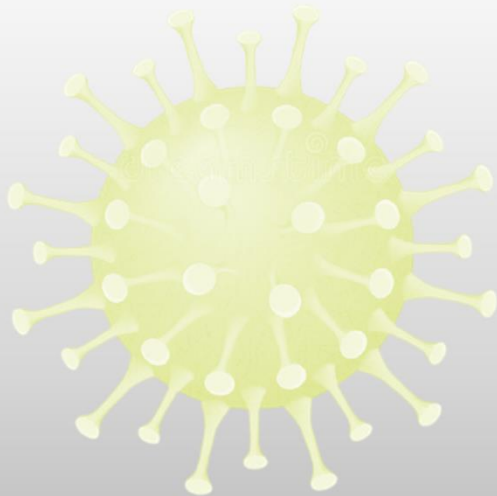




Abstracts Book

3rd International Conference on Applied Zoology-2020
(ICAZ-2020)



Organized By

The Applied Zoological Society of Pakistan,
Quaid-i-Azam University, Islamabad, Pakistan &
Government College University Faisalabad



ICAZ-2020 (7TH – 8TH DECEMBER, 2020)

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Welcome Remarks by AZSP Chairman



Prof. Dr. Muhammad Ali
Chairman AZSP

Dear Friends and Colleagues,

It is a great pleasure to welcome you all to the 3rd International Conference on Applied Zoology-2020 (ICAZ-2020), to be held from 7th to 8th December, 2020 under the shelter of The Applied Zoological Society of Pakistan in collaboration with Quaid-i-Azam University Islamabad.

In 2020, due to unfavorable circumstances, we have learned to do many things differently – such as our work in education and research – and this includes our annual scientific conference (ICAZ-2020)!

We will all join this new type of conference online on 7th -8th December, 2020. Our AZSP central organizing committee, Prof. Dr. Farhat Jabeen, Prof. Dr. Tayyaba Sultana, Prof. Dr. Salma Sultana and Dr. Azhar Rasul, in collaboration with local organizing committee from QAU, Islamabad has been working hard to adapt the program for online experience. They have managed to organize an exciting scientific program with multiple sessions for you to join in according to your interests. There will be opportunities to present your work and interact with other participants. This global meeting will feature highly respected internationally renowned speakers who will share, discuss, debate, and dissect significant new developments and scientific advancements that will impact the future of Applied Zoological Sciences, and related fields.

While we will miss catching up in person and the many other social aspects of the program, there are indeed some ‘silver linings’ to this new way of working! In particular, we hope that more people will be able to join this conference. I look forward to seeing you all online at the conference.

Prof. Dr. Muhammad Ali (TI)

Chairman, AZSP/Patron in Chief, ICAZ-2020

VC, QAU, Islamabad

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Welcome Remarks by Chief Guest



Prof. Dr. Shahid Kamal
VC, GCUF

First and foremost, I welcome you all on this prestigious scientific event organized by The Applied Zoological Society of Pakistan in collaboration with Quaid-i-Azam University Islamabad. I would like to appreciate the active participation of foreign delegates from UK, Malaysia, Iran, Italy, Turkey, Chile and Spain, and all the participants for joining us on this occasion. I want to thank all organizers of 3rd International Conference on Applied Zoology (ICAZ, 2020) for inviting me to address and officiate this conference.

We had planned to meet in the beautiful city of Islamabad this year but it was not possible under these COVID-19 pandemic circumstances. This 3rd ICAZ, 2020 provides an excellent platform to bring together leading academicians, researchers, postgraduate students as well as industrial folks. New knowledge and findings cannot be generated without any research and development (R&D) activities. This effort will undoubtedly provide a great platform to share interesting results and new knowledge in the field of Applied Zoology. I would like to express my appreciation at the joint adventure by the organizing committee members of ICAZ, 2020 from QAU, Islamabad and AZSP office bearers, Department of Zoology, GCUF for their hard work and relentless effort. Without their commitment and contributions, this event would not been possible and successfully delivered right this time. I wish you all a fruitful discourse.

Prof. Dr. Shahid Kamal

Vice Chancellor

Government College University,
Faisalabad

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Organizing Committee Members



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Patron in Chief/Chairman AZSP
Vice Chancellor
Quaid-i-Azam University, Islamabad



Prof. Dr. Shahid Kamal
Chief Guest
Vice Chancellor
Government College University, Faisalabad



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Patron / Vice Chairperson AZSP
Dean Faculty of Life Sciences
Government College University Faisalabad



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Prof. Dr. Salma Sultana
Convener/Joint Secretary AZSP
Associate Professor
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Government College University Faisalabad



Dr. Azhar Rasul
Organizing Secretary/Secretary AZSP
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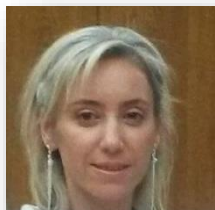


Dr. Syed Makhdoom Hussain
Associate Professor
Department of Zoology
Government College University Faisalabad



Dr. Samina Qamer
Assistant Professor
Department of Zoology
Government College University Faisalabad

Keynote Speakers



Prof. Dr. Zeliha Selamoglu
Professor / Chairperson
Department of Medical Biology,
Nigde Omer Halisdemir University, Turkey



Dr. Lai Ngit Shin
Institute for Research in Molecular Medicine,
Universiti Sains Malaysia, Pulau Pinang,
Malaysia

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Keynote Speakers



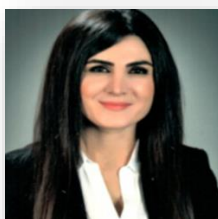
Dr. Antoni Sureda

Research Group on Community Nutrition and Oxidative Stress, University of Balearic Islands, Spain



Dr. Allah Bakhsh

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Dr. Ilknur Ucak

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Dr. Najib Altawell

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Dr. Fatima Kies

Department of Earth and Environmental Sciences University of Milano-Bicocca, Italy



Dr. Patricio DL Ríos-Escalante

Department of Biological Sciences, Faculty of Natural Resources, University of Católica Temuco,

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Oral Presentations



O-3/ICAZ-2020

Effect of the Covid-19 Pandemic on Nutritional Habits and Approach of Immune-boosting Nutrition

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Abstract:

The pandemic caused by the new coronavirus, also known as Covid-19 pandemic, devastates global morbidity and mortality. Therefore various measures are taken to prevent the spread around the world. While social distancing and isolation are at the top of these measures, there are also potential preventive and adjunct therapies, dietary and lifestyle modifications, and remedies that may boost the immunity. Several governments have obligated collective quarantine to contain the diffusion of infection. Quarantine induces stress, depression, and anxiety leading to unhealthy diet and reduced physical activity. The optimal status of specific nutrients are vital to keep immune components within their normal activity, helping to avoid and cope with infections. During pandemic, it is important to follow a healthy and balanced nutritional pattern containing a high amount of minerals, antioxidants, and vitamins. Especially vitamins A, C, E, D and minerals such as zinc, copper, iron, selenium have been investigated as nutritional components that strengthen the immune system. A strong immune response is a key factor against infection and avoiding reaching severe stages of the novel coronavirus disease. In this study, we review the effects of pandemic on nutritional behaviors and the importance of a healthy diet to protect and boost immunity.

Keywords: Covid-19, Quarantine, Nutritional Habits, Immune-boosting Nutrition



ppsPCP: A Plant Presence/absence Variants Scanner and Pan-genome Construction Pipeline

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ABSTRACT

The intra-species genetic variations, especially in the form of presence/absence variants (PAVs) are a key to natural and artificial selection. Whole genome re-sequencing data is usually mapped to a reference genome when genomes of different individuals are compared. However, one single reference genome is insufficient to epitomize genetic makeup of different individuals in the same species and often ignore some important genes and leads to inaccurate estimation of genetic diversity. To get a comprehensive map of genetic variations, phenotypic variations and genomic diversity, it is crucial to construct a pan-genome including all the specific PAVs. Since the idea of pan-genomics emerged several tools and pipelines have been introduced for prokaryotic pan-genomics. However, not a single comprehensive pipeline has been reported which could overcome multiple challenges associated with eukaryotic pan-genomics. To aid the eukaryotic pan-genomic studies, I developed ppsPCP, a novel pipeline which takes advantage of assembled plant genomes, screen PAVs from them and develops a completely annotated pan-genome. This ppsPCP pipeline is benchmarked with model cereal species rice and model dicot species *Arabidopsis thaliana*. In case of rice, ppsPCP constructed a 420 MB sized pan genome containing 43,082 genes. A total of 11,677 PAVs and 4,213 genes were screened and added to the rice pan-genome. In case of *A. thaliana*, ppsPCP constructed a 122 MB sized pan-genome containing 34,899 genes. A total of 7,480 PAVs and 1,432 genes were screened and added to the *A. thaliana* pan-genome. Furthermore, to evaluate the quality of developed pan genomes by ppsPCP, we compared our rice results with recently reported pan-genome developed from 3,010 diverse accessions of Asian cultivated rice and *A. thaliana* results with pan-genome of 19 ecotypes. In case of rice, only 2,650 genes out of 50,955 genes were found to be different, and 48,305 genes were fully mapped to ppsPCP constructed pan genome. While, in case of *A. thaliana*, the constructed pan-genome of ppsPCP contains 1,687 more genes compared to the pan-genome. All the input and output data can be downloaded from ppsPCP webpage. We believe with these unique features of PAV scanning and building a pan-genome together with its annotation, ppsPCP will be useful for plant pan-genomic studies and aid researchers to study genetic/phenotypic variations and genomic diversity.



Oral Presentations

O-7/ICAZ-2020

The effect of supplemental irrigation on yield and plant components of chickpea (*Cicer arietinum* L.) in different soil conditions

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ABSTRACT

This study was conducted in difference in soil conditions of Turkey's Nevşehir Avanos district and Siirt central. In the study, 3 different chickpea varieties (İnci, Hasanbey, Seçkin) were used. The study aimed to reveal the water use efficiency (WUE), Plant water stress index (CWSI) and chickpea yield, change on plant parameters of different irrigation applications. Irrigation treatment consisted of full irrigation (I₁₀₀), deficit irrigation (I₅₀) and no irrigation water (I₀).

At the end of the study, irrigation, variety, irrigation x variety interactions for both locations were found statistically significant at 1% level and some applications were found to be insignificant. The highest yield in Siirt and Nevşehir conditions was found in İnci variety, which is the subject of I₁₀₀ irrigation with a yield value of 2518 and 2717 kg ha⁻¹, respectively. The lowest yield was determined in Seçkin variety of I₀ irrigation treatment with 1774 and 1749 kg ha⁻¹ yield value. The highest WUE value in Siirt and Nevşehir conditions was found in İnci variety, which is the subject of I₅₀ irrigation with a value of 0,66 and 0,67 kg m⁻³, respectively. The lowest was determined in İnci variety, which is the subject of I₀ irrigation with a value of 0,52 and 0,53 kg m⁻³, respectively. The highest CWSI value at both locations was determined in the distinguished type of I₀ irrigation subject with the value of 0,57 and 0,62 respectively. I₁₀₀ irrigation treatment with the lowest value of 0,20 and 0,18 was found in İnci variety.

Keywords: Chickpea, Supplemental irrigation, WUE, CWSI, Yield and Plant component, Siirt and Nevşehir Provinces, Turkey

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Oral Presentations

O-10/ICAZ-2020

Identification of citrus peel extract as novel PKM2 inhibitor for the treatment of cancer

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ABSTRACT

A major metabolic aberration associated with cancer is a change in glucose metabolism. Pyruvate kinase (PK), a key enzyme that determines glycolytic activity, has been implicated in the metabolic phenotype of cancer cells, and specific pyruvate kinase isoform M2 (PKM2) has been reported to support divergent biosynthetic and energetic requirements of cells in tumors. Therefore, the aim of the present study was to identify natural agents against cancer via targeting PKM2. In order to find novel PKM2 modulators, target protein-based screening was performed by using agri-food waste biomass extracts library. For target protein-based screening, pET-28a-HmPKM2 was transformed to BL21 (DE3) E. coli strain. Protein expression was induced and rPKM2 was purified by using Ni-NTA columns. Using this purified rPKM2 protein, enzymatic assay system for the screening of PKM2 inhibitors was established and validated. Further, we have screened agri-food waste biomass extracts library in our established assay system for the identification of inhibitors of PKM2 protein. Out of 50 extracts screened, citrus peel extract was found to be potent inhibitor of PKM2. Citrus peel extract showed concentration-dependent inhibition against PKM2 at 90-360 µg/ml concentrations. The results of MTT assay demonstrated the anti-cancer activity of citrus peel extracts against cancer cells. Thus, we further generated dose-response curves for the evaluation of IC₅₀ values of hit extracts against cancer cells. Citrus peel extracts significantly inhibited the growth of cancer cells with IC₅₀ values of 105 µg/ml. The results of this study provide novel findings and possible mechanism of action of citrus peel extract against cancer via PKM2 inhibition. This study has laid the foundation for further investigation on citrus peel extract for the identification, characterization and bioassay-guided fractionation to identify active constituents specifically involved in PKM2 inhibition.

Keywords: Tumor metabolism, PKM2, Citrus Peel, NADH

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Oral Presentations

O-13/ICAZ-2020

Role of doxorubicin coupled Nanoceria in modulating TRAIL and NF-kappa B markers in leukemic rat model

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¹Department of Biochemistry, Quaid-i-Azam University, Islamabad

ABSTRACT

Nanoparticles have proven to be indispensable tools in disease monitoring and therapy. Their combination with chemotherapy has been recognized beneficial in multiple research studies. Given the context, this study was designed to identify the anticancer potential of cerium oxide (CeO₂) nanoparticles coupled with doxorubicin i.e. nanomedicine. Methodology: Chemical synthesis of CeO₂ nanoparticles and their loading with doxorubicin was verified by using different characterization techniques. Benzene was intravenously administered in Sprague Dawley rats for AML induction, which was later confirmed by hematological profiling. After administering leukemic rats with different experiment groups for 24 alternate days, Annexin V assay was performed to detect and quantify apoptotic cells, while RT-PCR was conducted for genetic expression profiling. Results: XRD exhibited average crystalline size of CeO₂-NPs at 18.57nm whereas FTIR, UV-Spectroscopy, and SEM confirmed successful loading of DOX on CeO₂-NPs with enhanced dispersion and stable suspension. Treating leukemic rats with doxorubicin coupled nanoceria restored the normal blood parameters along with hepatic and renal biomarkers. The size and weight of hypertrophic liver, kidney and heart was also recovered upon nanomedicine administration. Annexin V assay revealed significant apoptotic potential of nanomedicine at cellular level. Similar findings were obtained by RT-PCR at genetic level where nanomedicine down-regulated anti-apoptotic cFLIP along with pro-survival components of NF-kappa B pathway and up-regulated TRAIL pathway markers including DR5, Caspase 8, Cyt-c and Bax gene. Conclusion: These findings suggest that CeO₂-NPs may enhance apoptotic potential of doxorubicin both at cellular and genetic levels. It may also inhibit pro-survival NF-Kappa B pathway markers, as nanomedicine, rendering itself as a potential therapeutic agent against acute myeloid leukemia.



O-16/ICAZ-2020

Prevalence and hematological effects of haemonchus contortus in ghaljo sheep kohat

Nayab Khattak

Kohat university of Science and Technology, Pakistan.

ABSTRACT

Haemonchus contortus is one of the most important parasites that infects sheep and exerts its pathogenic effects by sucking blood, causing disturbances of organ-functions. The present study was conducted from June 2019 to September 2019 to examine the effect of Haemonchus contortus (H.contortus) infection on hematological and parasitological parameters. Total 203 fecal and blood samples were collected and examined at Research Lab to assess the prevalence and hematological effects of Haemonchus contortus and all infected Ghalju sheep appeared to be highly susceptible to the infection by H.contortus. The study revealed that H. contortus had an overall prevalence of (32.01%). The females showed higher prevalence (39.31%) as compared to males (22.09%). The hematological study was conducted on ghalju sheep to assess the hematological parameters of apparently normal and diseased sheep. Hematological studies revealed significant decrease in hemoglobin concentration, packed cell volume in infected Ghalju sheep. All Haemonchus infected Ghalju sheep showed a progressive and severe anaemia characterized by marked reductions of body weight, packed cell volume (PCV), haemoglobin concentration (Hgb) and red blood cell (RBC) compared to control animals with a significant difference ($P<0.01$).

Key Words: Haemonchus contortus, Ghalju sheep, PVC, RBC, Hgb.

O-19/ICAZ-2020

Co-delivery of chemotherapeutic agents for antileishmanial effect: in vitro & in vivo analysis

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ABSTRACT

Targeted and localized drug delivery via dermal route is an effective approach to improve drug delivery and reduce drugs associated toxicities, most particularly treating cutaneous leishmaniasis (CL), which otherwise are difficult to achieve via oral and parenteral routes. Aim of this study was to develop rifampicin (RIF) and vancomycin (VAN) co-loaded transfersomes (RVCT) and further incorporate them into chitosan gel to allow the retention of RVCT to the infected skin and reduce the drugs toxicity. The

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RVCT was optimized for lipid to surfactant ratio, stirring speed and injection rate. Further, RVCT were assessed for their particle size (PS), zeta potential (ZP), polydispersity index (PDI) and entrapment efficiency. Moreover, the RVCT gel was optimized for its deformability index, in-vitro drug release and ex-vivo drug permeation. The in-vitro and in-vivo anti-leishmanial activities were also performed. The optimized formulation was nano-sized (167.9 ± 1.6), with better %EE of both RIF ($58.51\% \pm 0.11$) and VAN ($86.18\% \pm 0.13$). RVCT get demonstrated sustained release of the incorporated drugs. Ex-vivo permeation study showed better skin permeation of RVCT and RVCT gel without using permeation enhancers. The anti-leishmanial activity on in-vitro leishmania cultures of promastigotes and axenic amastigotes of *L. tropica* exhibited a 2.4 folds and 2.3 folds increased activity against promastigotes and axenic amastigotes, respectively. The in-vivo antileishmanial study demonstrated significantly reduced lesion size when treated with RVCT gel. These results clearly indicated the potential of RVCT gel in achieving targeted co-delivery of RIF and VAN for the treatment of CL, after topical application.

O-20/ICAZ-2020

Reprotoxicity and abnormal steroidogenesis following oral exposure of tartrazine in mice and remedial potential of *Allium cepa*

Aqsa Arshad *, Ayesha Siddiqua, Memoona, Shaista Shabbir, Chaman Ara

Department of Zoology, University of the Punjab, Lahore, Pakistan

ABSTRACT

Tartrazine is one of the most common additives used in dietary products to make food attractive. The artificial additives are declared to pose consequential health problems. Keeping in view these controversies, it was inevitable to conduct research to explore the steroidogenic and reproductive toxicity of tartrazine dye. The current study was executed on 60 prepubertal male and female mice (both genders were kept separately), divided into three groups named as Control Group, Dose Group and Dose + Antidote Group. The control group was not treated exceptionally other than ad libitum food and water while other two groups were administered with prepared doses (0.140mg of tartrazine, 0.1ml of aqueous onion extract) via gavage respective to their body weights on daily basis for 30 days. The body and gonadal weights decreased in tartrazine-exposed group of both genders. A reduction in total sperm count and increase in number of abnormal sperms occurred. In addition, hormonal analysis exhibited significant decrease in testosterone, FSH, LH and Estradiol levels in respective group when compared with control. Histopathology of testes and ovaries showed major tartrazine instigated deteriorative effects like vacant

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lumen, degenerative leydig cells, reduced follicular cells and vacuolations of granulosa cells respectively. While above mentioned physical parameters, hormonal disturbances and histological defects were resolved to a great extent when onion was co-administration with tartrazine probably due to onion phytochemicals and flavanoids containing properties by elevating antioxidant potential mechanism. Above findings in our experimental proceedings inferred that antioxidant and protective capability of onion (*Allium cepa* L.) against tartrazine provoked reprotoxicity and steroidogenesis in male and female albino mice.

O-21/ICAZ-2020

Evaluation of Antimicrobial Properties in Fish Mucus Extract of Major Carps

Anila parveen¹, Farkhanda Asad¹, Memoona Fatima¹, Noor Tariq¹ and Zunaira Shaheen¹

¹ Department of Zoology, Government College University, Faisalabad, Pakistan

ABSTRACT

Fish is a diverse group of animals very specialized by their aquatic existence. Fish inhabit the aquatic medium which might have high concentration of pathogenic microbes. Fish skin mucus is a protective secretion that cover the outer surface and act as a first line of defense against pathogenic microbes. This experimental trial was aimed to evaluate the antimicrobial properties in fish mucus extract of major carps (*Labeo rohita*, *Cirrihinus mirrigala* and *Catla catla*) against two bacterial strains (*Staphylococcus aureus*, *Aeromonas hydrophila*) and two fungal strains (*Aspergillus niger*, *Fusarium oxysporum*). Antimicrobial activity of acidic mucus extract was examined by using agar well diffusion assay. Cefixime and gentamicin were used as positive control. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition (ZOI) in mm. The mucus from all fish species showed strong antimicrobial activity against all the bacterial and fungal strains, compared to control antibiotic. The maximum zone of inhibition was observed against the *S. aureus* (23 ±3mm) by *L. rohita* and minimum ZOI was observed by the *Catla catla* against *F. oxysporum* (13±1.5mm). The findings of the present experimental trial suggested that the acidic mucus extract of all fish exhibited the antimicrobial properties against the selected bacterial and fungal strains which might play an important function in fish defense against the pathogens. Thus it appears to the possible use of skin mucus of carps as a potential source of antimicrobial agents in alternative to antibiotics, against the fish and might be in human infections.

Key words: *Labeo rohita*, *Cirrihinus mirrigala*, *Catla catla*, antimicrobial properties, and microbial strains.



O-22/ICAZ-2020

Antifungal potential of selected plants extracts against *Aspergillus flavus*, isolated from Poultry feed.

Naghmana Andleeb, Tayyaba Ali, Asma Ashraf, Shabana Naz, Tayyaba Sultana

ABSTRACT

Poultry farming has gained much importance worldwide and has become one of the largest industries in south Asian countries due to increasing demand of protein. It is one of the well regulated and most dynamic sectors which contribute 26.8% to the total meat production, 5.76% to the total agriculture sector and 1.26% to the overall national GDP. Food safety has always been the main focus of attention while production. *Aspergillus flavus* are found more abundantly in poultry feed and cause maximum damage by producing Aflatoxin such as Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2). Aflatoxins are reported to be carcinogenic, teratogenic, and hepatotoxic in nature. A study was designed aimed to determine the anti-fungal potential of *Eucalyptus globulus*, *Ocimum basilicum*, *Medicago sativa* and *Aloe vera* against aflatoxin producing strain of *Aspergillus flavus*. Feed samples were collected from four different poultry farms of Faisalabad. *Aspergillus* specie was isolated by using three different culture media. It was identified microscopically by using lacto phenol cotton blue stain. Antifungal activity of these plants extracts were determined by agar well diffusion method. Ethanolic extracts of *Eucalyptus globulus*, *Ocimumbasilicum* showed positive antifungal activity. Their minimum inhibitory concentrations were 5mg/mL and 20mg/ mL respectively. *Medicago sativa* extracts showed negative activity. Only methanolic extract of *Aloe vera* was examined for its Antifungal potential and it showed negative activity. So *Eucalyptus globulus*, *Ocimumbasilicum* have potential to inhibit aflatoxin producing strain of *Aspergillus* and it can be used for subsequent addition in poultry feed.

O-23/ICAZ-2020

Antifungal Potential of Selected Plants Extracts Against the Ochratoxin Producing Strains of *Pencillium* Specie

Shama Ihsan, Tayyaba Ali, Shabab Nasir, Farkhanda Asad

ABSTRACT

Poultry industry has become the largest and fastest growing agricultural industry in the world and especially gained importance in South Asian countries. Poultry production is increasing day by day, because it is the cheapest source of protein as compare to beef and mutton. Feed also play an important

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role in the successful business of poultry and it gives better quality of meat and eggs. Poultry feed is contaminated by fungi and cause deleterious effect on the health of poultry and human. Penicillium is an important Ochratoxin A (OTA) producer predominantly responsible for the occurrence of OTA in cereals and cereal products. Ochratoxins are reported to be nephrotoxic, teratogenic and immunotoxic in nature. The present study was aimed to determine the genotoxic effects of poultry feed extracts on peripheral blood of chicken. Ochratoxin producing strain of Penicillium was isolated and identified from poultry feed. Antifungal potential of Eucalyptus globules, Ocimum basilicum, Medicago sativa and Aloe vera against Penicillium strain was determined. Feed sample was collected from four different poultry farms of Faisalabad. Genotoxicity was checked by comet assay. Penicillium specie was isolated by using Dichloran Yeast Extracts Sucrose Glycerol Agar media. Penicillium specie was identified microscopically by using lacto phenol cotton blue stain. Antifungal potential of these plants were determined by Agar well diffusion method. Ethanolic extracts of Eucalyptus globules and Ocimum basilicum showed positive result and their minimum inhibitory concentration was 10mg/ml and 20mg/ml respectively. Ethanolic extract of Medicago sativa and methanolic extract of Aloe vera showed negative result against Penicillium specie. Result showed that Ethanolic extracts of Eucalyptus globules and Ocimum basilicum has antifungal potential to kill Ochratoxin producing strain of Penicillium specie. So it can be used for subsequent addition in poultry feed.

O-24/ICAZ-2020

Effect of Chromium Chloride Hexahydrate Supplementation on Gelatinized and Non-Gelatinized Corn Based Diets in Catla catla

Zunaira Shaheen, Farkhanda Asad, Asma Ashraf, Shabana Naz, Azhar Rafique, Tayyaba Ali and Manahil Tasadaq

ABSTRACT

The effects of chromium chloride hexahydrate supplementation in gelatinized and non-gelatinized corn based diet on haematological parameters, growth and the enzyme activity (proteolytic and amylase) of liver and gut tissue of Catla catla for 90 days. Six experimental diets (Gelatinized and Non-gelatinized) using corn meal as carbohydrate source were formulated containing three chromium level (0.0, 0.6, 0.8 mg/kg). For each treatment two replicates (gelatinized and non-gelatinized) were used, each with twenty fingerlings. Before proceeding to the experiment, fingerlings were acclimatized for 2 weeks weaned on control diet (NG, 0.0 Cl₃CrH₁₂O₆) at the rate of 4% live wet body weight. At the completion of the experiment, Results showed that chromium fortified the experimental group T4 (G=0.6) with

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highest growth as compared to other treatments, while minimum growth was observed in T1 (NG=0.0) experimental group. Hematological aspects were determined by using hemocytometer, that hemoglobin contents and RBCs were at its peak in T4 (G=0.6) while WBCs and HCT % was maximum in treatment T1(NG=0.0). In case of RBCs indices, T2 showed maximum values of MCV and MCH while MCHC was maximum in T4. The activity of the amylase and protease was higher in all treatments fed with Gelatinized corn. Liver showed high amylase activity contrast to intestine. Overall findings of this work revealed that gelatinized corn along with chromium chloride hexahydrate has promising effect on hematology and growth of *Catla catla* when compared to non-gelatinized corn.

Key Words: Inorganic chromium, Haematology, Enzyme activity, *Catla catla*

O-28/ICAZ-2020

Cardiovascular And Hepatotoxicity Induced By Nickel And Therapeutic Potential Of *Punica granatum* In Male Swiss Albino Mice

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ABSTRACT

Nickel (Ni) is tremendously distributed in human surroundings and consumed in many applications as well as important for plants and micro-organisms normal growth rate. It is an important part of earth's core. The objectives of this investigation were to examine the toxicology of nickel chloride (NiCl₂) on albino mice heart and liver and protective potential of pomegranate extract against nickel toxicity. Eighty male mice (*Mus musculus*) used and divided into eight groups (n=10). One group used as the control group (without any treatment) and second group used as Dose-I (8mg/kg NiCl₂), third group as Dose-II (15mg/kg NiCl₂), fourth group as Dose-III (30mg/kg NiCl₂), fifth group as group as Antidote+ Dose I (500mg/kg pomegranate extract + 8mg/kg NiCl₂), sixth group as Antidote+ Dose II (500mg/kg pomegranate extract + 15mg/kg NiCl₂), seventh group as Antidote+ Dose III (500mg/kg pomegranate extract + 30mg/kg NiCl₂) and eight group as Antidote (500mg/kg pomegranate extract) respectively given



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to mice for 30 consecutive days and sacrificed on 32nd day, by collected blood in EDTA vials, for Liver functioning test (LFT) and Lipid profile and also for histological testing. Nickel chloride D-I (low), D-II (medium) and D-III (high) amounts increased (TG) triglycerides, (VLDL) very low density lipoprotein, (TC) total cholesterol and (LDL-cholesterol) low density lipoprotein-cholesterol levels and decreased (HDL-cholesterol) high density lipoprotein-cholesterol level while, caused heart injury, intercalated discs degenerations, hemorrhages and thrombus formation. At the same time, it leads to increase in bilirubin, ALT (Alanine aminotransferase) and ALP (Alkaline phosphatase) levels and produced histo-pathological effects such as, liver damaging, necrosis, fibrosis and congestions. Although, Nicl2 administration with pomegranate antidote do not showed any toxic histo-pathological effects. On the basis of studies, it can be concluded that nickel chloride effected liver and heart tissues. While, treatment with pomegranate extract is a good source to overcome toxicity level and greatly reduce damages.

Key words: Fibrosis, Necrosis, Amyloid formation, Hemorrhage, Intercalated discs degenerations, Congestion, Vacuolization, Cardio-myopathy, Thrombus formation

O-30/ICAZ-2020

Study on growth performance, carcass composition and nutrient digestibility of *C. carpio* juveniles fed on *Chenopodium quinoa* seed meal based diet

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ABSTRACT

Plant based protein sources are suitable and profitable for aquaculture. The protein sources based on plants by-products are cheap. *Chenopodium quinoa* was one of the pseudocereal plants and promising source of protein. A feeding trial of 60 days was conducted to evaluate the effects of *C. quinoa* seed meal (CQSM) on growth performance, carcass composition and nutrient digestibility of *C. carpio* juveniles. *Quinoa* seed meal was used in the diet by replacing fish meal in test diets-I, II, III, IV, V, VI at the levels of 0%, 10%, 20%, 30%, 40% and 50% respectively. Triplicate tanks were used and each tank will contain 15 juveniles that were fed at the rate of 5% of their live wet weight. Feces were collected to measure the digestibility. The results were maximum for weight gain (14.12), weight gain% (199.15), FCR (0.92) and SGR (1.21) at level-III (20%) replacement of fish meal with *C. quinoa* seed meal based diet. Similarly, the nutrient digestibility, the CP (28.43), CF (4.01), GE (4.23) were also maximum at 20% replacement of fish meal with CQSM. The results showed that the replacement of fish meal not more than 20% is best for fish growth, carcass composition and nutrient digestibility.

Key words: *C. quinoa* seeds, *C. carpio*, growth performance, carcass composition, nutrient digestibility

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Prevalence of Different Types of Endothelial Corneal Dystrophies by Age and Gender and visual acuity in Punjab, Pakistan.

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ABSTRACT

Endothelial Corneal Dystrophy (ECD) is characterized by a slowly progressive endogenous degeneration of corneal endothelium and is at least in a part of it due to genetic predisposition. A cross-sectional, analytical study was performed from September 2018 to June 2019 and patients of ECD dystrophy were examined by visiting various cities hospitals of Punjab including Okara, Sahiwal, Narowal, Kasur, Lahore, Gujranwala and Multan. The basic purpose of this research was to determine the prevalence of different types of ECD in population of different cities of Punjab. Data was collected on the basis of relevant parameters such as age, gender and visual acuity. The assessment of visual acuity was done with the help of ophthalmologists by using Visual acuity test and Snellen chart. Among 3000 patients, 6.6% (n=198) cases of ECD were recognized which includes more males 52.52% (n=104) as compared to females 47.48% (n=94). Maximum cases of ECD 31.82% (n=63) were observed in 41-50 age group. In all types of ECD, Fuchs endothelial corneal dystrophy (FECD) was observed most with 38.38 % (n=76) and x-linked endothelial corneal dystrophy (XECD) with 6.67% (n=13) was least. The prevalence of congenital hereditary endothelial dystrophy (CHED) and posterior polymorphism CD (PPCD) was 25.25% (n=50) and 29.79% (n=59) respectively. The results of recent research finalize that posterior corneal dystrophy is a rare disease prevailing in Pakistan with a proportion of only 6.6%. This study benefits in updating the data about predominance of posterior corneal dystrophy in Pakistan. Careful clinical evaluation, early diagnosis, genotyping, genetic counseling and proper treatment are necessary. For the restoration of optimum vision, this clinical examination and proper care are important and should be done to prevent outcomes induced by endothelial corneal dystrophy.



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Studies on the protective properties of Punica granatum (peel) in arsenic-mediated endothelial dysfunction and hypertension

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ABSTRACT

Arsenic is a copious environmental pollutant present in water causing endothelial dysfunction, and hypertension. Functional food including pomegranate is popular for their use to prevent, reverse, ameliorate and/or treat cardiovascular complications Objective: Present study has been designed to evaluate the protective properties Punica granatum (peel) against arsenic-induced endothelial dysfunction and hypertension.

Methodology: In the acute study, rats were exposed to Arsenic (1.5 mg /kg i.p. for 2 weeks) to induce endothelial dysfunction. The in-vitro vasorelaxant activity of peel extract was determined in isolated rat aortae using high K⁺ (80 mM) and phenylephrine (PE 1μM). In a chronic study, rats were exposed to arsenic (100 ppm in drinking water for 90 days) to induce cardiovascular complications like endothelial dysfunction, hypertension, and hyperlipidemia. Weekly (SBP), (DBP) and (MABP) were monitored using Powerlab data system. The serum samples and aortic tissues were isolated for lipid profile (TC, TGs, LDL-c, HDL-c), electrolyte concentration (Cl, Na, K), antioxidants enzymes (CAT, SOD, GSH) level, NO production, and MDA levels using kit and chemical-based assays were carried out. Histopathological investigation of aorta and heart tissues were carried out using H & E staining. Results: In the acute study, peel extract (5 mg) showed maximum relaxation naïve rat aorta against K⁺-induced and PE contractions. At week 13, the SBP, DBP, MABP (mm Hg) of arsenic administered rats were found significantly increased compared to control group. At week 13, systolic blood pressure (mm Hg) of rats was significantly reduced (p<0.001) in all treated groups compared to the disease group. TGs, TC, LDL-c were significantly reduced (p<0.001) in Ar+ST, Ar+T1, Ar+T2, and Ar+T3. HDL-c was significantly enhanced (p<0.001) in Ar+ST, Ar+T1, Ar+T2 and Ar+T3. Na, Cl, and K were significantly reduced (p <0.001) in Ar+ST, Ar+T1, Ar+T2, Ar+T3. Antioxidant (CAT, SOD, and GSH) and NO were significantly increased (p<0.001) in treatment groups. MDA was significantly reduced (p<0.001) in Ar+ST, Ar+T1, Ar+T2, Ar+T3. Conclusion: Punica granatum peel extract possessed vasoprotective, antihyperlipidemic, and anti-hypertensive properties against arsenic-induced endothelial dysfunction, hyperlipidemia, and hypertension. It was evident by significant attenuation in SBP, DBP, and MABP, hyperlipidemia and hypertriglyceridemia. Protective effects were found mediated possibly through promoting NO production, HDL-c, vasodilation antioxidant defense system (SOD, CAT, GSH) and reduction of lipid profile (TC, TGs, LDL-c, VLDL-c), electrolytes (K, Cl, and Na), and oxidative stress (MDA).

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Biogenic synthesis, characterization and therapeutic potential of copper nanoparticles using avocado seed extract

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ABSTRACT

Nanotechnology is one of modern material science's in most active areas of research. Usually referred to as particles in at least one dimension with lengths ranging from 1 nm to 100 nm. Avocado is an energetic fruit with a strong dietary value and is regarded a significant tropical fruit because it is rich in protein. The avocado natural product not exclusively is an amazing wellspring of monounsaturated oleic corrosive, yet contains significant lipid-dissolvable cancer prevention agents what's more, phytochemicals with abnormal states. Cu-nanoparticles were portrayed by a very enormous surface zone to volume proportion, and their properties were resolved essentially by the conduct of their surface. In FTIR the peaks at 3349.19 cm⁻¹, 3021.77 cm⁻¹, 1719.69 cm⁻¹, 1411.93 cm⁻¹ correspond to O-H carboxylic acid stretch, H-C-H symmetric alkane stretch, C-C-C Symmetric alkenes stretch respectively. In XRD spectral information of the face-centered cubic (FCC) Cu corresponding to two values of 44.64, 49.75. In CuNPs (SEM) images were obtained at different scales, i.e. 10, 3, and 5 micrometers, and these images were magnified at different resolution such as 4000X, 12000X, and 7000X. There were not very sharp edges, and CuNPs with avocado seed extract were agglomerated. These acquired copper nanoparticles have potential applications in the biomedical sector as they showed a significant antifungal, antibacterial, and anticancer activity.

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Aeroallergens and Food Allergens sensitization patterns in young adults

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ABSTRACT

The prevalence of aero allergy and food allergy has increased dramatically over the past few years. Allergens exposure is a strong risk factor for sensitization, development and severity of the

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allergic diseases, especially in the Asian pediatric population. Present study was conducted to investigate the the prevalence of aero allergy and food allergy has increased dramatically over the past few years. Allergens exposure is a strong risk factor for sensitization, development and severity of allergic diseases, especially in the Asian pediatric population. Present study was conducted to investigate the prevalence of food and aero-allergen sensitization patterns in Lahore. For this purpose, a total of 500 samples were collected from 5 different laboratories and IgE levels of patients were measured. Based on results of IgE, out of total 500 patients, an elevated prevalence of aeroallergy and food allergy was observed in adults and in children of different age groups. 75% of Infants of age 0-1 were allergens sensitized. In children of age 1-5, 72% were suffering from aero and food allergy while in children of age 6-9 87.50% were infected. Furthermore, children of age 10-15 47.61% were susceptible to aeroallergy and food allergy. However, children outgrow allergies with the age. In adults, 67.28% were allergens sensitized and only 32.71% adults were found normal. Hence, it is concluded that exposure to poor indoor and outdoor air quality is playing a major role in exacerbating the allergic diseases in adults. The elevated rate of aeroallergy and food allergy demands more public health facilities, and strict environmental regulations to manage allergic diseases in Pakistan.

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Bis-(2-ethylhexyl) Phthalate; A major bioactive metabolite produced by *Lactobacillus coryniformis* BCH-4 with antibacterial, antioxidant and mosquitoes larvicidal activities

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ABSTRACT

Emergence of diseases caused by various bacterial and vector borne pathogens becomes a significant cause of human morbidity and mortality. The control of such infections has been considered as a major task for global health. The present research was planned to assay antibacterial, antioxidant and larvicidal activities against *Culex quinquefasciatus* of bioactive metabolite, Bis (2- ethylhexyl) phthalate (BEHP).

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The resultant compound was isolated and purified from the culture filtrate of *Lactobacillus coryniformis* BCH-4. The detection of BEHP was observed by FT-IR spectra and further confirmed by GC-MS. The BEHP documented effective antibacterial activity against *Escherichia coli* (inhibition zone: 12.33±0.56mm) but moderate activity against *Staphylococcus aureus* (inhibition zone: 5.66±1.00mm). It also showed effective antioxidant activity of 68.86±1.02, 69.33±0.54, and 84.90±0.50 (%) in a concentration-dependent manner (25, 50, and 100µg/ml) respectively. Additionally, BEHP exhibited potent larvicidal activity against 4th instar larvae of *Culex quinquefasciatus* in a concentration and time dependent manners (50, 100, 150, 200, and 250ppm after 24, 48, and 72h respectively). The effective resultant compound showed 100% mortality at 250ppm after 72h with LC50 of 49.88ppm. Furthermore, after 72h, acetylcholinesterase inhibitory activity of dead larvae was recorded as 20.77, 28.08, 36.29, 44.10, and 55.43% at 50, 100, 150, 200, and 250ppm respectively. In comet assay, the mean comet tail length (14.18±0.28µm), tail DNA percent damage (18.23±0.06%), tail movement (14.68 ± 0.56 µm), Comet length (20.62± 0.64µm), head length (23.75± 0.27 µm), and head DNA percentage (39.19 ± 0.92 %) was observed at 250 ppm as compared to control. The results of this research suggested that such bioactive metabolites exhibited good antibacterial, and antioxidant activity. In addition, BEHP could also serves as a controlling agent of *Culex quinquefasciatus* larvae to combat the spread of vector borne pathogenic diseases.

Keywords: *Lactobacillus coryniformis* BCH-4, Bis (2- ethylhexyl) phthalate, FT-IR, GC-MS, *Culex quinquefasciatus*.

O-37/ICAZ-2020

Bellis perennis a common ornamental plant with a potency of lowering glycemia and associated health biomarkers

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ABSTRACT

Bellis perennis is a common European species of daisy, belongs to Asteraceae family. Due to the presence of many secondary metabolites such as triterpenoid, saponins, triterpenes, several anthocyanins, flavonoids and polyacetylenes it has been traditionally used as expectorant, diuretic, antiinflammatory, hypolipidemic and antioxidant. The aim of the present study was to evaluate the effects of *Bellis perennis* ethanolic extract as anti-hyperglycemic, antioxidant, immunomodulatory and hepatoprotective effects in streptozotocin-induced diabetic rats. For this,

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diabetes was induced in 32 rats by injecting streptozotocin intraperitoneally at the dose of 55mg/kg prior to 15 minutes of injecting nicotinamide in normal saline at dose of 110mg/kg body weight. Rats with blood glucose level ≥ 150 mg/dL, were divided into four groups with 8 rats in each group; Positive control (no treatment), standard control (55mg/kg body weight of standard drug Metformin), treatment 1 (100mg/kg body weight of *Bellis perennis* ethanolic extract) and treatment 2 (200mg/kg body weight *Bellis perennis* ethanolic extract) with a weekly measurement of body weight and BGL. A negative control group was consisted of eight normal rats with $BGL \leq 150$ mg/dL. On day 14th of the experiment, four rats from each group were evaluated for cell-mediated immunity by assessing the lymphoproliferative reaction to DNCB (DNCB assay). After treatment of 21 days all the rats were decapitated for blood collection. Serum was separated for evaluation of antidiabetic, antioxidant, hepatoprotective and immunomodulatory effects. Data was analyzed statistically by one-way analysis of variance (ANOVA). Results showed significant decrease in level of glucose in treatment 1 and 2 groups (147 ± 0.57 and 137.3 ± 3.38 mg/dl respectively) as compared to positive control group (354.6 ± 15.81 mg/dl) and significantly increase in level of insulin and glucagon in treatment groups as compared to positive control group in a dose dependent manner. Results also showed antioxidant, hepatoprotective and immunomodulatory activities of *Bellis perennis* ethanolic extract by reducing all the elevated blood serum markers induced by the streptozotocin treatment to significant levels in rats treated with the plant extract at both low and high doses ($P < 0.01$) and also after metformin treatment ($P < 0.01$). So, it is concluded that *Bellis perennis* has antihyperglycemic antioxidant, hepato-protective and immunomodulatory activities.

Keywords: *Bellis perennis*, Methanolic Extract, Antioxidant, Antidiabetic. Theme

O-38/ICAZ-2020

Fish protein: A novel dietary approach for managing diabetes-associated complications in diabetic wistar rat model

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ABSTRACT

Diabetes mellitus is a metabolic disorder associated with short term as well as long term undesirable complications caused by persistent hyperglycemia. Recently, there has been emerging evidence that natural foods and their bioactive compounds are the key contributors to the treatment of diabetes and associated complications. This study was designed to explore the therapeutic efficacy of fish protein-rich diet for managing diabetes and associated complications in the diabetic Wistar rat model. A high protein (HP) diet (45% and 55% fish protein rich in ω 3 fatty acids) was given to alloxan-induced diabetic rats for 28 days. Blood samples were collected for monitoring serum glucose, oxidative stress markers, lipid profile, kidney function markers, serum proteins, and liver function markers. Results indicated that there was a noteworthy control ($P < 0.05$) of serum glucose, oxidative stress, and lipid profile in HP diet treated diabetic rats. Treatment with 45% and 55% fish diet appreciably improved the concentration of serum creatinine, urea, uric acid and exhibited a vibrant improvement in renal functions. Our results confirmed that the HP diet restored total protein and albumin concentration in blood. The HP diet treatment also restored the normal serum AST and ALT concentration.

Keywords: Fish protein; novel dietary approach; diabetes-associated complications; alloxan-induced diabetic rat model

O-40/ICAZ-2020

Cytotoxic and Anti-Alzheimer Evaluations of Novel Lancifolamide Isolated from Methanol Extract of *Conocarpus lancifolius*

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ABSTRACT

Background: Combretaceae is a large family comprising of 500 species and 20 genera distributed in subtropical and tropical regions of world. *Conocarpus* genus is an ornamental tree native to coastal and riverine areas of East Africa and is planted as ornamental plant in different areas of Pakistan. This genus has proved medicinal value as cytotoxic, antibacterial, antiprotozoal, anti-leishmanial, antifungal and antidiabetic agent.

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Objective:

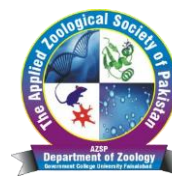
Current study was designed to screen the selected pharmacological attributes of a nitrogen containing novel compound isolated from *Conocarpus lancifolius* using series of in vitro assays and molecular docking techniques.

Materials and Methods: After collection and authentication of plant materia, methanolic extract was prepared from which various secondary metabolites were qualitatively examined. Compound was isolated using open column chromatography and structure was established with spectroscopic techniques such as UV-visible, infrared spectroscopy, proton nuclear magnetic resonance (¹H-NMR), ¹³C NMR (BB, DEPT-135, 90), two dimensional correlation techniques (HMBC, HSQC) and mass spectrometry respectively. *C. lancifolius* extract and isolated compound were studied for cytotoxic and antifungal potentials using in vitro Sulforhodamine B (SRB) and disc diffusion methods respectively. Molecular docking studies were conducted to check interaction of isolated compound with major oncogenic proteins.

Results: Qualitative phytochemical screening revealed presence of saponins, steroids, flavonoids, anthraquinones and cardiac glycosides while alkaloids were absent in *C. lancifolius* extract. Compound isolated was characterized as lancifolamide which showed cytotoxic activity towards variety of cancer cell lines including murine lymphocytic leukemia (P-388, IC₅₀ = 2.65 µg/ ml), human colon cancer (Col-2, IC₅₀ = 0.84µg/ ml), human breast cancer (MCF-7, IC₅₀ = 0.72 µg/ ml) while no cytotoxic activity was observed towards human lung cancer (Lu-1) and rat normal glioma cells (ASK, IC₅₀ = 11.6 µg/ ml) and human embryonic kidney cells (Kek293, IC₅₀ = 6.74 µg/ ml) respectively. Minimum inhibitory concentration (MIC) of Lancifolamide towards *Aspergillus fumigatus*, *Aspergillus nigar* (skin sample), *Aspergillus flavus* (pleural fluid) and *Candida albicans* (urine and blood sample) was found to be 54.5, 44.8, 43.5, 22.4 and 20.2 µg /ml respectively. Moreover, docking results are in strong agreement with our experimental finding which has identified lancifoliate to be more potent anti-proliferative agent than previously known compound ellipticine.

Conclusion: *C. lancifolius* extract and lancifolamide possess significant cytotoxic and Anti-Alzheimer properties. To best of our knowledge this is the first report that highlights isolation, identification and pharmacological activities of lancifolamide from *Conocarpus lancifolius*.

Keywords: *Conocarpus lancifolius*, Combretaceae, Cytotoxic activity, Anti-Alzheimer, Molecular docking



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Synthesis, characterization, antibacterial and thrombolytic activity evaluation of 2,5-disubstituted-1,3,4-oxadiazole derivatives

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ABSTRACT

Oxadiazole is reported to have high synthetic potential for synthesis of many biologically active heterocyclic compounds. Oxadiazole can be helpful in the design of novel highly effective pharmaceuticals with a broad spectrum of bio responses. Oxadiazoles constitute four different classes but 1,3,4-Oxadiazole is the most potent one. The different 2,5-disubstituted-1,3,4-Oxadiazoles possess a large number of biological activities like antifungal, anti-inflammatory and antibacterial [1,2,3]. A series of new N-substituted derivatives of naproxen were synthesized in three phases. The first phase involved the sequentially conversion of naproxen acid to ester, hydrazide and 5-benzyl-1,3,4-oxadiazol-2-thiol. In the second phase, N-substituted-2-bromoacetamides were prepared by reacting substituted aromatic amines with bromoacetyl bromide in basic media. In the third phase, 5-benzyl-1,3,4-oxadiazol-2-thiol was stirred with N-substituted-2-bromoacetamides in the presence of NaH/DMF to get the target compounds. Spectral analysis was used to confirm the structures of synthesized compounds. The synthesized compounds were evaluated for biological activity and were found to be relatively more active.

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Evaluation of antioxidant and antidiabetic potentials of *Tabernaemontana divaricata* and its therapeutic applications

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ABSTRACT

Diabetes mellitus is a disorder with a complex and multifaceted etiology. Oxidative stress plays a major role in diabetic physiopathology; hence, the interest of using natural antioxidants as therapeutic tools exists. *Tabernaemontana divaricata* (Crepe jasmine) is an economically important evergreen shrub grown in tropical and sub-tropical parts of the world. The aim of the study is to assess the various.



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antioxidant potentials and inhibitory effects of *T. divaricata* flower with some key enzymes which are involved in carbohydrate metabolism and anti-oxidative aptitude against free radicals. Antioxidant effects of the extract were manifested by TPC, TFC and DPPH assay in comparison with ascorbic acid and some enzymatic activities such as CAT, SOD and POD were also checked in vitro Cytotoxicity against red blood cells was also determined. Antidiabetic potential of methanolic extract was checked through α -amylase & α -glucosidase and glucose uptake by yeast cells. Flowers of *T. divaricata* were shade dried and powdered properly. Then methanolic extract was prepared by soxhlet extraction method. Results revealed that the methanolic extract of *T. divaricata* have TPC and TFC as 62.32 ± 4.02 mg GAE/g dw and 24.53 ± 0.61 mg QE/g dw respectively. DPPH assay in comparison with ascorbic acid, several enzymatic assays such as CAT, SOD and POD showed maximum antioxidant potential such as $15.9 \pm 2.33\%$, $65.57 \pm 13.4\%$, 3.02 ± 3.4 , 15.87 ± 0.5 , 0.74 ± 0.2 respectively. Extract show minute cytotoxic potential with reference to human RBCs. Glucose uptake by yeast cell, α -amylase & α -glucosidase showed maximum antidiabetic potential such as $75.11 \pm 1.44\%$, $41.81 \pm 3.75\%$, $35.9 \pm 1.24\%$ respectively. From the above results, it may conclude that *T. divaricata* flowers are potent source of natural antioxidants as well as antidiabetic activity and justifying its traditional use in green therapeutics.

Keywords: *Tabernaemontana divaricata*; antioxidant, antidiabetic, cytotoxic potential

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Efficacy of enzymes on growth performance and nutrient digestibility of *Catla catla* fingerlings fed cottonseed meal based diet

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ABSTRACT

Present research was conducted to investigate the efficacy of exogenous enzymes supplementation in improving growth performance and nutrient digestibility of *Catla catla* fingerlings fed cottonseed meal based test diet. Six test diets were formulated: including one control diet (without enzyme supplementation) four other diets, which supplemented with phytase, xylanase, protease, cellulase and sixth diet containing a mixture of these four enzymes supplementation. Chromic oxide (Cr_2O_3), an inert marker was added at a rate of 1% in all test diets. Three replicates having 15 fingerlings were stocked in V-shaped tanks specially designed to collect the fecal material. Fish fingerlings were fed at the rate of 4 % of live wet weight with their manufactured diets twice in a day. Fecal materials were carefully collected to prevent the breakage of feces to minimize the nutrients leaching in water. Supplementation of enzymes showed a significant ($p < 0.05$) improvement in growth performance and

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nutrients bioavailability of *C. catla* fingerlings. Results suggested that higher growth performance and nutrients digestibility of *C. catla* fingerlings were observed at mixture of enzymes level which is significantly different from control level. It was concluded that mixture of enzymes supplementation in cottonseed meal based diet was useful to develop a cost effective and eco-friendly environment.

Keywords: *C. catla*, phytase, xylanase, protease, cellulase, growth performance, nutrient digestibility.

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Antiadhesion and antibiofilm potential of *Fagonia indica* from Cholistan desert against clinical multidrug resistant bacteria

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ABSTRACT

High resistance to antimicrobials is associated with biofilm formation responsible for infectious microbes to withstand severe conditions. Therefore, new alternatives are necessary as biofilm inhibitors to control infections. In this study, the antimicrobial and antibiofilm activities of *Fagonia indica* extracts were evaluated against MDR clinical isolates. The extract exhibited its antibiofilm effect by altering adherence and disintegration of bacterial cell wall. *Fagonia indica* has antibacterial effect as minimum inhibitory concentration (MIC) values ranging from 125 to 500 $\mu\text{g mL}^{-1}$ and minimum bactericidal concentration (MBC) value was 500-3000 $\mu\text{g mL}^{-1}$ against multidrug resistant (MDR) clinical isolates. The extract exhibited its antibiofilm effect by altering adherence and disintegration of bacterial cell wall. *Fagonia indica* had antibacterial effect as minimum inhibitory concentration (MIC) values ranging from 125 to 500 $\mu\text{g mL}^{-1}$ and minimum bactericidal concentration (MBC) value was 500-3000 $\mu\text{g mL}^{-1}$ against MDR isolates. The maximum inhibitory effects of *Fagonia indica* chloroform extract on biofilm formation was observed on *Staphylococcus aureus* (71.84 %) followed by *Klebsiella pneumoniae* (70.83 %) after 48 hrs showing that inhibition is also time dependent. Our results about bacterial cell protein leakage indicated that MDR isolates treated with chloroform extract of *Fagonia indica* showed maximum protein leakage of *K. pneumoniae* (59.14 $\mu\text{g mL}^{-1}$) followed by *S. aureus* (56.7 $\mu\text{g mL}^{-1}$). Cell attachment assays indicated that chloroform extract resulted in a 43.5-53.5 % inhibition of cell adherence to a polystyrene surface. Our results revealed that extracts of *Fagonia indica* significantly inhibited biofilm formation among MDR clinical isolates, therefore, could be applied as antimicrobial agents and cost effective biofilm inhibitor against these MDR isolates.

Keywords: Biofilm inhibitors, Biofilm formation, MDR, Antibacterial activity

Comparative study of insecticidal activities by formulated fish emulsified bio fertilizer on growth and productivity of solanum lycopersicum l. (tomato)

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ABSTRACT

The purpose of this research is to achieve nutrient rich fertilizer for dealing insect pest infestation and faster growth of *Solanum lycopersicum* L. (tomato) by decomposing waste fishes and its comparison with normal organic fertilizer, chemical fertilizer, and controlled replicates. According to the observations and results it was observed that fish emulsion has high efficacy against insect pests because of anti-pest control activity of fish residues. This research was conducted in Jinnah University for Women from the months of June to December 2019. Preparation of fish emulsion fertilizer was arranged in laboratory of Zoology Department. There were some major and minor variations in the growth rate, leaves, flowers, and fruit emergence duration. Higher rate of insect infestation was observed in chemical fertilizers and controlled plant. Moderate rate of insect infestation was observed in organic fertilizer. Minimal mealy bug's infestation was recorded in fish emulsion than in chemical, organic and controlled plants. The number of leaves and fruits emergence was greater in fish fertilizer then in chemical, organic and controlled plants. Fruiting capability of chemical fertilizer was moderate because of higher mealy bugs infestation at early stage. The manure prepared from the utilization of fish waste can be used in large scale in agriculture (Radziemska et al., 2019). Fish emulsion fertilizer has resistivity to toxic substances like pesticides and salinity because of the essential elements that contributes in higher growth of plant.

Determination of Prevalence and Risk Factors of Infection with Babesia in Small Ruminants in Punjab, Pakistan

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ABSTRACT

In small ruminants babesiosis develops in RBCs of peripheral blood. Ovine babesiosis is the significant disease of sheep and goats and affects the health of ovine and caprine adversely. The study was carried out to assess the prevalence and the effect of ovine babesiosis in goat and sheep along with the risk associated with the pervasiveness of babesiosis. During 2018-19, a total of 892 blood samples were collected from 348 goat and 544 sheep in summer and winter season from three districts i. e. Faisalabad, Toba Tek Singh and Jhang. The blood samples were fixed, stained and examined under light microscope. DNA samples were extracted and amplified by polymerase chain reaction PCR and the nested PCR were performed to identify to Babesia spp. The results of microscopic examination showed that a total of 19.73% samples were positive for Babesia spp infection. The prevalence among goat and sheep were 10.73% and 14 % respectively. By using ELIZA technique the overall prevalence of Babesia spp. were 17.26 %. By molecular method, 14 % sheep and 10.73% goat blood samples were infected by Babesia. Totally, 13.73% were observed to have Babesia ovis. Our study have shown small ruminants Babesia ovis. While nested PCR detected 19.00% and 8.51% positive samples for B. ovis in both summer and winter season respectively. During summer season the prevalence of Babesia spp showed higher percentage. The hematological analysis showed that the values of RBCs, Hb, PCV, MCH, MCHC and MCV were significantly decreased as ($P < 0.05$) in diseased animals as compared to healthy goat and sheep. Moreover, the risk factors associated with the occurrence of babesiosis in ovine and caprine were studied and revealed that age (Adult), sex (female), housing (closed), tick association, dogs, non-cemented floor system, large herd size, poor body condition and ground feeding are the main risk factors causes the prevalence of babesiosis in goats and sheep.

Key words: Babesiosis, hematological, biochemical, PCR, nPCR

O-53/ICAZ-2020

Peripheral and testicular oxidative stress in varicocele patients may be responsible for the primary infertility

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ABSTRACT

Varicocele is a condition characterized by pathological vascular lesions of the pampiniform plexus within the spermatic cord. Varicocele is considered as the most common non-idiopathic treatable

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cause of male infertility. About 35% of males diagnosed with varicocele report primary infertility whereas 50% to 80% males reported with secondary infertility. Clinically varicocele causes marked decline in serum testosterone levels as well as decreased sperm output. The current study was conducted to evaluate the pathophysiology in the varicocele patients suffering primary infertility. A total of 30 patients (age 29-37) were examined. Blood samples were collected for the hormonal analysis (Testosterone, LH, FSH, Cortisol) as well as the total oxidant status (TOS) and total antioxidant capacity (TAC). Semen examination was also conducted including Semen Volume, Sperm Motility, Sperm Count & Sperm Morphology. Total antioxidant capacity (TAC) and Total oxidative status (TOS) of the semen was also examined. The results revealed high levels of plasma Cortisol as well as high levels of total oxidant status in the semen whereas the total antioxidant status of the semen was low. The plasma levels of testosterone, LH and FSH were also found to be low along with low sperm concentration and low to moderate sperm motility. The testicular volume was also found to be low in all the patients. Although the total number of patients (n=30) was less in the current study but our results highlights that oxidative stress might be a major factor in causing primary infertility in varicocele patients by the effecting the process of spermatogenesis. As the major effect of varicocele at the testicular level is lowering the overall blood supply so the lack of oxygen in the seminiferous tubules might cause the increase in the oxidative stress at the germ cell level.

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Biosynthesis of ZnO nanoparticles using *Bacillus subtilis*: Characterization and nutritive significance for promoting plant growth in *Zea mays* L

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ABSTRACT

Nano-fertilizer(s), an emerging field of agriculture, is alternate option for enhancement of plant growth replacing the synthetic fertilizers. Zinc oxide nanoparticles (ZnO NPs) can be used as the zinc source for plants. The present investigation was carried out to assess the role of ZnO NPs in growth promotion of maize plants. Biosynthesized ZnO NPs (using *Bacillus* sp) were characterized using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), X-ray diffraction (XRD) and Zeta potential. Different concentrations of ZnO NPs (2, 4, 8, 16 mg/L) were explored in pot culture experiment. Size of ZnO NPs ranged between 16 and 20 nm. A significant increase in growth parameters like shoot length (61.7 %), root length (56.9 %) and significantly higher level of protein was

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observed in the treated plants. The overall pattern for growth biomarkers including the protein contents was maximum at 8 mg/L of ZnO NPs. It was observed that application of biosynthesized ZnO NPs has improved majority of growth biomarkers including plant growth parameters, protein contents and leaf area. Therefore, biosynthesized ZnO NPs could be considered as an alternate source of nutrient in Zn deficient soils for promoting the modern agriculture.

Keywords: ZnO NPs biosynthesis, Zea mays L, Growth parameters, Zn deficiency, Nanofertilizers

Key Area: Environmental Biology, Microbiology

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Efficacy of anthelmintic properties of leaf extracts of moringa oleifera and azadirachata indica against nematodes of ovis orientalis orientalis

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ABSTRACT

The continuous and indiscriminate use of anthelmintic drugs has caused a growing problem of parasite resistance to conventional treatments. Essential active components of plant can be used as alternatives or in association with current anthelmintic treatment. The dried leaves of two indigenous medicinal plants i.e. *Moringa oleifera* and *Azadirachta indica* were primarily selected and aqueous, methanol and ethanol extracts were prepared for anthelmintic trial and determination of anthelmintic properties in vitro against the gastro-intestinal nematodes in wild sheep during the period from October 2018 to March 2019. Fecal samples of wild sheep of all ages and sexes were collected at the Wildlife Park Gatwala, Faisalabad from the ground after defecation at morning of the day. The anthelmintic activity of two plants: *M. oleifera* and *A. indica* extracts on egg hatching assay (EHA) and larval development assay (LDA) was examined by in vitro tests. The plant extracts were evaluated at six concentrations 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml. Ivermectin (1%) drug was used as positive control while untreated eggs in water was used as negative control. Both plant extracts showed the anthelmintic effects on EHA and LDA. The effect was dose dependent on both egg hatch assay and larval development assay. The results were expressed in terms of mean inhibition percentage and mean inhibition of larval mortality. Results revealed that methanol extract of *A. indica* was the most effected on EHA (LC50 = 1.75mg/ml) and LDA (LC50 = 1.89 mg/ml) which contribute to obtaining lower LC50 value. The ethanol extract of *M. oleifera*



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was most effected on EHA (LC50 = 1.89mg/ml) and LDA (LC50 = 1.97 mg/ml), which contributed to obtaining lower LC50 value. The overall findings of the current study indicated that the evaluated medicinal plants have potential anthelmintic effect and further in vitro and in vivo evaluation is indispensable to make use of these plants.

Key words: Medicinal plants, Anthelmintic, wild sheep, In-vitro

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Synergistic effect of *Moringa oleifera* leave and *Cinnamomum cassia* bark methanolic extracts against Bisphenol A induced hepatotoxicity in rats

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ABSTRACT

Bisphenol A (BPA) is a synthetic compound and [use](#) to make different plastic products. BPA-based plastic is strong and hard, and its common consumer products are included plastic [food storage containers](#), [water bottles](#), and [baby bottles](#). Thus, human exposure to BSA is widespread. It is indispensable to concern BSA-induced liver toxicity as the liver is the main organ of exposure. *Moringa oleifera* (*M. oleifera*) leaves (MLE) and *Cinnamomum cassia* (*C. cassia*) bark (CBE) methanolic extracts are reported to have significant anti-inflammatory, antihepatotoxic, and antioxidant properties. The present study aimed to assess Bisphenol A (BPA- induced liver damage in male rats Sprague Dawley rats. Furthermore, we investigated the synergistic effects of MLE (350 mg/kg per Body weight; BW) and CBE (225 mg/kg per BW) methanolic extracts against BSA induced liver damage. The study evaluated biomarkers of liver function and oxidative stress markers from the blood serum of rats. The rats (n=5) were randomly allocated to five groups (control and treated). The control (C) group remained untreated while vehicle control (VC) received olive oil (1 ml). The treated groups were assigned as CG1 (received CBE at 225 mg/kg per BW), MG2 (received MLE at 350 mg/kg per BW), CMG3 (co-administrated with CBE and MLE at 225 mg/kg and 350 mg/kg per BW, respectively) three times in a week for 59 days. After the trial, the animals were sacrificed and blood was collected in blood clotting tubes. Biochemical analyses found significant ($P<0.05$) elevation of liver biomarkers (ALT, AST, ALP, TB, and LDH) and biomarkers of oxidative stress (MDA, CAT, GSH, and LPO) in the BPA treated group. The co-administrated groups showed significant restoration of all the parameters than the individual supplementations of plant extracts. The study found promising synergistic effects of *M. oleifera* leave and *C. cassia* bark extracts against BPA induced liver damage. Our investigation will help to explore new avenues in terms of synergistic interactions of plant extracts at their effective concentrations for better medicinal effects.

Keywords: BPA, synergic, liver, *M. oleifera*, *C. cassia*, oxidative stress, inflammatory cytokinescs

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Effectiveness of probiotics supplemented linseed meal based diets on overall performance of *Labeo rohita*

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ABSTRACT

Seventy days trial fish feeding research work was carried out to check the effects of probiotics supplemented linseed based diet on growth, mineral absorption and nutrient digestibility of *L. rohita* fingerlings. Probiotics have positive effects on the host by inhibiting pathogenic microorganisms, improving feed intake, contributing enzymatically to digestion, secreting growth-promoting factors and improving the quality of pond water. By using various concentration of probiotics (0, 1, 2, 3, 4, 5 g/Kg) six test diets were prepared. All six groups were fed twice daily on their respective diets and samples of feces were collected from each tank on daily basis and stored for chemical analysis. Results of this research revealed that probiotics addition showed significant improvement in fish. Highest weight gain (20g), weight gain% (279%), specific growth rate (1.48) and best FCR (1.21) was found in fish fed 2 g/kg level of probiotics supplemented linseed meal based diet. Similarly, maximum nutrient digestibility (CP; 71% and Fat; 75%) and mineral absorption (P; 72%, K; 75% and Na; 76) were also analyzed in the fingerlings that were fed on the above said test diet. Whereas maximum digestibility of GE (74%) and minerals absorption (Ca; 73%, Al; 69%) was observed maximum at test diet IV that was supplemented with 3 g/kg linseed based diet. On the basis of these results, it was concluded that 2g/kg level of probiotics inclusion was optimum for the formulation of ecofriendly and economically affordable fish diet by using linseed meal based diet.

Keywords: *Labeo rohita*, Probiotics, Growth performance, Digestive Enzymes, Nutrient Digestibility, Mineral Absorption.

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Serum biochemical changes in liver and kidneys of male Albino mice after oral exposure of Zinc Oxide Nanoparticles

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ABSTRACT

Nanotechnology is one of the dominating technologies of recent era. Nanoparticles are extensively being used in several industries including food, cosmetics and medical instrument manufacturing industry. However, a lot of questions are being raised regarding the damaging effects of nanoparticles in living organisms. These particles possess high surface reaction due to the high percentage of atoms on their surface. Zinc oxide nanoparticles (ZnO-NPs) are also being consumed as essential dietary component but their excessive usage has led to deleterious effects in vital organs of body. Present study was designed to explore the toxicity of ZnO-NPs in liver and kidneys of mice. For this, 30 adult Swiss albino mice were taken and divided into 3 groups (N=10) i.e. Control (ctrl.), Zinc Oxide Nanoparticles Dose I (ZnO-NPs I) and Zinc Oxide Nano-particles Dose II (ZnO-NPs II). Sub-lethal doses of ZnO-NPs i.e. 15µg/g of B.W. and 50µg/g of B.W. were given orally for 35 days continuously. Dissection was made on 36th day, 24 hours after the last administration. Results showed that continuous administration of ZnO-NPs caused significantly decreased ($P < 0.05$) in the body weight as the concentration of ZnO-NPs increased in body. Blood serum analysis showed increased levels of ALT, AST, ALP and decreased creatinine concentration in blood serum of mice. From observations, it is concluded that ZnO-NPs can cause hepatic and nephric toxicity even if it is used at sub-lethal concentration in mice.

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Defensive potential of aqueous onion extract against tartrazine mediated hepatic and nephric toxicity in *Mus musculus*

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ABSTRACT

Tartrazine, an extensively used commercial food colorant is utilized in various food products, pharmaceuticals and cosmetics. Keeping in view the controversy about its toxicity, current study was conducted to evaluate the remedial potential of *Allium cepa* (onion) against tartrazine instigated in-vivo toxicity in male mice. 30 male mice, randomly divided into three groups (N=10) labeled as group I (control group), group II (tartrazine group), group III (tartrazine + onion extract group). Mice were orally administered with respective doses (0.140 mg of tartrazine, 0.1 ml of onion extract) once in a day for consecutive 4 weeks. After completion of dose administration period, mice were sacrificed. Blood was collected by intra-cardiac puncture and vice versa dissection was carried out for further analysis. Body weight gain comparison among different groups showed a remarkable decline in weight gain in tartrazine group, while decrease was less obvious when onion extract was co-administered with tartrazine. Moreover, an obvious decrease in weights of liver and kidney was observed in tartrazine treated group. While those mice administered with onion extract along with tartrazine showed tissue weight restoration comparable to control. Serum ALT, AST, ALP, total bilirubin, urea and creatinine tended to increase whereas concentration of total protein declined in tartrazine exposed mice against control. Histological lesions like hemorrhages, pyknosis, hypertrophy, necrosis, sinusoidal dilations, degenerations, glomerulosclerosis and tubular dilations in hepatic and renal tissues respectively were obvious in tartrazine exposed groups. Anyhow, coadministration of onion extract with tartrazine protects these biochemical alterations and histopathological lesions to a great extent in mice by probably activating the antioxidant defense system of body.

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Green Revolution: A Possible Solution to Air Pollution in Changing Climate of World

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ABSTRACT

A physical, chemical or biological alternation to the air in the atmosphere that may harmfully effects to living organisms is termed as air pollution. It is a key concern that has been impacting human health, plants, animals as well as the environment. Indigenous environmental or health organizations regularly make daily air contamination predictions for public awareness and for use in making decision concerning reduction methods in addition to the management air quality. Air pollution is responsible for 2.6 million deaths worldwide (4.7 % of the global) and about 33 % in south Asia in addition to its impacts on human health and well being. Burning of fossil fuel, agricultural activities, exhaust from factories and indoor activities such as household waste are major causes which resulted in respiratory disorders and heart problems, global warming, acid rain and depletion of ozone layer. About 135000 deaths per year are attributed to ambient air pollution making it a leading cause of sickness and death in Pakistan. Nearly 90 percent of air pollution related deaths occur in low and middle income countries with nearly 2 out of 3 occurring WHO's South East Asia and Western Pacific regions.

The concept of green revolution is based on plantation activities, increasing aforestation, decreasing deforestation, overgrazing etc and such processes enable to protect and benefit the economy, habitat, people and planet. Plants use CO₂ and other toxic materials which can persist in the environment. It is a strategic pathway to build a sustainable future the principles of green revolution involves the development of green belts and use of non toxic reagents. The findings of this study showed that plantation activities should be considered on large scale for air quality monitoring and modeling. As plants act as natural lungs of Universe and release oxygen providing good quality of air. It will take time to build robust integrated air quality and climate mitigation regimes but that patience will be rewarded. As every nation aspires to provide its citizens with the highest quality of life, including freedom from air pollution and the ravages that will result from a widely unpredictable, destabilized climate.

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Evaluation of environmental effects of heavy metals on biochemical profile and oxidative stress among children at brick kiln sites

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ABSTRACT

The present study was designed to study the health risks among children living at brick kiln industries. A survey was conducted, questionnaires were filled out, and demographic data were collected from Punjab, Pakistan (n=232). Heavy metals burden and BMI was calculated, hematological and enzyme analysis, comet assay and hormonal ELISA was performed. The results showed decreased BMI, RBC count, hematocrit in the exposed group. Nickel ($p>0.05$), cadmium ($p>0.05$), zinc ($p>0.01$) and chromium ($p>0.05$) concentrations in whole blood were detected among exposed children. Antioxidant enzymes ($p>0.001$) and growth hormone ($p>0.01$) concentration decreased, while reactive oxygen species ($p>0.001$) and cortisol level increased ($p>0.01$) in the exposed group. The comet assay findings showed decreased percentage DNA in the head ($p>0.001$) and increased in the tail region ($p>0.001$) among exposed group. It was concluded that children living at brick kiln sites experienced decreased BMI, altered antioxidant enzymes status and hormone levels and cellular DNA damage that pose a major threat on child health.

Keywords: Brick kiln, heavy metals, stress, hormone analysis, children

O-65/ICAZ-2020

Calotropis procera bestows ameliorating effect on the mechanically insulted sciatic nerve in a mouse model of peripheral nerve injury

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ABSTRACT

The sensorimotor functional loss as a result of peripheral nerve injuries leads to permanent disability and ultimately, physical dependency with huge cost and compromised quality of patient's welfare. Unfortunately, such complicated medical situations are still waiting for their first-line therapeutic agent/s. This study was designed to investigate the role of *Calotropis procera* (*C. procera*) in speeding up the functional retrieval after mechanically induced sciatic nerve injury in healthy albino male mice. After acclimnormal chow and Treatment (n=8) fed on *C. procera* powder (100mg/kg/body weight) uniformly mixed in chow since the day of sciatic nerve injury induction. A mechanical crush was induced in the sciatic nerve of the right legs of all mice. Behavioral analyses including grip strength and sciatic functional index for evaluation of motor function retrieval and pinprick and hot plate tests for assessing the sensory function reclamation were conducted at various time points. At the end of the experiment, the mice were sacrificed. Their serum was collected for biochemical analysis. Skeletal muscles were dissected out for quantifying muscle mass. The significantly earlier retrieval of both motor and sensory activities was seen ($p<0.05$) in the treatment group which suggest the positive impact of this plant for speedy recovery of functions. Moreover, the treatment group displayed a significant decrease in total oxidant status (TOS), and an increase in total antioxidant capacity (TAC) with significantly enhanced activity of arylesterase ($p<0.001$) and paraoxonase ($p<0.001$) suggested the oxidative stress combating effect of *C. procera*. These findings point towards the connection between the positive impact *C. procera* on hastening the functional recovery following nerve injury and its antioxidant role. Thus this plant can be considered as a potential candidate drug for further investigation to seek the bioactive compound/s that may actually responsible for ameliorative functional recovery following PNI.

Keywords: Peripheral nerve injury, Total oxidant status, Total antioxidant capacity, Arylestrase and Paraoxonase-1

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Efficacy of probiotics on growth performance, nutrients and mineral digestibility of *Cirrhinus mrigala* fingerlings fed sunflower oilseeds by-products meal-based diet

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ABSTRACT

The study was carried out to estimate the effects of probiotics supplemented sunflower meal based diet on growth performance, nutrients and mineral digestibility in *Cirrhinus mrigala* fingerlings. The experiment consisted of nine test diets. Diets were formulated by adding graded levels of probiotics (0 gKg⁻¹, 0.5 gKg⁻¹, 1 gKg⁻¹, 1.5 gKg⁻¹, 2 gKg⁻¹, 2.5 gKg⁻¹ and 3 gKg⁻¹) probiotics supplementation to a sunflower meal-based diet. Chromic oxide was added as an indigestible marker. Fingerlings were fed at the rate of 4% of live wet weight. The maximum growth performance, minerals, and nutrients digestibility value were observed in fingerlings fed diet supplemented with 2 gkg⁻¹ level of probiotics. These values were significantly different from fish fed the control and other test diets. It was concluded that the probiotics supplementation to a sunflower meal based diet at 2 gkg⁻¹ level is optimum to release sufficient amount of chelated minerals and nutrients for *C. mrigala* fingerlings. Our findings also suggested that probiotics supplementation can help in the development of sustainable aquaculture by reducing the feed costs and nutrient discharge through feces into the aquatic ecosystem.

Keywords: *Cirrhinus mrigala*, probiotics, Sunflower meal, Growth, Nutrient digestibility

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Amelioration by *syzygium cumini*, *fragaria ananassa*, *morus macroura* and *olea ferruginea* fruit extract on testes, seminiferous tubules and spermatid cells by ccl4 induced histopathologies in mice

***Syeda Nadia Ahmad, Khawaja Raees Ahmad, Iram Inayat, Sitara Shamim, Sadia Suleman, Muhammad Ali Kanwal, Tasleem Ishaq**

ABSTRACT

Male toxicology in terms of histopathology of testes and histometry of seminiferous tubules and various spermatid cells against CCl₄ exposure and their ameliorations upon post treatment of (*Syzygium cumini*, *Fragaria*×*ananassa*, *Morus macroura* & *Olea ferruginea*) fruit pulp extract were studied in mice. Sixty male mice were equally divided into six groups as {1. Vehicle Control (Vcon) group: received 0.1mL pure corn oil on day zero followed by the twice daily dose of 0.1mL mineral water on 1-6 days; 2. CCl₄ (C) group received single dose of 0.1mL of 0.2mL/kg CCl₄ solution in corn oil by gavage on day zero of study, followed by normal mineral drinking water on days 1-6 every 12hour/basis. The other 4 groups; 3. CCl₄+*Syzygium cumini* (CSc); 4. CCl₄+ *Fragaria*×*ananassa* (CFa); 5. CCl₄+ *Morus macroura* (CMm) and 5. CCl₄+*Olea ferruginea* (COF) were given single dose of CCl₄ on day zero followed by single dose of 0.1mL “Sc, Fa, Mm and Of” fruit pulp extracts was given on days 1-6 every 12 hour/basis. On day 7th of the study the animals were sacrificed to obtain testes and blood. The testes were processed for histology and histometry. Blood samples were used to obtain biochemical results of lipid profile e.g, and biochemical triglycerides, cholesterol, High Density Lipid, Low Density Lipid and Very Low Density



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Lipids. Results show drastic pathological changes in testicular tissue on chromium CCl₄ shrinkage of seminiferous tubules, disruption of the characteristic distribution of spermatogenic cells in these tubules and obliteration of interstitial tissue. Rupture of basement membrane of various seminiferous tubules, extremely scanty and scattered spermatogonia, primary spermatocytes and dislodged spermatogenic cells, lack of tail in many of the dislodged spermatozoa in CCl₄ treated group. Most of these pathological signs were recovered effectively in COF group where all dispositions of the seminiferous tubules and the interstitial tissue were mimicking the VCon group layout in general with slight variations including centrally placed debris of the spermatogenic cells or hollow caliber of the tubules in-lieu-of indicating the rehabilitation of the process of spermatogenesis. Histometric results give a strong evidence for the above mentioned histological results as it indicates gradual shrinkage of seminiferous tubule and significant loss of spermatogonia, primary spermatocytes; significant decrease in the relative abundance of spermatozoa in CCl₄ group than in Vehicle control group. All the histopathological, histometric results were supportive of these findings. Findings of this study indicate that CCl₄ has strong gonadotoxic (testes) effects whereas the post treatment of “Sc, Fa, Mm and Of” fruit pulp extracts lead to the improvement of the andro-pathological signs.

Keywords: *Syzygium cumini*, *Fragaria* × *ananassa*, *Morus macroura*, *Olea ferruginea*, CCl₄

O-71/ICAZ-2020

Occurrence of Multi-Drug Resistant *Mycobacterium tuberculosis* from patients of Pulmonary Tuberculosis in District Khanewal

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ABSTRACT

Mycobacterium tuberculosis is the causative agent of Tuberculosis (TB) and is included among the highly contagious diseases. Pakistan ranked at 6th among the 22 high-TB burden nations. Almost half a million individuals including ~15000 youngsters contracted tuberculosis in Pakistan every year along with ~70000 deaths. Further, Pakistan also ranked among the top listed countries with a high incidence of multi-drug resistance tuberculosis (MDR-TB). Hence, the present descriptive cross-sectional study was designed for the determination of the frequency of pulmonary TB as well as Rifampicin-resistant cases using the CBNAAT (Cartridge Based Nucleic Acid Amplification Test) based GeneXpert among the suspected patients attending District Head Quarters Hospital, Khanewal. For this purpose samples (n=200) were collected according to the guidelines of the World Health Organization. The samples were samples for TB. A total of 09



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also processed for traditional Ziehl-Neelsen stain (ZN staining). The ZN staining showed a total of 64 positive samples for TB, whereas GeneXpert detected a total of 80 positive (11.25%) samples were found resistant to Rifampicin (males=4 and females=5). Further, the results showed that the prevalence of TB was significantly higher among males, rural areas and among the age group of 26-50 years. In conclusion, GeneXpert is a highly sensitive technique for accurate diagnosis of clinical TB as well as Rifampicin resistant TB as compared to the traditional ZN staining technique.

Keywords: Mycobacterium tuberculosis, GeneXpert, Rifampicin

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DNA damage caused by chronic exposure of cadmium in the Catla catla

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ABSTRACT

During chronic exposure of cadmium, for 84 days, the fish peripheral erythrocytes were collected fortnightly to observe the dose and time dependent DNA damage in the *C. catla* through comet assay by following the method of Singh et al. (1988). DNA damage was measured in terms of damaged nuclei, genetic damage index (GDI), and comet tail lengths (CTL). Peripheral erythrocytes of *C. catla* exhibited significantly variable DNA damage after exposure to various concentrations (2/3rd, 1/3rd, 1/4th, and 1/5th LC50) of cadmium, negative and positive controls for 84 days, and sampling was done fortnightly viz. 14-, 28-, 42-, 56-, 70- and 84-days. The DNA damage was significantly maximum ($53.00 \pm 1.92\%$) at positive control among metallic ion doses while it was minimum ($1.33 \pm 0.73\%$) due to negative control. The GDI and CTL values varied significantly at different exposure concentrations that followed the order: positive control > 2/3rd LC50 > 1/3rd LC50 > 1/4th LC50 > 1/5th LC50 > negative control. Cd exposure caused significantly more serious damage to the nuclei as GDI values were significantly higher after 56 days of exposure (1.29 ± 0.70), while nuclei damage and GDI values were significantly lower after 14 days exposure period as $22.56 \pm 17.52\%$ and 0.96 ± 0.58 , respectively. However, after 28 and 70 days of exposure, the damage in the nuclei became non-significantly different at $p < 0.05$. During 84 days of Cd exposure, the CTLs of nuclei varied between 78.55 ± 43.03 and $110.38 \pm 62.06 \mu\text{m}$.



O-78/ICAZ-2020

First Report Regarding the Simultaneous Molecular Detection of *Anaplasma marginale* and *Theileria annulata* in Equine Blood Samples Collected from Southern Punjab in Pakistan

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ABSTRACT

The present study was designed to check the molecular detection of *Anaplasma marginale* and *Theileria annulata* in blood samples of horses and donkeys collected from Dera Ghazi Khan District in Punjab and to document their phylogenetic origin and their association with studied epidemiological factors (sex and age) and complete blood count parameters, if any. A total of 195 blood samples were collected from apparently healthy horses (N = 141) and donkeys (N = 54). *A. marginale* DNA was detected by PCR in 4.9% (7/141) horse and in 9.2% (5/54) of donkey blood samples. Prevalence of *T. annulata* was 5.6% (8/141) and 11.1% (6/54) in horse and donkey samples, respectively. While 1.4% (N = 2) horses and 3.7% (N = 2) donkeys were found co-infected with both parasites. Representative amplicon for both parasites was confirmed by DNA sequenced and partial DNA sequence of the major surface protein-1b encoding gene of *A. marginale* and cytochrome b gene from *T. annulata* were submitted to the GenBank database under the accession number MK792344- MK792348. Epidemiological data analysis revealed that female horses were more prone to *A. marginale* (P = 0.02) while female donkeys were more susceptible to *A. marginale* (P < 0.001) and *T. annulata* (P < 0.001) infection. It was observed that horse and donkey infected either with *Anaplasma marginale* or *Theileria annulata* had significantly disturbed red and white blood cell counts and their associated parameters. This is a first ever study regarding molecular detection of *A. marginale* and *T. annulata* in equine blood samples from Pakistan. We recommend that this multiplex PCR protocol should be used for the detection of *Anaplasma marginale* and *Theileria annulata* in livestock for their proper diagnosis and treatment.

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Molecular epidemiology of *Theileria annulata* infection of cattle in Layyah District, Pakistan

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ABSTRACT

Theileria annulata is the cause of tropical theileriosis in cattle in Pakistan, where it has a significant impact on the cattle industry. Here we report the molecular detection and seasonal prevalence and blood parameters of *T. annulata* infection in crossbreed, Holstein Frisian and Sahiwal breed in Layyah District in the Punjab. A total of 844 blood samples (Cross = 244, Holstein Frisian = 300, Sahiwal breed = 300) collected in 2017 and 2018 were tested. Blood smear screening revealed 125/844 (15%) of cattle positive for *Theileria* species. PCR amplification of cytochrome b gene indicated an overall *T. annulata* prevalence of 21% (174/844). The highest prevalence was observed in autumn (53%) followed by winter (20%), summer (14%) and spring season (3%). Crossbreed cattle were the most susceptible to *T. annulata* (28%) followed by Sahiwal (19%) and Holstein Frisian. Representative partial cytochrome b gene sequences of *T. annulata* revealed phylogenetic similarities with sequences submitted from India, Iran, China, Turkey and Spain. Small number of ticks including *Hyalomma anatolicum*, *Hyalomma excavatum*, *Rhipicephalus microplus*, *Haemaphysalis punctata* was identified from cattle but none of them was found PCR positive for the presence of *T. annulata*. Analysis of the hematology data indicated that red blood cell, hemoglobin, mean cell hemoglobin, mean corpuscular hemoglobin, lymphocyte (%), monocyte (%) and platelet count were significantly altered in *T. annulata* positive cattle of all three breeds. Screening of cattle by PCR for the detection of *T. annulata* is recommended for diagnosis and treatment.

O-82/ICAZ-2020

Evaluation of biocontrol activity of fungal isolates against hyalomma ticks in cattle

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ABSTRACT

Ectoparasites are one of the major health problems of cattle in Pakistan resulting in significant economic losses such as decreased productivity and mortality. Cattle production is affected due to parasitic nature of *Hyalomma* ticks and because of the fact they transfer important protozoan diseases such as babesiosis and theileriosis. *Hyalomma* is one of the most medically important tick genera in the Africa, Asia and Europe. The study was conducted to investigate the biocontrol of *Hyalomma* ticks

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through fungal spore-free culture from September 2018 to September 2019 in Microbiology lab of Kohat University of Science and Technology. Fungi were isolated from the dairy farm soil. The isolated fungi were processed to get spore free culture from them. The isolate have been evaluated for their ability to produce mortality against Hyalomma ticks. In order to select an isolate for the cattle tick control three fungi (Alteranaria, Aspergillus and Penicil) were Jisolated from the dairy farms soil. Different concentrations of selected fungal derived spore free culture filtrate were made. The filtrates were then added to adult and larvae mortality. All concentration on larvae and adult showed different results. Regarding larval mortality all the filtrate did not showed mortality in first three days. In diagnosis of larval mortality the higher concentrations i.e. 100% showed 100% results. It was observed that decreasing concentrations of filtrate lowers tick mortality. Minimum ticks mortality was recorded at the lowest fugal culture filtrate. It was concluded that the effect of fungal spore-free culture filtrate against Hyalomma ticks was time and dose, depended for their activity against adult and larvae mortality.

Key words: Ectoparasites, Hyalomma ticks, Adult, Larvae, dairy farm soil, fungal spore-free filtrate

O-85/ICAZ-2020

Toxicological effects of waterborne titanium nanoparticles in *Cyprinus carpio*.

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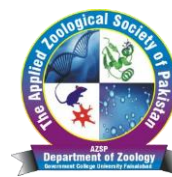
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ABSTRACT

Nanoparticles (NPs) have vast range of diverse resources which consist of particular materials having size fewer than 100 nm. Titanium nanoparticles were formed by sol-gel method and characterized by different methods such as, X-ray Diffraction, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy and Transmission Electron Microscopy. The individuality of nanoparticles was proved to be more poisonous as compared to their bulk forms. For determination of toxicological effects of titanium nanoparticles in common carp (*Cyprinus carpio*), juveniles of common carp having same weight and length were selected. Fish were acclimatized in cemented tanks for 15 days and exposed to different concentration of NPs under acute exposure. The physico-chemical parameters were also be calculated on daily basis during acute toxicity trials. The mean 96-hr LC₅₀ of titanium nanoparticles for *Cyprinus carpio* was calculated as 168.583±9.2377mg/L while lethal concentration was 289.249±25.6008mg/L respectively, determined by probit analysis method. Oxidative stress in terms of lipid per-oxidation and glutathione S-transferase was checked after acute exposure. After exposure, there were irregularities in the gills, kidneys, liver and muscles of carps due to toxicological effects of titanium

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nanoparticles. The effect on lipid peroxidation level and glutathione S-transferase activity at 96-hr concentration and lethal concentration was recorded in order as followed: Liver>gills>kidney>muscles.

Key words: Acute toxicity, Titanium nanoparticles, Toxicological effects, Lipid per-oxidation, glutathione S-transferase, *Cyprinus carpio*.

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Effects of Atrazine on Blood Biochemical and Haematological indices of Grass Carp (*Ctenopharyngodon idella*)

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ABSTRACT

The current study evaluated the toxic effects of Atrazine herbicide on blood Biochemical and Haematological indices of fresh water fish Grass Carp (*Ctenopharyngodon idella*), an economically important fish of Pakistan. Different concentrations of Atrazine were given to Grass Carp and the 96 hours LC50 value of Atrazine was calculated to be 150.5 µl/L at 25 °C. The LC50 concentration of Atrazine was further used for 24, 48, 72 and 96 hours for determination of its harmful effects. Rapid movements, nervousness in swimming, lack of balance in position and equilibrium, more mucous secretion and changed in their color of Grass Carp was observed dependent upon time. Haematological analysis showed significant decrease in Red Blood Cells (RBCs), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), whereas significant increase was observed in White Blood Cells (WBCs), Mean Corpuscular Volume (MCV) and Haematocrit (Ht) of Grass Carp after exposure to Atrazine. The biochemical analysis showed significant decrease in total protein, albumin, triglyceride and cholesterol level whereas significant increase was recorded in glucose level at different time points after Atrazine exposure. We concluded that Atrazine is highly toxic for Grass Carp that effect the blood biochemistry and haematology of Grass carp even during short exposure. Precautions may be taken in the use of Atrazine and/or alternate ecofriendly herbicides may be used in the agricultural fields.

Key words: *Ctenopharyngodon Idella*; Atrazine; Toxic effect; Biochemical; Haematological; LC50.



Optimum Dietary Calcium Requirement of Hypophthalmichthys molitrix Fingerlings

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ABSTRACT

The main objective of this study is to investigate the optimum dietary calcium requirement in the fingerlings of *Hypophthalmichthys molitrix*. Five purified diets containing different calcium levels in the form of calcium lactate at 0, 3, 6, 9 and 12 g/kg were added for the determination of optimum requirement of calcium of silver carp. Eighteen fish were stocked in each aquarium having three replicates. Experimental diet was given to fingerlings up to satiation for 6 days a week. Water quality parameters (pH, temperature, and dissolved oxygen) were kept constant throughout the feeding trial. The results showed that the growth performance and alkaline phosphatase activity (ALP) shows significantly increased with Ca supplementation up to 6 g/kg, while the rate of survival and feed intake showed non-significant ($p \geq 0.05$) results. In the body proximate analysis significant results were obtained. The crude protein and ash % were maximum in 6 and 9g/kg level of diet while crude fat and moisture % was decreased at this concentration of Ca in diet. The mineral content was also analyzed from different body tissue and showed significant results in the samples of scales, whole body, serum, and bones. The calcium concentration was increased when calcium level was increased in the diet while the phosphorus concentration was reduced with the increase in the level of calcium in test diet. The calcium and phosphorus ratio were also observed, and non-significant results were obtained in the Ca/P ratio of bones and serum. From serum samples the calcium and phosphorus also showed non-significant results. The other minerals like magnesium and zinc also showed a decreased concentration due to increased dietary level of Ca in diet. In short, it was concluded that the Ca supplementation in the form of calcium lactate should be beneficial for growth, proximate and mineral content from different body tissues and from results of broken line regression it was also observed that the optimum requirement of calcium for the fingerlings of silver carp is 8.1 g/kg.

Key words: Calcium lactate; Silver carp; Proximate analysis; Minerals; Alkaline phosphatase activity



Synergistic effects of Ginkgo biloba leaf extract and Panax ginseng root extract on carbohydrate and lipid metabolism gene expression in alloxan induced diabetic rats

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ABSTRACT

The present study aimed to evaluate the anti-diabetic properties of Ginkgo biloba leaves extract (GBE) and Panax ginseng roots extract (PGE) in different combinations. A total of 40 rats were fed on high-fat-diet for 14-days, then divided into five groups (N=8). Non diabetic group (NDG), Diabetic-group (DG), Mixed-group-1 (MG1), Mixed-group-2 (MG2), Mixed-Group-3 (MG3). Alloxan monohydrate (120-130 mg/Kg BW) was used as a diabetogenic agent. The data of blood glucose and body weight (BW) were monitored regularly weekly. Basal blood was collected from the heart for biochemical analyses. Skeletal muscle, hepatic, and adipose tissue were obtained for mRNA expression of genes. A Significant decrease in BW was found in all treated groups. A significant reduction in fasting serum glucose, AST, ALT, and creatinine were recorded in dose dependent-manner. The treatments showed up-regulation of GLUT-4 in the muscle (all treated groups) and hepatic tissues (MG3); IR in the muscle (MG3) and adipose tissue (MG3), and IRS-1 in hepatic (MG3) and adipose tissue (MG3). Our results showed that these herbs improve dyslipidemia and have strong antioxidant activities. We found significant down-regulation for SREBP-1c in dose-dependent manner in the liver and significant upregulation for FAS (MG2 & MG3) in the liver. Significant up-regulation was found for PPAR- α in muscles and PPAR- γ in adipose tissues in all treated groups. Significantly down-regulation for TNF- α seemed in all studied organs. In conclusion, GBE and PGE showed strong anti-diabetic, anti-hypercholesterolemia and anti-oxidative effects in combination by regulating the genes involved in carbohydrate and lipids metabolism.

Key words: Alloxan, Diabetes, Ginkgo biloba leaf extract, Metabolism, Panax ginseng root extract, Rats.

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A comparative analysis of *in vitro* antibiotic potential of selected medicinal plants against *E. coli* of poultry chicken

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ABSTRACT

Globally, the poultry industry has experienced massive growth in response to consumer demand for poultry products over the past few decades. In Pakistan, the poultry sector is playing an important role in bridging the gap between the supply and demand for protein. Despite of the scientific development, poultry industry is still in the grip of various diseases of bacterial, viral, fungal and parasitic origin. Avian colibacillosis caused by Avian Pathogenic *Escherichia coli* is an infectious disease of birds which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions. A lot of research is being conducted in the area for the discovery of novel antibiotics by utilizing extracts of medicinal plants. Swab samples were collected from naturally infected broiler chickens showing clinical signs of colibacillosis in various poultry farms. *E. coli* was isolated and biochemically identified by various methods. Medicinal plants were collected from natural habitats and aqueous extracts were prepared. *In vitro* assay was performed as per standard protocol. It was observed that *Allium sativum* revealed a remarkable antibacterial activity against *E. coli*, which was considered quite comparable with the positive control. *Aloe barbadensis*, *Calotropis procera* and *Kalanchoe daigremontiana* also showed good antibacterial activity against *E. coli*. In this study it was concluded that *A. sativum*, *A. barbadensis*, *C. procera* and *K. daigremontiana* exhibit antibacterial potential against *E. coli* of poultry.

Key words: Antibacterial activity, *Allium sativum*, *Calotropis procera*, *Kalanchoe daigremontiana*

Computer aided prediction and identification of phytochemicals as potential drug candidates against MERS-CoV

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ABSTRACT

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is the major leading cause of respiratory infections listed as blue print of diseases by World Health Organization that needs immediate research in the developing countries including Saudi Arabia, South Korea and China. It is a positive single stranded RNA virus that affects the host cell directly by binding to dipeptidyl peptide receptor 4 (DPP4). Still no vaccine has been developed against MERS-CoV therefore an effective strategy is required to overcome the devastating outcomes of MERS. Computer aided drug design is the effective method to find out potency of natural phytochemicals as inhibitors of MERS-CoV. In current study, molecular docking approach was employed to target receptor binding of CoV. Total 150 phytochemicals were docked as ligands in this study and found that most of the phytochemicals successfully inhibited the catalytic triad of MERS-CoV. The best 10 ligands found in this study were tannic acid, paclitaxel, hesperidin, naringin, rosavin, betaxanthin, quercetin, calamin, rutin and citromitin based on minimum S-score values hence these can serve as potential drug candidates to stop the viral load. The docking results brought novel scaffolds which show strong ligand interactions with Arg178, Arg339, His311, His230, Lys146 and Arg139 residues of viral domains. The study revealed that that these phytochemicals would serve as strong potential inhibitors and starting point for the development of vaccines and proteases against MERS-CoV. Further, in vivo studies are needed to confirm the efficacy of these potential drug candidates.

Keywords: MERS-CoV; betacoronavirus; computer aided drug designing; molecular docking; catalytic triad of MERS-CoV

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Recombinant dna technology for the production of industrially important enzymes

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ABSTRACT

Poultry sector is one of the major established industry of Pakistan that is committed to provide valuable meat to our community. Phytases, cellulases, xylanases and proteases are the main enzymes being added in the poultry feed. The addition of these enzymes is important because their addition in feed put a positive impact on the growth of poultry bird. Phytases are responsible for the availability of free phosphorus while the xylanases and cellulases are responsible for the availability of monomeric absorbable sugars for the growth of bird whereas proteases also involve for the improvement of digestion of proteins. In the absence of these enzymes the phytate, cellulose and xylan are not being digested by the poultry bird and these components of feed simply pass through the digestive track and are removed from the body with manure and contribute in environmental pollution.

Recombinant DNA Technology is a very good tool for the production of industrially important enzymes/proteins. In the current study the phytase, cellulase and xylanase genes from hyper-thermophilic bacterium were amplified by PCR, cloned in pTZ57R/T and the recombinant vector was utilized for the transformation of E. coli DH5 α cells. The expression of phytase, cellulase and xylanase genes were analyzed in E. coli BL21 cells using pET 21a as expression vector. Recombinant proteins were purified through different chromatographic techniques and their molecular masses were determined through SDS-PAGE and gel filtration methods. The recombinant proteins were characterized and these locally produced recombinant enzymes were utilized for supplementation of poultry feed to examine their effect on the growth of poultry birds. The supplementation of poultry feed with locally produced enzymes showed significant growth enhancing effect on poultry birds and improved the feed uptake and feed conversion ratio.

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Effect of *Moringa oleifera* leaves against phenyl hydrazine hydrochloride induced anemia in Albino rats

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ABSTRACT

There are many medicinal plants in traditional medicine which are used to prevent, control, and treat iron deficiency problems especially anemia. *Moringa oleifera* is known for its therapeutic effects as it has been long used in treatment of many diseases. *Moringa oleifera* leaves extract have gained much importance due to presence of antioxidant compounds e.g potassium, calcium, phosphorous, iron, vitamin C, vitamin A, vitamin E, flavonoids and many other compounds. Current study was actually designed to analyze the iron status and effect of *Moringa oleifera* leaves against anemia. In this study, 30 rats were used. Phenyl hydrazine hydrochloride for 2 days at 40mg/kg was used to induce anemia in rats. The rats were divided into six groups, including normal control, negative control, positive control and three other groups receiving the *M. oleifera* extract at 250, 500 and 650 mg/kg concentrations. At the end of treatment, the animals of all groups were weight and then sacrificed. The blood samples were drawn immediately to analyze the hematological and biochemical parameters. All groups of *M. oleifera* (especially G-6 given 650mg/kg doze) significantly ($p \leq 0.05$) normalizes the concentrations of iron, ferritin, transferrin, ALP, AST, ALT, bilirubin and improved the levels of WBC, lymphocyte, monocytes, platelets, haematocrit, RBC, Hb, etc. as compared to the untreated group. In conclusion, ethanolic extract of *M. oleifera* leaves have anti-anemic potential as it improves haematological indices and iron deficiency thus, it can be used as a nutritional supplement and therapeutic agent leading to normalizing body functions.

Key Words: Iron deficiency, *Moringa oleifera*, haematological parameters, ferritin, anemia



O-100/ICAZ-2020

WNT10A mutation associated with a complete Odonto-Onycho-Dermal Dysplasia syndrome

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ABSTRACT

WNT signalling is one of a few pathways that are crucial for controlling genetic programs during embryonic development as well as in adult tissues. WNT10A is expressed in the skin and epidermis and it has shown to be critical for the development of ectodermal appendages. A nonsense mutation in WNT10A was recently identified in odonto-onycho-dermal dysplasia (OODD; MIM 257980), a rare syndrome characterised by severe hypodontia, nail dystrophy, smooth tongue, dry skin, keratoderma and hyperhydrosis of palms and soles. We identified a large consanguineous Pakistani pedigree comprising six individuals affected by a complete OODD syndrome. Autozygosity mapping using SNP array analysis showed that the affected individuals are homozygous for the WNT10A gene region. Subsequent mutation screening showed a homozygous c.392C>T transition in exon 3 of WNT10A, which predicts a p.A131V substitution in a conserved α -helix domain. We report here on the first inherited missense mutation in WNT10A with associated ectodermal features.

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Effects of Moringa oleifera leaf meal (MOLM) based diet on hematological indices in Cirrhinus mrigala fingerlings

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ABSTRACT

A 90-day feeding trial was carried out to investigate the effects of Moringa oleifera leaf meal (MOLM) on hematological indices in Cirrhinus mrigala fingerlings by replacing costly fish meal (FM) at the levels of 0%, 10%, 20%, 30%, 40% and 50%. Fingerlings (avg. wt. 6.35±0.04g) were randomly distributed into triplicate tanks and each tank contained 15 fingerlings which were fed at the rate of 5% of live wet weight. The hematological indices of fingerlings fed 10% MOLM based diet were found to be significantly different (p<0.05) from the fish fed control diet. From the current research work, it was indicated that the red blood cells (RBCs 2.50×10⁶mm⁻³), white blood cells (7.50 ×10³ mm⁻³), hemoglobin (8.47 g/100 ml) and mean corpuscular hemoglobin concentration (MCHC 33.94%) of fish

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showed a significant ($p < 0.05$) inverse correlation with increase in MOLM in diets whereas platelet (PLT 58.96) followed by 50.47 in the fish fed on control diet, packed cell volume (PCV 25.47 %), mean corpuscular hemoglobin (MCH 25.37 pg) and mean corpuscular volume (MCV 85.53 fl) in fish fed 10% MOLM diet which showed an increasing trend as MOLM increased in the diet. The current study showed that MOLM has good prospective for use as FM replacement in *C. mrigala* diet up to 10% level without hematological disturbance whereas the inclusion of MOLM above 20% in the diets showed the hematological disturbance. From the results it was suggested that FM can be replaced with MOLM up to 10% in the diets to increase the nutritive values of *C. mrigala* fingerlings.

Keywords: *C. mrigala*, Hematology, MOLM, FM, Replacement

O-102/ICAZ-2020

Effects of Protease Enzyme on Growth and Body Composition of *Cirrhinus mrigala*

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ABSTRACT

Aquaculture is the fastest food growing industry in the world and plays a significant role in the economical account of a country. Nowadays enzymes are use in the fish feed to get many benefits as they accelerate the chemical reactions and increase metabolic rate. An experimental trial was conducted to study the impact of protease enzyme on growth and body composition of *Cirrhinus mrigala*. Duration of trial was 90 days. Fingerlings of *Cirrhinus mrigala* were subjected to two treatment groups (PT1: Protease 0.1mg/kg, PT2: Protease 0.2mg/kg) and one control group. Prior to the experiment fish fingerlings were acclimatized for two weeks to control diet. Food was given at the rate of 4% of live net body weight. At the end of experiment, result showed that experimental group PT1 showed maximum growth. In the case of body composition, maximum deposition of fat and gross energy was found in PT1 while maximum value of crude protein and dry matter was found in PT2. These results showed that the protease supplementation in fish feed improved the growth performance and have significant effect on the body composition of *Cirrhinus mrigala*.

Key words: Protease enzyme, Growth, Body composition, *Cirrhinus mrigala*



Valorization of indigenous waste plant leaves into optimized biosynthesis of tannase and gallic acid by solid-state fermentation: A Cost-effective approach

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ABSTRACT

This research was conducted to evaluate the potential of various indigenous plant leaves for biosynthesis of value-added products including tannase and gallic acid (GA) by *Aspergillus oryzae* in solid-state fermentation. Tannic acid-rich indigenous plant leaves of guava, black plum, Eucalyptus, Banyan, lemon, Sacred fig, Orange and Mango were separately used to explore their potential as substrates for the production of tannase and GA. Among all substrates assessed, black plum leaves produced the most promising effect resulting in a significantly ($p < 0.05$) higher yield of tannase (138.34U/g) and GA (0.565mg/g). Various influential physicochemical parameters were optimized to further improve the production of both compounds using the black plum leaves as optimal substrate. Results revealed that black plum leaves at a substrate water ratio of 1:2, pH 5.5, and temperature 30 °C yielded the best production after 72 h of incubation period. Supplementation of glucose and $(\text{NH}_4)_2\text{SO}_4$ to basal media increased the products yield to tannase (179.95U/g) and GA (0.986mg/g). Furthermore, the GA produced was extracted by soxhlet apparatus and identified by FTIR. The purity of GA produced was 98.5% as quantitatively analyzed by HPLC. The presented data is the first report on the consumption of various local plant leaves to synthesize tannase and GA. Optimization and scaling up of key fermentation parameters exhibit a potential to biosynthesize in high levels to meet their increasing industrial demands.

Keywords: Gallic acid; Tannase; Black plum leaves; *Aspergillus oryzae*; Solid state fermentation; Process optimization



O-108/ICAZ-2020

Genetic variations among different sub species of *laudakia pakistanica* from different localities of pakistan

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ABSTRACT

Agamidae is morphologically and ecologically a diverse saurid family consisting of more than 330 contemporary species in 54 genera which is the fourth largest lizard family and is widely distributed throughout various regions. The genus *Laudakia* comprises about 20 species, mainly occurring in highland and mountainous regions of the central and southern Asia. The aim of the study is to identify *L. pakistanica* from northern areas of Pakistan as well as its genetic variations among the sub species of *L. pakistanica*. A total of 120 blood samples *L. pakistanica* were collected from Chitral, Gilgit, Besham, Hunza, Skardu, and district Swat. The samples were subjected to DNA extraction followed by PCR with proper designed protocols. After confirmation through 1.5% gel electrophoresis the PCR amplified products were subjected to sequencing. The sequenced data was analyzed through BioEdit, Mega 7 and Clustal W showing that the *L. pakistanica* and the phylogenetic tree was constructed through neighbor joining method showing that most of the *laudakia* species were slightly similar to the neighboring countries, Afghanistan, Bangladaish. From the current study it was concluded that the sub species of *Laudakia pakistanica khani*, was found in Kohistan, district Swat, *Laudakia pakistanica auffenbergi* was found in Gilgit, Besham and Swat while the *Laudakia pakistanica pakistanica* were found in Gilgit (Jaglot) only. The current study may be followed for other *laudakia* sub species in different hilly areas of Pakistan especially the Indus built.

Keywords: *L. pakistanica*, DNA, PCR, *Laudakia pakistanica pakistanic*, *Laudakia pakistanica khani*, *Laudakia pakistanica auffenbergi*

O-113/ICAZ-2020

Parasitic threat to houbara bustard: an endangered bird species in pakistan

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ABSTRACT

Houbara Bustard (*Chlamydotis macqueenii*) is one of the endangered and old species of birds present in Asia. Although they can survive almost all over the world in desert areas. These bird species can be differentiated by their geographical, behavioral, and hereditary characteristics. These birds tend to migrate in winter season for a period of 6 months in plain desert areas of Pakistan including: Sindh, Southern Punjab, Baluchistan and down to South Arabian Peninsula. But, due to hunting from Royal Arabs it stopped migrating beyond Pakistan. In Pakistan, the killing of Houbara bustard is banned. However, when Royal Arabs came to Pakistan, they are being allowed to hunt the bird. The number of Houbara bustard migrates to Pakistan are estimated about 4,746. Apart from the fact, this species comes under the category, which is going to extinct, this species is under the threat of many disease-causing organisms including parasites e.g. chewing louse (Phthiraptera: Philopteridae) and louse fly (Diptera: Hippoboscidae), ticks (*Hylomma* spp.) and mites etc. which transmits various diseases like Paramyxovirus type 1 (PMV-1), Newcastle disease, Mycobacteriosis and neoplasia etc. while parasitic diseases include endoparasites *Fasciola*, *Ascaris*, *Ascaridia*, *capallaria* Spp., *Hartertia rotundata*, *Allodapa* spp, *Otiditaenia conoideis*, *Hispaniolepis falsata*, *Centrorhynchus lancea* and *Mediorhynchus taeniatus*. Keeping in view the parasitic threat to Houbara bustard, the investigation of intestinal parasitic fauna of Houbara bustard is direly needed. The identification of parasitic fauna will help in making drug of choice to control gastrointestinal parasitism in migratory Houbara bustard species in Pakistan.

Keywords: Epidemiology, Houbara bustard, Endangered species, Parasitism, migratory birds

O-114/ICAZ-2020

Blood parasites of owned dogs: an insight into potential diagnostic methods to identify the threat

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ABSTRACT

Dogs are prone to many parasitic infections particularly with those of blood parasites. Among significant parasitic infections blood parasites are most lethal for dog population. Severity of the infection varies from subclinical infection to death of animal. They flourish in blood and the major reason of erythrocyte destruction. Blood parasites for example *Babesia*, *Theileria*, *Trypanosoma*, *Dirofilaria* and

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Hepatozoon canis are known to infect dogs. Distribution of dog blood parasites varies from region to region across the world. Globally, 40.1-57.3% dog population is declared positive for different blood parasites. Studies have been conducted in Faisalabad, Punjab on morphological basis also notified high risk of blood parasites in dogs. They are transmitted by different arthropod vectors. Control of blood parasites is a challenge that requires measures focused on arthropods vectors and environment based on the epidemiology of these parasites in the given area. Because parasite depends upon vector for its life. There is a dire need to utilize molecular and serological based methods like PCR, ELISA, Loop-mediated amplification, Nested PCR, Reverse line blot hybridization, Quantitative fluorescent resonance energy transfer-PCR to identify infectious microorganisms (such as piroplasm) which are unable to be identified with classical method. So that the control strategies can be made with relevant to epidemiology of blood parasite and to control their arthropod vector.

Key words: Blood parasites, Molecular based assays, Arthropods vectors

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***Macrolepiota procera*: Dietary mushroom as a potent therapeutic agent**

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ABSTRACT

Macrolepiota procera common parasol mushroom of family “Agaricaceae”. This is well known edible mushroom cultivated worldwide but mostly cultured and widespread in Turkey, Europe and north western America. This has high proteins, vitamins, amino acids, less fat contents & other nutritional compounds. So, mushrooms are very popular as oriental food stuff in Europe, America and other western countries. Besides all these nutrients this mushrooms also have other compounds such as Anti inflammatory, antioxidant, immunomodulatory compounds, antiangiogenic, CDK’s inhibitors, topoisomerases and apoptosis inducers. Due to having this versatility of compounds these mushrooms could be used for medicinal purposes. It is investigated that mushrooms have antimicrobial, antioxidant and anticancer potential. That’s why this was first study to investigate antimicrobial, antioxidant and cytotoxic potential of *Macrolepiota procera* against lung cancer cell line A549 cells. Antimicrobial activity was measured through well diffusion method and DPPH scavenging assay was performed to determine antioxidant potential.



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MTT assay was established to check the cytotoxic effect of both mushroom extracts. Presence of Tannins, steroids, alkaloids, saponins and flavonoid were confirmed by phytochemical assays. In antimicrobial activity *M. procera* extract exhibited significant results for each bacterial and fungal strain with MIC value $\leq 187.5 \mu\text{g/ml}$. This mushroom also exhibited significant free radicle scavenging activity in with $\text{IC}_{50} 214\mu\text{g/ml}$. Cytotoxicity of this mushrooms also gave significant results against A549 cell line with $\text{IC}_{50} 6.18\mu\text{g/ml}$. Overall results showed suggest the use of these mushrooms and their derivatives as novel natural drugs for bacterial, fungal related diseases and also could be used against lung cancer.

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Growth evaluation of *Acanthopagrus latus* (Dandya) found in Indus River, Sindh-Pakistan

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ABSTRACT

Acanthopagrus latus commonly known as Yellowfin Seabream (Dandya) is one of important specie in fisheries and aquaculture. *Acanthopagrus latus* occurs in shallow coastal waters and enters river mouths and estuaries. While, there is a great effect of environment (estuarine and marine) on the growth of any individual. In present study a total 103 specimens collected from Indus River Thatta, Sindh Pakistan to evaluate the growth. An average length and weight of *Acanthopagrus latus* was recorded 15.67 cm and 75.64 gm. respectively. The correlation of length and weight was found in the range $r=0.939$. The length weight relationship and condition factor was calculated for the population collected from Indus River. The Length weight relationship was estimated by $W=aL^b$. Where $W=75.642\text{gm}$, $L=15.667$, $a=0.318291$ and while b (exponent) was obtained $b=2.519$. The condition factor were calculated by leCren (1951) $K_n = 1.937$ and Fulton's $K=1.967$. The maximum and minimum weight was recorded about maximum weight $243.4(\pm) \text{ gm}$. and minimum weight $47.4(\pm) \text{ gm}$. with an average weight of 75.642gm respectively. While the maximum and minimum length was recorded maximum length $28(\pm) \text{ cm}$ and minimum length $13.2(\pm) \text{ cm}$ with an average length 15.667cm respectively. Length weight relationship helps in estimation of the growth. Present estimation suggests that the *Acanthopagrus latus* is the suitable candidate of aquaculture in freshwater system.

Keyword: Migratory, *Acanthopagrus Latus*, Morphometric, length weight condition factor Indus River.

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Importance of heat shock proteins in livestock sector

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ABSTRACT

Heat stress is one of the major issues encountered by the livestock sector, particularly for dairy animals. Its negative effects includes reduction in feed intake, quality of milk, milk production and fertility. Other physical effects includes increased respiratory rates, heart rates, peripheral blood flow, panting activity and sweating, etc. With the increasing global warming such concerns have risen even more in recent years and dairy industries are struggling to maximize milk production. Heat shock proteins (HSPs) are produced by the cells under stressful conditions. First, they were reported concerning the heat shock but later also found to be linked with cold shock and other stresses like UV light, tissue healing, wound remodeling, etc. These proteins are also produced during normal cell functions like growth and differentiation. Many members of this family are known as molecular chaperons because of stabilizing, correcting and refolding damaged proteins. Characterization of thermotolerant genes has been performed in dairy and beef cattle which shown that it is more of a quantitative trait than qualitative. The target genes which are important for thermoregulation are present at genomic regions under influence of this trait. In mammals heat shock response at the cellular level is mediated by heat shock factors shortly referred to as HSFs at the transcriptional level. HSP70 family is highly conserved among all other HSPs and HSP70 gene has been characterized in different livestock species i.e. buffalo, cattle and goat that contains 1,926 base pairs in open reading frame. Under heat stress, cells lost their functions like replication of DNA, transcription, translation, and formation of new polypeptides. Heat shock proteins are triggered in this condition and save the cell from all these damaging effects. Heat shock genes can be used as a potential biomarkers for the selection of thermotolerant animals.

Keywords: Heat shock proteins, Livestock, Heat stress, Thermotolerance

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The role of mammalian target of rapamycin (mTOR) in Aging

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ABSTRACT

Aging is a physiological / pathological phenomenon marked by a steady degradation in neuronal functions, supported by modifications in many molecular mechanisms, culminating in an enhanced sensitivity of the cell to damage. Popular denominators of aging in diverse species are multiple cellular pathways, including genomic instability, telomere erosion, epigenetic changes, proteostasis depletion, deregulated nutrient-sensing, mitochondrial dysfunction, stem cell fatigue and altered intercellular signal transduction. The mammalian target of rapamycin (mTOR) is an evolutionarily maintained protein kinase sensing nutrient that controls growth and metabolism in all eukaryotic cells. Studies in flies, worms, yeast and mice support the theory that in modulating aging, the mTOR signaling network plays a pivotal role. As the most robust mediator of the preventive effects of diverse types of dietary restriction, mTOR has arisen, which has been shown to prolong lifespan and delay the onset of age-related diseases across species. The use of mTOR inhibitors in healthy people is currently precluded by the presence of side effects, so these can be commonly used to delay ageing and mitigate age-related diseases. At the same time, though, playing with mTOR, a key switch in cellular metabolism, may be useful and risky. While mTOR inhibitors have been used for more than ten years in the clinical setting, we have not yet learned how to take advantage of their beneficial acts without paying an unnecessary fee for their side effects. Ultimately, their clinical use may shed light on some important biological issues regarding the relationship between metabolisms, immune response, and aging.

Key Words: Aging, Immune response, Inhibitors, Metabolism, Rapamycin

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Cryopreservation and quality assessment of semen in Kajli, Lohi and Thalli sheep

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ABSTRACT

Fresh and frozen semen quality parameters were compared in Lohi, Kajli and Thalli rams. A visual macroscopic evaluation was done for color, volume and consistency of the ejaculates, and a microscopic evaluation was done to measure mass activity, motility percentage of spermatozoa for individual motility, motility after dilution and post thaw motility under the microscope. Concentration of spermatozoa in each ejaculate was measured by spectrophotometer. A General Linear Model was used to analyze data in MiniTab_17 software. Results showed the color of semen in all rams was creamy white, Mean volume of Lohi, Kajli and Thalli rams was 0.98 ± 0.12 ml, 1.53 ± 0.11 ml, and 0.97 ± 0.12 ml, respectively. Spermatozoa average concentration per ml of the semen for Lohi, Kajli and Thalli rams were 2047.00 ± 116 million, 1704.80 ± 66.3 million and 1700.10 ± 74.8 million, respectively. Mass activity average score for Lohi, Kajli and Thalli rams were 3.00 ± 0.16 , 2.69 ± 0.10 and 2.81 ± 0.09 , respectively. Similarly, average percentage of individual motility $79.06 \pm 0.50\%$, $79.06 \pm 0.68\%$ and $80.00 \pm 0.00\%$ for Lohi, Kajli and Thalli rams, respectively. Average post thaw motility of spermatozoa was $50.00 \pm 2.67\%$, $53.13 \pm 3.13\%$ and $49.38 \pm 3.33\%$ for Lohi, Kajli and Thalli rams, respectively. The semen of Kajli, Lohi and Thalli rams is successfully cryopreserved and used for artificial insemination to pregnant the ewes.

Key words: Semen, Ejaculates, Spermatozoa, Concentration, Motility

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Antibacterial Potential of Silk Extracted from Genus Argiope (Araneae: Araneidae).

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ABSTRACT

Both the human health and economy are being negatively and continuously affected by bacterial diseases. Many drugs are available against harmful bacteria but these microbes are developing resistance against applied chemicals. So, new antibiotics with environment friendly nature must be available persistently. Living things are more suitable to get these products than synthetic ones. In the present work, silk recovered from spiders belonging to genus *Argiope* was checked for its antibacterial activity, if any. Two types of silk, 'Egg silk' and 'Web silk' were collected from these spiders and then dissolved in six different solvents i.e. urea solution, ethanol, acetone, methanol, distilled water and 5% NaOH. 3.5% solutions of both types of silk were formed separately in each described solvent and analyzed for their antibacterial activities against four different bacterial species i.e. *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Klebsiella pneumoniae* (gram negative). Further, 1.5%, 1% and 0.5% dilutions of web silk in 5% NaOH were also analyzed against mentioned bacterial species because the silk dissolved more in this solvent physically. Results were analyzed in terms of inhibition zone's diameters on bacterial culture plates by following disk diffusion method. Statistical analysis showed none of these bacterial species were actively inhibited by any of the applied treatment. However, the inhibition zones of positive control were much larger than negative control and treatments. Treated groups also showed zones of inhibition but these were due to the effect of solvents instead of silk. So, the silk extracted from genus *Argiope* has no antibacterial effect against any of the tested bacterial species.

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Antibiogram and biofilm formation potential of pseudomonas aeruginosa isolated from hospital environment

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that commonly colonizes human tissues and is involved in biofilm formation. This biofilm formation contributed to the nosocomial infections in animals and humans, which include dermatitis, soft tissue infection, cystic fibrosis, mastitis, and gastrointestinal infections, etc. This study was designed to isolate the *P. aeruginosa* and to determine its biofilm formation potential and antibiotic susceptibility. For the detection of *P. aeruginosa* environmental swab samples (catheter, sink, tap water, floor, door or cafeteria) were collected from Allied hospital Faisalabad. The Samples were inoculated on MacConkey agar. For the confirmation of *P. aeruginosa* Gram staining and biochemical tests were performed. The Antibiotic susceptibility test was done through the Disc diffusion method. most of the isolates of *P. aeruginosa* exhibited multidrug resistance. The highest resistance was shown to Tetracycline, Piperacillin or Gentamycin. Some isolates were found to be susceptible to Ciprofloxacin. Crystal violet microtiter plate assay was carried out for the determination of biofilm formation potential. Using OD value, the biofilm quantitative analysis of *P. aeruginosa* was done by spectrophotometry. Spectrophotometric measurement of OD of adherent cells enabled us to classify our isolates collection into three categories; weakly (OD 0.062-0.124), moderately (OD 0.248) and strongly (OD >0.248). 15 isolates studied, in which 9 were showed result as a strong, 4 were as moderate and 2 as weak adherent.

The result of this study indicated that Biofilm forming strains showed high ability of these strains to persist in hospital environment which increases the risk of disease development in hospitalized patients.



O-135/ICAZ-2020

Assessment of Synergistic antibacterial activity of ten indigenous plants against locally isolated MRSA

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ABSTRACT

Staphylococcus aureus responsible for many serious hospitals-acquired bacteremia and is a major cause of high morbidity and mortality rates around the world. Methicillin-resistant Staphylococcus aureus (MRSA) is a gram positive bacteria that cause deadly infections through open wounds and invasive devices. These are resistant to many antibiotics and a cause of concern for

pharmaceuticals. Plants are a rich source of phytochemicals and have been used to treat different infections as a part of traditional medicine. In this study, ten indigenous plants were selected and their extracts were screened for antibacterial activity. Synergistic effect of these plant extracts was assessed using the agar plate well diffusion assay. Antibacterial effect was observed against all tested MRSA isolates. The largest zone of inhibition measured 22mm against an individual concentration of 10 µg/µl of each extract. The cost effectiveness, ease of accessibility, lesser adverse effects and environment friendly nature of plant derived antimicrobials, render it necessary to explore the targets of plant antimicrobials and study them at the molecular level. Synergistic effect of plant extracts and synthetic antibiotics may provide fruitful results that are ultimately beneficial to the community. Furthermore, the antibacterial extracts can also be applied as preservatives in processed foods by preventing microbial growth and prolonging product shelf life. So, these plants could replace the use of potentially carcinogenic chemical-based food preservatives. The antioxidant and antibacterial potential of these plants also helps them to meet with and overcome the food security issue by preserving food naturally, raising the nutritive value of the food products and up surging the availability of the food.

Further studies can be based on isolating the active phytochemicals/components from each extract exhibiting antibacterial activity which may be further processed to antibiotics used in treating resistant bacterial strains.



Effects of atrazine on survival, behaviour and biochemical aspects of silver carp (*Hypophthalmichthys molitrix*)

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ABSTRACT

The current research was conducted to evaluate the acute toxicity of the widely used herbicide Atrazine on the biochemical and behavioral responses in brain, gills, liver and muscle tissue of fresh water fish Silver carp (*Hypophthalmichthys molitrix*). Silver carp was exposed to different concentrations of Atrazine for different time periods. High mortality rate was observed with increase concentration of Atrazine and time of exposure. Estimated 96 hours LC₅₀ of Atrazine was found to be 13.7µl/L at 25 °C. After further exposure of Silver Carp to LC₅₀ of Atrazine at different time points, the

fish showed fast swimming, loss of equilibrium and balance, gulping, pale body color and excess amount of mucous particularly that covered the buccal cavity and gills. Total Protein Contents, Lipid Peroxidation, Antioxidant enzyme Catalase (CAT), Peroxidase (POD) and Glutathione Reductase (GR) were measured in the brain, gills, liver and muscle tissues at different time points. Atrazine exposure resulted in a significantly time dependent decrease in total protein content in liver, gills, brain and muscle tissue of Silver carp compared to the control group. Slight increase was observed in the activities of antioxidant enzyme like Catalase (CAT) and Peroxidase (POD) in the control group, whereas slight decrease was observed in the ATZ-treated groups from 24 to 96 hours. The Glutathione reductase (GR) activity was significantly increased in the ATZ treated groups while GR activity was slightly increased in the control group. Likely, Lipid Peroxidation level was also increased in liver, gills, brain and muscle tissue with both the time and concentration while its level was decreased in control group. The obtained results reveal that Atrazine is toxic to Silver carp acts as enzyme inhibitor and alters total protein and lipid peroxidation level in Silver carp. Therefore, the extensive and indiscriminate use of Atrazine can harm and reduce the Silver carp population in the natural water bodies.

Key words: Atrazine; Sliver Carp; Toxicity; LC₅₀; Biochemical Analysis; Total Protein.

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Antibiotic susceptibility of bacteria causing subclinical mastitis in camel population of southern khyber pakhtunkhwa

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ABSTRACT

Mastitis is an inflammatory disease of the mammary glands of lactating animals, including camels causing decrease in the milk production and leading to huge economic losses. *Staphylococcus aureus* is the most common causative agent of mastitis worldwide.

Objective: The aim of the study was to determine the antibiotic sensitivity profile of bacteria causing mastitis in camels of the Southern Khyber Pakhtunkhwa.

Materials and Methods: A total of 87 milk samples were collected from camel population in three districts (Lakki Marwat, Dera Ismail Khan, and Tank) of Khyber Pakhtunkhwa, Pakistan. The camel population was screened for sub-clinical mastitis using Surf Field Mastitis Test (SFMT). The bacteria were isolated by multiple streaking and characterized biochemically using coagulase and catalase tests. Disk diffusion method was used to check the antibiotic susceptibility of *S. aureus*.

Results: Overall 32.18% (28/87) of Multi Drug Resistance (MDR) *S. aureus* were reported from sub-clinical mastitis milk samples. Non-significantly higher prevalence of MDR was noted in district D. I. Khan (38.23%) compared to district Lakki Marwat (29.03%) and district Tank (27.27%). Oxacillin, Cefoxitin and Ampicillin faced 90% resistance while Vancomycin, Cefixime and Cefotaxime had >72% of resistance from MDR isolates of the study area.

Conclusions & Recommendations: The study concluded higher prevalence of MDR *S. aureus* in camel milk and also reported some good response of the antibiotics tested against *S. aureus*.

Keywords: Mastitis, Camel, *S. aureus*, Khyber Pakhtunkhwa.

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Phylogeny of *Laudakia tuberculata* Reptilia: Agamidae from Northern Pakistan

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ABSTRACT

The genus *Laudakia* comprises about 20 species, mainly occurring in highland and mountainous regions of the central and southern Asia. Agamid lizard *Laudakia tuberculata* (*L. tuberculata*), commonly known as Kashmir Rock Agama, is distributed mainly in the North Pakistan, Nepal which is known for its beautiful coloration, adaptability and instincts with its foraging activity. This species inhabits holes, crevices, and rocky structures near water streams and around human habitations and has been observed to hibernate in the crevices during winter. In the current study a total of 115 blood samples of *L. tuberculata* were collected from Azad Jammu and Kashmir, Chitral and Kumrat, and were brought to the laboratory of molecular parasitology and virology, department of Zoology, Kohat University of Science and Technology, Kohat for further process. The DNA was extracted through Qiagen DNA extraction kit with prescribed protocol. The extracted DNA was subjected to PCR using ND4 gene specific primers followed by gel electrophoresis. The PCR amplified products were sequenced through genetic Analyzer. The sequenced data was analyzed by Mega 7 and the phylogenetic tree was made through neighbor joining method. Most of the samples were similar to the previous published sequences of *L. tuberculata* and *Laudakia papenfussi* species of China. From the current study it was concluded that *L. tuberculata* is present in northern areas of Pakistan. The current study may helpful for the molecular identification herpetofauna of Pakistan.

Keywords: *L. tuberculata*, herpetofauna, PCR, ND4 gene

O-146/ICAZ-2020

ND2 gene based DNA barcode for genetic characterization of Pakistani Pigeons

Dr. Sehrish Firyal

ABSTRACT

Being an integral part of an ecosystem, avian species are one of the populous forms of life on the earth. Incredible number of birds species revealed amazing evolutionary pattern and adaptations. Pakistan

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has its unique and diversified avian fauna. Among them the Pigeons are most common usage birds belonging to family Columbidae. Traditionally, species of pigeons have been identified on the bases on their phenotypic characteristics but is less authentic than genetic approaches. Genetic approaches are more reliable for species identification. Among them mitogenome is one of the most suitable marker for species identification. In the present study total of 20 birds were selected belonging to four species of Pakistani pigeon. Mitochondrial genome was extracted followed by ND2 gene sequencing. Bioinformatics analysis revealed five unique polymorphisms in ND2 gene. These SNPs can serve as reliable molecular marker for pigeon breeds identification. This work can provide the genetic data for better pigeon conservational activities in future. SNP based identified barcode can assist the avian fanciers to authentically identify the pigeon breeds. This would also solve the forensics issues regarding blood and meat identification of pigeon breeds.

O-149/ICAZ-2020

In-vitro antileishmanial activity of Pakistani Bee propolis Extracts

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ABSTRACT

The current chemotherapeutic agents for leishmaniasis are expensive, toxic and with frequent inefficiencies. The discovery of new active compounds with antileishmanial potential is essential for development of new approaches for leishmaniasis therapy. Propolis is a natural substance produced by honey bee from the exudates of different plants. The aim of this work was to evaluate the antileishmanial activity of methanolic and ethanolic extracts of Pakistani bee propolis.

Methodology: Propolis sample collected from district Kohat and dried in the dark until processing. The dried propolis was dissolved in ethanol and methanol solvent. The extract was concentrated by evaporating the solvent with the aid of rotary vacuum evaporator to obtain the final extract. A serial dilution of extracts and control compound in dimethyl sulfoxide (DMSO) was prepared. Stationary phase promastigotes of *L. tropica* were incubated in methanolic and ethanolic extracts in vitro. Amphotericin

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B was used as a positive control. After 48 hours the antileishmanial activity of extracts was measured, using XTT assay. Experiments were performed in triplicate.

Finding: The IC₅₀ values were 198.8 µg/ml and 134.7 µg/ml for methanolic and ethanolic extractions, respectively. The IC₅₀ value for Amphotericin B was 2.52 µg/ml. Although there was little difference but ethanolic extract of propolis showed more activity as compared to methanolic extract. Both the extracts showed less antileishmanial potential compared to amphotericin B.

Keywords: Propolis, DMSO, leishmaniasis, in vitro, XTT.

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Insecticidal effects of Neem leaf Extract on control of Khapra beetle (*Trogoderma granarium*), a stored grain pest

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ABSTRACT

Infestation by different insect pest to stored cereals and their products storage is a serious problem. Khapra beetle (*Trogoderma granarium*) is one of the most common devastating insect pests of stored grain products. It causes both the nutritional and economical losses to stored cereals. This present study work were conducted to evaluate the toxicity of acetonic extracts of Neem plant against the *Trogoderma granarium*. For neem leaf extract, dose rates were 5%, 10%, 15%, 20%, 25% and 30% and leaves were collected from University of Agriculture Faisalabad, Pakistan. The acetonic extracts from neem leaves were prepared that showed remarkable toxicity and 30% dose rate caused significantly higher mortality rate in in Khapra beetle. The toxic effect was found to be dependent on dose and duration. Summarising, these plant extracts could have likely practical utilizations in protection of stored grains products against the attack by *Trogoderma granarium*.

Keywords: Khapra beetle; pest; stored grain; neem leaf extract

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Screening of natural products against human triple negative breast cancer cells

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ABSTRACT

Natural products act as the endless reservoirs of chemical scaffolds which come up as the eminent bioactive compounds against a number of diseases. Triple negative breast cancer (TNBC) stands out as a highly aggressive as well as resistant type of cancer due to the absence of therapeutic targets. Here, we have screened a plant extract library (comprising of 50 plant extracts) and natural compounds library (comprising of 100 natural compounds) against human TNBC (MDA-MB231) cells, to check the anticancer potential of aforementioned natural products. The results of the study indicated that plant extracts: *Mangifera indica* (seeds) *Mangifera indica* (bark), *Eucalyptus camaldulensis* (bark), *Aloe barbadensis* (whole plant) and *Caltropis procera* (leaves) showed significant inhibitory potential against human TNBC while the natural compounds: eriocalyxin B, resistomycin, brevicarine and emetine showed remarkable antibreast cancer activity. Two natural compounds, eriocalyxin B and resistomycin were found to selectively target MDA-MB231 cells, hence, providing the potential drug candidates for developing novel therapeutics against human TNBC.

O-158/ICAZ-2020

Macrophage targeting for the treatment of cutaneous leishmaniasis; Using novel technology and drug delivery approach

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ABSTRACT

The purpose of this study was to develop novel carbopol-based miltefosine-loaded transfersomal gel (HePCTG) for efficient targeting of the leishmania infected macrophages in order to treat cutaneous leishmaniasis (CL). Miltefosine loaded transfersome (HePCT) were prepared by ethanol injection method followed by their incorporation into carbopol gel to develop HePCTG. Physicochemical characterization like mean particle size, polydispersity index (PDI), zeta potential, TEM, deformability index (DI) and elasticity of HePCT was investigated. Similarly, viscosity, pH, homogeneity, spreadability and extrudability of HePCTG was evaluated. Moreover, in-vitro evaluation of HePCT including release, permeation, skin irritation and antileishmanial activity against infected macrophage model were also performed. The optimized HePCT had a particle size of 168 nm with 92% entrapment efficiency (EE). HePCTG showed suitable viscosity, pH and sustained release of the incorporated drug. Further, both the HePCT and HePCTG demonstrated higher permeation than drug solution. The results of macrophage uptake study indicated the improved drug intake by passive diffusion. Low IC₅₀ value, selectivity index and high CC₅₀ value of the prepared formulation as compared to that of HePC solution demonstrated the improved anti-leishmanial efficacy and non-toxicity of the formulation. Thus, HePCTG can be an effectively used for the treatment of tropical diseases most particularly for CL.

Key Words: Cutaneous leishmaniasis, transfersomes, macrophages, IC₅₀, deformability.

O-161/ICAZ-2020

Determination of antimicrobial activity of date palm pits against food spoilage bacteria

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ABSTRACT

Globally antibiotic resistance is one of the major phenomena. Food spoilage bacteria create resistance against antibiotics that's why more observation has been applied to extract the active compounds from plant species used in phytomedicine. Date Palm (*Phoenix dactylifera*) considered as a part of Arabian diet as a low cost of food that is cultivated for eatable sweet fruit and the date pits having phenolic compounds such as phenolic acids, flavonoids depending on these compounds it has been

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Observed that it has effective impacts on human health like antioxidant activities and anti-inflammatory activities. Antimicrobial activity of date fruit pit extract was determined against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *salmonella*, major food spoilage organisms. The current work was conducted to estimate the consequence of date palm pits as antimicrobial agents against food spoilage bacteria. Antimicrobial activities of the ethanolic extract was determined by agar disc diffusion method. Zone of inhibition of the seed extract was 20.4 mm against *Pseudomonas aeruginosa* and 15mm against *E.coli*. As same the zone of inhibition against *staphylococcus aureus* and *salmonella* was 9.5 mm and 11.5 mm respectively. Minimum Inhibitory Concentration (MIC) and Maximum Bactericidal Concentration (MBC) were determined by broth micro dilution Method. Result showed that MIC and MBC value of pit extract against *E.coli* and *Pseudomonas* were higher than *Staph.aureus* and *Salmonella*. Date fruits were collected from the nursery of Horticultural department, University of Agriculture Faisalabad. Procured food spoilage bacteria were confirmed through gram staining and biochemical tests. Through ethanol solvent, date palm pits were extracted. Agar diffusion method was used to find out the antimicrobial activities of the ethanolic extraction of date palm pits. Results were analyzed statistically. It was found that pit extract of date fruit was most effective against *Escherichia coli* and *Pseudomonas* and less effective against *staphylococcus aureus* and *salmonella*.

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Evaluation of Rice Husk as a Substrate for the Growth of *Lactobacillus* Species

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ABSTRACT

Rice (*Oryza sativa*) is the most produced and consumed cereal. Rice husk is composed of 11% hemicellulose with xylan being the main sugar subunit. Xylan is the most abundant carbohydrate in lignocellulosic biomass which is the precursor of Xylooligosaccharides (XOS), used as prebiotics. XOS as an ideal prebiotic suitable for diabetic patient, antioxidant, drug adjuvant, cytoprotective and dietary supplement. Toxic products and activity of pathogenic bacteria are decreased by xylooligosaccharides which enables the growth of probiotic bacteria. In the present study, the by-products of rice were used as a substrate for the growth of *Lactobacillus* species. Two step auto-hydrolysis method was used for using rice husk as a renewable resource of prebiotic. The liquor contained xylooligosaccharides which was refined by Soxhlet condensation process. The non-digestible oligosaccharides XOS was predominant in acid extract when sulfuric acid was 2% (w/w), temperature and time was 100°C and 0.5 hours respectively. Prebiotic efficacy of XOS was checked with *Lactobacillus* strains grown in MRS broth for in-vitro evaluation. The OD600 was super high with the liquor extract of rice husk instead of other sugar.

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These results illustrated that xylan from agriculture waste can produce XOS, which is an emerging prebiotic with a promising low-cost strategy of production.

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Evaluation of sugarcane bagasse as substrate for the growth of *Lactobacillus* species

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ABSTRACT

Probiotics are microorganisms, non-pathogenic in nature with beneficial outcomes to human health, when consumed in a suitable amount. Probiotics primarily derive from two genera i.e. *Lactobacillus* and *Bifidobacterium* LAB, these probiotics need prebiotics to grow, proliferate and stimulate their activity. Prebiotics are non-digestible ingredients for human consumption, different type of prebiotics are consumed by probiotics such as galactooligosaccharides, isomaltooligosaccharides, and xylooligosaccharides. Lignocellulosic biomass is the richest source of xylan a carbohydrate which is the precursor of xylooligosaccharides. Gastrointestinal disorders are prevented when xylooligosaccharides are used as prebiotic, providing health benefits to livestock and humans. The goal of This research was to consume by-products and utilize cheap resources for xylooligosaccharides (XOS) production such as sugarcane bagasse (SCB). The production of XOS was carried out utilizing different treatment which included mechanical treatment, then chemical treatment via alkali extraction followed by enzymatic hydrolysis of xylan-rich hemicelluloses. HPLC technique was used for quantification of XOS from hemicellulosic mixture. Then the efficacy of XOS was evaluated using *Lactobacillus* strains grown on MRS media then determined the growth by using a spectrophotometer.

The results of this study indicated that Sugarcane bagasse is a good source for producing xylooligosaccharides. The XOS was found to have good results on growth of *Lactobacillus* strains and can be utilized as prebiotic after their purification



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Population dynamics of pests and their natural enemies in sugarcane crop

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) considered an important commercial crop and provide major raw material for many other sugar products. The crop yield has been influenced due to several abiotic and biotic factors. Among these, arthropods play significant role in the sugarcane field ecosystem, regarding the crop yield and the quality of the products. In the recent years use of pesticides and moderns agronomics practices has significantly affect the insect pest population in the sugarcane crop. In the current study, population dynamics of pests and their natural enemies in sugarcane was assessed from the crop, planting till harvesting. The trial was conducted in the entomological research field area (in sugarcane variety SPF-245) near Young-wala, Department of Entomology, University of Agriculture, Faisalabad. The field was divided into different blocks and sub blocks using RCBD layout. The population dynamic was recorded twice a week by using random sampling method. From each block observations of insect pests was done from selected spots. From each spot five plants was selected for population observation. The collected specimens were brought into the laboratory for the identification. The results showed that the population of pests recorded was more than natural enemies. The pest population mainly on its peak during end of July to mid of September 2019 and the population of natural enemies was on peak during September to end of October. Similarly, the current study showed the attack of sugarcane borers on sugarcane variety SPF-245 was less than the other sugarcane varieties. The population of pyrilla was highest during 3rd week of September 868 specimens, 144 specimens of Acrididae during July to October, and population of beet webworm highest during 2nd to 3rd week of August. Similarly, natural enemies green lacewing 34 specimens was recorded and population peak during 3rd week of September to 2nd week of October, the lady bird beetle population on peak during end of October and total 127 specimens was noted. On average the pest population is high (302 Specimens) than natural enemies (107.6 specimens). The study will be useful for the future sustainable management of insect pests of sugarcane in changing environmental conditions.

Keywords: Population dynamics, Pests, Natural enemies, Sugarcane crop

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Identification and characterization of clostridium perfringens in the sheep and goats population of district abbotabad

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ABSTRACT

Clostridium perfringens (*C. perfringens*) is a rod-shaped, gram-positive, spore forming and anaerobic pathogenic bacterium that causes infections like food poisoning in human beings, gastrointestinal tract's (GIT) diseases and enterotoxaemia in domestic animals. *C. perfringens* replicates and produces efficient toxins that adversely affect their host. *C. perfringens* Toxinotype D causes enterotoxaemia also called as pulpy kidney disease in goats and sheep. In order to isolate and characterize *C. perfringens*, a total of 256 samples comprising of 121 (47.26%) fecal samples from infected goats and 135 (52.73%) fecal samples from infected sheep, each sample weighing 2 grams were obtained from eight separate topographic localities of Abbottabad. The total numbers of infected goat samples were 15 fecal samples of goats from Qalander Abad, 16 from Sajikot, 13 from Havelia, 11 from Amirabad, 13 from Sherwan, 19 from Sadiqabad, 18 from Dhamtour and 16 from Khutyala. While the total number of infected Sheep fecal samples were 15 from Qalander Abad, 16 from Sajikot, 14 from Havelia, 16 from Amirabad, 15 from Sherwan, 18 from Sadiqabad, 24 from Dhamtour, and 17 from Khutyala. In Thioglycollate broth the *C. perfringens* was grown. A total of 49 goat's samples and 53 sheep samples were identified through both Perfringens agar base and gram staining as positive samples for *C. perfringens*. While through Gelatine liquefaction test a total of 28 goat's samples and 41 sheep samples were positively identified for *C. perfringens*.

The PCR was performed to detect the alpha toxin gene of *C. perfringens*, showing 23.14% in goats and 30.38% in sheep resulting 53.52% as overall prevalence in district Abbottabad. From the current study it was concluded that the prevalence of *C. perfringens* toxinotype D is significantly high in goats and sheep in district Abbottabad. The present study will help in toxoid vaccines development through Animal Research Centers against *C. perfringens* strains.

Key words: Small ruminants; Clostridium perfringens; PCR; Enterotoxaemia; Alpha toxin; Type D

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Zoological gardens :a research gate for conservation of wildlife

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ABSTRACT

Zoos and zoological gardens are practicing major role in conservation measures of wildlife. As the extinction rate is at its peak in this century. It has become a need of the hour to make a reasonable effort for the conservation of natural ecosystem and wildlife diversity . Many governmental and nongovernmental organizations are accomplished for a great contribution to save our nature for future generations. There are many success stories regarding the reintroduction of many threatened specie into their natural habitat. As the rate of specie extinction is increasing drastically. And the zoological gardens and aquaria are becoming a mean to conserve the extinction rate. Conservation strategies applied in Zoos and aquarias are a mean to meet the extinction related problems. Research perspective of nature is also flourishing in these places. Research tools are enhanced for saving our nature in the direction of new challenges. Animal testing are playing prodigious role in medicine. Animals are broadly used in modern research. And the conservation strategies are vastly used in zoos and zoological gardens. Ex sito breeding methods are actually applicable in the journey of conserving the wildlife. It has a broad opportunistic value for the conservation methodology. The conservation standards are raising as new challenges are coming in meanwhile. So zoological gardens are really an essential part for modern studies of wildlife rehabilitation. Zoos are going to play a vital role to spread awareness among the people. As in modern zoos and aquarias majority of people visit and accomplish the awareness methods. People have a broad perspective about saving our natural ecosystem and biodiversity. In the zoos People go throw with their nature and their earth friends. People get a chance to understand their natural habitat and its basic factors. Zoos play their role significantly in the modern sciences. The research labs in these AZAs have vast research material to produce significant knowledge about the rehabilitation of threatened habitats and wildlife community. It is a need to have more research houses as like AZAs and other type of gardens so as to meet the real challenges of biodiversity loss and to rehabilitate our ecosystem for our natural biodiversity.

Keywords: Zoological Gardens ,Conservation, Diversity, Rehabilitation.

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Aluminium oxide nanoparticles inhibit EPS production, adhesion and biofilm formation in multidrug resistant *Acinetobacter baumannii*

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ABSTRACT

Acinetobacter baumannii is a biofilm forming multidrug resistant (MDR) pathogen that is responsible for urinary and respiratory tract infections. In the present study aluminium oxide nanoparticles (Al₂O₃ NPs) were chemically synthesized and characterized by Transmission electron microscopy (TEM) and Energy dispersive X-ray (EDX). The results revealed that synthesized nanoparticles were more or less spherical in shape with diameter < 10 nm. Broth microdilution assay indicated that minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for *A. baumannii* were 125 µg/mL and 1000 µg/mL respectively. Cell viability assay confirmed that no significant reduction in the percentage viability was observed after exposure of bacterial cells to sub-inhibitory concentrations of Al₂O₃ NPs. Exposure of bacterial cells to NPs caused cellular membrane disruption as indicated by an increase in leakage of cellular proteins, DNA and sugars. The percentage biofilm inhibition was found to be 11.64-70.2 %, whereas the attachment of bacterial cells to polystyrene surfaces was reduced to 48.8-51.9 % in the presence of Al₂O₃ NPs. Nanoparticles also significantly reduced the biomass of 24, 48, 72, 96 and 120 hours old biofilms. In the presence of NPs, a decrease in extracellular polymeric substance production was also observed. Our data revealed the non-toxic nature of Al₂O₃ NPs upto the concentrations of 120 µg/ml in HeLa cell lines. These results demonstrate an effective and safer use of Al₂O₃ NPs against multidrug resistant *A. baumannii* by targeting biofilm formation, adhesion and EPS production.

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Effects of phytase and citric acid supplementation on growth performance and nutrient digestibility of *Cyprinus carpio* fingerlings fed on canola meal based diet

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ABSTRACT

This research work was conducted to study the synergistic effects of CA and PHY enzyme supplementation on growth performance and nutrient digestibility of *C. carpio* fingerlings fed on canola meal (CM) based diet. Fish fed with sixteen graded levels of PHY supplementation from 0 and 750 FTU kg⁻¹ whereas CA at levels of 0% and 2.5%. Chormic oxide was added as an inert marker. Fifteen fingerlings were fed twice a day at the rate of 5% of their live wet weight. Results of current research work clearly illustrated that PHY along with CA enhanced growth performance and nutrient digestibility of *C. carpio* fingerlings fed on CM based diet. Maximum weight gain (267g) and the best FCR (1.32) was recorded when fish fed on T12 having 2.5% CA and 750 FTU kg⁻¹. Similarly nutrient digestibility (crude protein 75.06%, crude fat 83.31% and gross energy 67.45%) were also recorded at the same CM based diet. It was concluded that canola meal based diet supplemented with PHY 750 FTU kg⁻¹ and CA 2.5% improved the growth performance and nutrient digestibility of *C. carpio* fingerlings.

Keywords: *Cyprinus carpio*, growth, nutrient digestibility, canola meal, phytase, citric acid

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Evaluation of functions retrieval ameliorating effects of indigenous flora following an induced insult to a peripheral nerve

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ABSTRACT

Injury to peripheral nerves is emerging as one of the most serious health issues of this age and growing number of traffic accidents is thought as a chief cause of this frightening condition. Numerous other reasons such as congenital anomalies, trauma, infections, metabolic disorders, injuries, and chemical agents can also cause a mild to severe injury to the fibers of both PNS and CNS. These damages perturb

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motor, sensory, or both functions. An eternal disability or partial loss in the body functions is the eventual consequence in the majority of the cases. The foremost emphasis in such conditions is given to the sensory and motor functions restoration. The functional retrieval happens when injured axons regenerate and reinnervate but this rate of rehabilitation depends on timely and appropriate management. Pharmacological approaches are preferred to surgical procedures due to allied complexities. Moreover, these pharmacological agents are also impregnated with side effects therefore, the purpose of function refurbishment remains unsatisfied. In this age, natural molecules have gained ample consideration. Several plants have been found effective to accelerate the rate of nerve regeneration and improve nerve outcomes. Here, we evaluate the possible role of a well-known indigenous flora on the peripheral nerve injury. Based on the available pieces of information, it can be taken as a fact that plants parade antioxidant activity and plays an important role in scavenging free radicals. Here, we explore the regeneration and functions regain sponsoring effects of common plants in a rodent model of induced nerve insult. We assessed the restoration of motor and sensory functions by evaluating behavioral response, systemic biochemical markers, and hematological markers. We observed a boosted function with a shortened period to achieve the muscle functions in the treated group. The sensory response was ameliorated in treated animals and a trend of reduced oxidative stress was noted in response to treatment. These results provided initial evidence that our local flora is helpful in accelerating the functions recovery after an injuring to the sciatic nerve in rodent model. The present findings have paved the way to establish affordable and easily accessible remedies against traumatic brain and nerve injuries that have been considered incurable.

Keywords: Nerve injury; Oxidative Stress; Functional Recovery; Phytochemicals

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Efficacy of *Euphorbia helioscopia* in context to a possible connection between Antioxidant and Antidiabetic Activities: A comparative study of different Extracts

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ABSTRACT

Euphorbia helioscopia, conventionally known as sun spurge, has been used as a traditional medicine to treat different diseases owing to its reported antitumor, antiviral and antioxidant activities. The current research was formulated to assess in-vitro antioxidant and antidiabetic ability of Euphorbia helioscopia subsequent to the phytochemical analysis of its various extracts. For this purpose, methanol, ethanol and aqueous extracts were prepared using the whole dried plant. Phytochemical analysis of the extracts was done to evaluate the total flavonoid components (TFC) and total phenolic components (TPC) in the extracts. A total of seven phenolic and three flavonoid contents were documented and quantified using HPLC. Antioxidant values were found by DPPH• assay, FRAP and ABTS assays. Antidiabetic potential of the extracts was evaluated by the inhibition ability of the activity

of enzymes α amylase and α glucosidase. After analyzing statistically, the results showed that methanolic extract possess the highest TFC and TPC values while aqueous extract encompassed the lowest level of these contents. In vitro results showed that methanolic extract of the Euphorbia helioscopia has the maximum antioxidant capability since it showed the highest scavenging ability towards the DPPH• (IC₅₀ value = 0.06 ± 0.02 mgml⁻¹), FRAP (758.9 ± 25.1 μ MFe⁺²/g), and ABTS (689 ± 25.94 μ MTEq/g) due to the presence of high TPC (24.77 ± 0.35 mg GAEq/g) and TFC (17.95 ± 0.32 mgQEq/g) values. Antidiabetic activity in terms of inhibition potential of α amylase and α glucosidase activity was also observed maximum in methanolic extract having lowest IC₅₀ value (0.4 ± 0.01 mgml⁻¹ and 0.45 ± 0.01 mgml⁻¹ respectively) and minimum in the distilled water extract (IC₅₀ value = 0.57 ± 0.02 mgml⁻¹ and 0.76 ± 0.1 mgml⁻¹ respectively). The outcomes of the experiment have shown that the Euphorbia helioscopia extracts used in current study, contain the antioxidant and antidiabetic activities however it is highest in its methanolic extract. The presence of the same trend towards the highest antidiabetic activity of the methanolic extract in terms of maximum inhibiting activity of α amylase and α glucosidase enzymes, suggests a close association of TFC and TPC in minimizing diabetes.

Keywords: Euphorbia helioscopia, Methanolic Extract, Antioxidant, Antidiabetic.

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Systematic analysis of fungicidal activity of silver nanoparticles against *Rhizopus species*

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ABSTRACT

In this research, Silver nanoparticles (SNPs) were used against bread fungus (*Rhizopus species*) for systematic analysis of fungicidal activity of SNPs. Fungus was isolated for pure culture, identified and then used for further experiments. potato detox agar (PDA) was used to culture the fungus. Morphological identification of fungus after lactophenol cotton blue staining, was done under the microscope. Silver nanoparticles were synthesized by classic citrate reduction method. UV-vis spectrophotometry and SEM were used for characterization of silver nanoparticles. Fungus incubated with different concentrations of SNPs at 37°C for 7 days and growth was recorded on day three and day seven. The results indicate that SNPs have significant antifungal activity and this activity is concentration dependent. This research article is in the process of publication.

Key words: Silver nanoparticles, potato detox Agar, bread fungus, morphological identification, UV-vis spectrophotometry. SEM

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LLY-507 Loaded Iron Oxide Nanoparticles for Anticancer Drug Delivery Applications

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ABSTRACT

Background: Cancer incidence and mortality rate is increasing worldwide. In cancer treatment, targeting the tumor tissues without damaging healthy tissues is the major issue as chemotherapeutic drugs produce unwanted cytotoxicity. Nanoparticles-based drug delivery is an efficient and promising vehicle for cancer therapy, minimizing the severe toxicity associated with conventional chemotherapy. The unique physiochemical properties of iron oxide nanoparticles (IONPs) make them suitable carriers for targeted chemotherapeutic applications.

Materials and methods: This study emphasizes the loading and release profile of methyltransferase inhibitor as an anticancer drug on PVA functionalized IONPs (PVA-IONPs) for targeted drug delivery system. The iron oxide nanoparticles are prepared by the reformed co-precipitation method and characterized to verify several properties like size and composition. PVA functionalization of IONPs and drug loading is carried out through the immobilization method. UV-Vis spectroscopy is being used to measure the drug loading and release kinetics.

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Results: Characterization results show the average size of IONPs ranges between 44-100nm and weight percentage of iron and oxygen is 36.41%, 36.30%, respectively. The maximum loading efficiency and loading capacity of PVA coated IONPs for LLY-507 is 97%, 4.4%. The release profile of LLY-507 is 80%.

Conclusion: Methyltransferase inhibitor as an anticancer drug coupled with nanoparticle drug delivery systems could effectively reduce the systemic drug load and adverse effects of chemotherapy with controlled and targeted delivery.

Keywords: Cancer, Chemotherapeutic drugs, Iron oxide nanoparticles, LLY-507,

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Effects of Carvacrol and Menthol Supplemented *Moringa oleifera* Leaf Meal Based Diets on Growth Performance and Body Composition of *Oreochromis niloticus* Fingerlings

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ABSTRACT

With increasing world population, the demand for fish is growing thus there is a need to identify products with potential to increase the efficiency of fish production. Fishmeal is a superior aqua-feed and pet-food ingredient due to the high content of protein and nutritive value of lipids and other constituents. Fish meal is getting expensive day by day, which urges scientists to use alternative protein sources. Phytonics are among the products being investigated as potential naturally derived growth promoters. The aim of this study was to identify phytonic compounds like carvacrol and menthol individual and combined effect on the growth performance and whole body composition of *Oreochromis niloticus* by feeding *Moringa oleifera* leaf meal (MOLM) based test diets. To accomplish it, 10 experimental diets including control (without supplementation) were formulated with graded levels of carvacrol and menthol at 200, 300 and 400 mg/kg first individually and afterwards in combination of 1:1. Fingerlings were fed at the rate of 5% of their live wet weight for the period of 60 days. The differences among means were compared by Tukey's Honest Significant Difference Test. The results showed that combination of

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carvacrol and menthol at 200mg/kg had statistically ($p < 0.05$) considerable improvement in weight gain (17.15g), weight gain% (262.07%), Feed Conversion Ratio (1.25), Standard Growth Rate (1.42), protein (55.56%) and fat contents (13.94%) compared with the control and other test diets. These findings suggest that supplementation of carvacrol and menthol in the diet of Nile tilapia can be done up to 200mg/kg, so that improved growth parameters and bodily composition can be perceived.

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Histopathological Evaluation of Gills and Liver of Fish Treated With Different Doses of Silver Nanoparticles

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ABSTRACT

Nanotechnology has multiple uses in aquaculture and fisheries but it may have toxic impact on organisms' health if it is used in excessive amount. This study was aimed to investigate the effect of AgNPs on freshwater fish, *Labeo rohita* (*L. rohita*) which is a valuable and common source of food in Pakistan. Histological changes in gills and liver were investigated in AgNPs treated fish in comparison to the control untreated fish. Fish were exposed to AgNPs for 14 days. The fish were divided into 4 groups, control, T1 (1mg/L AgNPs), T2 (10mg/L AgNPs) and T3 (20mg/L AgNPs). The AgNPs were directly mixed into aquarium water containing treatment groups in alternate days. It was found that AgNPs induced alterations in histological parameters in a dose dependent manner. In gill tissues, it induced aneurism, partial lamella fusion, and severe lamella fusion, congestion, lifting of epithelium, inflammation and hyperplasia. In the liver tissues, AgNPs induced different abnormalities, i.e. necrosis, congestion, vacuolization, inflammation, accumulation of condensed nuclei and severe hepatocyte degeneration. It was concluded that AgNPs are toxic to aquatic organisms and induce histopathological alterations at higher concentrations.

Key Words: Nanotechnology, AgNPs, Histopathology, Gills, Hyperplasia.

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Effects of Zeolite Supplemented Guar meal Based Diets on Overall Performance of *Oreochromis niloticus* Fingerlings

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ABSTRACT

Natural zeolites are a family of minerals of volcanic origin that are made of crystalline aluminosilicates. The active ingredients in zeolites include antibiotics, minerals and vitamins etc... It is estimated that in year 2030, the global aquaculture production will increase up to 109 million tons, due to rapid increase in world population. In past, the fishmeal was mainly used as a major protein source in aqua feeds, but nowadays it is very expensive because of its growing demand. One of the most useful strategy to reduce the fish feed cost is the use of plant proteins instead of fish protein. Guar meal is relatively inexpensive and can successfully substitute the fish meal in fish feed because it has good amino acid profile and a higher protein content. Present study was planned to determine the effect of zeolite supplemented guar meal based test diets on overall growth performance and carcass composition in *Oreochromis niloticus* fingerlings. To achieve this, guar meal based test diets were formulated with graded levels of zeolites viz. 0%, 1%, 1.5%, 2%, 2.5% and 3%. Fingerlings were fed at the rate of 5% of their live wet weight for the period of 60 days. The differences among means were compared by Tukey's Honesty Significant Difference Test. Diet containing zeolites at 1.5% level showed statistically ($p < 0.05$) significant improvement in weight gain (19.06g), weight gain% (270.71%), Feed Conversion Ratio (1.21), Standard Growth Rate (1.40), protein (51.61%) and fat contents (16.3%) compared with the control and other test diets. These findings suggest that supplementation of zeolite in the diet of Nile tilapia can be done up to 1.5%, so that improved growth parameters and bodily composition can be observed.

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Biodegradation of isoproturon by a soil bacterium *Sphingobium* sp. S29 in soil and aqueous media

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ABSTRACT

Isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea (IPU)], one of the phenylurea herbicides, is extensively used across the globe in agricultural fields to overcome the pre- and post-emergence of broad leaf weeds in cereal cultures. Intensive use of IPU is becoming a source of ground and surface water contamination. Several problems including cancer, endocrine disruption and inhibition of growth in humans, animals and plants are related to IPU toxicity. Keeping in view the environmental concerns associated with IPU, there is a need to develop the strategies for removal of IPU from our environment. In this regard, the present study was conducted to isolate the bacterial strain having the capability for biodegradation of this herbicide through enrichment culturing using IPU as a sole source of C and N. In this study, a bacterial strain designated as *Sphingobium* sp. S29 was found to have the potential for biodegradation of isoproturon. The strain S29 had the potential to degrade known metabolites of IPU including monodemthyl-isoproturon, didemtheyl-isoproturon and 4-isopropyl aniline as well as other structurally related phenylurea herbicides including diuron and chlorotuluron. The biodegradation of IPU by this strain was optimal under shaking at 30°C and at 8.0 pH. The bioaugmentation study of the isolated bacterial strain carried out in soil microcosms revealed that this strain has a good potential for biodegradation of isoproturon in the soil in the presence of 24% moisture content and at 30 °C. Based on the findings of this study, it might be concluded that the strain *Sphingobium* sp. S29 can serve as a potential candidate for devising the strategies for bioremediation of the soils contaminated with isoproturon.

Keyword: Phenylurea herbicides; Isoproturon; Biodegradation; *Sphingobium* sp.; Metabolites



O-187/ICAZ-2020

Neonatal Calf Diarrhea (NCD): A Potent Reservoir of Multi-Drug Resistant Bacteria and Public Health Hazard in Pakistan

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ABSTRACT

Though emergence of multi-drug resistant bacteria demonstrated a worldwide phenomenon, limited research is reported about the prevalence of antibiotic resistant bacteria in fecal ecology of neonatal calf diarrhea (NCD) diseased animals in Pakistan. The present study aimed to identify and assess the prevalence of bacterial pathogens and their resistance potential in the fecal ecology of NCD diseased animals of Pakistan.

Methodology:

A total of 51 bacterial isolates were recovered from feces of young diarrheic animals (n=10), collected from 6 cities of Pakistan and identified on the basis of 16S rRNA gene sequence and phylogenetic analysis. Selected isolates were subjected to antimicrobial susceptibility by means of ceftazidime (30 µg), cefpodoxime (10 µg), cefotaxime (30 µg), cefotaxime/ clavulanic acid (40 µg), imipenem (10 µg) and augmentin (30 µg) while minimum inhibitory concentration (MIC) analysis to quinolones (i.e. ofloxacin, levofloxacin and ciprofloxacin), sulfonamides (i.e. sulfamethoxazole) and penicillins / penicillin like (i.e. ampicillin / amoxicillin) antibiotics classes. Additionally, polymerase chain reactions (PCRs) were utilized to characterize the *blaTEM*, *blaNDM-1*, *blaCTX-M*, *mcr-1* and *qnrS* antibiotic resistance genes.

Results:

Based on the 16S rRNA gene sequence all isolates were identified and their DNA sequences were submitted to NCBI DNA database (**Accession numbers: LC488898 to LC488948**) that belonged to seventeen genera with the highest prevalence rate for genus Bacillus (23%) and lowest for genus Kurthia (2%). Antimicrobial susceptibility by disc diffusion method explained the prevalence of resistance in isolates ceftazidime (27.7%), cefpodoxime (27.7%), cefotaxime (11.1%), cefotaxime / clavulanic acid (22.2%), imipenem (0%) and augmentin (27.7%). MICs demonstrated that almost 90% isolates were

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multi-drug resistant (against at least three antibiotics), specially against ciprofloxacin, with the highest resistance levels for *Shigella* sp. NCCP-421 (MIC-oflo up to 20 µg/L, MIC-lev up to 10 µg/L, MIC-cipro upto 75 µg/L, MIC-smx up to 20 µg/L, MIC-amp up to 50 µg/L, and MIC-amox up to 20 µg/L).

PCR-assisted detection of antibiotic resistance genes showed that 7 isolates were positive for *blaTEM*, 1 isolate was positive for *blaNDM-1*, 1 isolate was positive for *blaCTX-M*, 6 isolates were positive for *mcr-1*, 3 isolates were positive for *qnrS* whereas some isolates exhibited coexistence of antibiotic resistance genes.

Conclusion & Significance:

These preliminary findings conclude the presence of multi-drug resistant bacteria some of which are reported zoonotic agent and antibiotic resistance genes harboring in diarrheic animal feces pose the potential public health risk thus neonatal calf diarrhea (NCD) disease may be included in the surveillance program of cattle infectious diseases in Pakistan. The data obtained from this study also suggested that levofloxacin (MIC \geq 10 µg/L) could be effective against diarrheic infections. Results of these studies also showed the presence of some candidate novel species of bacteria that may be validated as a new species from Pakistani ecology by poly-phasic taxonomic characterization and would be available for future studies.

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Acute toxicity assessment in *Labeo rohita* and *Cyprinus carpio* exposed to tannery effluents Asma Noureen¹, Saba Manzoor², Arshad Javid³, Bushra Ayub⁴ and Khurshied Ahmad Khan⁴

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ABSTRACT

Tannery industries produce great amount of effluents containing diverse pollutants such as heavy metals and the discharge of such industrial waste in untreated form have negative impacts on the health of aquatic ecosystem. The aim of the present study was to calculate the acute toxicity of tannery effluents with respect to 96-h LC₅₀ and the lethal concentrations for two species of fish viz. *Labeo rohita* and *Cyprinus carpio* at constant levels of water temperature (29°C), pH (7.40) and hardness (205mgL⁻¹) with three replications for each treatment. In order to conduct this study water samples contaminated with

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tannery effluents were collected from industrial zone Multan and fish were collected from Dhrama Fish Seed Hatchery, Dera Ghazi Khan. Fish were transported to wet laboratory at Department of Zoology, Ghazi University, Dera Ghazi Khan, acclimatized and exposed to different concentrations of tannery effluents and dead fish were collected from test aquaria. During acute toxicity trials, the physico-chemical characteristics of water were analyzed on 12 hourly basis for each test concentration of the tannery effluents for both fish species. Probit analysis method was used on fish mortality data to determine the acute toxicity of tannery industry effluents with 95% confidence intervals. Analysis of variance and Tukey's tests were applied to determine statistical differences existing among various variables. Correlations among various physicochemical variables and metal concentrations were computed to determine their relationship. The 96-hr LC₅₀ of tannery effluents for *Labeo rohita* and *Cyprinus carpio* were determined as 11.02±1.18 and 15.18±2.50 mgL⁻¹, respectively. However, the tolerance limits of the two fish species for the industrial effluents (tannery) in terms of lethal concentrations were calculated as 22.67±2.20 and 34.93±3.67, respectively. From the findings of this study it is concluded that tannery effluents proved extremely toxic to both fish species as they caused significant fish mortality even at low concentrations so there should be proper treatment before their release into natural water bodies to minimize their harmful effects on aquatic fauna.

Keywords: Tannery effluents, *Labeo rohita*, *Cyprinus carpio*, acute toxicity

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The role of fish in the treat of COVID-19 patients

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ABSTRACT

To suppress and ultimately stifle the spread of the global COVID-19 pandemic, the world's scientific and medical communities are turning to treatments containing omega-3 fatty acids and fish oil for help. Clinical trials are underway by various pharmaceutical companies to test the efficacy of a new drug based on eicosapentaenoic acid in fish oil on symptomatic patients with inflammatory cytokines.

A new formulation of free fatty acids eicosapentaenoic acid (EPA-FFA) very pure (refined) in gastro-resistant capsules and to ensure maximum absorption, the optimal pH to be delivered to the intestines. The fish oil used in this formula is produced from pelagic species. EPA as a free fatty acid is very promptly absorbed and incorporated into the body's phospholipid membranes and then starts to act very quickly. Once incorporated into cell membranes, EPA-FFA significantly affects the production of pro-inflammatory mediators such as IL-6 and leukotriene B4 that play a crucial role in starting and maintaining the inflammatory process in the lungs. This formula to not only modify the COVID-19 disease process, reducing harmful, excess inflammatory responses but also to do without suppressing the immune response to the virus which are vital to seroconversion giving the patient ongoing protection against continued viral challenge. The goal is to minimize the symptoms of infection to reduce the risk of coronavirus complications.

Keywords: Coronavirus patient, Omega-3, Fish oil, Eicosapentaenoic acid, Lung



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The Effects of Aromatherapy on COVID-19 Patients

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ABSTRACT

COVID-19 is a member of the Coronavirus family that spreading the worlds and due to lack of drugs at present. Medicinal and aromatic plants, especially those frequently used in traditional medicine, play a very important role, as they contain bioactive compounds which could be used to develop new formal medicines to relieve patients with symptoms of COVID-19 with no or minimal adverse effects, Essential oils (EO) from aromatic plants have the main active molecules that have shown promise as antiviral agents against several pathogenic viruses. In the many types of research that have been done, essential oils have been screened against several pathogenic viruses including influenza and other respiratory viral infections. The oils of thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*) were tested in viruses. Thyme essential oil presents thymol, p-cymene, γ -terpinene and *Cinnamomum zeylanicum* leaf oil is characterized by eugenol. Aromatic and medicinal plants remain an alternative to contain and prevent this pandemic in their way. A systematic and advanced search, using keywords on this thematic, was carried out in the following Databases: Google Scholar, Pub Med, Science Direct, Web of Science, Scopus, Springer in addition to a standard search using the search engines. In the pandemic COVID-19 the thyme is advised to use frequently as a spice and in some foods as a preventive measure to decrease the viral load of coronavirus]. A recent study has shown the effectiveness of cinnamon with other ingredients (Gene-Eden-VIR) to exert a variety of antiviral effects on beta coronaviruses and SARS-CoV, by inhibiting of cell entry and infection, inhibiting of replication and inhibiting the viral proteases, to improve the antiviral immune response and reduce the formation of virulent quasi-species. These compounds may be effective in decreasing and preventing the contamination risk as well as treating some symptoms of COVID-19.

Keywords: Coronavirus, COVID-19, Aromatherapy, Essential oils



The Influences of COVID-19 on Marine Mammals

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ABSTRACT

In the current century, the world is faced with unprecedented pandemic not only on humans but also on aquatics, especially marine mammals. The coronavirus subfamily; Coronavirinae is divided into four genera, alpha, beta, gamma, and delta (α , β , γ , and δ) coronavirus. The γ coronaviruses are found in birds and aquatic mammals including bottlenose dolphin and beluga whale. It has been seen that human-to-marine mammal transmitted during an MRSA outbreak in dolphins and walrus that. Some studies identified that many species of whale, dolphin and seal, as well as otters, are predicted to be highly susceptible to infection by the SARS-CoV-2 virus. There are three indirect impacts of this virus on marine mammals including reducing marine vessel traffic, impacting of plastic pollution and changing in citizen science collection. A consequence of poor wastewater management is the discharge of untreated wastewater carrying infectious SARS-CoV-2 into natural water systems that are home to marine mammals. Thus, this research is going to highlight the potential for reverse zoonotic transmission of COVID-19 and its impact on marine wildlife; impacts that can be mitigated with appropriate action to prevent further damage to these vulnerable populations.

Keywords: Marine mammals, COVID-19, Virus, Zoonotic transmission



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Artemia (*Artemia urmiana*) enriched for Persian sturgeon fish (*Acipenser persicus*) larvae

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ABSTRACT

A series of experiments was carried out to evaluate the effects of nutritional enrichment of Artemia by oils in different concentrations with or without different levels of vitamin C during two enrichment periods (12 and 24h) on survival, growth rate and salinity tolerance in Persian sturgeon larvae reared in tanks. Four types of oils (ICES30/4, sturgeon ovary, cod liver, and linseed oils) were evaluated. Artemia nauplii eicosapentaenoic (EPA), docosahexaenoic acids (DHA) contents and DHA/EPA and $\omega 3/\omega 6$ ratios significantly increased ($P < 0.05$) as highly unsaturated fatty acid (HUFA) concentration and enrichment period increased. Artemia arachidonic acid (ARA) content, however, increased significantly ($P < 0.05$) as HUFA concentration decreased and enrichment period increased. 300 ppm ICES30/4 plus 20% vitamin C with 24h enrichment period resulted in significantly higher ($P < 0.05$) vitamin C content ($1063.8 \pm 48.00 \mu\text{g/g DW}$) in Artemia nauplii than other combinations. HUFA oil source with or without vitamin C did not affect the growth rate of Persian sturgeon larvae. Nevertheless, survival rates and chemical compositions were significantly improved ($P < 0.05$) only in fish larvae fed Artemia enriched HUFA oil supplemented with vitamin C. ICES30/4 was the best oil source for improving the chemical composition including fatty acids in Persian sturgeon larvae only when it was supplemented with vitamin C. Lower levels of vitamin C (less than 30%) and longer enrichment period (24h) were more effective ($P < 0.05$) in improving on chemical composition of sturgeon fish larvae. When the sturgeon larvae were exposed to salinity test, those fed with HUFA enriched Artemia showed tolerance only up to 6 ppt while those fed HUFA+ vitamin C enriched Artemia demonstrated high tolerance ($\geq 90\%$ survival) up to 12 ppt for 120h. Although the enrichment of *Artemia urmiana* with 300 ppm ICES30/4 and 30% vitamin C gave the best salinity tolerance at 12ppt (100% survival), the enrichment with 300 ppm sturgeon ovary oil and 10% vitamin C was economically affective in increasing the salinity tolerance of the Persian sturgeon fish larvae (98 % survival).

Key words: *Artemia urmiana*, Enrichment, Persian sturgeon fish, *Acipenser persicus*, Chemical composition, Salinity tolerance

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The Negative Impacts of COVID-19 on the Aquatic Processing Industry

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ABSTRACT

With regional and international food and agricultural value chain severely disrupted, seafood is caught in the aspect of the COVID-19 storm. The global responses to this worldwide pandemic have had wide-ranging consequences for seafood sector. Fish and fish products are a vital part of a healthy diet. Misleading perceptions in some countries have led to decreased consumption of fish and fish products. The possibility that processing and transport business will be forced to reduce, stall or completely slow down, will result in reduced levels of food security and increased issues related to malnutrition. Many seafood processing businesses had to reduce production or close down completely in response to COVID-19 and lockdown restrictions. It is expected that the production of seafood and its trade value is reduced. Demand for packaged and frozen products has had a significant impact on retail sales as household demand for non-perishable food has increased. The labor shortage caused by the epidemic has affected everything from initial production to the end of the product chain. Harvesting of aquaculture products is also delayed and storage targets are drastically reduced. As a result, various measures to curb the spread of COVID-19 have led to a stagnation in the food industry and tourism, a reduction in the transportation industry, delays in cross-border procedures, and the like, confusing domestic and overseas supply chains. Live fish, fresh fish, and chilled fish, which account for 45% of the marine products consumed, are extremely fragile, which makes logistics even more difficult. If the seafood is not delivered to the fish processing plant through the cold chain at the right time, it will cause a lot of food loss. Also, fisheries processing plants suffer from lack of protective equipment and clothing for hygiene management.

Keywords: Seafood, COVID-19, Cold chain, Procedure, Fish processing

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Dried brown Seaweed as natural biosorbent of Nitrogen (N) and phosphorous (P) of Aqueous solution at laboratory condition

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ABSTRACT:

Aquaculture is source of significant amount of wastes, which generally leads to deterioration of water quality. Removal of two important ions, Nitrogen (N) and phosphorous (P), from aquaculture wastewater is an important environmental challenge. A simple alternative to this is use of biosorbent to reduce the nutrient load. Hence, the collected dried different sizes and amounts of marine macro algae *Sargassum ilicifolium*, from Chabahar-Bay, Sistan and Baluchistan province, IRAN, as low-cost adsorbent was screened for its nutrients absorption capacities, at various initial ions concentrations and contact period at different pH of 100ppm aqueous solution nitrate and phosphate separately at laboratory condition. The experiment on nutrient uptake by seaweed inferred that the maximum phosphate removal was obtained (98-98.2%) at pH 9 where initial phosphate concentration of 0.2 ppm was finally reduced to 0.004 ppm at 60 min. when 2g dried, crashed to 0.5mm *S.ilicifolium* as biosorbent was used. The concentration of nitrate decreased (52.1-59.2%) significantly at pH 9 where initial nitrate concentration of 25 ppm declined finally to 10.2 ppm at 7min. by using 4g seaweed, 0.5mm size. Increasing the concentration of phosphate caused higher reduction of this ion in aqueous but no any differences was found in nitrate reduction when its concentration increased. Although absorption of these two ions increased when the amount of seaweed increased but it was conversely decreased when the size of seaweed increased. Using the acid and base washing out methods of seaweed after absorption has not any effect on output increasing biosorption potential.

Key words: Uptake, Nutrients, Waste water treatment, Seaweed, Bioremediation

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Recent applications of nanoparticles-based lateral flow immunoassay as a novel diagnostics tool for aquatic animals' infectious diseases

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ABSTRACT

The development of rapid disease diagnostic methods is necessary since the increasing number of bacterial and viral pathogens leading to threatening diseases in aquaculture systems. Rapid, accurate diagnosis of disease and fast removal of infected fish are critical for effective outbreak control. Prompt action in the early stages of any disease problem can have an enormous impact on the scale of the outbreaks. As a result, the design of rapid, accurate and sensitive methods has been considered by researchers to replace with other complex and costly immunoassay methods like Radioimmunoassay and ELISA in recent years. Most of these methods are based on agglutination, immunochromatography (ICG) and some of the molecular techniques. ICG tests are used for the qualitative and quantitative detection of a wide variety of antigens and antibodies in hospitals, clinics, physician offices, and clinical laboratories. Immunochromatography assay or lateral-flow immunoassay is based on the flow of the infected sample along a cellulose membrane via capillary action. Major advantages found on this technology is inexpensive, simple to use, portable, short assay time (with results in minutes), does not require skilled operators and complicated equipment. Herein, we summarized the principle of the assay and recent advancements the nanoparticles-based lateral flow as an on-side diagnostic tool for infectious agents and diseases.

Keywords: Immunochromatography; Lateral-flow immunoassay; Rapid diagnosis

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The Effect of Iron Nanoparticles (FeNPs) on Some Biological and Hematological Parameters in Albino Rats

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Abstract:

The aim of the current study was to evaluate the risk of using iron oxide nanomaterials as food additives for animals to increase its utilization compared to bulk size particles of the same material. The study included the effect of two different nano-sized iron oxides (25 nm and 50 nm) at two doses (1 mg/kg feed and 10 mg/kg feed) on some biological and Hematological parameters in male albino rats. The work was carried out in Advanced Physiology Research Lab, at the University of Zakho. The parameters studied included rat's performance such as growth rate, mortality, and food conversion ratio. In addition, it also included some hematological parameters such as red blood cell count (RBC), platelet count (PLT), and white blood cells (WBC). The results related to the rat's performance were non-significantly affected by IONPs. Most of the hematological parameters in all treated groups were not affected by IONPs except AB group which showed just a significant difference ($P < 0.05$) in the percent of granulocytes compared with the control. It can be concluded from the results of the current research that iron oxide NPs of different sizes and doses did not affect their performance, growth rate and hematological parameters with no sign of toxicity.

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Possibility of COVID-19 in animals and its control and prevention

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ABSTRACT

COVID-19, as pandemic disease, originated in China and spread rapidly all over the world and despite tremendous efforts to its control, the disease is still spreading. Human-to-human transmission of the disease has been proven, but there are differing views in the case of animal disease, animal-to-human transmission or animal-to-animal. According to doing studies, here provides some information on the risk of COVID-19 for animals.

Following the outbreak of Covid-19, reports of infection of some animals such as cats in Hong Kong, New York Zoo tigers and mink on Dutch farms prompted researchers to investigate virus-susceptible species and their possible transmission to humans. Also, the susceptibility of some animals to COVID-19 has been investigated. Some reports also indicate that dogs, pigs, chickens, and ducks are less sensitive to COVID-19 than cats and ferrets. According to laboratory experiments, hamsters, rabbits and common marmosets are also susceptible, but there have been no studies of other animals like livestock. To date, there is no evidence that animals play a significant role in spreading COVID-19 and based on the limited information, the risk of spreading COVID-19 from animals to humans is rare but despite the non-confirmation of this way, control and prevention of the spread of the disease are considered important. Anyway, it should study more and future researches will determine lots of information for Covid-19 transmission and animal management in its control.

Keywords: COVID-19, Animals, Transmission, Prevention

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Evaluation of lateral flow immunochromatographic strip test for diagnosis of aquatic animal disease

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ABSTRACT

Diseases are one of the major threats to expansion and intensification of sustainable aquaculture. Therefore, rapid detection of disease to stop the spread of infectious pathogens is necessary. Several rapid methods have been developed for the detection of pathogens in fish, shellfish, molluscs via immuno- and molecular diagnostics. Currently, Lateral flow test, also called Immunochromatographic assays, is widely used in the rapid diagnosis of infection agents in domestic animals and humans. This technology has many advantages over conventional detection methods; it is easy to use, relatively inexpensive to make, Long-term stability over a wide range of climates, provides prompt results in shorter times (with results in 10min), and does not require skilled operators or expensive equipment. These features make strip tests ideal as a pond-side test. The typical lateral flow assay test strip is composed of different parts of which are assembled on a plastic backing. These parts include a sample pad, a conjugate pad, nitrocellulose membrane and adsorption pad. Actually, the format in Lateral flow test is similar to ELISA in which nitrocellulose membrane is as a base substrate with two discrete zones; test and control lines. Lateral flow test strips have different detection formats; the two predominant approaches to the tests are the sandwich and competitive reaction format. Commercially available ISAV and WSSV (Shrimple®) immunochromatographic diagnostic test kits have been developed for the detection of the infectious salmon anemia virus and white spot syndrome virus from fresh samples. This review intends to provide an analysis of lateral flow assays for rapid detection of disease.

Keywords: Diseases, detection, Lateral flow test, nitrocellulose membrane

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Mutagenicity evaluation of medicinal plants collected from different localities of Pakistan

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ABSTRACT

Different ailments are treated by medicinal plants as herbal remedies. Plants have been carefully chosen and used empirically as drugs for centuries owing to their carcinogenic and mutagenic effects besides their therapeutic effects. Current project has been conducted to evaluate the cytotoxicity and mutagenicity of plants having therapeutic potential. To pilot screen phytochemicals by adopting Standard biochemical assays, extracts of leaves of medicinal potent plants (*Azardirecta indica*, *Calatropis procera*, *Cassia fistula*, *Nicotiana-N- tobacum*, *Pinus sylvestris*, *Solonum nigrum*) were prepared sequentially by ultrasonic extraction using different solvents like methanol, ethanol, petroleum ether and Methanol/ethyl acetate. The proximate parameters of medicinal plants were determined. Antimicrobial (Antibacterial and antifungal) potential of these extracts was determined against different bacterial (*Bacillus subtilus* and *E. coli*) and fungal (*Aspergillus niger* and *Penicillium notatum*) strains. Cytotoxicity and mutagenicity was determined.

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Fishmeal Alternative Protein Sources in Aquaculture Diets

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ABSTRACT

As the human population is going to be increased continuously, one of the most crucial challenges faced by all over the world is to find the means to feed the people. Almost more than 50 % of the world population is suffering from under nutrition or malnutrition. The importance of aquaculture can be observed in current scenario as it ensures a rapid and consistent supply of aquatic species for food to overcome on this drastic challenge. Most of the high cost of feed arises from extensive reliance on protein sources such as fish meal. Increasing fishmeal prices motivated the scientists to identify the cost-effective alternatives of fishmeal and when we search for alternatives of fish meal then we find plant by-products are the best alternative protein sources. The advantages of using plant proteins lie not only in the availability and economic benefits but also that these plant products also have less amount of phosphate and nitrogen than animal protein, therefore, reducing the chances of eutrophication in fish ponds. Plant proteins considered as the most viable alternative for economic fish production in most of the developing countries. In this manner, it has become an inevitable trend of replacing fish meal with less expensive and locally available plant protein sources such as canola meal, cottonseed meal, sunflower meal, moringa by-products etc. To overcome the problems facing during the use of plant by-products in fish feed, various supplements may be added in the fish feed such as enzymes, probiotics, nanoparticles and organic acids. It was concluded that we may hope that fish meal will no longer be a part of the fish diets in future. Although there is a major challenge in the expansion of plant ingredients, it is justified by many researchers that through proper dietary tactics fish can be fed with excess plant protein without any negative performance. Use of plant by-products may lead to a way for better coordination of fisheries with agriculture and can achieve a goal of better aquaculture production in an eco-friendly, cost effective and more sustainable way.

Key words: Fish meal, Alternatives, Plant by-products, Supplements, Fish production

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Hesperidin: A promising drug candidate against COVID-19

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ABSTRACT

SARS CoV-2, a rapidly spreading new strain of coronavirus, has affected almost all the countries of world and received worldwide attention. The lack of specific drugs or vaccines against SARS CoV-2 is a major hurdle towards the prevention and treatment of COVID-19. Thus, there is an urgent need to boost up research for the development of effective therapeutics against COVID-19. The crystallized form of SARS CoV-2 main protease (Mpro) was demonstrated by a Chinese researcher Liu et al. (2020) which is a novel therapeutic drug target. This study was conducted to evaluate the efficacy of customized dietary-nutrients based virtual library against COVID-19 virus Mpro by molecular docking study. Molecular docking investigations were performed by using Molegro Virtual Docker 7 to analyze the binding interactions of these compounds against COVID-19 virus Mpro. COVID-19 virus Mpro (PDB ID: 6LU7: Resolution 2.16 Å) was docked with 80 flavonoid compounds and the binding energies were obtained. According to obtained results, hesperidin, rutin, diosmin, and apiin have been found as more effective against COVID-19 virus Mpro than nelfinavir (positive control). This study will hopefully pave a way for doing advanced experimental research to evaluate the real medicinal potential of these compounds to cure COVID-19.

P-25/ICAZ-2020

The Impact of Solar Radiation and Balanced Diet on Induced Breeding in Freshwater Fish *Labeo rohita*

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ABSTRACT

Effect of solar radiation and balanced diet has been studied in *Labeo rohita* that maintained in 0.19Acre pond at stocking density 1000kg/Acre during February. The survival rate of rohu is more than others. Solar radiation has beneficial effect for all aquatic organisms. Balanced diet contained fish meat 10%, groundnut oilcake (GOC) 35%, soyabean oilcake (SOC) 20%, wheat flour 10%, rice bran 24.8%, trace mineral mix 0.1% and vitamin mix 0.1%. Balanced diet has more powerful effects in the maturity and health of fish. Fish divided into two batches, one batch exposed to radiation after 50 days while the second batch remains unexposed. Exposed batch have more protein concentration than the unexposed batch. Radiation and balanced diet more important in induced breeding in carp. The results concluded that high hypophysation with the combination of solar radiation and balanced diet 100% induced success in rohu (females 0.3-0.4 ml/kg; males 0.1-0.2 ml/kg). The *L.rohita* yielded 103 liters of fertilized eggs with 95% fertilization showing excellent performance of the brood fish.

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Wound healing assessment in mice using novel biomedical chia gel loaded with methanol extracts of *Oxystelma esculentum*

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ABSTRACT

Chia (*Salvia hispanica* L., Lamiaceae) is an annual herb, and chia seed gel (CSG) is widely use particularly in food and confectionaries since centuries across the world. CSG contains variety of peptides, higher oil contents (omega-3 fatty acid), vitamins (B-complex), plenty of fibre contents, antioxidant potential and huge moisture contents. Owing to these biological properties, current study was designed to formulate chia gel as a novel drug carrier biomedical material and its wound healing potential in mice. In this study methanol extracts of *Oxystelma esculentum*, were used in a mixture form with the CSG to formulate a new biomedical material. Material characterization was confirmed by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). CSG was applied alone, and with plant extracts (*Oxystelma esculentum* L.) in laceration wound mice models. CSG alone showed significantly higher wound healing activity as compared to other

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groups. The groups treated with drug loaded and plant extract loaded CSG exhibited good wound healing potential than their controls which indicated the drug delivery capacity of CSG. However, plant extract loaded CSG showed less significant wound healing response compared with drug loaded CSG group. Chorioallantoic membrane (CAM) assay confirmed the angiogenic activity of CSG, hence proved the wound healing capability of CSG. The current study provided the strong evidence to use CSG as a biological material with drug carrier and wound healing capacity in mice. However, further detailed studies are recommended prior its use in clinical trials.

P49/ICAZ-2020

Comparative study on arsenic effects in selected species of earthworms.

Hira Khan

ABSTRACT

Arsenic (As) is widely spread in soil and is poisonous to plants, animals and humans. Arsenic contamination from both anthropogenic and natural origins are one of the biggest problems around the globe. The present research was aimed to find the bioaccumulation of arsenic in selected species of earthworms. To examine the comparative bioaccumulation, *Pheretima posthuma* and *Lumbricus terrestris* were exposed to arsenic contaminated soil having doses of 210, 270 and 330mg arsenic per 3kg of soil. Two control experiments, each for one species and six treatment experiments with three replicates were carried out for one month. The accumulation of metals in digested samples were analysed by using atomic absorption spectrophotometer. The size of earthworms, morphological characteristics and weight were examined and contrasted with different species of earthworms along with initially recorded parameters as well. The biomass (length and weight) of the earthworms was increased in the control group and decreased in treated groups. Dark color patterns were observed in the skin that shows accumulation of metals. Cocoon production reduced in the treated groups. The arsenic compounds showed more toxicity against *Lumbricus* species as compared to *Pheretima* species. All the parameters used in this study showed arsenic toxicity in both species of earthworms. No change was seen in the behavior of earthworms after trial. No mortality occurred during the said period. Treatments were examined through Analysis of Variance and T-test. It was inferred that the bio-accumulative ability of earthworms was directly proportional to the concentration of arsenic in the soil where they lived.

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Effect of weeds and seed treatment on pests and their natural enemies in maize crop

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ABSTRACT

Maize (*Zea mays* L.) is considered a significant grain crop in Pakistan. It has been cultivated for grain food, feed processing and as biogas production. Moreover, it is an important source of nutrition as well as phytochemical compounds. The low production of this crop has been attributed to many biotic and abiotic factors. Insect pests are the key factor that affect the crop both quantitatively and qualitatively. In addition to insect pests, weeds play an important role in crop yield by harbouring insect pests and plant pathogens. So keeping in view the significance of weeds, the current study was conducted to assess the effect of weeds and seed treatment on pests and their natural enemies in maize crop in research area Young Wala Department of Entomology, University of Agriculture Faisalabad. The pesticide treated hybrid maize seed was planted using normal agronomic practices. Insect pests monitoring was done on regular basis using fixed sampling method in weeds and weed free treatments. There were four replications and each sampling site was considered as replication by using RCBD layout. Insect specimens were brought into the lab for identification. The results showed that in weedy plot maximum density of arthropods lady bird beetle (10) was recorded followed by ash weevil (9) maize stem borer (8) shoot bug (7) flea beetle (6.2) grasshopper (6.2) shoot fly (6.1) syrphid fly (6) bristle beetle (5) white fly (5) fall army worm (4). While in weed free plot arthropods density decreased, maximum density of bristle beetle (10.77) was recorded followed by maize stem borer (8.87), shoot bug (5.45), flea beetle (5.19), syrphid fly (3.62), grasshopper (6.26) and ladybird beetle (6.63). Pesticide seed treatment also minimized the infestation by pests. The study will be useful for the future sustainable management of pests of maize crop in changing environmental conditions.

Keywords: Maize crop, Weeds, Seed treatment, Pests, Natural enemies



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Exposure to variable doses of Nickel oxide nanoparticles disturbs serum biochemical parameters and oxidative stress biomarkers from vital organs of albino mice in a sex specific manner

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ABSTRACT

This study was designed to report the biological effect of nickel oxide nanoparticles (NiO NPs) in albino mice. Material and methods: Five weeks old albino mice of both sex were intraperitoneally injected either with 20mg (low dose) or 50mg/ mL saline/ Kg body weight (high dose) of NiO NPs for 14 days. Saline treated controls were maintained in parallel. Complete blood count, selected serum biochemical parameters and oxidative stress biomarkers from vital organs were determined in all subjects. Results: Male mice treated with NiO NPS had increased blood urea nitrogen, elevated superoxide dismutase (SOD) in liver elevated MDA in liver, kidney and heart and reduced catalase activity in heart and kidney. Female mice treated with NiO NPs had significantly reduced serum albumin and total proteins, SOD in lungs and elevated MDA in liver. Discussion: We are reporting that intraperitoneal injections of NiO NPs for 14 days drastically affect blood serum parameters and oxidative stress biomarkers from vital organs of albino mice. Conclusion: Toxic effects of NiO NPs were dose and sex dependent and they were more pronounced at higher dose and in male mice.

P77/ICAZ-2020

Microemulsion based synthesis of Strontium Hexaferrite Cobalt Iron Oxide nanoparticles and their biocompatibility in albino mice

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ABSTRACT

Present study was conducted to account for the synthesis, characterization of strontium hexaferrite cobalt iron oxide nanoparticles and to report their biocompatibility in albino mice.



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Nanoparticles were synthesized by normal micro emulsion and XRD analysis confirmed the single and SEM revealed average particle size ranged between 30 to 50 nm. Nine week old male mice were intraperitoneally administered with 50mg/ml solvent/Kg body weight of Strontium hexaferrite cobalt iron oxide nanoparticles for 22 days. Control group was maintained in parallel. A series of neurological tests (rota rod, light and dark box, open field and Morris water maze) were conducted in both groups. Blood samples were collected from direct cardiac puncture and parameters of complete blood count, serum biochemical parameters and antioxidant were determined in liver and brain tissues of all subjects. Analysis of result revealed that all studied neurological test performances varied non significantly ($P > 0.05$) between the two treatments except clockwise rotations during open field test that were significantly reduced ($P = 0.05$) in Strontium Hexaferrite Cobalt Iron Oxide nanoparticles treated male albino mice than control group. All studied complete blood count and serum parameters varied non significantly ($P > 0.05$) between two treatments. It was observed that superoxide dismutase concentration was significantly higher ($P = 0.05$) in liver of nanoparticle treated male mice. In conclusion, we are reporting that applied dose of Strontium hexaferrite cobalt iron oxide nanoparticles is affecting the exploratory behaviour and antioxidant metabolites of male albino mice.

P126/ICAZ-2020

Population dynamics and community structure of pests and their natural enemies in rice crop

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ABSTRACT

Rice is considered an important cereal crop and staple food and earns a lot of foreign exchange as an export commodity. The rice yield has been affected by insect pest species from planting till harvesting. The population of arthropod is known to change with the growth of the rice crop and play a significant role in crop yield. In the current study, community structure and synchronization of pests and their natural enemies were assessed from the crop transplanting till harvesting. The trial was conducted in the Entomological research field area Youngwala, Department of Entomology, University of Agriculture, Faisalabad. The field were divided into blocks and sub blocks using RCBD layout. The population dynamic was recorded thrice a week by using fixed sampling method. Sampling plants was considered as replication. The collected specimens were brought into the laboratory for the identification. The insect pest community structure was developed up to family and order level. The data regarding population dynamics of pests and their natural enemies was collected and subjected to descriptive statistical tools using Microsoft excel for analysis. The results showed that pest population was recorded more than their natural enemies. The pest population was recorded maximum during the month of September to end of

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October and the population of natural enemies was on peak in the start of September but reduced at the mid to end of October. The population of rice leaf folder was maximum during the 4th week of September and total 629 specimens was observed, 369 specimens of *Pyrilla* and population during the 3rd week of September was on its peak, and population of grasshopper was highest during the 3rd week of September and total 203 specimens was noted. The population of beat webworm moth was recorded maximum during the first half month of September then decrease in the start of October and total 227 specimens were recorded during the experimental period. Similarly, the population of natural enemies such as lady bug was recorded of about 69 specimens and population peak during the end of 3rd week of September, the dragonfly population on peak during the 3rd to 4th week of September and total 250 specimens was noted. The population of yellow jacket wasp which act as parasitoids was noted maximum during the 3rd week of September and remained minimum during the month of October and total 230 specimens was noted. The current study will be useful for the future sustainable management of insect pests of rice in changing environmental conditions.

Keywords: Rice crop, Population dynamics, Natural enemies, Rice pests

P127/ICAZ-2020

Effect of Lead and Copper on Hematological Parameters and Serum Biochemistry of Bighead Carp

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ABSTRACT

Aquatic environment face serious problems and damages due to heavy metals found in low or high range. The heavy metals are the main cause of contamination which cause adverse effects on fish, disturb the metabolic pressure and fish come into stressed condition. The *H. nobilis* is nutritionally significant fish in fresh water was used to regulate the consequence of dense metals. The objective of this research work was to check the “effect of lead and copper on hematological parameters and serum biochemistry of Bighead carp”. The experiment was performed in triplicate. The experimental fish were exposed to different concentrations of lead and copper to check 96-hr LC50 after acute exposure. During experiment, physico-chemical parameters like total hardness, dissolved oxygen, temperature, total ammonia, pH, calcium, magnesium and carbon dioxide were checked. Under the exposure of treated heavy metals, the hematological parameters i.e. RBC's, WBC's, Hct, Hb, MCV, MCHC, MCH and serum biochemistry parameters like total cholesterol, glucose, total protein, alanine aminotransferase, aspartate aminotransferase and albumin were observed. Results showed significant alterations in hematological



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parameters and serum biochemistry during acute exposure of selected metals then control group. The toxicity effects of selected metals followed the order: Copper > Lead. The present research work explained that there was a close relationship among metal toxicities and in physico-chemical parameters of water.

Key Words: Heavy metals, Acute toxicity, LC50 exposure, Lead, Copper, Metals mixture, Hematological parameters, Serum Biochemistry.

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Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* in integration with new chemistry insecticide against red cotton bug (*Dysdercus koenigii*) ([Fabricius](#))

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ABSTRACT

The red cotton bug (*Dysdercus koenigii* F.) is considered a serious cotton pest particularly after boll opening. Both adults and nymphs decrease plant productivity and crop yield by sucking cell sap from seeds especially from opened bolls. Affected fruiting body resulted in stained lint that become unfit for seed or oil extraction. Traditionally, it has been controlled by synthetic insecticides that resulted in high residual effects and natural enemies disturbance. As an alternate the use of biocontrol agents can be a potential management technique. In the current study we used entomopathogenic fungus *Beauveria bassiana* and *Metarhizium anisopliae* in combination with new chemical insecticide Movento and tested against red cotton bug, under lab conditions. The study conducted under controlled conditions of temperature $25\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ relative humidity using CRD layout in the Department of Entomology University of Agriculture Faisalabad. Different concentrations of fungi applied alone or in combination with recommended dose of Movento. Data was recorded on regular basis. The data obtained was subjected to ANOVA and treatment means with noticeable differences distinguished using LSD test $p \leq 0.05$. The results of the experiment showed that maximum nymphal mortality of *Dysdercus koenigii* (100%) was observed in case of integrated use of highest *B. bassiana* concentration. Bb2: 1×10^8 conidia/ml) and Movento (1%) (Mov: 240 SC) The integrated applications of lower dose rate of *B. Bassiana* and Movento caused higher nymphal mortality of *D. koenigglei* (90%) as compared to lower dose of *M. anisopliae*. The results of the experiment showed that maximum adult mortality of *Dysdercus koenigii* (96.7%) was observed in case of integrated use of highest *B. bassiana* concentration Bb2: 1×10^8 conidia/ml) and Movento (1%) (Mov: 240 SC) The integrated applications of lower dose rate of *B. Bassiana* and Movento caused higher adult mortality (83.3%) as compared to lower dose of *M.*

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anisopliae (Ma: 1×10^6 conidia) (80%). The study will be useful for the sustainable management of red cotton bug in integration with other pest management strategies.

Keywords: Red cotton bug, *Beauveria bassiana*, *Metarhizium anisopliae*, New chemistry insecticide

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Conception rates using single and double dose of cryopreserved semen in Kajli and Lohi sheep

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ABSTRACT

Pregnancy rates in Kajli and Lohi sheep was compared after insemination with single and double dose of cryopreserved semen. After conformation of heat, in half of the animals, single insemination was done and in other half of the ewes, double insemination was done at morning-evening intervals by cervical deposition method with the help of a duck speculum. Pregnancy was detected after 3 months of insemination by manual pregnancy test and blood serum progesterone level test using progesterone ELISA Kit. A Logistic regression model was applied for statistical data analysis using SPSS 2012 software. Manual pregnancy test showed 68.33% and 60.34% conception rates and progesterone EIA kit showed 70% and 68.96% conception in Kajli and Lohi ewes, respectively. The single insemination remain more effective, as the double insemination does not improve the conception rates as compare to single insemination in both Kajli and Lohi ewes.

Key words: Artificial insemination, Pregnancy, Progesterone, ELISA, Conception rate.

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The Contribution of Mitochondrial Calcium Homeostasis to Neutrophil Extracellular Traps (NETs) Formation

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ICAZ-2020 (7TH– 8TH DECEMBER, 2020)

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ABSTRACT

Neutrophils fight with invading pathogens through various mechanisms including degranulation, phagocytosis, production of cytokines, generation of reactive oxygen species (ROS), and the release of neutrophil extracellular traps (NETs). We designed this study to investigate the NETs-inducing potential of Formyl-methionyl-leucyl-phenylalanine (FMLP), a synthetic bacterial peptide, in human neutrophils and to determine the role of mitochondrial Ca^{2+} homeostasis in this process. Isolated neutrophils and a whole-blood preparation of neutrophils were pre-treated with mitoxantrone (MTX), a pharmacological blocker of mitochondrial Ca^{2+} uptake, and then stimulated with FMLP. Field's-stained smears were observed through brightfield microscopy for morphological characterization and quantification of neutrophils. The release of cell-free DNA (cfDNA) was also measured for determining neutrophil damage. Our data demonstrated NETs-like changes and a greater cfDNA release upon stimulation with FMLP which was negatively associated with the presence of platelets in whole blood preparation. Intriguingly, MTX pre-treatment significantly reduced FMLP-triggered NETs-like alterations and cfDNA release. Metformin, a known inhibitor of NETs formation, also decreased the FMLP-induced changes in neutrophils. In addition to the NETs-inducing potential of FMLP, this study unveils an exciting contribution of mitochondrial calcium uptake in FMLP-induced NETs formation in human neutrophils making it an interesting therapeutic target to cure diseases caused by excessive NETs formation.

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Green Approach for synthesis of Zinc Oxide nanoparticles from *Hadera nepalensis* leaf extract and evaluation of reproductive toxicity in male rats.

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Abstract

Nanotechnology is a rapidly developing concept in various fields of science like medical, agriculture and physical sciences. Nanoparticles (NPs) are nano-scale sized particles with enhanced thermal conductivity and chemical stability due to their large surface area to volume ratio. Green approach for nanoparticles' synthesis is an alternative to conventional techniques (Physical and chemical methods). The use of green approach for nanoparticle synthesis is gaining importance due to its cost-effectiveness, and reduced production of toxic chemicals. Zinc Oxide nanoparticles (ZnO NPs) are used in production of various daily used materials including dyes, pigments, medical equipment, sunscreens,

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cosmetics and health care product. Current study was designed to check and compare toxicity of ZnO NPs dissolved in distilled water, methanol and ethanol on reproductive system of male rats. Medicinally important plant *Hedera nepalensis* was used for the synthesis of ZnO NPs. Stock solution of different doses (5mg/kg and 50mg/kg) of ZnO NPs was prepared. Synthesized ZnO NPs were subjected to various characterization techniques UV-visible spectroscopy, FTIR, SEM and EDX spectroscopy. In present study adult male Sprague Dawley rats (n= 49) were divided into seven group. First group served as control and received distilled water by oral gavage. Second, third and fourth group received low dose (5mg/kg) of ZnO NPs in distilled water, methanol and ethanol while the other three groups received high dose (50mg/kg) of ZnO NPs in distilled water, methanol and ethanol for fourteen days. Body weight and glucose level was checked at 1st, 7th and 14th day of treatment. Animals were sacrificed at day 15 post treatment. Testicular tissue and blood samples were collected for histology and biochemical assay. High dose (50mg/kg) of ZnO NPs in distilled water resulted in significant reduction in body weight, testicular and epididymal weight as compared to the control, while the animals treated with high dose (50mg/kg) of ZnO NPs prepared in methanol and ethanol did not show that significant effect on body weight and reproductive organ weight as compared to control. High dose (50mg/kg) of ZnO NPs in distilled water caused significant reduction in plasma and intra-testicular testosterone concentration as compared to control but this reduction was less pronounced in animals treated with high dose (50mg/kg) of ZnO NPs prepared in methanol and ethanol respectively. Histomorphological studies revealed significant degenerative changes in rat testes in animals treated with high dose (50mg/kg) of ZnO NPs prepared in distilled water as compared to methanolic and ethanolic preparation. The results of present study showed that green synthesized ZnO NPs from *Hedera nepalensis* leaf extract in distilled water caused pronounced reproductive toxicity in adult rats' testis as compared to ZnO NPs in methanol and ethanol. It can be precluded that this toxicity might be due to poor capping of phytochemicals present in the leaf extract of *Hedera nepalensis*.

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Cross-talk of Mitochondrial Calcium Homeostasis with mPTP Opening in Mouse Brain Hemispheres

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ABSTRACT

Prolonged accumulation of calcium into mitochondria may cause opening of high conductance channel known as Mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane that is involved in cell death. The opening of the clogged brain artery after stroke referred to as reperfusion may lead to the so-called ischemia reperfusion injury (I/RI) which undermines the beneficial effects of reperfusion. I/RI triggers mPTP opening due to increased ROS and calcium in mitochondria matrix. This channel causes the mitochondrial swelling leading to apoptotic or necrotic neuronal death. Calcium entry into mitochondria by mitochondrial calcium uniporter (MCU) is one of the major stimulators of mPTP. But, the association of MCU and its component with mPTP opening in the right and left hemisphere of the brain remains poorly explored. Therefore, this study was designed to link the level of MCU to the calcium-dependent mPTP opening in the cerebral hemispheres of BALB/c albino mice. For this purpose, mitochondria from mice cerebral hemispheres were isolated, mitochondrial viability was determined by MTT assay and mPTP opening was measured using a spectrophotometric time-lapsed assay. Furthermore, the level of MCU components was also assessed by quantitative real time PCR (q-RT-PCR). Interestingly, our results depict the fact that left cerebral hemisphere experienced less mPTP opening as a result of exogenous addition of calcium in the isolated brain mitochondria. Our data further demonstrate that all the components of MCU (MCU, MCUb, MICU1, MICU2 and EMRE) are expressed in both the cerebral hemispheres. Notably, the mRNA level of EMRE, a regulator of MCU, tended to be low in the left cerebral hemisphere which goes in line with the reduced mPTP opening in this part of brain. However, there was no considerable difference in the mRNA level of other MCU components. In brief this study suggests that mitochondria isolated from the cerebral hemispheres behave differently in mPTP opening which may not be associated with the mRNA level of MCUC components.

Keywords: Mitochondrial Permeability Transition Pore (mPTP), Mitochondrial Calcium Uniporter (MCU), Brain Hemispheres.