

Conference Proceedings

Abstracts of technical session lectures delivered during International Conference on Applied Biosciences (ICAB2021) held in Mohammad Ali Jinnah University, Karachi, Pakistan during December 30-31, 2021.

Preface

Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan organized 2nd International Conference on Applied Biosciences (ICAB2021) during December 30-31, 2021. Hundreds of scientists, post-doctoral fellows and graduate students from Pakistan and other countries attended this conference. In this conference, an array of topics related to applied biosciences including Biochemistry, Molecular Biology, Bioinformatics, Metabolomics, Biotechnology, Cancer Biology, Genomics, Metagenomics, Proteomics, Health Biotechnology, Immunology, Infectious Diseases, Microbiology, Genetic Engineering, Molecular Medicine, Neurosciences, Virology were discussed.

In this issue of PJBMB, we are publishing abstracts of technical session lectures presented during the conference. All abstracts submitted to ICAB2021 for presentations in technical sessions were peer reviewed. Editorial board of PJBMB is grateful to the organizing committee of ICAB2021 for providing abstracts of plenary presentations for publication in PJBMB.

Editorial Board

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SESSION LECTURES IN ICAB2021

EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS REDUCE OXIDATIVE STRESS IN RAT INTERVERTEBRAL DISC DEGENERATION MODEL

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ABSTRACT

Intervertebral disc degeneration (IVDD) is a progressive degenerative disc disease characterized by nucleus pulposus cells (NPCs) loss due to inflammation-driven

cascade in the disc, results in nerve compression that leads to lower back pain. Oxidative stress is mainly associated with pathogenesis of IVDD, it is a potent trigger of notochordal cells senescence, autophagy and apoptosis. As a result, the number of functional and viable cells decreases significantly due to persistent oxidative stress. Mesenchymal stem cells (MSCs) derived EVs have shown promising effects in tissue regeneration and immunomodulation. EVs gain tremendous attention as a novel tool for cell-free therapies. Aims: The aim of the study is to investigate the role of EVs derived from human umbilical cord mesenchymal stem cells (hUC-MSCs) for in vitro rat notochordal cells induced oxidative stress model to evaluate their regeneration potential. Methods: EVs were isolated from hUC derived MSCs, purified, size and

surface charge were determined by dynamic light scattering, additionally tagged with fluorescent probe (DiI) and visualized under fluorescence microscope. The topography and elasticity of EVs was determined by atomic force microscopy (AFM), and western blot analysis was performed to examine EVs proteins marker such as annexin V, and CD81. NPCs were isolated from rat coccygeal disc, propagated and characterized. The in-vitro effects of EVs on the migration, cell growth and survival of NPCs were determined by scratch assays, ROS measurement, and qPCR analysis of oxidative stress genes. Fluorescence microscopy was used to examine the uptake of DiI labeled EVs by NPCs. Results: MSCs were characterized by immunocytochemistry. The phenotypes of isolated EVs showed that the size of EVs was 403.3 ± 85.94 nm, and the zeta potential was -0.270 ± 4.02 mV. The topographic images revealed size distribution of EVs by AFM and labeled EVs were visualized by fluorescence microscopy. The protein expression results showed that EVs were CD81 and annexin V positive. EVs treatment reduced the H₂O₂ induced oxidative stress in NPCs by decreasing ROS as compared to NPCs under oxidative stress. qPCR analysis showed that EVs significantly reduced the expression of oxidative stress genes, and promoted cell proliferation. Scratch assay showed that EVs significantly increased proliferation, and migration of NPCs. Co-culturing of NPCs with DiI-labelled EVs showed internalization and uptake of EVs by NPCs. Conclusion: It is concluded from this study that EVs protected NPCs from H₂O₂

induced oxidative stress by reducing the intracellular ROS generation, and improved NPCs proliferation and migration. These findings will enhance our understanding for the cell-free therapy for IVD degeneration.

Key Words: Extracellular vesicles, Oxidative Stress, Stem Cells, Regeneration

VULNERABILITY OF CARNIVORE SPECIES TO HABITAT FRAGMENTATION ALONG INDUS RIVER

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ABSTRACT

Carnivores belonging to order carnivora are important component of every ecosystem because they control the population of other animals through predation. The members of this order are more threatened to extinction in comparison to their prey species because of having larger home ranges and smaller population size. Current study was designed to check the effects of habitat fragmentation on distribution of carnivore species in the Indus Riverine delta. For this purpose we reviewed previous literature and conducted questionnaire survey of fifteen different sites for fifteen different carnivorous species. Data collected from scientific papers and field survey was grouped into three categories (a, b and c) and then analyzed using betapart (an R-package for studying beta-diversity). Sorenson dissimilarity (β_{sor}) shows a regular

change in beta-diversity from before 1990-uptill now both in nestedness and species turnover. But when we measure nestedness dissimilarity index (β_{sne}) alone, it shows a relatively small change from 1990-2010 but after 2010 there was an abrupt change and same is the case with turnover dissimilarity index (β_{sor}). Site wise turnover dissimilarity (β_{sim}) for all three categories shows that with the loss of some species diversity among study sites is decreasing and they are becoming more correlated with each other. While nestedness dissimilarity for all three data sets also shows gradual variation and increased dissimilarity. I also compared square root transformed β_{sim} and β_{sne} components of β_{sor} for data before 1990 and before 2010, before 2010 and current data and at the end before 1990 and current data. Results show that during 1990 to 2010 there was gradual increase in the species nestedness but after 2010 due to flooding fragmentation of habitat resulted in species loss. It proved that fragmentation has negative impact on survival of species.

Key words: Carnivore; Habitat; Fragmentation; Indus River; Vulnerability.

IN SILICO APPROACH TO EXPLORE THE MOLECULAR INTERACTION OF NNAT GENE VARIANTS WITH THERAPEUTIC COMPOUNDS OF ANOREXIA NERVOSA

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ABSTRACT

Introduction: Anorexia nervosa (AN) – a perplexing heritable, psychiatric eating disorder (ED) with high prevalence in younger age adults. Identifying the specific role of gene(s) that predispose an individual to AN is still needed. Objectives: To explore the structural role of NNAT (neuronatin) gene pathogenic SNPs (single nucleotide polymorphism) with therapeutic compounds of AN. In 2019, it was proposed that constitutionally reduced levels of NNAT wild-type or expression changes may serve as the basis to increase the susceptibility towards AN. Methodology: Gene sequence, SNPs was extracted from public databases. Gene to gene interactions, human tissue-specific expression analysis in the brain and computation of transcript level functional impacts of SNPs using machine learning in silico algorithms were used to investigate the pathogenicity and protein stability. Ab initio 3D-modeling of wild-type, validation and recognition of druggable binding pockets of NNAT through various in silico tools were performed. Alternate model prediction for NNAT variants through residue specific mutation approach was generated using UCSF Chimera program. The 3D compounds involved in the management of AN were extracted from the DrugBank database. Energy minimization, rule of drug-likeness were investigated, and docked with pathogenic variants to estimate the drugs'

binding free energies. Results & conclusion: 10 missense SNPs were obtained as rare coding variants of NNAT gene. The functional impact analyses of SNPs showed risk pathogenicity that cause the change in protein function in mutation. The 3D-modeling analysis of AN drugs' binding energies indicated lowest binding free energy (ΔG) and smaller inhibition constant (K_i) with mutant models, which strengthen that NNAT variants strongly interact with AN drugs. Therefore, NNAT mutations may be associated with having susceptibility to ED such as AN, by exhibiting highest drug binding affinity for AN drugs, employed to manage the respective psychiatric illnesses.

Keywords: Anorexia nervosa, mutations, neuronatin, wild-type.

MULTIPLEX-ARMS PCR FOR OF VITAMIN D RECEPTOR GENE VARIATIONS IN TYPE 2 DIABETIC PATIENTS IN PAKISTAN

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ABSTRACT

Variations in vitamin D receptor (VDR) gene have been extensively correlated with diabetes mellitus (type 1 and 2). A multiplex ARMS-PCR technique was developed to probe SNPs present in VDR gene. These

include BsmI, ApaI and TaqI. This observational study included 182 healthy subjects and 192 type 2 diabetic (T2D) patients. Results indicate that the TT genotype of TaqI SNP was frequently present in T2D patients and was significantly associated with T2D ($p < 0.001$). TaqI polymorphisms with TT genotype in VDR gene may be associated with the onset of T2D in the study group. The results of this study indicate the necessity to undertake further studies on a large sample size.

Keywords: Multiplex ARMS-PCR; Haplotype analysis; Diabetes type 2; Vitamin D receptor polymorphisms.

EVALUATION OF ANTIFUNGAL RESISTANCE AND VIRULENCE FACTORS OF CANDIDA ALBICANS ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

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ABSTRACT

Candidiasis is a versatile group of fungal infections which is frequently emerging as opportunistic infections with various clinical manifestations ranging from mild mucosal or cutaneous infections to severe life threatening invasive infections. Approximately 90% cases of Candidiasis are caused by *Candida albicans*. There are certain factors which contribute to the pathogenesis of this specie such as different virulence and

antifungal resistance mechanisms. This study was designed to determine the production of extracellular hydrolytic enzymes such as, esterase, coagulase, gelatinase and catalase by this fungi as well as determination of its antifungal resistance profile. A total of three hundred and four clinical *C. albicans* isolates obtained from different clinical specimens were identified by conventional diagnostic protocol. The antifungal susceptibility of *C. albicans* strains was determined by disc diffusion technique against commercially available antifungals such as Nystain 50ug, Amphotericin B 100 unit, Fluconazole 25ug, Itraconazole 10ug, Ketoconazole 10ug, and Voriconazole 1ug. Furthermore, these isolates were also screened for the production of virulence factors. The qualitative and quantitative assessment of biofilm formation was determined by the tube staining assay and spectrophotometry respectively. Similarly, Gelatinase, Coagulase, Catalase and Esterase enzyme production was detected by using standard techniques. A total of 66.1% (201/304), 37.5% (114/304) and 28.9% (88/304) of *C. albicans* strains showed sensitive dose dependent (SDD) against Nystain, Ketoconazole and Itraconazole while among all of the antifungal drugs *C. albicans* strains showed high resistance against Ketoconazole 24.7% (75/304). Similarly in case of virulence factors, majority of the *C. albicans* strains 88% (267/304) formed biofilm while, the quantitative mean values is 0.1762 (SD \pm 0.08293), ranging from 0.012 to 0.378. Although the strains of *C. albicans* also produced hydrolytic virulence enzyme such as Esterase 52% (157/304), Catalase 100% (304/304), Coagulase 69% (211/304), and Gelatinase 65% (197/304). The growing incidence of antifungal resistant and virulent species of *Candida albicans* in population

poses a threat for health care community and need prompt management.

Keywords: *Candida albicans*, opportunistic infections, Candidiasis, extracellular hydrolytic enzymes

NOVEL SYNERGISTIC COMBINATION TREATMENT FOR TRIPLE NEGATIVE BREAST CANCER

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ABSTRACT

Breast cancer is one of the most alarming health issues among women throughout the world. Nearly, 2.3 million new cases of breast cancer were identified in 2020, across the globe with approximately 685,000 lives lost. One out of every nine women in Pakistan is suffering from Breast cancer, making Pakistan the country with the highest incidence rate of Breast cancer in Asia. Triple negative breast cancer (TNBC), the most frequently occurring breast cancer in Pakistan, shows resistance against hormonal therapy and chemotherapy hence it is an aggressive type of breast cancer with poor prognosis. Approximately, 19% of the Pakistani population was diagnosed with TNBC with a majority of younger females. Mammograms at stages I, II, or III are performed to diagnose breast cancer, however due to inefficient diagnosis and absence of early biomarkers, breast cancer

has a very high mortality rate. Surgical resection, chemotherapy and radiation therapy are the most common choices of treatment used against breast cancer. During recent years combination therapy is being investigated for better therapeutic effects. The purpose of our study was to investigate the role of Mevalonate (MVA) pathway intermediates in TNBC. MVA is a complex metabolic cascade which is essential for the production of cellular end products and growth of cell cycle. In the present study, we induced Bisphosphonate (MVA pathway inhibitor), in combination with Temozolomide (alkylating agent), to stimulate cytotoxic effect as therapeutic strategy for TNBC. Gene expression analysis was performed in both treated (single agent and drugs combination) and untreated breast cancer cells to identify the changes in pro apoptotic, apoptotic and MVA pathway enzymes. Moreover, proteomic analysis was performed to identify the differential expression of proteins after incubation of combination therapy in TNBC cells. Our study may provide an insight to understand the role of novel drugs combination as effective anticancer regimen which could be beneficial for TNBC treatment.

Keywords: Triple Negative Breast Cancer, Bisphosphonate, Combination Therapy, Anti-tumor Effect

THE BROAD FIELD OF FORENSIC PHARMACY: AN IMPORTANT AND EVOLVING RECOGNITION NEED IN PAKISTAN

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ABSTRACT

A pharmacist has an expert knowledge as well as working experience in the subjects of therapeutics, pharmacokinetics, and toxicology along with exposure to subjects such as forensic medicine during Pharmacy education. All these knowledge domains can be applied and act as an interface to the forensic situations. The skills and expertise of a forensic pharmacist can be useful in a large and diverse number of legal cases. With an ever increasing incidence of criminal and civil cases in Pakistan, the development and inclusion of forensic pharmacist in the judicial system of country are the need of the hour. The research in pharmacy has witnessed great technological advancement that allows it to expand its scope beyond the domain of therapeutics. Differing pharmacokinetics and pharmacodynamics of drugs in living and dead, drug interactions, abuse of drugs, personal injury or death due to drug exposure leading to medico-legal issues, environmental exposure to chemicals, and doping and forensic pharmacovigilance are the diverse aspects of Forensic Pharmacy.

IN VITRO SCREENING OF FDA- APPROVED DRUGS AGAINST SARS-COV-2 ISOLATED IN PAKISTAN

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China in late 2019 and soon became a pandemic severely burdening healthcare systems of even well developed countries. The first case of COVID-19 in Pakistan was reported in February 2020 and as of 20th December 2021 the total number of reported cases and deaths are 1,291,467 and 28,878 respectively. This necessitated investigating anti-SARS-CoV-2 potential of already approved FDA drugs or drugs locally in use for treating other illnesses so that to reposition them for clinical management of COVID-19 patients. Eleven such drugs were tested and it was found that eight drugs inhibited SARS-CoV-2 in vitro with varying degrees of efficacy. Remdesivir, Nitazoxanide and Niclosamide were found to be highly and selectively effective against the virus. Other promising drugs included Chloroquine Phosphate, Azithromycin, Daclatsvir, Hydroxychloroquine and Ivermectin.

Keywords: Coronavirus, SARS COV 2, Drug Repurposing, Antiviral

METAGENOMIC ANALYSIS OF DRINKING WATER SAMPLES COLLECTED FROM TREATMENT PLANTS OF HYDERABAD CITY AND MEHRAN UNIVERSITY EMPLOYEES COOPERATIVE HOUSING SOCIETY

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ABSTRACT

The quality assessment of water, supplied to the end user, is an essential part to assess the physical, chemical, and biological status of water, which impacts on human health. For the quality assessment of drinking water treatment plants and distribution systems of Hyderabad City and Mehran University of Engineering and Technology, Jamshoro, Pakistan, 13 surface drinking water samples were collected from three treatment plants, two of Hyderabad City, including WASA treatment plant and its distribution system (n = 5), Hala Nakka treatment plant and its distribution system (n = 6), and Mehran University Employees Cooperative Housing Society (MUECHS) treatment plant and its distribution system (n = 2). Physicochemical parameters of all drinking water samples were in the range compared to EPA and WHO guidelines, except in L-12 sample. Notably, no free chlorine was detected in all samples. In metagenomics analysis, targeting V3-V4 hypervariable region of 16S rRNA gene, in QIIME2 environment, high bacterial prevalence was observed in all samples. On average, 348 OTUs were observed per sample. Among all samples, treated water sample from the Hala Nakka Treatment Plant (HNTR) was the most diverse sample in bacterial composition (Shannon 7.51 and Simpsons reciprocal indices 0.98). Overall, Proteobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia, and Actinobacteria were the five most abundant phyla (relative abundances of 43.6, 37.9, 8.5,

2.5, and 2.4 percent, respectively). Notably, Cyanobacteria are well-known toxin producers which effect the human, and animal health. At genus level, Flavobacterium (4.86%) and Aquirestis (3.77%) were the most abundant genera. Functional predictions, based on 16S rRNA gene by PICRUSt, predicted 6909 KEGG ontologies, relating to 245 KEGG pathways. Among the predicted pathways of KEGG ontologies, pathways to human infections were also found. In conclusion, this study gave a deep insight into bacterial contamination in drinking water samples of Hyderabad City and MUECHS treatment plants and water quality status in Hyderabad and Mehran University of Engineering and Technology.

Keywords: Metagenomics. Bioinformatics. Next generation sequencing. Drinking water distribution system (DWDS). Water quality. PICRUSt.

**SEED NANO-PRIMING WITH
ZINC OXIDE NANOPARTICLE
ENHANCES BIOMASS,
PHYTOCHEMICAL
CHARACTERISTICS AND
AGRONOMIC FEATURES OF
BITTER GOURD (MOMORDICA
CHARANTIA L.) PLANTS**

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ABSTRACT

The current agronomy is under tremendous pressure to produce higher yields due to escalating human population. The old

methods in agriculture, uneconomic landholdings and limited supply of agricultural inputs are further threatening agricultural commodities. In modern times, nanotechnology holds the pledge to provide another green revolution in horticulture and crop science. Current study is a field study set out to elucidate the potential of Zinc oxide nanoparticles (ZnONPs) to enhance growth, phytochemical profile and yield of bitter gourd plants. The seeds of bitter gourd were soaked in 25, 50, 75 and 100 ppm aerated solution of ZnONPs taking 0ppm priming treatment as control and then they were planted. Seed nano-priming resulted in higher growth rates in terms of plant height, number of leaves and branches per plant, plant dry weight and leaf area. It was found that 75ppm priming treatment proved the best among all treatments raising the number of leaves per plant by 81% and plant height by 115%. The nano-primed seeds produced plants with higher yields in terms of number of fruits per plant, fruit length, fruit diameter and number of seeds per fruit. Seed nano priming with 75ppm ZnONPs increased fruit diameter by 48%. Furthermore the phytochemical profile of bitter gourd was enhanced in terms of increased leaf charantin contents i.e., 214% as compared to control and total flavonoids contents i.e., 150% as compared to control. The findings of the study suggest that seed nano-priming with ZnONPs is operatively beneficial in improving performance of bitter gourd crop.

Keyword: Nano-priming of seeds, growth and yield, charantin, bitter gourd, vegetables

**GENPRO: AN ANDROID
APPLICATION FOR THE
ANALYSIS OF GENOMIC AND
PROTEOMIC DATA**

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ABSTRACT

For the analysis of genomic and proteomic data, various tools and databases are available. The currently available biological databases and bioinformatics tools support Windows, Linux and MAC operating system. The bioinformatics tools that support smartphone operating systems are generally not available. Nowadays, smartphones and tablets are extensively used so there is a dire need to develop bioinformatics applications (app). We have developed GenPro, which is an android based app which was designed with a novel strategy in which Python scripting, Kivy language and Buildozer tool have been used to generate Android Package file. The GenPro app takes DNA sequences as input, transcribes and translates the sequence and calculates GC content. Moreover, this app is able to import, analyze and visualize Variant Calling Format (VCF) files, a widely used variant calling format in genomics. This android app will be available on Google Play store to serve the biomedical research community for sequence data analysis.

NEURO-OCULAR DEFECTS MODELLING IN DROSOPHILA MELANOGASTER BY MTX

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ABSTRACT

Drosophila melanogaster is an effective model for studying human disease biology. This is primarily due to short generation time and fast breeding of the organism. Additionally, 60 % total genomic similarity and 75% orthologous relationship with human disease genes further represent its suitability as model organism. In this study we aimed to develop fly models of anatomical and functional human neuro-ocular defects by chemical mutagenesis. Briefly, flies were bred in different concentrations of Methotrexate (MTX) and F1 progeny was assessed for ocular developmental defects. Stereomicroscopic and scanning electron microscopy showed variation in the surface topology and number of ommatidium upon exposure of MTX. UV-Visible spectroscopy (300-1000 nm) of organic solvent extract of eye also revealed variations in the absorbance especially between 400nm to 550nm. Consistently, TLC and HPLC profile showed variations in the proportion of 2 & 7 different kinds of ommochromes and pteridines, respectively that constitute the total pigmentation of eye. Functional manifestations of these changes are also noticed in phototaxis assay where by and large flies developed under MTX stress lacks the normal positive photo-tactic behaviour. Out of 31 genes that regulates the expression of eye pigment, ommochrome and pteridine, 29 were found orthologous to humans. Perturbation in some of these genes leads to eye defects like retinitis pigmentosa, anterior segment dysgenesis and early infantile epileptic encephalopathy.

Additionally, module distribution in system network of the *D. melanogaster* and human gene interactome share noticeable similarities in biological functions.

LONGEVITY IN DROSOPHILA MELANOGASTER MODEL ADVERSELY AFFECTED BY CHRONIC ANTIBIOTIC EXPOSURE

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ABSTRACT

Infectious diseases share a considerable portion of the total disease burden around the world. Amongst the therapeutic interventions, antibiotics are assessed with profound efficacy. However, ill-advised and self-prescribed usage of antibiotics are much common in the developing countries including Pakistan. In this study, we have assessed the effect of chronic usage of antibiotics on the longevity of organisms using *Drosophila melanogaster* as model organism. In short, 100 male and 100 female *D. melanogaster* were exposed to different concentrations of antibiotics namely, Streptomycin, Tigecycline, Doxycycline and Norfloxacin and metronidazole. Each the flies were count for their survival and Kaplan-Meier survival curve were plotted and analyzed statistically. The findings showed that tigecycline and norfloxacin did not impact of the survival of the flies for the duration of 30 days, 60 days and as the

natural span of life. Conversely, streptomycin adversely affected the life span of the flies irrespective to gender and concentration. Metronidazole dwindled the life span of the flies in concentration dependent manner, whereas doxycycline exposure has shown to reduce the life span of male flies relatively faster compared to female flies. Additionally, bioinformatics analysis showed that out of the 89 commonly linked aging related genes, 67 were found as orthologues in *D. melanogaster*. These genes shared considerable similarities in the modular and functional attributes in the total interactome. The interactome also refers to key hub genes that could be explored further for the expressional changes to explore the underlying mechanism of antibiotic driven aging.

DETECTION AND CHARACTERIZATION OF CANDIDA SPECIES FROM HEAD AND NECK CANCER PATIENTS BY CHROMAGAR AND PCR TECHNIQUES

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ABSTRACT

Head and neck cancer is one of the most common malignancies in the world. Fungal infections, mainly by *Candida* species, are common in individuals with head and neck cancer. Changes in oral and/or general environment due to malignancy, radio- and

chemotherapies result in overgrowth of oral mycoflora, leading to variety of clinical fungal infections. This study was designed to detect the presence of oral *Candida* associated with head and neck cancer patients using CHROMagar *Candida* and PCR techniques. Primers based on the genomic sequences of DNA topoisomerase II of *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis* were designed and their specificities tested in PCR-based identifications. A total of 145 yeast cultures were isolated from cultures tests of 110 saliva samples, taken from head and neck cancer patients undergoing chemotherapy and radiation therapy. The most frequent fungal species were *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*. These results suggest that the use of Chromagar technique is suitable in resource limiting environment. Whereas, species-specific primers of the *Candida* DNA topoisomerase II gene, is more reliable and rapid detection technique for identification of *Candida* at the species level in advanced labs equipped with PCR instrumentations.

Key Words: *Candida*, CHROMagar, Head and Neck cancer, Rapid identification, PCR

**EFFECT OF PROCESSING
PARAMETERS ON FORMATION
OF SELF-ASSEMBLED
POLYELECTROLYTE
NANOCOMPLEXES: AN
OPTIMIZED NANOCARRIERS
SYSTEM FOR ORAL PROTEIN
DELIVERY**

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ABSTRACT

The most of peptides and proteins drugs are administered through parenteral route because of their degradation in the gastrointestinal tract and reduce oral bioavailability. The objective of present study was to optimize and use polyelectrolyte complexation phenomena to overcome oral protein delivery barriers. Bovine Serum Albumin (BSA) and nanogels (NGs) were used to prepare self-assembled polyelectrolyte nanocomplexes (BSA/NGs-PEC). Parameters BSA:NGs ratios, pH, incubation time and stirring rate were optimized. Formulations were evaluated for particle size (PS), polydispersity index (PDI), zeta potential (ZP) and percentage of entrapment efficiency (%EE). SDS-PAGE and esterase activity assay of BSA was performed. Intestinal permeability was investigated using Franz diffusion cell equipped with intestinal segment of goat. Optimized BSA/NGs-PEC (BF15) formulation had PS, PDI, ZP and %EE of 287.87 ± 8.86 nm, 0.201 ± 0.06 , 12.60 ± 0.98 mV and 89.32 ± 4.36 %, respectively. Intestinal permeation assay exhibited that the amounts of BSA released from BSA/NGs-PEC was 1.5-fold than pure BSA. SDS-PAGE and esterase activity assay revealed that primary structure and bioactivity, respectively, of BSA was intact. Results demonstrate that present PEC method improve intestinal permeability and preserve structural integrity and bioactivity of loaded peptides and protein drugs.

Key words: Bovine serum; Oral protein delivery; Nanocomplex; Polyelectrolytes; Nanogels;

EVALUATION OF ANTIBACTERIAL ACTIVITY OF PLANTS EXTRACTS PREPARED FROM COMMONLY FOUND PLANTS IN KARACHI AGAINST STRONG BIOFILM FORMING CLINICAL ISOLATES OF S.AUREUS

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ABSTRACT

Introduction: Biofilms are communities of microbial cells that underpin diverse processes including sewage bioremediation, plant growth promotion, antibiotic resistance, chronic infections and industrial biofouling. The cells residing in the biofilm are encased within an extracellular exopolymeric matrix that commonly comprises lipids, and proteins that frequently exhibit amyloid-like properties, eDNA and exopolysaccharides. This matrix fulfils a variety of functions for the community, from providing structural rigidity and protection from the external environment to controlling gene regulation and nutrient adsorption. Naturally microorganisms occur as surface attached cells than as planktonic or free-floating cells. In stressed conditions, they develop survival strategies and tend to attach to available surfaces to form biofilms. **Material and Method:** A total of 150 gram-positive isolates were collected from various clinical specimen like pus, urine, blood, HVS (high

vaginal swab), and wound from Clinical Laboratory Memon Medical Institute (MMI) Hospital, Karachi, Pakistan. Among those 63 were identified as gram positive. Identification and differentiation of the bacterial isolates was performed on the basis of their colonial, biochemical and morphological characteristics. Biofilm forming activity of isolates was tested with Congo Red Agar method. Screened organisms were sub-cultured and their qualitative analysis was done by measuring the absorbance through Trypticase Soya Broth (TSB) method in 96 well microtiter plate and organisms were categorized as Strong, Weak and Moderate biofilm formers. Antibacterial activity of *Fagonia* spp commonly known as Suchi Boti and *Ricinus Communis* commonly known as Caster Oil Plant was tested against strong biofilm forming isolates. Antimicrobial activity of extracts was measured by well diffusion method. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration was measured by two-fold serial broth dilution. **Results and Discussion:** Zone of inhibition showed activity against the clinical *S.aureus* isolates. Bacteria forming biofilm show more resistance to antibiotics due to an exopolymeric matrix which protects bacteria in several ways. Synergistic treatment of such organisms with herbal extracts and antibiotics can give better results.

Key words: Biofilm, Antibiotic resistance, *S.aureus*

PREVALENCE OF TUBERCULOSIS AND RIF RESISTANT CASES IN TAULKA GAMBAT DISTRICT KHAIRPUR MIR'S SINDH BY USING GENEXPERT INSTRUMENT

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ABSTRACT

Background: Mycobacterium is the causative agent of Tuberculosis (TB), and it's a contagious infection spread from person to persons. WHO suggests one TB positive may spread infection more than 16 persons whose living surrounding per year, its serious threat to public health and huge challenge for us to diagnose early at the stage. Aim: Investigation of Mycobacterium Tuberculosis (MTB) and MTB/rifampin (Rif) resistant patients using advance GeneXpert technology. Methodology: Mycobacterium Tuberculosis (MTB) was amplified by using sophisticated real time polymerase chain reaction GeneXpert (Cepheid GeneXpert® USA). Before this Acid Fast Bacilli (AFB) test was also performed. Results: Total 338 cases were investigated on the basis of TB suspected, among them 24 samples were smear positive and 21 samples smear negative, and without

smear samples were performed 137 in new diagnosed patients. It was found that 22 cases were justified MTB, 1 Rif resistant and 1 was negative, thus smear negative 6 cases were MTB with 1 Rif resistant and 15 were negative, while samples without smear 33 MTB and 1 Rif resistant. Although treated with TB medicine were 9 with AFB positive, 19 with AFB negative and 128 were without AFB. Among them 8 with MTB, 1 was negative, negative AFB 4 were MTB, 15 were negative, while 19 MTB with 2 Rif and 109 cases were confirmed negative. It was found that 182 new cases were performed among them 59 MTB (32%), 3 Rif (4.83%) and 120 were negative. Although previous treated 164 were examined and observed that 35 MTB (21.34%), 2 (5.71%) with Rif resistant and 127 was conformed negative. Conclusion: Current situation determined that not only common TB in the locality area, but also high burden of Rif resistant which will alarming condition for surroundings, and its need to eliminate this contagious infection.

Key words Mycobacterium, Tuberculosis, GeneXpert, Rifampin

THREE DIMENSIONAL STRUCTURAL INVESTIGATION OF LEAD MOLECULES AGAINST NEISSERIA MENINGITIDIS PATHOGENIC FACTORS; A STEP TOWARDS DRUG DESIGNING

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ABSTRACT

The Gram negative human pathogenic bacterium *Neisseria meningitidis* is responsible for causing meningitis and septicemia worldwide. A substantive question in bioinformatics analysis of bacterial genome is to ascribe a three-dimensional structure as well as a biologic role to all the coding regions. Here we performed structural bioinformatics analyses and docking studies of important *N. meningitidis* pathogenic factors involved in protein biosynthesis including methionyl-tRNA synthetase, 16SRNA methyltransferase, translation elongation factor-Tu and putative RNA methylase. Homology modeling of these important drug targets was carried out with better templates by MODELLER software and evaluated by Prosa and Procheck standalone software while docking was performed by Autodock Vina. The study not only provided detailed three dimensional structures of important proteins required for *N. meningitidis* pathogenesis but also presented potential lead molecules which can be used as their potent inhibitors. The present study has opened a door to achieve effective and safe drugs against the deadly meningoencephalitis.

Key words: *Neisseria meningitidis*, Homology modeling, Protein-ligand

docking, Pathogenic factors, protein biosynthesis

IDENTIFICATION AND STRUCTURAL INVESTIGATION OF POTENTIAL NOVEL DRUG CANDIDATES AGAINST LETHAL HUMAN PATHOGEN

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ABSTRACT

Neisseria meningitidis is responsible for causing meningococcal meningitis along with acute septicaemia in human beings. Functional genomics strategies proved cruciality of certain genes/proteins in *Neisseria meningitidis* pathogenesis. During the present studies, three important *Neisseria meningitidis* proteins i.e., Dead box RNA-Helicase, Polyribonucleotide nucleotidyl-transferase PNPase and Ribonuclease-III were targeted for homology modeling and protein-ligand docking studies not only to determine their three-dimensional architectures but also to identify their potential novel inhibitors. The Biscoumarin, malonitrile and indole derivatives showed the best inhibitory mode against all of the three enzymes. Since, these enzymes are assembled in Gram-negative bacteria to form RNA degradosome assembly therefore their inhibition will definitely shut off the

degradosome assembly and ultimately the decay of RNA, which is an essential life process. This is the first ever structural investigation of these drug targets along with identification of potential novel drug candidates. We believe that these small chemical compounds will be proved as better drugs and will provide an excellent barrier towards *Neisseria meningitidis* pathogenesis.

Keywords: *Neisseria meningitidis*, homology modelling, transcription. RNA degradosome, molecular docking

ASSOCIATION OF AVIAN DIVERSITY WITH WATER AT PATISAR LAKE, LNSP: A CASE STUDY IN SOUTH PUNJAB

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ABSTRACT

Birds are important indicators of ecosystem life, also known as farmer's allies, which have great economic value as a good source of protein, essential nutrients, pollen and add beauty to the environment. Present study was conducted to check the association of avian fauna with water and how it relates to avian diversity, record the current status of bird's distribution, richness and their population at Patisar Lake (LSNP) South Punjab, Bahawalpur Pakistan. Data was collected from October 2020 to March 2021(12 surveys) at Patisar Lake, usually at 9am to

12pm. Direct observations and indirect techniques of birds sampling were utilized to gather information. Lake was divided in to the four sites that were selected randomly and four water samples were collected from those sites. The line transect method was used to analyzed the abundance, diversity of avian fauna at Patisar Lake. DSLR camera (lenses 180mm, 75-300mm) and Vivo cellphone camera was used for photographs. Water samples were analyzed in laboratory for identification of different parameters such as PH, Odor, Color, Taste, Turbidity, Iron, TDS and synthetic chemical such as Calcium, Magnesium, Chlorides, its hardness as well as its conductivity. We found 45 birds belong to 28 families and 14 orders. Maximum number of species were falls in the Resident (19 birds) category followed by the second highest number of species, which were fall in migratory (7 birds), winter migrant (7) and summer breeder (7), category, while the lowest number of species richness were found in year around visitor (5). We concluded from analysis that conductivity, TDS, Chlorine, Calcium and Alkalinity is inversed in relation to avifauna. Other parameters were in direct relation to avian fauna such as Magnesium, Hardness, Turbidity and pH.

Keywords: Birds; Line transect; Migratory; Patisar Lake; Resident; Species-water association; Water Parameters

ECOBIOLOGY OF MONITOR LIZARD (VARANUS BENGALENSIS) IN CHOLISTAN DESERT BAHAWALPUR, PUNJAB

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ABSTRACT

Recent study was conducted to gather information on the ecology and behavior of monitor lizard (*V. bengalensis*) in Cholistan desert Bahawalpur during May 2017 to April 2018. Selected areas were eight in number with eight different habitats for monitoring, these habitats comprised on; Desert, Agriculture crop land, Canal side, Forest area, Ponds & Lakes, Orchards, Nurseries, and Human settlements. The procedure opted for survey was visual encounter followed by line transect. Focal sample method was also used for observing all the activities of an individual lizard from dawn to dusk. Preferred habitat observed was dry land or deserted areas followed by forested areas. Its diet comprised of crustaceans, earthworms, arthropods, beetles, fishes, frog and vegetables & fruits. Breeding period starts from end of February to mid of May in this area. Eggs laid in month of April but eggs could be found till end of May in felled logs or in termite mounds. Incubation period ranges between 5-6 months. Hatchlings emerged in the months of August and September. Out of total 126 eggs total eggs hatched were 39. According to our

calculation there were~ 50-70 individual in each of eight study sites and approximately total 700 number of individual of *V. bengalensis* found in the area of Cholistan desert. Due to illegal hunting or poaching, the population of *V. bengalensis* in these areas is at decline; long term study has been recommended to know the trend of monitor lizard population throughout its range.

Key words: Cholistan desert; Ecobiology; Lizards; *Varanus bengalensis*; Visual encounter survey method (VES)

GENETIC ASSOCIATION OF GJB2 VARIANTS IN PAKISTANI POPULATION

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ABSTRACT

Hearing loss (HL) is a sensory defect with major global health challenge for care providers. An estimated 15% of the world's population is suffering from some degree of hearing loss with 17% having severe hearing impairment (>60db). HL badly impact social, emotional and physical attributes. Gap Junction Beta 2 (GJB2) is the gene of interest in hearing loss as mutation in this gene results in faulty auditory processing. Sequence variations in GJB2 chromosome accounts for 50 % of the genetic causes and more commonly NSHL. There are some pathogenic variants of GJB2 which are more prevalent in Pakistani populations i.e. 231A>G (W77X) and 71A>G (W24X).

Studies found difference in frequency distribution of GJB2 linked HL among different ethnic groups of Pakistan. Consanguinity is other important factor which also determines prevalence of HL in any population. A total of 26 samples from Pakistani population were recruited after obtaining their informed consent. Blood samples were collected and then subjected to DNA extraction followed by DNA quantification, concentration and purity assessment. Two sets of primers were designed and optimized. After PCR amplification, DNA fragments were separated by gel electrophoresis and was visualized to compare findings with positive control. Interestingly, these two variants were not detected in our studied samples. Ethnic diversity, high consanguinity rate, regional differences and very limited source of studies do signify that other possible genes might be involved or even similar GJB2 gene with different mutation sites are at interplay in Pakistan.

Keywords: Hearing Loss (2) Gap Junction Beta 2 Protein (3) Polymerase Chain Reaction

EFFICACY OF BIOCONTROL AGENTS AGAINST PHYTOPATHOGENIC FUNGI IN GREEN HOUSE CONDITION

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ABSTRACT

This study was designed to isolate bacterial strain from rhizosphere and phyllosphere

with broad-spectrum antagonistic activity against phytopathogenic fungi. 360 sample of crops rhizosphere/phyllosphere were collected from different area of Quetta. 20 isolate showed antagonistic activity against phyto-pathogenic fungi, three isolates were selected on the basis of broad-spectrum antifungal activity and plant growth promoting activity. The antifungal activity of isolates were analyzed in in-vitro and green house conditions. The plant growth promoting activities such as cellulose, HCN, ammonia, siderophore, IAA and phosphate solubilization efficiency were monitored. The selected bacterial isolates were identified on molecular basis. The 16srRNA sequences deposited in genbank and accession numbers were acquired. The greenhouse assay on wheat was performed against *Fusarium oxysporum*, causative agent of fusarium wilt. The isolates BA, GL-1 and 5a showed more than 70% inhibitory activity against different fungi, they were positive for IAA, HCN and cellulose enzymes production. The two isolates, BA and GL-1 were positive for siderophore while isolate BA was positive for ammonia production. On the basis of 16s rRNA sequence and phylogenetic analysis the selected isolates were identified as *Bacillus* sp. furthermore, the isolate BA showed significance escalation shoot and root height, fresh weight and dry mass and decrease disease severity caused by *Fusarium oxysporum* by 64.97% on wheat (FD-08) seedling in green house assay. The wide-ranging antagonistic action of *Bacillus velezensis* isolated from the phyllosphere of wheat crop showed promising fungicidal and plant growth promoting capabilities with significant greenhouse results.

Key words: biocontrol, phyllosphere, antifungal, rhizosphere, antagonistic, plant growth promoting, *Bacillus* sp.

PAN-GENOME ANALYSIS OF STENOTROPHOMONAS MALTOPHILIA: AN INSIGHT OVER PATHOGENICITY AND DRUG RESISTANCE

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ABSTRACT

Stenotrophomonas maltophilia is a pathogenic bacillus, causes respiratory tract and many other infections in man and animals. Many pathogenic genes are found to involve in its pathogenicity. Pan-genomic analysis is performed to show genes distribution among the shared, unique and accessory genome. Genomic data of 538 strains was taken from NCBI Refseq database. Twenty-seven pathogenic proteins were selected as query sequences to run reciprocal BLASTp. Out of 538 strains 116 were selected for running Pan-Genome Analysis Pipeline. Gene locations were identified using Gview and phylogenetic analysis of 116 strains was also performed using online tool iTOL. BLASTp shows that maximum hits are found to be 17/27 in GCF_009676545 and GCF_006149095 and minimum hit is six in strain GCF_003751565, within the criteria of 90-100% identity. Genes; GspD, Gsp, rmlC HlyIII, smeF, smeT and virB11 are mostly occurring pathogenic genes. Whereas sull, sul2, omp54, virD4, xanB are present in minimum number of strains. PGAP analysis reveal that it is an open pan-genome with 447 core cluster count, shared 14,672, unique 15,550 and total 30,403 cluster count. Pathogenic genes 6/27 lie in the core region,

rest of genes are in the shared part. Strains from same location were clustered into same groups showing their close evolutionary relationship.

Keywords: Pan-genome Analysis Pipeline, BLASTp, Horizontal Gene Transfer.

PAN-GENOME ANALYSIS OF MULTIPLE STRAINS OF MYCOPLASMA PNEUMONIAE

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ABSTRACT

Mycoplasma pneumoniae is one of the simplest, smallest bacterial pathogens. The evolution increases the virulence and causes respiratory, urogenital infections and other mucous membranes related infections comprising extra-pulmonary manifestations with mild to severe cases. To explore the intra-specific variations and evolution of *M. pneumoniae*, a pan-genomic analysis was performed on 150 strains. The analysis for the most potential pathogenic strains within *M. pneumoniae* species was carried out through reciprocal BLAST (blastp). The visualization of reciprocal blast and gene count of 150 strains was carried through seaborn and matplotlib libraries of python. Pan Genomic Analysis was performed through a typical Pan-Genome Analysis Pipeline (PGAP) with a Bio-Linux-based environment. Pan Web Visualizer (PanGP) was used to visualize the results of PGAP. Comparative Neighborhood Gene Analysis was performed using GView and the Phylogenetic tree was constructed using iTOL. This species is potentially pathogenic as 134 out of 150 strains shows

almost all pathogenic factors with above 90% homology except 16 strains which belong to Spain, the USA, China, and Japan. *M. pneumoniae* pan-genome is an open pan-genome that showed total genes of 103174 in which 70359 (68.19%) core genes, 32241(31.24%) shared genes, and 574 (0.55%) unique genes. Adhesin P30, Protein TopJ, CARDS toxin, GLPF, GLPK, GLPO, GLT, and IgG-blocking protein M are part of the core genome. Adhesin P1, HMW1, and HMW2 genes are part of the shared genome. HMW3 and P65 are part of the unique genome. Therefore, comparative neighborhood gene analysis revealed many different neighbor genes pattern for HMW3 and P65. Phylogenetic analysis was exhibited in two main clades and also identified major ancestral lineage within *M. pneumoniae* species.

Keywords: *M. pneumoniae*, Pangenomic Analysis, Reciprocal Blast, Phylogenetic analysis, Pathogenesis.

TO DETERMINE IF AN ATHLETE'S DIET CHANGES THE HORMONE TESTOSTERONE IN THE ORGANISM

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ABSTRACT

Annotation: Testosterone is a hormone that increases the effectiveness of exercise, improves the physiological state of the athlete, and is essential for muscle function. Testosterone is also popular among athletes

as a "muscle-building" hormone. Because testosterone is the main androgen hormone. While on the WADA list, when testosterone levels naturally increase in the body, strength and power increase, and exercise efficiency increases. So we decided to explore ways that athletes can naturally increase testosterone. In humans, the normal level of testosterone in the blood plasma averages 7-50 pk / ml, which varies with age. This hormone is extremely important for athletes involved in strength and endurance-based sports, including bodybuilding, athletics, weightlifting, wrestling, and cycling. Because they increase muscle mass and strength, they are also important hormones for success in sports. For this reason, male athletes consume steroid hormones to increase the level of testosterone in their blood plasma.

This causes testosterone to rise above normal and you are not allowed to compete. That's why athletes try to walk at the highest testosterone levels. However, natural ways to increase testosterone have not yet been implemented in our country, so far they have been able to keep testosterone high in the body through the use of prohormones, or testosterone itself. In this way, the hormones in the body rise above normal and are excluded from the competition process. That's why we've studied diets that increase testosterone in the body without hormones, hormones, steroids, and naturally improve hormone synthesis.

In this section, we explore foods that affect testosterone. We selected 10 non-athletes, 10 weightlifters and 10 players for the experiment. We determined the amount of testosterone in their constant quiescent state. The results show that people who do not exercise have lower testosterone levels than those who do not. In weightlifters, the mean

testosterone level was 21.8 ± 03 pk / ml. This is 6.37 pk / ml higher than the players. One of the reasons for this is that just as the hormone testosterone shapes muscle activity, muscle mass and exercise also affect testosterone levels. The hormone testosterone is involved not only in the production of masculine traits in the body, but also in the metabolism of the body for a healthy lifestyle, especially in the metabolism of hormones. Testosterone is also a steroid hormone that is important for the normal functioning of the musculoskeletal system and bone processes. As can be seen in the histogram above, the amount of testosterone recorded a pass that was lower than normal testosterone levels. It can be seen that the size of the force is correspondingly lower than that of other controls. The average weight gain per kilogram in the non-sporting study was 36.3 kg. That's almost twice as many weightlifters as weightlifters. These indicators alone show a link between endurance, testosterone and physical activity. However, we explained that the lack of strength of both testosterone and endurance in players compared to weightlifters.

**THE MAIN DIRECTIONS OF
PHYSIOLOGICAL SELECTION
AND ORIENTATION USED IN
THE ORIENTATION OF
CHILDREN WITH
DISABILITIES IN SPORTS IN
UZBEKISTAN AND ABROAD**

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ABSTRACT

Annotation: Sports result is determined by the totality of the athlete's motor abilities. Motor ability is a property of a person that makes him fit for the successful completion of any task in sports activities. To actively influence the course of vocational guidance and selection, it is necessary to know the requirements that this sport makes for athletes and the makings that an athlete should possess. To identify the requirements of a sport, it is customary to test athletes of the highest qualifications. The resulting arithmetic mean values of test indicators are called model characteristics. Determining in tests the degree of influence of heredity (inclinations) on achievements or on other measurable indicators is one of the most difficult tasks of selection. Of great importance are the works related to the determination of the influence of sports training or physical activity on morpho-functional restructuring, since by the degree of intensity of restructuring processes, one can get an idea of hereditary characteristics and the degree of influence of environmental factors. Great prospects are opened by the method of studying the skin relief of the fingers of the hand or sole. The skin relief formed in the embryonic period remains unchanged throughout life. And with the help of the dermatoglyphics method, which has wide practical application in orientation and selection, to prove that there is a close connection between hereditary skin reliefs and the characteristics that determine sports inclinations.

Methods for studying pedigree and studying statistical data on the relationship between the motor abilities of children and parents have a low predictive ability. Since long-term breeding work is required to obtain stable genetic similarity, the application of these methods is almost impossible. The functional capabilities of a person are predetermined by

the morphological characteristics of his organs and tissues, therefore, hereditary characteristics are supposed to be present in the structure of organelles, cells, organs. This is a strategic path for the development of the science of selection, but the insufficient amount of fundamental facts, the lack of knowledge of many mechanisms of inheritance of structural rearrangements in the body of a growing person contribute to empirical research. You can give a list of recommended indicators obtained from the data of generalized genetic studies. 1) Morphological indicators: body length, body weight, longitudinal body dimensions, active body weight, skeletal muscle fiber composition. Physiological indicators: a) vital capacity of the lungs, their relative value is especially important; b) minute volume of respiration per 1 kg of body weight; c) resistance to oxygen deficiency and sensitivity to the concentration of CO₂ in the blood; d) heart rate at rest (bradycardia); e) the reaction of the cardiovascular system to physical activity of submaximal power; f) maximum oxygen consumption. 2) Indicators of motor skills: speed of movement (temping test, etc.), flexibility (tilt of the trunk forward, etc.), vestibular stability, orientation in space, relative muscle strength, calculated for 1 kg of body weight). Physiological indicators: features of the central nervous system (strength, poise, mobility), features of temperament and personal characteristics (stable emotional states, extra - or introverted personality orientation), etc.

THE EFFECTIVENESS OF PURPOSEFUL ACTIVITY AMONG STUDENTS IN THE EXAM SITUATION

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ABSTRACT

The objective of this study was to identify the physiological and psychological correlates of differences in the effectiveness of purposeful activity when testing students in a situation of exam stress. 40 young students were examined during regular school days and immediately before the exam. The degree of intro-extraversion and neuroticism was determined by Eysenck, personal and situational anxiety by Spielberger. An electroencephalogram (occipital, central and frontal cortical regions) was recorded in a state of calm wakefulness with open and closed eyes, followed by processing using the Braingam software package. To assess the effectiveness of students' activities, a modified test "Establishing patterns" by B.L.Pokrovsky and the speed of processing visual information were used. The assessment received by students on the exam was negatively correlated with the number of mistakes made during the test. In turn, applicants with high personal anxiety made large numbers of mistakes before the exam. The performance of the test before the exam positively correlated with the severity of the alpha rhythm in the EEG spectra of the frontal and occipital cortex regions and negatively with the values of the delta EEG index of the frontal and beta EEG index of the occipital cortex regions. The greatest severity of the delta rhythm in the EEG spectra of the central and frontal regions of the cortex in the pre-examination situation was observed in students with high personal anxiety. Thus, it was found that the effectiveness of

purposeful activity of students in a stressful pre-examination situation, determined using the test. The "establishment of patterns" directly correlating with the assessment received at the exam is associated with both psychological (personal anxiety) and physiological (the severity of delta, alpha and beta rhythms of the EEG spectra) characteristics of students.

**EVALUATION OF THE
ACTIVITY OF ENZYMES OF
THE ANTIOXIDANT SYSTEM IN
THE LEAVES OF THE
ORNAMENTAL TREE
AÉSCULUS HIPPOCASTANUM
L. ON THE EXAMPLE OF
TASHKENT IN THE CONTEXT
OF URBANIZATION**

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ABSTRACT

Polluted environmental conditions have a negative impact on the condition, growth and development of plants, as well as their functional activity. The aim of our research is to determine the antioxidant system (AOS) of the leaves of *Aésculus hippocastanum* L., which grows in different conditions in the Botanical Garden of the National University of Uzbekistan and near the highway. In order to achieve this goal, the activity of AOS enzymes - catalase and superoxide dismutase was studied. The research was conducted in the summer. At the beginning of the season, SOD activity in chestnut leaves growing in the botanical garden was 1.55 μmol , in July it increased to 1.7 μmol , during which time the

catalase activity decreased by 1.3 times (from 57% to 45%). By August, AOS enzymes - catalase - increased 1.04 times (from 45% to 47%), SOD decreased 1.1 times (from 1.7 μmol to 1.51 μmol). SOD activity in the leaves of *Aésculus hippocastanum* L., growing near the highway, decreased 1.2 times compared to the beginning of the season in July (from 1.8 μmol to 1.65), catalase increased from 53% to 68%. In August, SOD activity reached 0.85 μmol , while catalase activity decreased 1.1 times (63%). It can be concluded from the results that unfavorable climatic conditions lead to the activation of antioxidant system enzymes in plants, and in this process SOD and catalase work in conjunction with each other, i.e. catalase becomes inactive when SOD activity increases, or vice versa. Thus, due to the activation of both AOS enzymes - SOD and catalase, *Aésculus hippocastanum* L. successfully adapts not only to the semi-arid conditions in Tashkent, but also to the additional adverse effects of the urbanized environment. Keywords: antioxidant system, catalase, superoxide dismutase.

**STUDY OF THE EFFECT OF
PREPARATIONS BASED ON
GLYCYRRHIZINIC ACID ON
NITRIC OXIDE IN
CARDIOMYOCYTES AND
BLOOD OF RATS WITH
EXPERIMENTAL
MYOCARDITIS**

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ABSTRACT

Nitric oxide (NO) is one of the most important regulators of various physiological processes in the body. Because of vasodilating properties and the ability to suppress the generation of O₂-phagocytic cells, NO plays a key protective role in ischemic myocardial damage. It is known that a decrease in the concentration of NO in the endothelial cells of the heart vessels causes spasm of the latter and, as a consequence, a violation of microcirculation of the heart, ischemic damage and the development of a heart attack. The search for compounds with NO - inducing activity will make it possible to use these substances as effective agents that improve the condition and function of blood vessels and the heart. The aim of the study is to study the NO-inducing activity of compounds, which is created based on glycyrrhizin acid and acetylsalicylic acid (GLAS) on a model of experimental myocarditis (EM). The study of the content of the end products of the metabolism of nitric oxide (nitrates and nitrites) in the blood serum and myocardial homogenate of rats with EM showed that with this heart pathology this indicator decreases by about 50 and 59%, respectively. The administration of GLAS at a dose of 2.5 mg / kg to animals with myocarditis increased the level of nitric oxide in the blood serum and in the cells of the rat cardiac muscle. It can be assumed that in animals with myocarditis, disturbances of the NO - ergic system occur in the tissues of the heart and in the blood. The administration of GLAS to sick animals had a stimulating effect and promoted the restoration of the level of NO, which had not yet reached the control value under the influence of the drug.

Keywords: Nitric oxide, glycyrrhizin acid, experimental myocarditis

STUDY OF THE EFFECT OF PHYTOECDYSTEROIDS ON CELL VOLUME REGULATION IN VITRO CONDITIONS

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ABSTRACT

Phytoecdysteroids are a large class of polyhydroxylated steroid compounds present in plants and are similar to zooecdysteriodes in their chemical structure. Phytoecdysteroids have been shown to have a wide range of beneficial pharmacological effects (normalizing tone, adaptogenic, antidiabetic, antimicrobial, and accelerating wound healing, etc.), but their effects on RVD (regulatory volume decrease) and ion channels involved in this process have not been elucidated. This determines the relevance of this study. Studies were performed using the cell volume control method using 6–8-week-old thymocyte cells isolated from rat thymus at concentrations of in experiments 0.1 μM, 1 μM, 10 μM, and 100 μM phytoecdysteroids (siasterone, turkesterone, ecdysterone, polypodine B., integresteron A and α-ecdysone). The effect of thymocyte cells on volume regulation was studied. In this isotonic environment (normal Ringer's solution), the volume of thymocytes remained unchanged for 15 min (n = 3). When the cells were exposed to a hypotonic

medium (control solution), they initially swelled rapidly (passive response), recovered to a level close to the isotonic volume (active response), and the RVD rate was $88.7 \pm 1.02\%$ ($n = 4$). The results showed that at a concentration of $0.1 \mu\text{M}$, polypodine B, integristeron A, siasterone and turkesterone had an inhibitory effect, while α -ecdysone and ecdysterone were not observed. Phytoecdisteroids at a concentration of $1 \mu\text{M}$ did not have a statistical effect on the recovery of thymocyte volume. At a concentration of $10 \mu\text{M}$, ecdysterone was close to control, and integristerone A, polypodine B, and α -ecdysones had an inhibitory effect, siasterone and turkesterone did not affect RVD. In our next experiment, we increased the concentration of the substance to $100 \mu\text{M}$, resulting in a partial inhibition of siasterone, integristerone A, α -ecdysone, and polypodine B RVD. When the effects of phytoecdysteroids on thymocyte cell volume reduction (RVD) under hypoosmotic stress at $0.1, 1, 10,$ and $100 \mu\text{M}$ were studied, polypodine B, integristeron A, siasterone, and turkesterone also showed inhibitory effects at low concentrations. α -ecdysone has been found to have a significant effect on this process only at high concentrations. Under the studied conditions, the substance ecdysterone did not affect the decrease in cell volume of thymocytes.

Keywords: phytoecdisteroids, thymocyte, turkesterone

DETECTION OF CHANGES IN THE AMOUNT OF CERTAIN ELEMENTS IN THE SOIL AS A RESULT OF AIR POLLUTION.

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ABSTRACT

Today, scientific and technological progress, on the one hand, has led to general development, and on the other hand, the deterioration of natural conditions leads to a violation of the ecological balance. Air pollution negatively affects plants, animals and the human body. At the same time, it significantly affects the amount of macro and microelements in the soil. In particular, this is the basis for laboratory studies on soil analysis carried out in farms of Sariosi, Uzun and Denau districts of Surkhandarya region. The study showed that the macro- and microelement composition of the soil, carried out in these areas, significantly differed from soils under normal conditions. The quantitative indicators of sodium and potassium, which are considered the most important for living organisms, were 2-3 times lower than the control ones. The sodium and potassium content of the soil, especially in the Denau region, was low. Thus, on the basis of the results obtained, it was established that the deterioration of the state of atmospheric air has a significant effect on the content of macro- and microelements in the soil.

STUDY OF GENE EXPRESSION OF CHLORIDE CHANNELS IN PARIETAL CELLS OF RAT STOMACH

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ABSTRACT

Chloride channels have different physiological functions. In particular, transport of chlorine ions plays an important role in the secretion of hydrochloric acid in the stomach. It was suggested that intracellular chloride channels (CLIC) are involved in this process. The aim of this work is to study the gene expression for different chloride channels in rat stomach epithelial cells and purified parietal cells. The standard techniques were used in the study. Bioinformatic analysis of the transcriptome of rat stomach cells was performed using the GeneSpring software and Excel 2013. Of 17 transcripts of gamma-aminobutyric acid A receptor in the microarray, Gabarap11_predicted, Gabarap, Gabrr2 and Gabrq showed the highest expression intensities and among 10 CFTR transcripts of chloride channels for Abcc5 the highest expression in the parietal cells was found. Of the 25 transcripts of the CLC channel families, the highest expression levels were found for CLcnkb and Dncl1. Of the 6 intracellular CLIC channels, the high levels of gene expression were found in CLIC6 in the stomach and purified gastric parietal cells. The obtained results of transcripts of chloride channels using oligonucleotide microarray analysis were confirmed by qPCR. Comparing the transcripts of purified parietal cells and total epithelial cells (PC/ST ratio), parietal cell specific genes, such as Gabarap1_predicted, ClcnkB, Mclc, Clcn4-2 and CLIC6 were found. Further studies of the expression of CLIC and other proteins in the stomach epithelial cells makes it possible to elucidate the functions of those proteins in the peripheral regulation of the transport of chloride ions in the gastric acid secretion of gastrointestinal tract.

Keywords: parietal cells, transcriptome, oligonucleotide microarray, chloride channels