium circumference is reduced below –4SD with preserved cerebral architecture and simplified gyral pattern. Genes encoding proteins

implicated in MCPH etiology are core components of centriole, mitotic spindle poles, kinetochore, and chromatin remodeling complex. Recently, cleavage furrow and mid body component (CRIK) is reported to be implicated in pathogenesis of the disease. This study was aimed to quest the involvement of novel MCPH genes and their functional exploration. Four unrelated families, 2 from Pakistan, 1 from Saudi Arabia and 1 from Germany were subjected to linkage analysis and whole exome sequencing to identify the causal variants. We identified 3 homozygous mutations in *KIF14* (NM_014875.2; c.263T>A; p.Leu88*, c.2480_2482delTTG; p.Val827del, and c.4071G>A; p.Gln1357) as the likely cause in Pakistani and Saudi families. Whereas in patient of German family, we identified compound heterozygous missense mutation in *KIF14* (NM_014875.2; c.2545C>G; p.His849Asp and c.3662G>T; p.Gly1221Val). Analyses at RNA as well as protein level were performed to functionally explore the consequences of identified mutations. Three of the 5 identified mutations impaired splicing, and 2 presumably resulted in a truncated protein. KIF14, a microtubule motor protein, interacts with CRIK at midbody to complete the cytokinesis in mitotic cells. All identified mutations impaired the localization of KIF14 as well as CRIK at midbody in patient fibroblasts. Other abnormalities observed in KIF14 deficient cells were significantly increased number of binucleated and apoptotic cells as consequence of cytokinesis failure. Thus, our findings suggest the KIF14 as novel cause of microcephaly by impairing the cytokinesis.

MESENCHYMAL STEM CELLS DIFFERENTIATION INTO HEPATIC-LIKE CELLS IN THE PRESENCE OF HISTONE DEACETYLASE INHIBITORS

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Liver diseases are one of the main causes of death worldwide. Currently, Liver transplant (LT) is the only available option. However, it has limitations in term of high cost, shortage of liver donors, long term use of immunosuppressive drugs and surgical complications. In recent times, stem cells become more attractive candidates to cure liver diseases. Stem cells differentiated into hepatic-like cells using hepatic modification medium. However, efficient method of hepatogenic differentiation of stem cells is yet need to be investigated. Histone deacetylase inhibitor (HDACi) suppresses the activity of histone deacetylase (HDACs) thus promotes the access of transcription factors to chromatin and enhance gene expression. The aim of this study was to investigate the role of HDACi in the differentiation of stem cells into hepatic-like cells. To conduct this study MSC were isolated from rat bone marrow and characterized on the basis of the presence of cell surface markers by immunocytochemistry. In order to initiate the hepatogenic differentiation, MSCs were treated with HDACi. Analyses of hepatogenic potential of MSCs treated with HDACi were done by RT-PCR and immunocytochemistry. The HDACi treated MSCs have shown increased expression of different hepatic genes and proteins. These hepatic-like cells may proliferate and contribute to the regeneration of damaged liver and may have an expended future applicability in drug screening.

SYNERGISTIC SACCHARIFICATION OF CELLULOSIC SUBSTRATES BY PROCESSIVE TCEL5A1 AND β-GLUCOSIDASE (BGLA) OF *THERMOTOGA MARITIMA*

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The implementation of the enzymatic cocktail for improved saccharification of cellulose substrates to yield glucose as a major product is found to be a very cost-effective procedure for biofuel production. This study reports the synergism between β-glucosidase (bglA) and tCel5A1 of *Thermotoga maritima* during the hydrolytic reaction of insoluble cellulose substrates. Results of this study indicated that combined action of β- glucosidase and tCel5A1 exhibited cellulose conversion rate of 49% with amorphous cellulose, 44% with avicel, 65% with rice straw, liberating 16%, 20%, 25% glucose respectively. Although the liberated glucose inhibited the β- glucosidase but still the synergistic actions of two enzymes produced pronounced cellulose hydrolysis as compared to individual enzymes. Evaluation of the end product effect demonstrated that glucose inhibited bglA at 400mM concentration while induced the activity of tCel5A up to 3 folds at 200mM concentration. Increased activity of tCel5A1 released cellobiose which in turn stimulated bglA. The stimulatory effect of glucose on tCel5A1 is responsible for increased cellulose conversion rate and the higher glucose yield in reaction makes it a good enzymatic cocktail for the saccharification of lignocellulosic biomass.

IDENTIFICATION OF A GENETIC BIOMARKER SPECIFIC FOR THE HETEROZYGOSITY OF FANCONI ANEMIA INDIVIDUALS IN PAKISTANI FAMILIES

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Fanconi anemia (FA), with an onset at a median age of seven years or more, is a rare autosomal recessive syndrome which includes various congenital abnormalities and physical anomalies including hematological manifestations with predisposition to cancer. The underlying deficiency in FA patients is the ability to repair DNA Inter-strand Cross Links (ICLs). FA cells are hypersensitive to DNA crosslinking agents such as Diepoxybutane (DEB) and mitomycin C which provide a valuable laboratory test for supporting clinical diagnosis. Genes which are reported to play vital roles in prognosis of this syndrome include *FANCA*, *FANCC* and *FANCG*, among which *FANCA* is the one which harbors two thirds of all pathogenic variants. *FANCA* is the most frequently mutated gene with biallelic variation of approximately 65%, while *FANCC* and *FANCG* account for 20% of all the mutations in FA patients as well as in carriers of the disease. The aim of this study is to analyze and screen the mutations which are found in carriers of the disease. This study will be approved by the Institutional Review Boards of both the institutes and the hospital. Blood samples will be collected randomly from affected families after taking written informed consent from the participants. Cytogenetic analysis will be carried out for the diagnosis of patients. Extracted DNA through phenol chloroform method will be amplified by conventional PCR followed by sequencing and sequence analysis. Mutations will be analyzed using bioinformatics tools. Results will be interpreted on the basis of comparison of mutations in patients and their families with the association of consanguinity and pedigree analysis. This study may provide strong evidence to identify silent carriers of FA and may helpful for the prevention of further births with FA in Pakistan. The findings of this study may also lead to increased awareness of susceptibility to FA in Pakistan.

ASSOCIATION OF GENETIC VARIATION(S) IN *PGRN* GENE WITH LEISHMANIASIS

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Leishmaniasis is a neglected tropical disease caused by intracellular protozoan parasite *Leishmania*. It is transmitted by the bite of infected female *phlebotomus* sandfly. World Health Organization has reported Leishmaniasis as seventh most important tropical diseases. In humans, the host for the parasite are macrophages that play an important role in immune system. It has also been reported that activation of TNF-α pathway promote the killing of parasite by producing microbicidal nitric oxide molecule. Inhibiting TNF-α pathway by blocking its receptors may increase the chance of parasite survival in macrophages. Progranulin (*PGRN*) is a naturally occurring growth factor gene which is highly expressed in epithelial cells and has been involved in inhibition of TNF-α signaling pathway by blocking its receptors. The aim of the study is to investigate the variation(s) in *PGRN* gene with its susceptibility towards Leishmaniasis. The procedures will be approved by the regulations of institutional ethical committees of concerned institutes for the use of human subjects in research. Total 200 individuals, 100 patients and 100 healthy controls will be included in the study. Blood samples will be collected after taking written informed consent from the subjects. DNA will be extracted by standard phenol-chloroform method. Specific regions of *PGRN* gene will be amplified by PCR. DNA sequencing will be done for the identification of genetic variations. Identified variations from the sequencing will be further checked through restriction fragment length polymorphism (RFLP). Results will be analyzed by using bioinformatics and statistical tools. Genetic variations in *PGRN* gene might be helpful in understanding etiology of Leishmaniasis. This may also be helpful in finding pathogenic associations of *PGRN* gene related to Leishmaniasis.

EFFECTIVENESS OF MAGNESIUM SUPPLEMENTATION ON 2,4-DICHLOROPHENOXYACETIC ACID INDUCED RENAL TOXICITY USING EXPERIMENTAL MODEL

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2,4-Dichlorophenoxyaceticacid (2,4-D) is an extensively used herbicide primarily to control broad leaf weeds and secondarily act as a plant growth regulator in the field of agriculture. Its vast application induces toxicity, health effects, and ecological obstacles on both human and animals. Magnesium (Mg) supplementation having antioxidant properties plays a vital role by directly influencing the metabolic and physiological processes. The main objective of present study was to investigate the antioxidant effectiveness of Mg supplementation on 2,4-D induced renal toxicity in rat model. Rats were randomly divided into four even groups (n=10). Group I: served as a control; no treatment, Group II: received 2,4-D (150 mg/kg body weight), Group III: received Mg supplement (100 mg/kg body weight), Group IV: received 2,4-D (150 mg/kg body weight) and Mg supplement (100 mg/kg body weight) simultaneously. The effects of 4 weeks oral administration showed the preventive effects of Mg supplementation against 2,4-D induced renal dysfunction as manifested by decrease renal biomarkers (plasma urea and creatinine) and Malondialdehyde (MDA) levels. Further, Mg caused improvement in antioxidant enzyme activities including Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Reductase (GSH). This study concluded that supplementation with Mg plays an essential role in the prevention of renal toxicity provoked by 2,4-D.

ROLE OF 5-HT1A RECEPTORS IN THE REINFORCING EFFECTS OF MIDAZOLAM

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An important role of 5-hydroxy tryptamine (5-HT; serotonin)$_{1A}$ receptors is there in the pathophysiology of anxiety and addiction. A supersensitivity of these receptors may impair adaptation to stress and lead to depression. Whereas, a desensitization of these receptors is suggested to be helpful for adaptation to stress and attenuation of addiction. Present study was designed to monitor midazolam-induced dependence in rats after giving the challenge of 8-OH-DPAT (5-HT$_{1A}$ agonist) on day 13. Experiment was conducted in two phases and midazolam was experienced repetitively in conditioned place preference paradigm for 12 days. Activities in novel and familiar environments, as well as daily food intakes and body weights were recorded. Role of 5-HT$_{1A}$ receptors was monitored on midazolam-induced behavioral effects, by injecting 8-OH-DPAT on day 13. 5-HT syndrome was monitored. Findings from the present study could be implicated to increased therapeutic utility of midazolam and related benzodiazepines.

CONSTRUCTION OF RECOMBINANT FUSION PROTEINS OF *MYCOBACTERIUM TUBERCULOSIS* FOR ENHANCING SENSITIVITY OF TB SERODIAGNOSIS

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Tuberculosis (TB) is a major global health problem mostly prevalent in developing countries like Pakistan and causes high rates of human mortality and morbidity worldwide. For effective TB control there is an urgent need for the development of more

rapid, econommical and sensitive diagnosis. Serodiagnosis of *Mtb* antigens is obstructed by the variable antibody responses in TB patients. The sensitivity of serodiagnostic tests can be enhanced by developing fusion molecules consist of two or more *Mtb* antigens containing multiple epitopes for relative antibody interactions. To achieve this goal, fusion proteins were cloned and soluble expression achieved by joining AgX (heat shock protein) at N-terminus of the fusion molecule. Polyclonal antisera were raised against each native antigen and immunogenicity was checked through ELISA. Furthermore, immunoreactivity of fusion proteins was evaluated through Western Blotting. The serodiagnostic potential of native and fusion antigens will be analyzed through ELISA. Data from the present study will be useful in the development of highly immunogenic molecules for diagnosis of TB.

POINT MUTATION(S) ANALYSIS IN CHECKPOINT KINASE 2 (*CHEK2*) GENE: AN ASSOCIATION WITH BREAST CARCINOMA

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Breast cancer is the second most frequent type of cancer reported around the world. According to "Cancer Facts Sheet 2018" published by WHO, breast cancer is 4th common cancer responsible for cancer related deaths. Diagnosis of breast cancer at early stage is almost impossible, that's why the treatment of breast cancer at later stages not entirely possible. Nowadays genetic biomarkers are used to diagnose the breast cancer at onset stage. Genetic inconsistency of these biomarkers makes it difficult to diagnose breast cancer in every population with same biomarkers. Several genes are found to be associated with breast cancer and most of them regulate the cell cycle, DNA repair mechanism and apoptotic pathway. Checkpoint Kinase 2 (CHEK2) activate by Ataxia Telangiectasia Mutated (ATM) protein is response to DNA damage. If DNA damage is minor it activate the DNA repair

mechanism, if DNA damage is major then apoptotic pathway activate which facilitate the apoptosis of DNA damaged cell. Mutations in CHEK2 gene disrupt these pathways and facilitate the continuous growth of mutant cell. The aim of the study is to analyze the point mutations in CHEK2 gene responsible for breast cancer progression. Blood samples of breast cancer patients will be collected in the study. The samples will be compared with age and sex matched controls. Extraction of DNA will be done by standard phenol chloroform method. Tetra-Primer Amplified Refractory Mutation System (t-ARMS) PCR will be performed by using allele specific primers. Genotypic analysis will be performed to find point mutations in targeted gene and their association with breast cancer. DNA sequencing will be used for further validation. The current study might be helpful in finding diagnostic biomarker to improve the genetic diagnosis which may be an integral tool to elaborate the etiology of breast carcinoma.

APOMORPHINE-INDUCED SENSITIZATION IN RATS EXPOSED TO RESTRAINT STRESS: RELATIONSHIP WITH ADAPTATION TO STRESS

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Drug abuse and impaired adaptation to stress are inter-related. Drug abuse is more potentiated upon exposure to stress and an impairment to cope with stress may lead to depression. On the other hand, use of addictive compounds increase the vulnerability to depression by inhibiting the adaptation to stress. The current study was designed to monitor association between tolerance to repeated restraint stress and sensitization induced apomorphine. Prior or later injections of apomorphine were given to monitor effects of restraint stress episode on apomorphine-induced sensitization and place preference. Apomorphine-induced sensitization and place preference were enhanced when

apomorphine was experiencing during restraint stress. Conversely, sensitization and place preference were attenuated when apomorphine was experiencing after restraint stress. It exhibits that if experienced during restraint stress, apomorphine, potentiates sensitization. Sensitization produced by apomorphine was inhibited in rats injected with apomorphine after restraint stress termination. Results therefore, tend to show that drug of abuse could serve as treatment options for stress-induced depression but could not be used for the prevention of very same.

MTHFR C677T GENETIC VARIATION IN CONGENITAL SEPTAL DEFECT PATIENTS IN KARACHI, PAKISTAN

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Development of heart is the primary biological process and first indicator of life. Congenital heart defects (CHDs) refer to the defects in the structure of the heart and great vessels present at birth, affected by both genetic and environmental factors. Among CHDs septal defects accounts for 40%, include Atrial Septal Defects (ASD), Ventricular Septal Defects (VSD) and Atrioventricular Septal Defects (AVSD). Methylenetetrahydrofolate reductase (MTHFR), acts as a crucial enzyme for the metabolism of folate, *MTHFR* gene is located at 1p36.3, the missense mutation, C677T (rs1801133), results in a thermolabile variant of the *MTHFR* with reduced enzymatic action. *MTHFR* C677T has strong association in adult cardiac diseases. The aim of the study is to identify the association of *MTHFR* C677T variants with congenital septal defects. Genotypic and phenotypic correlation will be established. Samples (150) will be collected from National Institute of Cardiovascular Diseases (NICVD) Karachi, of non-syndromic, non-familial patients after the confirmation of pediatric consultant and control (150). DNA samples will be extracted, and mutation screening will be done through high resolution melting (HRM). HRM is a relatively new PCR based method to analyse DNA melt

curves. Genotyping of known mutations can be done with high specificity and sensitivity. This study can be further applied in genetic diagnostics.

ROLE OF OXIDATIVE STRESS IN BREAST CARCINOGENESIS

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Globally in 2018, rate of cancer incidence and related deaths elevated up to 18.1 million and 9.6 million respectively. It is estimated that the frequency of the disease will rise to 29.5 million by 2040. Including all types of cancer, breast carcinoma (BC) is the most common cause of incidence (11.6%) and mortality (6.6%) among females worldwide. Discovery of precise biomarker is immediately required to identify novel diagnostic and therapeutic targets. Pakistan has highest rate of BC in Asian region. Improved quality of life and disease-free survival is not achieved by current available therapies. It may be due to inaccurate molecular mechanism that defines genetic vulnerability to BC. Among various risk factors oxidative damage pose great stress in development of breast malignancy. Oxidative stress is the accretion of reactive oxygen species (ROS) weakly encountered by antioxidant defense system. Oxidative stress plays a potential role in accelerating irregular cell multiplication. Amount of ROS that are generated in normal metabolic conditions are increased during disease condition. It affects normal function of signaling pathways, which in turns trigger abnormal cell division. Gene polymorphism of Paraoxonase-1 (PON), an antioxidant enzyme has been the focus of study in many life-threatening diseases. The purpose of current study is to examine possible association of an antioxidant enzyme with the increased incidence rate of BC among local population. The correlation of PON and lipid peroxidation is measured in BC patients as compare to normal individuals. L55M gene polymorphism was also observed. These findings deliberate the association of oxidative stress in the progression of BC that may contribute to develop better therapeutic strategies.

GENOTYPE EXTENDED FRAMEWORK OF MDR *PSEUDOMONAS AERUGINOSA*: A POTENTIAL CAUSE OF NOSOCOMIAL INFECTION

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Pseudomonas aeruginosa is considered as one of the major adaptable pathogens that is associated with severe and life-threatening conditions especially in immunocompromised individuals. Infections caused by *P. aeruginosa* are particularly difficult to treat, as the organism is both intrinsically resistant and capable of acquiring resistance (through mobile genetic elements) to most antibiotics. According to Center for Disease Control and Prevention (CDC), in U.S approximately 6,000 patients are infected with *P. aeruginosa* annually. *P. aeruginosa* develops resistance against almost all antibiotics by several mechanisms like multi drug resistance efflux pump, resistance genes and biofilm formation. Furthermore, exposure to broad spectrum antibiotics have added to the rapid increase in isolation of resistant strains. This study is designed to analyze the genetic variants of *Pseudomonas aeruginosa* by sequencing. The procedures will be approved by the regulations of institutional ethical committees. Samples will be collected from associated diagnostic laboratories and tertiary care hospitals in Karachi. All collected samples will be biochemically characterized based on biochemical markers. Antimicrobial susceptibility test will be performed followed by DNA extraction. Sequencing will be carried out for targeted genome sequencing and gene expression profiling. To validate our results bioinformatics analyses will be carried out. The findings of the study may provide precarious information related to the discovery of potential drug targets and vaccine epitopes in multi drug resistant *P. aeruginosa*.

NEUROPHARMACOLOGICAL AND NEUROCHEMICAL STUDIES ON METHYLPHENIDATE INDUCED LEARNING ACQUISITION AND MEMORY RETENTION IN RATS

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Psychostimulants are a class of drug whose pharmacological effects such as anorexia, enhanced arousal and improved performance are therapeutically important. The therapeutic use of these drugs (e.g. methylphenidate) as cognitive enhancers is limited because of abuse potential associated with long term use. Serotonin and dopamine are neurotransmitters which are known to have a role in the modulation of synaptic transmission in the central nervous system and are also involved in cognition and addiction. The brain regions such as prefrontal cortex and hippocampus play important role in processing information associated with learning and memory. The present study is designed to monitor the effects of methylphenidate (2.5mg/kg and 5mg/kg) on spatial memory in Morris water-maze test. Levels of dopamine (DA), Dihydroxphenyl acetic acid (DOPAC), Homovanillic acid (HVA), 5-Hydroxytryptamine (5-HT), 5-Hydroxyindole acetic acid (5-HIAA), and Noradrenaline-hydrochloride (NA-HCl) are monitored in the hippocampus and prefrontal cortex. It is found that clinically relevant doses of methylphenidate (2.5 mg/kg) improved memory acquisition and its retention but higher dose (5 mg/kg) impaired both. Food intake and body weight changes are not affected by methylphenidate administration due to short-term administration of the drug. Result shows an increase in 5-HT metabolism in prefrontal cortex of rats treated with 2.5mg/kg methylphenidate as well as hippocampus. An effect of DA neurotransmission is higher in case of 2.5mg/kg methylphenidate treated rats as compare to 5 mg/kg methylphenidate treated rats. HVA levels are decrease both in hippocampus and prefrontal cortex for both doses. Initial study will help to find out signal transduction mechanism associated with

learning acquisition and memory retention and to develop non-addictive therapeutics for cognitive impairment.

OXIDATIVE STRESS: A CONTRIBUTOR FOR PROGRESSION OF OSTEOARTHRITIS IN PAKISTANI POPULATION

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Globally, more than 150 musculoskeletal conditions have been diagnosed that distress the locomotor system. Osteoarthritis, rheumatoid arthritis, fragility fractures, back and neck pain are amongst the most disabling musculoskeletal conditions that affect the wide range of age group from adolescent to elderly people. Osteoarthritis (OA) is a degenerative joint disease with major socio-economic impact. In Pakistan, It has been reported that 25% rural and 28% urban population suffered from knee osteoarthritis with the ratio of 1:4 male to female respectively. Diagnosis and treatment at initial phase of infirmity are still hot area of research. However non-curative interventions such as exercise and psychological remedies might be helpful to manage the sicknesses. The risk factors for developing OA are mechanical stress, joint injury, age, gender**,** obesity and genetics. Among these factors oxidative stress is another focused feature found to be associated with the disease. Mean of access through which reactive oxygen species (ROS) contribute to OA pathology is still unknown and it requires further investigation. There is a need to study the role of antioxidants in OA since they may be supportive to provide significant insight in the origination and development of disease in local population. The rationale for the present study is to explore the association of oxidative stress as a contributing factor for the progression of disease. Focus of the current study is the determination of single nucleotide polymorphism in gene of antioxidant enzyme along with the level of Malondialdehyde (MDA). We have observed a possible association between L55M polymorphism and osteoarthritis. Level of MDA, a potential

biomarker for lipid peroxidation was measured and found increased in lipid peroxidation products in OA patients as compared to healthy individuals.

ANALYSES OF GENETIC VARIATIONS OF LONG NON-CODING RNA (lncRNA) IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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Oral cancer is the eighth most common cancer in the world and third most common in Asia. The incidence rate of oral cancer in Asia is much higher due to chewing habits of paan, chaliya, gutka, raw tobacco, mainpuri and niswar. It has been reported that genetic alteration such as changes in gene expression of tumor suppressor genes and oncogenes lead to the development of several cancers. A long non-coding RNA (lncRNA) named as HOXA distal transcript antisense RNA (*HOTTIP*) has emerged as a molecule involved in human carcinogenesis. *HOTTIP* is located at 5' end of HOXA cluster. *HOTTIP* plays an important role in cancer by regulating the expression of its neighboring HOXA genes. The aim of the study is to investigate the genetic alterations of *HOTTIP* with oral squamous cell carcinoma (OSCC). The procedures will be approved by the regulations of institutional ethical committees of concerned institutes for the use of human subjects in research. Samples will be collected from patient after taking written informed consent from different hospitals of Karachi. Blood and tissue samples of 250 patients will be included in the study. The samples will be compared with aged and sex matched controls. DNA will be extracted by standard phenol chloroform method. Tetra primers amplified refractory mutation system (t-ARMS) PCR will be performed to identify targeted genetic variations. Direct DNA sequencing will be used for further verification. Results will be analyzed by bioinformatics and statistical tools. The findings of this research might improve the understanding of the genotypic association of HOXA genes cluster in critical regions. This may also

be helpful in possible pathogenic implications of the unusual findings related to OSCC.

COMPARATIVE ANALYSIS OF DEPRESSION, COPING SELF-EFFICACY AND WAYS OF COPING IN A RECOVERED CASE OF POLYCYSTIC OVARIES SYNDROME: A CASE STUDY

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Depression, a psychopathological state involving a triad of symptoms with low or depressed mood, anhedonia, and low energy or fatigue, has been observed in females afflicted with Polycystic ovarian syndrome (PCOS). Polycystic ovarian syndrome is a health problem that affects women of child bearing age which is characterized by hormonal imbalance in terms of altered Follicle stimulating hormone (FSH) with an array of other problems leading to infertility. The present case study employed Zung self-rating depression scale, coping self-efficacy scale and the Ways of Coping scale to assess the depression, perceived ability to cope stress and coping mechanisms respectively before and after the treatment in a 23-year old Polycystic Ovarian Syndrome participant. Consent based participation of the candidate revealed that her score for depression was towards the upper bound of the Normal range (25-49) during the phase of Polycystic ovarian syndrome that further lowered after the treatment. Though, Polycystic ovarian syndrome is accompanied by the depressive symptoms but the participant's high coping self-efficacy that further enhanced after curing of Polycystic ovarian syndrome determine her effort, persistence, and strategy in the accomplishment of tasks thus preventing her to fall prey to depression. During the phase of Polycystic ovarian syndrome, the participant's coping process is predominantly characterized by self-blame followed by seeking social support and problem focused coping. The improved self-efficacy after treatment is supported by the shift of coping process to majorly focusing on the positive followed by seeking social support and wishful thinking.

In the multi-disciplinary era, the present case study helps to elucidate the importance of psychological wellbeing to combat and manage the biochemical changes while facing diseases like polycystic ovarian syndrome.

SALIVARYPROTEASE ACTIVITIES IN CARIES HEALTH AND DISEASE

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Caries is a common problem of oral cavity. It can result due to poor oral hygiene causing increase in microbial load and activity. Saliva is the oral fluid that harbors all valuable biomolecules of oral cavity hence is a reflection of oral states, health or disease. In the cavity the proteins such as proteases are derived from human glands and the oral microbial flora or originating from oropharyngeal mucosae and cervical fluid. These enzymes are important as they play varied important roles in metabolic activities making the study of proteases meaningful in the oral cavity. In this study we analyzed the protease activity based on polyacrylamide gel electrophoresis. The saliva from healthy and caries patient were collected. After protein estimation equal amounts are applied to zymographic investigation. The band patterns were observed and compared for interpretation of outcomes.