Centre of Excellence in Molecular Biology
University of the Punjab, Lahore

International Conference on

Trends and Challenges in Health Sciences

March 1-2, 2023

Venue:
Centre of Excellence in Molecular Biology (CEMB),
University of the Punjab
87- West Canal Bank Road, Thokar Niaz Baig, Lahore, Pakistan

Themes

- Stem Cell & Regenerative Medicine
- Molecular Virology
- Biopharmaceuticals
- DNA Sequencing
- Forensic Sciences
- Cancer Genomics
- Genetic Diseases

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Registration Fee

Students	2000 PKR
Academia	3500 PKR
Industrialists/ Others	5000 PKR

Accommodation (If Required)

Sharing based: 1500 PKR (per night)
Individual Room 3000 PKR (per night)
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Link for Online Application forms:

https://tinyurl.com/ICHS-2023

Call for Abstracts Oral Presentation/ Posters is open till 6th Feb 2023.

For correspondence and abstract submission:
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Symposium Language

English would be official language of symposium.

Oral Presentation

Time allocated for each talk is 15 minutes including discussion. Speakers are urged to prepare their talks in power point.

Abstract & Full Papers

The organizing committee cordially invites researchers across world to submit their abstracts for oral talks. The abstract must not exceed 250 words.

Poster Presentation

The organizing committee cordially invites researchers worldwide to submit their abstracts for poster presentations. The abstract must not be exceed 250 words.

Poster Dimensions:

Poster size should be 5X2 feet. All participants should bring their own standees for poster display.

ALS (ISN 2310-5380)

All the oral and poster abstracts will be published in Proceedings in the international HEC recognized journal "Advancements in Life Sciences".

Plenary Talk

Receptor tyrosine kinase-mediated resistance to PARP inhibitors in human cancer and therapeutic strategies to overcome it

Hirohito Yamaguchi*

Poly (ADP-ribose) polymerase (PARP) is a critical regulator of DNA single-strand break repair, and several inhibitors for PARP have been developed and currently used for treatment of breast and ovarian cancers. In particular, PARP inhibitors show higher response rate in patients carrying BRCA mutations, which are crucial for homologous recombination (HR). However, still high percentage of patients with BRCA-mutated cancers do not respond to PARP inhibitors. Therefore, in order to develop strategies to make PARP inhibitor treatment more effective and to identify biomarkers to stratify patients, it is critical to understand the underlying mechanisms of PARP inhibitor resistance. In our previous study, we have identified that receptor tyrosine kinases (RTK) including c-MET and EGFR directly phosphorylates PARP, which causes PARP inhibitor resistance. Moreover, we recently showed that another RTK, ALK is also involved in PARP inhibitor resistance through the upregulation of HR-related genes. Importantly, the inhibitors for these RTKs are currently used in clinic for various cancer types, and the combination of PAPR inhibitors and the RTK inhibitors can overcome the resistance induced by these RTKs. Moreover, expression and activation of these RTKs would serve as biomarkers to select the appropriate drug combination. Thus, our study is expected to provide the potential treatment strategy to overcome PARP inhibitor resistance.

Clinical translation from transcriptome pattern recognition to an oral cancer diagnostic test

Muy-Teck Teh*

All cellular processes are tightly regulated by a complex network of interacting biomolecules. Given that mRNA transcription precedes protein translation, the change in gene expression often precedes visible pathological manifestation. Hence, transcriptome instability, measured as differential gene expression (DEG), may contain gene signatures that could be exploited to predict or detect disease initiation and progression. With an aim to identify molecular signatures for early detection of oral cancer, Dr Teh explored the transcriptome profiles of normal oral keratinocytes, dysplastic and oral squamous cell carcinoma cells to identify DEG biomarkers. This led to the identification of a panel of 16 genes which were subsequently translated into a molecular diagnostic test - "quantitative Malignancy Index Diagnostic System (qMIDS)" for early oral cancer detection. The qMIDS test involves using reverse transcription quantitative PCR (RT-qPCR) to measure 16 genes implicated in the regulation of the cell cycle, genomic stability, chromatin maintenance, stem cell regulation, matrix and immune modulation. An algorithm then converts gene expression levels into a clinically meaningful quantitative malignancy index for cancer risk stratification. In 2013, we validated the first version of qMIDSV1 test for quantifying the aggressiveness of oral, vulva and skin squamous cell carcinoma patients from the UK and Norway. Subsequently, qMIDSV1 was independently validated in China on 68 OSCC patients. Due to mediocre diagnostic performance of the original qMIDSV1, we re-formulated the biomarker panel generating an improved qMIDSV2 with significantly better diagnostic performance (>90%). In 2022, we validated our qMIDSV2 through an international multi-cohort validation study involving 535 clinical tissue specimens from geographically diverse ethnic groups from Europe and Asia. This talk demonstrated a journey from basic research to clinical translation that resulted in a novel molecular diagnostic test that could potentially revolutionise early oral cancer diagnosis and patient management.

Oral Presentation

Advanced innovative approaches in drug discovery and development

Usman Ali Ashfaq*

Drug development is a lengthy, complex, and costly process, entrenched with a high degree of uncertainty that a drug will succeed. To tackle these issues, game-changing computational approaches are used that offer enthralling diagnostic novel therapies to lessen the burden and provide "Good Health and Well-being" to people all over the world. Nowadays, the use of biotechnology and bioinformatics is getting popular across all facets of life sciences. Recently, it has been seen as an outbreak of emerging sequencing technologies that enable researchers to make ground-breaking discoveries in the domain of drug discovery. These novel approaches can integrate a vast amount of multiple chemical and biological data into a single modeling equation. Opportunities to apply these in silico approaches occur in all stages of the drug discovery process. Examples include molecular docking, virtual screening, Quantitative structure-activity relationship (QSAR), and pharmacophore mapping. Following that, current single-target approaches in drug discovery have become increasingly inefficient. While single-target strategies might prove a useful approach for single-gene disorders, however, for complicated diseases that are caused by the interaction of multiple genes, multi-target drugs are more fruitful. Regarding this, network pharmacology defines disease mechanisms as networks best targeted by multiple, synergistic drugs which have a potential impact on the treatment of complex diseases. In short, our research can act as a virtual shortcut, helping in the expedition of a long process and potentially reducing the cost of research and development. It tends to prove a weapon of choice to bisect complex biological systems at different dimensions. With the current successes, we believed that there is a promising future for computational methods to aid in the discovery of more therapeutic options in the future to meet the public health challenge that imposes serious economic burdens all over the world. This presentation will cover advanced in-silico, in-vitro, and in vivo approaches in drug discovery against viruses, cancer, diabetes, and alzheimer (neurological disorders).

Network pharmacology approach elucidates the multi-target mechanism of *Cannabis sativa* against Alzheimer's disease

Muhammad Shareef Masoud*

Alzheimer's disease (AD) being a neurodegenerative disease is characterized by the loss of memory and cognitive abilities and ultimately causing the death all around the globe. Despite of the progress made in health, an appropriate treatment for AD is still lacking. Cannabis sativa L. is an herbaceous flowering plant endowing from central and eastern Asia, constituents of which are the main focus for biomedical research due to their psychotropic effects. In this study Network pharmacology approach is actualized to enunciate endeavouring mechanism of C. sativa in the treatment of AD. Chemical constituent were obtained from databases like IMPPAT 2.0, KNApSAcK Family Database and super TCM and then screened out on the principles of oral bioavailability and drug-like properties. In this study, potential targets of 14 compounds of C. sativa were integrating with the disease targets, and 388 intersecting targets were the potential targets of C. sativa for the treatment of AD. These targets were then subjected to Network pathway analysis, Protein-Protein interaction analysis, Gene ontology and KEGG pathway analysis. Key targets were and molecular docking was performed to validate the actions of candidate constituent on them. 7-O-Allylapigenin, 6-Prenylapigenin, Cannflavin B, Cannflavin A, Cytisoside and Cannabivarinic Acid are highly active compounds and binds well to key targets namely AKT1, EGFR, TNF and MAPK3. The synergetic effect of active components of C. sativa on the treatment of AD predicts the conceivable mechanism of action and provides basis for further study.

Dissecting patho-mechanisms of genetic conditions through genomic Investigations and in vitro Disease Modelling

Ambrin Fatima*

While progress in genetics has been important for diagnostics, our understanding of disease mechanisms and neuropathophysiology lags behind. Model systems that are translatable into humans is a limiting factor for research progress in biomedicine, notably on neurodevelopmental disorders. There is now a great need to fill this gap in knowledge to eventually develop strategies for targeted intervention to alleviate disease progression. The introduction of induced pluripotent stem cell (iPSC) technology has circumvented the limited access to biological material and animal models to faithfully recapitulate human brain development. We use state of the art human iPSCs with DNA editing techniques such as CRISPR/Cas9 for disease modelling of neurodevelopmental disorders. Isogenic lines of patients are differentiated in to 2D and 3D neuronal cultures followed by proliferation, apoptosis, image analysis for migration and neurite formation, and high through-put molecular analysis (transcriptomic). In this talk, I will focus on generation of iPSCs and subsequent disease modelling of patients with several neurodevelopmental disorders. The technology of iPSC offers countless new possibilities of disease modelling and will become a standard procedure in medical research to unveil previously inaccessible aspects of neurobiology.

Formulation, characterization, and in-vivo evaluation of Duloxetine loaded solid lipid nanoparticles

Imran Khan[1], Fahad Hassan Shah[2], Song Ja Kim[2], Fouzia Qamar[3], Fazal Subhan[4], Saad Salman*[4]

The current study's goal was to create duloxetine (DLX)-loaded solid lipid nanoparticles (SLNs) utilizing a nano-template engineering approach. The approach was chosen because of its benefits in terms of simple preparation, lack of organic solvent use, and high shear mixing. The resulting nanoparticles had a particle size of 141 ± 9.4 nm and 80 ± 4.16 % drug contained inside their core. The zeta sizer was used to project a unimodal graph with a polydispersity index of 0.29 and particles with a zeta potential of -18.2 mV. Thermal and crystalline behavior assessment revealed that the produced nanoparticles were amorphous, and no chemical interaction was discovered through FTIR, Powder XRD, DSC, DTA and DTG. For the release profile, an *in vitro* dissolution analysis revealed a sustained pattern, whilst *in vivo* pharmacodynamic models; forced swim test and tail suspension test used for behavioral testing revealed a reduction in immobility time. ELISA revealed a significant rise in BDNF levels in both the brain and the blood. TNF-α and COX-2 levels were shown to be lower after DLX-SLNs administration, according to immunohistological tests. As a result, it is possible that incorporating DLX into SLNs serves as an effective carrier for improved antidepressant efficacy.

Selective COX-2 inhibitors affect cartilage regeneration and healing

Asma Sajjad Khawaja*, Muhammad Saad Ilyas, Amer Aziz, Uruj Zehra

Retrograde intramedullary (IM) nailing is commonly done to stabilize distal femoral shaft fractures but may lead to iatrogenic injuries to the knee articular cartilage. Articular cartilage has limited regenerative capabilities that may be further hindered by intake of non-steroidal anti-inflammatory drugs commonly prescribed in musculoskeletal injuries. The current study was designed to see the effects of selective-COX2 (Celecoxib) and non-selective-COX inhibitor (Diclofenac sodium) on cartilage after retrograde intramedullary nailing. Retrograde IM nailing was done in thirty-six adult male Wistar rats (200-400gms) and were divided into three groups of 12 each, group A &B was given Diclofenac sodium and Celecoxib respectively while C was taken as control. Six animals from each group were sacrificed at 15th and 49th postoperative day and histological samples of the knee articular cartilage were taken. Cartilage regeneration was assessed using histological parameters including articular cartilage structure, cellularity, tidemark integrity and proteoglycan contents according to the Modified Mankin Scoring for cartilage degradation. Mean modified Mankin scores for cartilage degradation were increased in the experimental group taking COX-2 inhibitor at 2nd and 7th week of healing ($p=0.024$, $p=0.006$ respectively). There was a significant reduction seen in the cellularity of articular cartilage at 2nd and 7th week of healing ($p=0.003$, $p=0.046$ respectively) along with the loss of proteoglycan content ($p=0.004$, $p=0.002$ respectively) in these animals. The results of current study reveal that intake of COX-2 inhibitor may hamper the cartilage regeneration/healing process by decreasing the number of chondrocytes and loss of proteoglycan content.

Heterologous expression and biochemical characterization of a novel alkaline lipase gene from *Bacillus thuringiensis* for detergent industry

Asma Zafar*, Ziaur Rahman, Madood Makhdoom, Nida Maqsood, Zeenat Mahmood, Sadia Zakir

Present study was aimed to heterologous expression of a lipase gene from Bacillus thuringiensis in E. coli BL21. Recombinant purified enzyme was purified up to 48.6 purification folds by affinity chromatography with specific activity of 126.36 U mg−1. Molecular mass of recombinant lipase enzyme was 29 kDa as measured by SDS-PAGE. Purified enzyme showed up to 90% stability at 80°C and retained stable in a wide pH range (8–11). Enzyme activity was reduced in the presence of EDTA, but metal ions seemed to enhance the lipolytic activity of the enzyme. Treatment of purified enzyme with SDS, PMSF, and urea resulted in reduction of enzyme activity, but organic solvents did not show considerable impact on it even at higher concentrations. Purified recombinant lipase enzyme was also found to be compatible with commercial laundry detergents and showed very good stability (up to 90%). All these properties proved the lipase enzyme from B. thuringensis a significant addition in detergent industry.

Poster Presentation

Bone Healing is influenced by non-selective COX NSAIDs

Maria Zafar*, Muhammad Saad Ilyas, Amer Aziz, Uruj Zehra

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for musculoskeletal injuries. The existing literature presents controversial findings concerning its role during fracture healing and no known systematic studies have been reported comparing the role of selective COX2 and nonselective COX NSAIDs. The current study is designed to identify and compare the histological changes during femoral fracture healing in rats taking non-selective-COX (Diclofenac sodium) and selective COX-2 (Celecoxib) NSAIDs. Thirty-six adult male Wistar albino rats (200-400gms) were operated to induce fracture at middiaphyseal region of the femur and were divided into three groups A, B & C of 12 animals each. The experimental groups A and B received diclofenac sodium and celecoxib respectively, group C was taken as control. Six animals from each group were sacrificed at 15th & 49th postoperative day and fractured bone were taken for analysis. Histology was scored on 5µ thin Alcian-blue-orange-G stained sections based on presence of cartilage, newly formed bones, hypertrophic chondrocytes, fibrosis and bone defects. The data was analyzed using One-way ANOVA. The total healing scores in controls were significantly higher at both 15th & 49th post-operative day ($p=0.01$& $p=0.008$ respectively). Fibrosis was seen significantly increased in animals taking diclofenac sodium at 15th post-operative day ($p=0.03$), while at 49th post-operative day bone formation significantly decreased ($p<0.001$) and bone defect ($p=0.03$) was increased in the group taking diclofenac sodium. NSAIDs especially non-selective-COX can be a significant risk factor for bone healing, and their administration should be avoided in high-risk patients.

Regeneration of pancreatic beta cells using preconditioned mesenchymal stem cells

Javeria Masnoon, Aisha Ishaque, Irfan Khan and Asmat Salim*

Type 1 diabetes mellitus is an autoimmune disease characterized by the hyperglycemic condition due to the loss of pancreatic beta cells. The diabetes load is rising around the globe with an estimation to become 7th leading cause of death worldwide by 2030. Beside insulin administration, there is no such treatment available for type 1 diabetes. Stem cells have gained much popularity since past few decades due to their unique properties. The present study aims to explore the role of potential of Wharton's jelly mesenchymal stem cells preconditioned with naphthoquinones for the treatment of type 1 diabetes in rats. MSCs were isolated, cultured and characterized prior to the further experiments. MSCs were treated with optimized dose of naphthoquinones for 24 hours and injected twice with 2 weeks interval into STZ-induced diabetic rats via tail vein. Rats were observed for FBS and body weight for 4 weeks. Later, pancreas was harvested for histology and transcriptional profile analyses. The H&E staining showed the successful regeneration of the islets of Langerhans in the treated group compared to the untreated MSCs group. Immunohistochemical analysis showed the positive expression of insulin in the treated group while weak expression of insulin in the untreated MSCs group. Furthermore, the genetic expression analyses showed significant higher expression of pancreatic beta cell markers and low expression of the proinflammatory genes in the treated groups compared to diabetic and untreated MSCs group. Together these results suggest the promising role of preconditioning of MSCs for the reversal of type 1 diabetes.

Current Trends and Improved Technologies: The Launch of Novel Biopharmaceuticals

Asma Arqam Tahir, Irfan Ullah, Fouzia Qamar*

The production systems comprising of microbes and mammalian cells for biopharmaceutical production are competing for their commercial dominance. During the past decade the mammalian cells predominantly CHO cells have attained a dominance, reason attributed to the fast growing antibody based therapy usage. Since scaffolds and mimetics have shown their scope as future proteins of significant interest, microbes especially E.coli has become a center of attraction for the production system of interest. Availability of various expression vectors/ strains make E.coli an ideal candidate for industrial applications due to easy protein folding mechanisms and bioprocessing techniques. However due to the absence of some very crucial biological processes, the production of comparatively complex recombinant biopharmaceuticals has got restricted. Present day advancements through the introduction of genetically engineered microbes have allowed the potential microbial candidates to acquire capabilities like glycosylation of heterologous proteins and expression of complex antibodies. Present effort summarizes the major breakthroughs and advancements through a comparative approach to elaborate the future scope of various expression systems for production of complex therapeutic proteins.

Relative frequency and antibiotic resistance profile of gram-positive and gram-negative uropathogens isolated from the Faisalabad region

Tuba Iqbal*, Kinza Rani , Beenish Ehsan , Sadia Liaquat , Asma Haque

Urinary tract infections (UTIs) are among the most prevailing infections in the human population. Early diagnosis and treatment of UTIs can reduce the rate of morbidity. A rapidly increasing resistance among uropathogens against commonly used antibiotics is a great concern, especially in developing countries like Pakistan. Due to increasing UTI cases being reported in Faisalabad, Pakistan, this study was outlined to determine the relative frequency of Gram-positive and Gram-negative uropathogens along with their drug resistance profiles. Forty different drugs belonging to fourteen antibiotic classes were used to check the spectrum of antimicrobial susceptibility. Out of 300 samples, 209 (69.67%) were found positive for UTIs with a prevalence of 68.42% in female patients, and 28.70% in male patients. Escherichia coli were the most frequently isolated gram-negative bacteria (52.15%). Other isolated bacteria had not much significant UTI burden as compared to Escherichia coli. Enterococcus faecalis (18.1%) was the second most frequent bacterium. Gram-negative bacteria posed the highest resistance (90%) against Amphenicol whereas Gram-positive bacteria showed the highest resistance against Quinolones (72.2%). Carbapenems (Imipenem, Meropenem) were the most effective drugs against both Gram-negative and Gram-positive bacteria (22.5% and 3.3% respectively) which strongly recommends its usage for empirical UTI therapy in this population. The unchecked prescription of routinely used drugs should be strictly monitored which leads to the emergence of highly resistant bacterial agents. A broad nationwide survey of antibiotic resistance is highly required for the implementation of better strategies.

Anti-bacterial activity of *citrus limon* juice and peel on selected bacterial strains

Naila Riaz*

Medicinal plants are quiet important as whole world is taking benefit of them. They have different kinds of bioactive agents. Plants express antibacterial activity because of the existence of different substances. These substances are formed during the secondary metabolism of plants. Antioxidants as well as antimicrobial characteristics are possessed by medicinal plants due to the presence of antimicrobial agents such as terpenoids, flavonoids, tannins, as well as alkaloids. Due to the secondary metabolites, plants can bear the abiotic stresses such as UV radiations. Antibacterial activity of Citrus limon juice and peel is investigated against selected bacterial strains named as Escherichia coli, Bacillus cereus, Staphylococcus aureus and Bacillus subtilis. From the concentration of 50mg/ml to 250mg/ml, both Citrus limon juice and peel extract were applied on various bacterial strains. Lemon juice extract expressed more effective results as compared to lemon peel extract. At MIC of 50mg/ml, aqueous solution of lemon juice expressed more antibacterial activity against bacillus species and E. coli. Methanolic extract of lemon peel expressed maximum zone of inhibition of 2.0mm at the concentration of 200 mg/ml against Bacillus subtilis. The antibacterial activity of Citrus limon aqueous solution was also evaluated by at varying temperatures and PH at the lowest and highest concentrations. The different temperatures and PH were 40°C, 60°C, 80°C, 100°C, and 110°C as well as 3PH, 5PH, 7PH, 9PH, and 11PH respectively. Aqueous solution of lemon juice at 50mg/ml and 250mg/ml expressed maximum zone of inhibition of 2.6mm at 60°C and 100°C as well as 2.5mm at 100°C against Bacillus subtilis respectively. Methanolic extract of lemon peel at 50mg/ml and 250mg/ml expressed maximum zone of inhibition against Bacillus cereus and Bacillus subtilis respectively. Aqueous solution of lemon juice at 50mg/ml and 250mg/ml expressed maximum zone of inhibition of 1.2mm and 1.8mm at 3PH against Staphylococcus aureus respectively. Methanolic extract of lemon peel at 50mg/ml and 250mg/ml expressed maximum zone of inhibition of 1.1mm and 1.3mm at 3PH against Staphylococcus aureus respectively. One-way ANOVA is used to compare the means of all inhibitory zones.

Bacteriological analysis of drinking water in twin cities, Rawalpindi and Islamabad, Pakistan

Misbah Nawaz, Laraib Khan, Shoaib Khan, Sobia Kanwal*

Water is one of the most important elements on Earth and also, for our body. This study investigates the quality of drinking water through physicochemical and microbial Analysis. For this purpose, the water samples were collected from ten different location of Twin cities, Islamabad and Rawalpindi. There are following three types of drinking water included: spring water, ground water and filter water. For each type there are three water samples were collected from three different point of location. A total 10 water samples of 200 ml each were collected in sterile bottles and transferred to microbiology laboratory of department of biology AIOU Islamabad. Most probable number (MPN) technique was used to detect microbial contamination in these water source. Only two water samples were free from any microbial contamination. These two water samples were from house wells. Out of eight samples three samples were fecal coliform positive with MPN greater then WHO limits that is 2MPN/100ml of water. Remaining 5 samples have bacterial count, less then WHO standard limits. Total 17 bacterial colonies were isolated from all water samples. Biochemical tests (Methyl Red, Voges Proskauer, Indole, and Catalase, motility test and citrate utilization test) were performed for these isolates. From all the isolated bacteria four bacterial genera were identified namely Escherichia coli, Klebsiella, Staphylococcus and pseudomonas. In study area of Twin cities, conducted the research on drinking water, out of two types of drinking water three are safe for drinking purposes and other one (surface/filter water) is domestic use. So from the study it can be concluded that there must be a proper plan for the management of drinking water in order to reduce the risk of water borne diseases. There is a need for emergency steps to stop the deterioration of water quality.

Computational and Reverse Vaccinology Approaches to design m-RNA based vaccine against multidrug resistant *Candida auris*

Muhammad Munib[1]*, Hamza Jamil[1], Abdur Rehman Dar[1], Muhammad Aqib Shabbir[1], Muhammad Hammad[1]

Candida auris is a fungus responsible to invasive candidiasis, an infection of the central nervous system, internal organs, and blood. This illness, which has emerged as a severe danger to global health, affects 30% to 59% of people worldwide. Candida auris generally responds inadequately to the antifungal drugs commonly used to treat Candida infections. In order to create an mRNA-based multi-epitope vaccine against Candida auris, the current study relies on a reverse vaccinology approach. For the purpose of choosing potential epitopes for vaccine construction, three proteins were shortlisted. Ten B-cell, eleven MHC-I, and ten MHC-II epitopes that are highly antigenic, non-allergenic, and non-toxic is chosen for the mRNA- and peptide-based multi-epitope vaccine design. 96.67% of the world's population is covered by the combined MHC-1 and MHC-2 coverage. A vaccine constructed by combining all of the selected epitopes with the appropriate linkers to enhance epitope presentation. The physiochemical characteristics, secondary and tertiary structure were predicted. A high affinity connection between the immune cell receptor and the vaccination is verified by molecular docking. Better antigen expression is also made possible by codon optimization, nucleotide changes, and the availability of appropriate vectors. The outcomes of the immunological simulation confirmed the vaccine's ability to trigger a healthy immune response. We believe that this vaccine candidate may also prove to be an effective in vitro vaccine against *C. auris.*

Physicochemical properties and molecular insights of Favipiravir and Roflumelast fixed dose combinations for COVID-19 treatment

Abdul Rauf[1], Fahad Hassan Shah[2], Song Ja Kim[2], Sana Haider[3], Shehla Akbar[3], Saad Salman*[3]

Fixed dose combinations (FDC) of drugs benefit patients and the healthcare system. COVID-19 is a viral pandemic and is considered as most drastic and fatal pandemic which exponentially spread throughout the whole world. Multi-drug formulation containing a Favipiravir (an anti-viral drug) and a Roflumilast (a highly selective long-acting inhibitor of the PDE4 isoenzyme) can be a promising combination for treating COVID-19. The physicochemical nature and dissolution chemistry of the proposed multi-drug formulation is critical in making it purposeful and efficacious. Essential attributes of proposed formulations are studied by evaluating their nature of compatibility of APIs with excipients, solid amorphous dispersions, physical attributes of the tablet, content uniformity, and dissolution properties in both immediate releases as well as controlled release matrix. The method for quantification of Roflumilast and Favipiravir is developed and validated in accordance with international guidelines. Both layers of this FDC are with different drug release mechanisms. Immediate release is optimized with the use of Klucel EXF, and a controlled release matrix is formed with Klucel HXF and is further controlled with the use of Compritol 888 ATO. API and excipient compatibility is evaluated by combining the excipients in fixed ratios of API and there are founds to be no deterioration in drug contents or in physical parameters. Roflumilast was observed with acceptable dissolution with variable quantities of Klucel EXF and showed a comparable drug release in both formulations in the first 30 minutes. It is feasible to develop a novel fixed-dose combination FDC including Roflumilast and Favipiravir with acceptable physicochemical characteristics and a stable dissolution profile for both drugs. It can be a stable fixed dose combination and might be a more pharmaco-economical, efficacious, and superior alternative to traditional remedies for COVID-19.

Molecular aspects of most common european phenylalanine hydroxylase (PAH) gene R408W variant

Umair Mahmood*[1] , Shazia Anwer Bukhari[2], Huma Arshad Cheema[3], Saqib Mahmood[4], Muhammad Farooq Khalid[1], Umar Farooq[1], Muhammad Auon[1]

Phenylalanine hydroxylase (EC: 1.14.16.1) consisting 452 aminoacids catalyzes the conversion of L-phenylalanine to L-tyrosine in the presence of oxygen O2 and tetrahydrobiopterin BP4. The PAH enzyme is located in cytosol and variants in phenylalanine hydroxylase (PAH) gene is responsible for phenylketonuria (PKU) inherited in autosomal recessive pattern and its most common recessive form in amino acid metabolism with range of 1:4,500 in Italy to 1:125,000 in Japan. Out of 758 PAH gene variants, c.1222C>T (p.Arg408Trp) is the most common European variant in the classical form of phenylketonuria. The comparative molecular analysis of wild and variant R408W was determined by using Chimera software to check the effects on hydrogen bonds of protein and number of contacts/ clashes. By applying tryptophan rotamer to arginine at 408 of PAH, the number of hydrogen bonds reduced to 748 rather than 753 located in wild type of PAH protein. The number of contacts and clashes were also been observed as 41 and 25 with adjacent amino acids, respectively. Due to these increasing clashes and reduction of number of hydrogen bonds in R408W variant of PAH protein ultimately affects the 3-dimensional protein structure and physiological function. This in-silico study shows the molecular affects of protein and correlation of genotype and its particular phenotype.

Whole exome sequencing identifies a novel missense variant of UBE3A gene in a patient with Angelman syndrome in Pakistan: A case study

Sana Khawer*[1], Mohammad Ismail[2], Sitwat Zehra[1]

Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by intellectual disability, expressive speech impairment, movement disorder, epilepsy and a happy demeanor. According to the National Organization of Rare Diseases, 1 in 15000 live births are affected globally. This rare neuro-genetic disorder is caused by a deficiency of the imprinted and maternally expressed UBE3A gene. Although de novo genetic and epigenetic imprinting defects of UBE3A genomic locus account for the majority of Angelman diagnoses. The aim of the study was to identify the cause of the silent seizures and regression in intellectual abilities of the autistic patients. This case study reports a 5.5years old female proband, diagnosed with autism spectrum disorder (ASD) at 2.5years of age. Despite early intervention, the patient did not show signs of improvement in her language skills, cognition, motor and movement milestones. The Whole Exome Sequencing (WES) was performed on the patient sample which revealed a novel missense mutation in UBE3A gene which justified the patient's phenotype. The UBE3A missense variant c.1744A>G p. (Ile582Val) is located at the N-terminus of the E6AP protein and it is found in the catalytic cleft of the enzyme by the protein prediction tools. Conclusively stated, the patient initially diagnosed with autism spectrum disorder (ASD) was a case of Angelman Syndrome (AS). However, a parental genetic screening is required to identify the genetic aberrations in the family which will help predict if the mutations were de novo or maternally imprinted.

Vitamin d receptor gene polymorphism is associated with spinal TB & severity of spinal phenotypes

Shamaila Ejaz, Amer Aziz*, Uruj Zehra

Vitamin-D receptor (VDR) gene polymorphism is a promising risk factor which increases the susceptibility for spine TB (STB) in different populations. However, this association in Pakistani population is still unknown.The current study was designed to see the association of VDR gene polymorphism with STB and severity of phenotypes in Pakistani population. The demographic, relevant clinical profile and blood samples were obtained from forty-three adult STB patients undergoing spine surgery, and from same number of age and gender matched healthy controls after informed consent for polymerase chain reaction (PCR) and sequencing to analyze Fok-1 and Apa-1 polymorphisms of VDR gene along with an additional rs11574113 polymorphism which was found while analyzing sequence chromatogram. Radiographs, T1-T2 W magnetic resonance images (MRI) of STB patients were assessed for spinal changes while post-surgical tissue samples were analyzed for histological parameters. Results showed that STB patients had significant association with heterozygous (Ff) & homozygous recessive (ff) genotype of Fok-1 (p=0.003) and with heterozygous (Rr) genotype of rs11574113 (p=0.02). Wild type (FF) Fok-1, homozygous recessive (aa) Apa-1 and heterozygous (Rr) genotype of rs11574113 polymorphisms were significantly associated with severe spinal phenotypes on imaging such as higher number of vertebral involvement (p=0.01), complete vertebral bodies (p=0.001) & intervertebral disc collapse (p=0.002) and higher Pfirrmann grades (p=0.03). Apa-1 polymorphism was also significantly associated with well-formed granuloma (p=0.01) on histological analysis. The risk of STB may increase with Fok-1 and rs11574113 polymorphism of VDR gene and polymorphism may cause severe forms of STB.

Efficacy of soluble dietary fiber guar gum for the treatment of enteric ulcer in mouse model

Hajra Bibi, Iqra Shahid, Sadia Mushtaq, Asima Tayyab, Soumble Zulfiqar*

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most prescribed medicines for their efficacy to reduce pain, fever, and inflammation. Advanced endoscopic techniques such as capsule endoscopy revealed the ulcerogenic effect of NSAIDs in small intestine. Through many scientific evidences, lansoprazole which is a proton pump inhibitor is manifested for the treatment of NSAIDs induced enteropathy. As therapeutic options are limited, soluble dietary fibers have been considered very potent for the treatment of NSAIDs induced enteric ulcer. In the current study, efficacy of dietary fiber guar gum was assessed and compared with that of lansoprazole. Histological analysis of intestine showed high level inflammation in the injury group (administered with indomethacin, an NSAID) that was partially healed in the treatment groups (administered with guar gum and/or lansoprazole). Relative expression of pro-inflammatory genes: TNFα, COX-2, eNOS; antioxidant gene: GPX-2 and gene for mucosal integrity: MUC-2 was determined by real time PCR using GAPDH as housekeeping gene. The histological and molecular investigations indicated that soluble dietary fiber guar gum exerted similar effect as that of lansoprazole. So it has potential be used as inexpensive therapeutic tool having no side effects to treat NSAIDs induced enteropathy.

Long-term use of nsaids affects collagen maturation and regeneration of skin in wound healing in wistar-albino rats

Safia Ghufran*[1,2], Ferdose Sultana[2], Muhammad Saad Ilyas[3], Uruj Zehra[1]

Use of non-steroidal anti-inflammatory drugs (NSAIDs) is controversial in skin wound healing and its long-term effect on skin regeneration is not explored yet. The current study is designed to identify and compare the histological changes occurring in the skin during wound healing after giving NSAIDs. Thirty-six adult male Wistar albino rats were given full-depth skin incision in mid-thigh region and divided into three groups of 12 animals each. The experimental groups received diclofenac sodium and celecoxib while the third group was taken as control. Six animals from each group were sacrificed at 2nd & 7th postoperative week and skin tissues were taken for analyses. Histology was scored on 5µ thin sections using H&E and modified Masson's trichrome for inflammatory-infiltrate, collagen fiber orientation, maturity & regeneration of collagen, and expression of collagen IV in basement membrane on immunohistochemistry. Increase in inflammatory-infiltrates, neovascularization (P=0.012,P=0.039) and lower healing scores (P=0.003) were seen in the control group at 2nd week. In 7th week the inflammatory-infiltrate was seen to be still raised in diclofenac group (P=0.04), and maturation of collagen was significantly lower (P=0.001) in both experimental groups. Immunohistochemical expression of collage IV was significantly lower in celecoxib group as compared to other two (P<0.001). In acute Phase, NSAIDs especially celecoxib has reduced the inflammatory response and neovascularization, which affected the expression of collagen IV in the basement membrane which ultimately affect skin regeneration. In chronic phase, maturity of collagen was decreased in both experimental groups as compared to control, so long-term use of NSAIDs may affect the collagen maturation and regeneration of skin during wound healing leading to hypertrophic scar formation.

Vertebral endplate and the intervertebral disc degeneration is linked with each other- A human cadaveric study

Amber Salman*, Uruj Zehra

Intervertebral disc and vertebral endplates undergo degenerative changes and are highly associated with low back pain and disability. Studies have reported the degenerative features of both structures but how these two correlate with each other is not very clear. The current study was designed to identify and correlate the degenerative features in the intervertebral discs and vertebral endplate both macroscopic & microscopically. Fifty-nine lumber motion segments (L1-L5) comprising of intervertebral disc with adjacent vertebrae were collected from thirteen cadavers aged 20-80 years. Macroscopic scoring of disc and vertebral endplates was based on visual examination of mid-sagittal sections using subscale that yielded a maximum score of 32 for endplate and 64 for discs. Microscopic scoring was based on H& E stained histological sections taken from discs & endplate, using subscale scores that totalled 42 for endplates and 30 for discs. Linear regression analyses and ANOVA was applied for statistical analysis. Macroscopic and microscopic scores of endplate and intervertebral discs were highly correlated with each other ($r= 0.67, 0.64, p<0.001$). The overall macroscopic and microscopic scores were also highly correlated with each other ($r=0.9, p<0.001$). All scores of degeneration significantly increased with age, however, spinal level did not have any effect on degeneration. Correlation of intervertebral disc and endplate degenerative changes indicate that both structures are critically linked to each other. It's important to establish a cause and effect relationship so that pathogenesis can be properly understood.

Health hazards associated with Cd-contaminated soil and its remediation using plant growth promoting bacteria

Sara Rehmat and Ambreen Ahmed*

Generally, industries release their effluents without any treatment in the environment (soil, air and water). These effluents contain many hazardous chemicals including heavy metals. Among heavy metals, cadmium is on the top of the list. Soil contaminated with cadmium has serious effects on living organisms such as acute inhalation exposure (high levels over a short period of time) to cadmium can result in flu-like symptoms (chills, fever, and muscle pain) and can damage the lungs. Chronic exposure (low level over an extended period of time) can result in kidney, bone and lung disease such as plant growth. It has serious effects on plant growth as well. Cadmium contaminated soil causes stunted growth in plant and ultimately leads to plant death. Bioremediation of cadmium contaminated soil is an economical approach using plant growth promoting rhizobacteria (PGPR) to minimize hazardous impacts of Cd on soil. These microbes enhance the plant growth under Cd- stress by various mechanisms. In order to study the effect of these bacterial isolates as monocultures 3a, Th1, Tp8, Th6, A9G and mixed cultures 3a+Th1, 3a+Th6, 3a+Tp8, 3a+A9G, Th1+Th6, Th1+Tp8, Th1+A9G, Th6+Tp8, Th6+A9G and Tp8+A9G on growth as well as biochemical parameters on the Zea mays L. plants, plant growth experiment was conducted. Plants of Zea mays L. were grown in the presence (200, 400, 600 and 800µg/ml Cd-stress) as well as absence of cadmium stress. Results indicated that bacterial isolates significantly enhanced the shoot length, root length, number of leaves, fresh weight and chlorophyll and protein content under cadmium stress. However, in some cases, reduction was also observed in these parameters. Among all bacterial isolates, the monoculture using the isolate A9G showed the maximum enhancement in growth parameters under Cd-stress. Moreover, mixed cultures A9G+3a, A9G+Tp8 and A9G+Th6 showed best beneficial effect on biochemical parameters. So these cadmium resistant bacteria can be used as a potential source of bioremediation for cadmium contaminated soil.

Cardiac stress induced by depression leads to atrial fibrillation, cardiomyopathies, and heart failure in mice

Shahzadi Arhum[1], Muhammad Mustafa[2*]

Stress is a word that is used to describe challenging experiences that could be physiological or emotional. It is an adverse condition that disturbs the homeostasis of the body and triggers adaptation responses. Emotional stress is one of the most prevailing health issues, and cardiovascular health is directly associated with it. Acute emotional stress is known to trigger multiple cardiovascular diseases by inducing cardiac stress that lead to various cardiac complications including heart toward failure. In this study we observed that depression like behavior in mice induces cardiac stress leading towards cardiac failure via atrial fibrillation, dilated cardiomyopathy, and tachycardia-induced cardiomyopathy. In this study C57 black mice are undergone a forced swim test and their behavioral analysis is done by tail suspension test while, cardiac stress and complications are studied at molecular levels by analyzing gene expression of Ankrd1, Fhl1, Ttn, Myl4, Hcn4, and Myh7 gene. Also, the histopathological analysis is done to analyze the morphological changes in the cardiac tissue and biochemical analysis was done to study the impact of chronic emotional stress on Hb, MCH, platelet count, and cardiac enzymes level including CPK, Ck-MB, LDH, SGOT, and ProBNP(N-T) to confirm the onset of cardiac failure. This study proves that the mouse undergoing emotional stress is more prone to cardiac failure and these genes can be used as potential biomarkers for the diagnosis of various complications.

Expression profile of Circular RNA (circRNA_0000096) in patients infected with hepatitis C virus and its therapeutic response

Fatima Butt, Umm e Habiba, Fiza Farooq, Muhammad Shahid*, Iram Amin, Samia Afzal, Muhammad Idrees

Hepatitis C virus (HCV) is a prime cause of chronic liver illnesses. This single-stranded RNA virus mainly targets liver cells. Chronic infection of this virus disturbs various processes and pathways of the body including immune responses and metabolic pathways. Almost one in every twenty Pakistani has an infection of HCV. In this research, we intended to evaluate the expression of circRNA_0000096 in the whole blood of HCV 3a patients of the Pakistani population and to compare the gene expression profile between Sustained Virological Response (SVR) and Relapsed (R) patients. This contributed in identifying novel circRNA molecules that can serve as diagnostic as well as prognostic biomarkers for HCV infection and can also serve as potential therapeutic targets. For this research, blood samples of 142 HCV patients and 20 healthy controls were collected. Out of 142 HCV-positive samples, 85 were included in this study for expression profiling. Sample collection was followed by total RNA extraction and cDNA synthesis. Relative gene expression was then measured in both HCV patient samples and controls by Real-Time-PCR. Comparative analysis of blood-based gene expression revealed upregulation of has_circ_0000096 in case of HCV infection. According to our understanding, circRNAs can provide us with valuable insight into the mechanism involved in HCV infection and progression and can thus serve as potential biomarkers. Our findings highlight the significance of gene expression profiling as a diagnostic and therapeutic tool in the case of Hepatitis C infection. This research will not only contribute to knowledge concerning the gene expression profile of Pakistani HCV patients but will also help in getting insight into the genes that are associated with the treatment response.

Association of interleukin-10 gene with DAAs treatment response in Hepatitis C virus patients

Hira Raheem Akbar, Samia Afzal*, Iram Amin, Muhammad Shahid, Muhammad Idrees, Kausar Malik

Hepatitis C virus is one of the most infectious known viruses, which leads to hepatitis C infection in humans around the whole world including in Pakistan. Due to the unavailability of any FDA-approved vaccine against HCV, direct-acting antivirals are given to HCV patients. DAAs (Direct-acting anitivirals) therapy is more effective and reliable than older treatments such as interferons. The sustained virological response rate of DAAs is 90%. This study explores the relationship of viral and host factors with response to DAAs therapy in HCV patients in the Pakistan population. 95 DAAs treated HCV patients were enrolled in the study who were treated with sofosbuvir-400mg, Velpatasvir-100mg, and Declatascir-60mg. 41 patients had 3 months DAAs treatment while 29 patients had 6 months DAAs treatment. SVR occurred in 71.1% of patients while relapse occurred in 28.1% of patients who developed HCV infection again even after having a complete course of DAAs therapy. There is a strong association between single nucleotide polymorphisms and genetic variations in human immunoregulatory genes named IL-10(Interleukin-10). This gene is accountable for viral clearance from the body, so genetic mutations in the genes can affect the person's immune response to the viral infection. In this direction, the study was performed to detect genetic mutations in IL-10/672 gene. Human genomic variations of IL-10/672 SNPs (rs1878672) was recognized and it was concluded that IL-10 SNP rs1878672-T/A, C/A, and G/C genotypes were found to be strongly associated with relapse patients only and not found in SVR patients. Data related to genotype C/A is reported in the literature, however, data related to genotypes T/A, and G/C is not reported in literature. Thus, it was concluded that the predominance of IL-10 rs1878672-T/A, C/A, and G/C genotypes, is strongly associated with high IL-10 gene expression, may contribute to the HCV persistence and decompensation with cirrhosis.

Depression promotes hepatic gene expression and induces liver damage which promotes the progression of type 2 diabetes mellitus in mice.

Abeer Asif*, Muhammad Mustafa

Diabetes remains amongst the most prevalent diseases in Pakistan, affecting over 537 million people worldwide, and around 33 million Pakistani adults. Type 2 Diabetes Mellitus (T2DM), a sub-category, is engendered by both external and internal factors (2021). One of the external factors reported to accelerate its progression is stress. External stress instigates oxidative and mitochondrial stress, as well as an amalgam of inflammatory markers leading to possible liver damage. Eight-week-old C57 male mice were treated with a forced swim stress model to assess the effect of stress on the emergence of T2DM. We measured this progression using housekeeping, and hepatic genes that undergo changes in T2DM. Data was collected using a complete blood count, Liver function test (LFT), Hematoxylin and Eosin staining (H & E), and gene analysis. H&E showed swelling in hepatocytes, ballooning degeneration, and nuclear enlargement in some focal areas of mice who underwent forced swim stress. This change was reinforced through chemical test reports which showed a significant increase in ALP, ALT, and MCH values in experimental mice when compared to control mice showing a possible trend towards liver damage. We also found an up-regulation of Crp, Cyp2e1, and Irs-2. These genes, especially Crp, can be used as potential biomarkers for not only the detection of T2DM but also as indicators of psychologically stressed conditions. Moreover, Irs-2 was revealed to have an initial protective effect against liver damage. Early detection can help us diagnose and alter lifestyle conditions in time for the reversal of T2DM.

Endogenous IL-29 biotherapeutic cargo loaded exosomes to harness amplified in vitro cytotoxic effect against cancer

Nao Akusa Fujimura[1], Seerat E Fatima[1], Nadeem Ahmed[1]*, Muhammad Akram[2], Saad Tahir[1], Mohsin Ahmad Khan[1], Imran Amirzada[3], Tariq Nadeem[1], Hamid Bashir[2], Kausar Malik[1]

The threat of cancer continues to pose a major challenge worldwide, with high mortality and morbidity rates. Efforts to improve treatment methods have led to advances in immunotherapy, including the use of IL-29, which shows promise as a targeted therapy for specific cancer cell types. However, IL-29 has limitations in terms of bioavailability and half-life, reducing its effectiveness as a treatment. To overcome these limitations, exosomes have been proposed as solutions for targeted drug delivery. A recent study aimed to evaluate the impact of both purified IL-29 and IL-29 encapsulated exosomes on cancer cell proliferation. The IL-29+pET-28a construct was transformed into an E. coli expression strain, which was utilized for the large-scale production of IL-29. Exosomes were isolated from established human cancer cell lines using the Total Exosome Isolation reagent and loaded with IL-29 through sonication. Exosome isolation was verified by western blotting for core protein signatures and RT-PCR for specific miRNA profiles. The drug-loading efficiency and release kinetics of IL-29 encapsulated exosomes showed a stable release of the recombinant drug. When treated with 10 µg/mL IL-29, approximately 50% of the cancer cells survived, whereas less than 10% survival was observed when treated with 10 µg/mL IL-29 loaded exosomes. It was concluded that IL-29 loaded exosomes have a more significant cytotoxic effect on cancer cells, which can be attributed to the sustained drug release, better targeting efficacy, ability to utilize endogenous intracellular trafficking pathways, improved half-life, and enhanced biocompatibility of the exosomes.

Preventive and curative anticancer effect of fagonia arabica l. against induced hepatocellular carcinoma (hcc) in *Oryctolagous Cuniculus L.* (carcinomic rabbits model)

Shazia Kanwal Malik[1]*, Fouzia Yasmeen[2], Maqsood Ahmed[3], Farah Khan[1]

Ethanolic extraction of F. arabica was performed. Photochemical screening determined Flavonoids (++), Phenols (++), Terpenoids (+++), Quinine (+), Saponins (+), Glycosides (++), Alkaloids (++). Antioxidant analysis (in-vitro DPPH free radical scavenging) resulted in 98.4%. 26 Bioactive compounds including 11 known and 1 novel anticancer compound were determined by GCMS, FTIR and UV-Viz spectroscopy. For cytotoxic screening of plant extract against 3T3 cell line MTT assay was performed showing 16% inhibition. For HCC O. Cuniculus animal model, baseline values of physiological (body temperature, body weight, aggressive mode), Biochemical (pH of saliva, blood glucose, CBC, LFT's) and Tumor marker Alpha Fetoprotein (AFP) were recorded. HCC was induced by CCl4. Afterwards, these values were again noted. HCC was suspected by changes in psychological findings, raised transaminases indicating liver damage, and confirmed/diagnosed by Histopathology of the liver. Comparison of three agents - plant extract, n-Hexadecanpic acid (Terpene), and sorafenib (Nexavar) for preventive and curative effects of anticancer activity was made. All the above perimeters were analyzed and the results of our plant extract were compared with that of n-Hexadecanpic acid (Terpene) and sorafenib (Nexavar) acting as standards. Statistical analysis was done by one-way ANOVA using SPSS showed remarkable preventive and curative anticancer effect of our plant extract.

Genetic Polymorphism of 22 Autosomal STR Loci in Sindhi Population of Pakistan

Ruhma Arshad, Muhammad Shafique*, Syeda Bariyyah Hasnain, Abida Shehzadi, Muhammad Shahzad, Ahmad Ali Shahid , Kausar Malik

This study was designed to determine the genetic polymorphism of 22 autosomal STRs of 201 individuals from Sindhi population of Pakistan using PowerPlex® Fusion 5C system. Population genetics software's such as PowerStat v1.2, PowerMarker v3.25 and Arlequin v3.5.1.2 were used to statistically analyze the samples for genetic variation. As a result, the study revealed that a total of 236 polymorphic alleles were obtained in Sindh population. The locus 'TPOX' showed the highest allele frequency 0.428 at allele number 8. The most polymorphic locus was Penta E having 19 alleles in this population with maximum power of discrimination as 0.9812 and maximum value of polymorphism information content (PIC) as 0.9043. The maximum value of paternity index was 4.02 and the maximum value of power of exclusion was 0.7459 at Penta E locus. The combined power of discrimination (CPD), combined power of exclusion (CPE) and combined matching probability (CMP) were obtained as 0.999999999999999999999999997572, 0.999999953105559 and 2.4282 x 10-27 respectively. Differentiation test revealed that no significant differences were observed in Bangladeshi, Azad Kashmir, Pakhtun, Balochi, Punjabi, and Saraiki populations. It was concluded that based on forensic efficiency parameters, these 22 STRs would be useful for the identification of individuals in paternity cases, crime scene investigation, missing persons, and database development for Sindh population of Pakistan.

Genetic diversity of 22 autosomal STR loci in Azad Jammu and Kashmir population of Pakistan

Syeda Bariyyah Hasnain, Muhammad Shafique*, Ruhma Arshad, Abida Shehzadi, Muhammad Shahzad, Ahmad Ali Shahid, Kausar Malik

The aim of this study was to characterize genetically 22 autosomal STRs, gender discriminating marker (Amelogenin for both genders) and male specific Y STR in 200 unrelated individuals of Azad Jammu and Kashmir (AJK) using PowerPlex® Fusion 5C System. Capillary electrophoresis was performed on ABI3730xl genetic analyzer to genotype the DNA samples. Forensic statistical analysis was executed using PowerStats v1.2, PowerMarker v3.25, and Arlequin v3.5.1.2 software's. A total of 229 alleles of 22 STR loci were determined in the AJK population. Among 22 STRs, D22S1045 expressed the highest allele frequency 0.425 at allele 15. Highest polymorphic loci were observed to be D1S1656, Penta E and FGA in the AJK population having 17 alleles. The maximum power of discrimination and maximum value of polymorphic information content was 0.981 and 0.903 at Penta E respectively. All the loci were in coordination with the Hardy Weinberg equilibrium at $p<0.005$ except for the one locus (D16S539) whereas no significant deviations were observed after Bonferroni's correction ($p<0.002$). Differentiation test revealed that significant differences were observed in the populations of Vietnam, Japan, Jordan, Italy, Paraguay, Estonia. The combined values for power of discrimination, power of exclusion and matching probability were calculated as 0.99999999999999999999999995701, 0.999999994851914 and 4.299x10-27 respectively. It is concluded that based on forensic efficiency parameters, these 22 STRs would be helpful to identify individuals in criminal investigation, mass disaster victims, paternity issues, and database development.

Haplotype Diversity of 23 Y-Chromosomal STRs in Kalasha Population of Pakistan

Imran Shahid, Muhammad Shafique, Abida Shehzadi*, Faqeeha Javed, Jaweria Munsif, Ahmad Ali Shahid, Kausar Malik

We examined haplotype diversity of 23 Y-chromosomal STRs (DYS576, DYS389II/I, DYS19, DYS448, DYS393, DYS458, DYS385a/b, DYS456, DYS390, DYS391, DYS392, DYS437, DYS438 (Penta), DYS439, DYS481, DYS549, DYS533, DYS570, DYS635, DYS643 (Penta), and Y-GATA-H4) using Power Plex® Y23 System in 98 unrelated males of Kalasha population residing in Kalash valley (commonly called as Kafiristan) of district Chitral in Khyber Pakhtunkhwa province. Forensic efficiency parameters were scrutinized leading to identification of 52 haplotypes. Among these, 34 haplotypes were found unique with diversity value of 0.9928. Among single copy and multi copy Y-STRs, the most polymorphic loci were DYS635 (76%) and DYS385 (79%) respectively. Analysis of Molecular Variance (AMOVA) and Multidimensional Scaling Plot (MDS) were generated by using tools at YHRD (Y-Chromosome STR Haplotype Reference Database). However, the results showed that the Kalasha population is genetically and geographically distinct from rest of Pakistani populations.

An Organic Green strategy to Alleviate Malnutrition

Rida Rasheed*, Ambreen Ahmed

Wheat is a staple food crop and a significant source of dietary energy worldwide. Due to inadequate intake of micronutrients like Zn, over 2 billion people worldwide suffer from malnutrition, which can cause severe physical and mental abnormalities. The biofortification method has been found to be the most effective method for adding minerals to grains. A study was carried out in both wire house and laboratory to evaluate the efficacy of ZSB to upgrade Zn content and enhance wheat growth and yield. The findings showed that foliar Zn application combined with ZSB improved plant growth and Zn content. In a lab setting, plants treated with Burkholderia cepacia (FS1), in the presence of 100 g/ml Zn, showed a 28% increase in fresh weight. Using atomic absorption spectrometry, the maximum Zn concentration, 2.68 ppm, was noted in plants augmented with Pseudomonas aeruginosa (DS4) as a result of foliar spraying 200 g/ml Zn under wire house conditions. Hence, biofortification of crops using Zn and ZSB is recommended as a cost-effective, organic-green solution, not only to increase mineral concentrations in edible crops and treat malnutrition, but also to boost plant development and yields.

The Role of CYP1B1 in Primary Congenital Glaucoma Pathogenesis among the Pakistani Population

Tazeen Zahid[1], Aymn Zulfiqar[1], Muhammad Umer Khan*[1], Muhammad Usman Ghani[2]

Primary congenital glaucoma (PCG) is an idiopathic, non-syndromic, innate developmental malfunction of anterior chamber angle and cupping of optic nerve head that results in triad symptoms, including photophobia, blepharospasm, and epiphora and is characterized as a significant cause of irreversible blindness in the world. Its incidence is linked with the high ratio of consanguinity. The sequence variations in cytochrome P450-1B1 (Cyp1b1) gene is a usual genetic predisposition linked with primary congenital glaucoma. The purpose of this study was to identify the genetic variants associated with PCG in Pakistani population using bioinformatics tools. Five hotspot mutations (F231L, A119S, R48G, R390H, E229K) in CYP1B1 gene segregating in Pakistani consanguineous families were included from previously reported studies. These mutations were identified by fluorescence based chain terminator (dye-deoxy) sequencing of coding exons using genomic DNA extracted from affected patients. In silico analysis was performed to understand the effect of CYP1B1 mutations on their protein structure, function, and 3D model, sequence conservative properties, and to identify dynamics and biochemical changes. Missense mutation, Arg390His, was the most common deleterious mutation observed in Pakistani population. In silico analysis revealed the genetic heterogeneity and molecular pathogenicity, thus confirming the deleterious effect of detected mutations on the protein function, structure and stability. Similar investigations will be significant in acknowledging the biological function of CYP1B1 gene and the impact of these mutations on the enzymatic and regulatory activities of CYP1B1 that cause primary congenital glaucoma.

Biosafety study of mosquito larvicidal transgenic algae (*C. reinhardtii*) using adult Zebrafish and its embryos

Fareeha Amjad, Mohsin Ahmed Khan, Kausar Malik, Rashid Bhatti*, Nadeem Ahmed, Hamza Khan, Sidra Ayyub, Islam Khan.

Mosquito-borne diseases are a persistent risk to humanity all over the world. The pathogenic agents that cause illnesses like Malaria, Dengue fever, Chikungunya, Yellow fever, and West Nile virus are carried by mosquitoes. Previously in CEMB Algal Biotechnology Laboratory a genetically engineered mosquito larvicidal algae was developed. The objective was to check its toxicity assessment on non-target organisms. We evaluated the biosafety of this transgenic algae, *Chlamydomonas reinhardtii* on Zebrafish over the course of a 90-days test period. Zebrafish embryos were also used in toxicity assay. A total of 140 Zebrafish were used in the experiment, divided into seven groups, to which different feed combinations were given. Zebrafish were routinely examined for any signs of death, allergenicity, or moribundity. The data was statistically analyzed by ANOVA and correlation, and the results revealed no statistically significant difference. Hematology, molecular analysis, and other clinical pathology characteristics were assessed, and it was determined that there was no physiologically significant difference between any of the experimental groups. Necropsy analysis was conducted in which Zebrafish's organs morphology, weight, and histopathology of vital organs (liver, heart, gills, brain, intestine) were evaluated and there was no substantial difference found among any of the groups. In conclusion, there were no adverse effects found on the overall health of adult Zebrafish and its larvae feeding on mosquito larvicidal alga producing the protein. The fish embryo acute toxicity test indicated no toxic effect on the Zebrafish larvae as they developed normally and showed no abnormality. This study proved that the transgenic mosquito larvicidal algae did not cause any adverse effect on the non-target organisms i.e. Zebrafish and its larvae.

Physicochemical, Functional, Toxicological and Pharmacological properties evaluation of *Prunus armeniaca* gum: Introducing a new landscape for its applications

Shazia Noureen[1], Sobia Noreen[1]*, Shazia Akram Ghumman[2], Fozia Batool[1], Tusneem Kausar[3]

Hydrocolloids offer a lot of applications in food and non-food industries since ages. Plan-based gum hydrocolloids are acquiring a high demand due to their structural and functional diversity. The present study was intended to purify and characterize the gum obtained from Prunus armeniaca. The gum was extracted in distilled water and precipitated using ethanol. Micro and macro-elemental composition of PAG was determined by using laser induced breakdown spectroscopy (LIBS). Gum was evaluated for physicochemical, functional, toxicological and pharmacological properties. Percentage yield of purified gum was 29.54±1.15. Hygroscopicity, water holding, oil holding, foaming capacity, foam stability, emulsification capacity, and emulsion stability determined were determined. Toxicity study showed that gum is non-toxic up to 2000 mg/Kg body weight in rabbits. Aqueous solution of PAG showed various pharmacological activities with significant value of antioxidant, antibacterial, anti-nociceptive, anti-inflammatory and thrombolytic activities. It can be inferred from these results that Prunus armeniaca gum has good flow and functional properties along with non-toxic and various pharmacological effects, which make Prunus armeniaca gum, a suitable natural candidate for the food and pharmaceutical industries.

Management of a patient having COVID-19 along with type 1 Diabetes and comparative treatment with a non-diabetic patient--- First case report from Pakistan

Rabia Ayub , Rabia Nawaz* , Umer Farooq Khan , Anum Ajmal , Usman , Nimra Saqib , Ammara Ahad , Zohal Hassan , Attia Razzaq* , Uqba Mehmood

Coronavirus disease 2019 (COVID-19) is a newly emerged contagious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In this study, we report a case of a 53-years old immunosuppressed person with an underlying condition of chronic type I diabetes. We also report its differential management from a nondiabetic young presumably horizontally infected patient. We concluded that although comorbidities complicate treatment adaptations in patient management but is achievable with cautious choice of regimen and continuous monitoring. This complexity poses a greater challenge with novel diseases like COVID-19 where available information might be strictly limited. We have shared complete treatment regimen of successful management of a complicated case of COVID-19 added DM1 with limited and easily available resources of a developing country like Pakistan. Our study also supports a few prefigured facts, like COVID-19 severity variations related to age group and underlying medical conditions.

Euphorbia pilulifera subfractions protect against CCL4 induced damage in HepG2 cells via regulating NF-κB/Nrf2/Akt/TGF-β1 pathway.

Ammara Shams, Koloko Brice Landry, Faiza Shams, Ayesha Malik , Kausar Malik, Bushra Ijaz*

Hepatic diseases are a major global health concern; particularly acute liver damage is associated with significant death rates. The limitations of currently existing therapeutic options for hepatic diseases demand the discovery of innovative and economical therapies. In modern medicine, plants and their constituents have been utilized for a long time as beneficial and protective therapeutic substances against liver diseases. This study was anticipated to assess the hepatoprotective and anti-inflammatory effects of Euphorbia pilulifera by modifying the NF-κB/Nrf2/Akt/TGF-β1 pathway. Among essential inflammatory pathways, the NF-κB cascade is a potentially major target in liver fibrosis and may be addressed to recover liver injury. Briefly, E. pilulifera and its the fractions (n-hexane, chloroform, ethyl acetate, butanol, and aqueous) were found effective in reversing the impacts of CCl4 -induced liver damage via downregulation of the NF-κB/Akt/TGF-β1 pathway. Particularly, ethyl acetate fraction revealed 90% hepatoprotective activity against CCl 4 - induced toxicity in HepG2 cells. Moreover, the antioxidant potential of the plant was assessed through ferric-reducing power assay and DPPH radical scavenging activity. In addition, the anti-inflammatory potential and quantitative phytochemical profile of the plant extract were analyzed. Furthermore, an acetylcholinesterase inhibition test was also executed to analyze Euphorbia pilulifera therapeutic activity against Alzheimer's disease. Conclusively, the sub-fractions of the plant were found equally potent against inflammation and acetylcholinesterase. Therefore, further evaluation of Euphorbia pilulifera plant extract may assist in producing novel, ethical, and economic substances that might effectively contribute to developing therapeutic remedies against hepatic disorders.

Co-culturing effect of normal and osteoarthritic chondrocytes on hypoxic knee cells

Aisha Tarar, Sumera Rashid, Nadia Naseer , Umar Sajjad , Kausar Malik, Sheikh Riazuddin, Noreen Latief*

The aim of the study was to firstly estimate the difference in growth characteristics of normal and osteoarthritic chondrocytes. For this purpose, we prepared female wistar rat model of osteoarthritis (OA) by intra-articular injection of collagenase B. Female rats were selected because the incidence of osteoarthritis in humans is higher in females as compared to men. Growth rate of normal and osteoarthritic chondrocytes was estimated by calculating population doubling time, cell proliferation rate, LDH released, ALP levels, GAGs content and by glucose levels. Secondly, comparison of normal and osteoarthritic chondrocytes against hydrogen peroxide induced injury was analyzed using co-culture technique. Equal number of chondrocytes from normal, OA and combination of both normal and OA were co-cultured with hydrogen peroxide (H_2O_2) stress induced chondrocytes. We used this co-culture system to study the effects of paracrine factors released by normal, OA and combined normal and OA cells on stress induced chondrocytes *in-vitro*. The effects of co-cultured groups were analyzed by proliferation rate, proteoglycan content, cell viability, gene expression analysis and immunostaining results. It is concluded that normal cells group and combined normal and OA chondrocytes group increased the proteoglycan contents and viability of H_2O_2 induced injury with a concomitant decrease in apoptosis and cell damage. This study also provides an insight about the therapeutic potential of normal and OA chondrocytes for the treatment of osteoarthritis.

Molecular characterization of *Toxoplasma gondii* in human population and therapeutic efficacy of silver nanoparticles against *Toxoplasma gondii* infection in mice model

Zunera Shafiq*

Toxoplasmosis infection is caused by T. gondii that may lead to serious health consequences. Recently nanoparticles is considered as the most promising technology in the field of biomedical sciences. Present study will aim to identify the T. gondii strain in human population and determine the therapeutic efficacy of AgNPs against Toxoplasma infection induced in mice. For this purpose general health status and possible risk factors will be assessed through a designed questionnaire. Blood samples will be collected randomly from study subjects of age ranging between 18-60 years to confirm the Toxoplasma infection through LAT. For the identification of T. gondii strain DNA extraction from seropositive blood samples, amplification of SAG2 gene by PCR and RFLP will be done. Efficacy of AgNPs with Nigella sativa will be assessed to control T. gondii infection in experimental mice. AgNPs will be synthesized and characterized. Infection will be induced in mice (n=40) and confirmed by MAT. Total five groups of mice will be formed. Three groups (n=10 each) will be given optimum silver nanoparticle dose upto 5 weeks for treatment and two groups (n=10 each) will be used as untreated positive and negative control group. Mice blood will be used to analyse various endocrine hormones and interleukin-10 level by ELISA to reveal the parasitic influences. Hematological changes will be determined by CBC test. For behavioural analysis the level of dopamine will be determined by using HPLC. Liver, heart and gonads will also be processed to evaluate the histopathological changes caused by the infection. Statistical analysis will be done. Present study will help to identify the parasitic strain and determine the optimum dose of AgNPs against T. gondii infection induced in mice that may lead to improve public health.

Isolation, purification and characterization of bacteriophages for the bio-control of multidrug-resistant uropathogenic Escherichia coli

Zoha Masood*, Kuasra Malik, Muhmmad Hamza Hashmi

Antimicrobial resistance is a global-level concern faced by almost every country. It is a great threat encountered by humanity presently. If antimicrobial resistance specifically, antibiotic resistance hasn't been controlled yet it will result in 10 million death by 2050, a huge loss of humanity. There is a hefty ratio of people, who die frequently from infections caused by resistant pathogens like UTI, meningitis, enteritis and other nosocomial infections. Bacteriophages have been discovered 100 years ago which can kill and destroy bacterial cells. These are 'bacterial eaters' who completely rely on bacteria for their lifecycles. We can treat infections caused by antibioticresistant strains through phage therapy, which gives new hope to save millions of lives. Thus, we isolated phages from natural dirty water sources against resistant Uropathogenic E.coli. The main purpose was to isolate a bacteriophage with a narrow range, so it won't disturb the normal microflora of the body. The phages isolated showed a lytic cycle and checked their stability at different temperatures and pH ranges. We also performed PCR for molecular characterization of the MCP gene, three phages were isolated that belongs to different family against UPEC. Further, TEM and whole genome sequencing were the main goals for the complete characterization of isolated phages. The main objective of the study was to find out an alternative to antibiotics to treat various infections caused by MDR bacterial strains. Different phage cocktails can be made and used for treating MDR bacterial infections. We can use phage as a mainstream medicine as an alternative to old resistant antibiotics.

Production and exosome encapsulation of recombinant human IL-2 to study its comparative in-vitro anticancer potential in various cancer types

Seerat-e-Fatima* , Nadeem Ahmed

Interleukin-2 was the first interleukin to be discovered. It was discovered as a glycosylated molecule that was involved in mediating various immune responses. It acts in activation and growth of the NK cell and T-cells. In this way, they play a part as an anticancer therapeutic agent. It was also the first FDA approved drug for the treatment of cancers like Metastatic melanoma and renal cell carcinoma. Thus, its clinical implications make it important to produce the recombinant human IL-2 protein. Moreover, there was also need to increase the biodistribution of the protein in cancer cells. Exosomes are the extracellular vesicles ranging in size from 30-200nm and being studied for their functioning as natural nanoparticles. The main objective of this study was to produce IL-2 by recombinant technology and load them onto exosomes and study their enhanced anticancer potential. Therefore, we isolated exosomes from two different cell lines (Hep-G2 and HCT-116) and characterized their contents, structure, stability, drug release and loaded them with recombinant human IL-2 and studied their in-vitro anticancer activity. The loading by sonication method resulted in considerable loading efficiency and sustained drug released pattern. Importantly, the encapsulation of rhIL-2 into exosomes increased their chemotherapeutical activity in different cell lines including Hep-G2, HCT-116, MD-MB-231 and H1-Hela. We concluded that exoIL-2 holds a significant potential for increasing the delivery and biodistribution of rhIL-2 in various cancer cell lines.

Cross-sectional study on different psychological distress among university students

Fatima*, Khalid Hussain Rind

Psychological distress one of the major health concern leads to chronic disease development. The aim of study was to investigate mental health or psychological distress among university students. The cross-sectional study was conducted from August to November, 2022, at Shaheed Benazir Bhutto University Shaheed Benazirabad. Individuals' mental health was evaluated utilizing the survey questionnaire. Overall, 364 respondents were participated with questionnaire , out of them, 173 marked their physical health average, 143 excellent, 25 somewhat poor, 12 poor and 11 were not sure about their physical health. On the other hand, 152 marked their mental health average, 133 excellent, 34 somewhat poor, 29 poor and 16 where not sure about their mental health. Even though individuals with average and excellent mental physical health get stressed by family issues. Due to disturbance in mental health, dietary habits also get affected as 62 felt no change, 83 said yes, I eat more often, 107 said yes, I do not feel hungry, and remaining 112 said they feel change but not much. 181 students tend to feel positive towards their lives according, 12 never, 68 often, 21 rarely, 82 sometimes 36 have done mental health examination less than 6 months, 24 said they done mental examination 6 months ago, 20 individuals have done their examination a year ago, 29 said it's been more than a year to their mental health examination and 255 never went for mental health examination it shows that people do not give importance to mental health issues.