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(Online International Conference)

BOOK OF ABSTRACTS

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A compilation of abstracts of all research papers presented in Annual Conference on Toxicology Research & Development organized by Zoology Department & RUSA on 11th-12th March, 2022

SOPHIA COLLEGE (AUTONOMOUS)

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About Sophia College

Sophia College, founded in 1940, is affiliated to the University of Mumbai. The Governing Body of the Society for the Higher Education of Women in India runs the College. The religious sisters of the Society of the Sacred Heart of Jesus that was founded in France in 1800, by St. Madeleine Sophie Barat, and lay staff form the staff of the College. The main goal of Sophia is the relentless search for Wisdom, through an education that is holistic and transformative. The College has achieved an A++ Grade in its third NAAC cycle with CGPA of 3.70 on the scale of 4.0. In the academic year 2018-19, the college has been granted Autonomous status. Sophia College (Autonomous) offers Bachelor Degree programmes in Arts, Science, Mass Media and Information Technology, and Postgraduate M.Sc. programmes in Life Sciences, Microbiology, and Chemistry as well as Postgraduate diploma course in Quality Assurance in Food & Pharmaceutical Industry. The college has a well-equipped Suman Tulsiani Research Centre which is recognized for Ph.D. programme in Applied Biology, Microbiology and Zoology. Sophia has illustrious alumnae from different walks of life as it offers a wide scope for girl students to explore their full potential through an academically rich, value-based education enhanced with many co-curricular and extra-curricular activities.

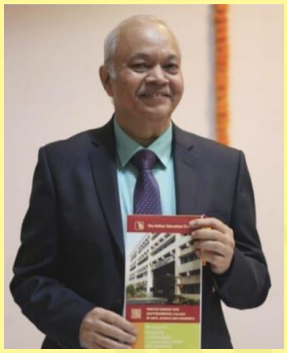
About Zoology Department

The department of Zoology was started in 1952. Zoology is offered as one of the subjects upto SYBSc level and the students subsequently major in Microbiology or Life Sciences at their TYBSc level. The department encourages students to actively participate in various academic as well as the co-curricular activities under Excellence in Science Program and DBT STAR Status by undertaking mini research projects, literature reviews and seminar presentation. The department also organizes environment related activities in order to sensitize the students towards the conservation of ecosystem and understanding the importance of biodiversity. Innovative pedagogies used by the department faculty members such as collaborative learning, enquiry-based learning, invited guest lectures, experiential learning through field-based studies offer an exciting and an enriched academic experience to the students. The syllabus under autonomy has incorporated a blend of basic as well as applied topics in order to give students a sound foundation knowledge of zoology. The department has spacious UG laboratory and is also a recognized research centre for Ph.D. in Zoology with well-equipped infrastructure facilities.

About The Conference

Toxicological research has been of great significance in making the life safer not just for humans but for all the living organisms found in various ecosystems. The field of toxicology research and development has also contributed to the development of selective toxicants that could serve beneficial to humans such as pesticides, anticancer and other clinical drugs. Thus, toxicants can have a dual role to play and scientific interventions are necessary to strike a balance between their excessive usage and mitigation of their harmful effects. This conference therefore, aims at exploring the toxicological studies carried out in various fields which may also be mutually connected. The conference has been planned with an objective to orient the UG and PG students to toxicology as one of the dynamic research fields. It will also offer a platform for the researchers to present their research work in this field whereby an exchange of scientific knowledge could be facilitated.

Plenary Session Speakers:



Keynote Address: 11th March 2022
Topic: Medical Toxicology
by
Dr. Bharatbhushan B. Sharma
Principal (Retd.), Advisor, Kelkar Education Trust's
V. G. Vaze College (Autonomous), Mumbai.

Plenary Session-I: 11th March 2022
Topic: Regulatory Toxicology
by
Dr. Sasikumar N. Menon
Director, Institute of Advanced Research in Interdisciplinary Sciences (TDM Lab.);
Associate Professor, Pharma Analytical Sciences. KAUSHAL Kendra, Ramnarain Ruia Autonomous College, Mumbai.

Plenary Session-II: 12th March 2022
Topic: Venomics - The Current Perspective
by
Dr. Aldon Fernandes
Vice President - Research and Development Biologicals, Bharat Serums and Vaccines Limited, Navi Mumbai

Plenary Session-III: 12th March 2022
Topic: *Chironomus* as a model organism in Toxicology and Environmental Biomonitoring System
by
Dr. Bimalendu B. Nath
Director, MIE SPPU Institute of Higher Education (Overseas campus of Pune University), Doha-Qatar;
Emeritus Professor, Centre for Advanced Studies, Department of Zoology, SPPU, Pune

Session Chairs for Oral Presentations:

- **Dr. Hemalatha Ramchandran**, Associate Professor, Sophia College
- **Dr. Vinod Narayane**, Associate Professor, B. K. Birla College

CONTENTS

EVALUATION OF SILVER NANOPARTICLES ON ANTIOXIDANT ENZYMES OF ZEBRAFISH, *DANIO RERIO*.

Dr. Abhay D. Shelke. (Associate Professor and Head)

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Objective: The objective of the present study was to evaluate the effect of Silver nanoparticle on antioxidant enzyme activities in liver, gills and muscle tissues of Zebrafish, *Danio rerio*.

Methods: Chronic toxicity (LC50) test of silver nanoparticles on adult fish, Danio rerio were conducted in a static water renewal experiment, according to the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals (OECD 1992). Catalase (CAT) was estimated by method prescribed by (Sinha, 1972). Superoxide dismutase (SOD) was estimated by method (Das et al., 2000). Glutathione Peroxidase (GPx) was estimated by method (Rotruck et al., 1973). One way analysis of variance (ANOVA) was applied to test the significance of data. All the data are expressed as means (n=5) ± standard deviation (SD) and differences were considered significant at $P<0.05$.

Results: In chronic toxicity study adult fish, Danio rerio were divided in to two groups. First group was experimental group in which fish were consecutively treated with a graded series of 0.3, 0.6, 0.9 mg/l an average 60 nm. PVP coated AgNPs. Second group were kept as a control which was free from AgNPs exposure. Treatment were given for 21 days at the end of experimental period, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in gill, liver and muscle tissues were assayed. The levels of catalase (CAT), was found to be decreased were as the superoxide dismutase (SOD) and glutathione peroxidase (GPx) were found to be increased significantly in gill, liver and muscle tissue of AgNPs treated fish.

Conclusion: Silver nanoparticle AgNPs treated group showed decreased CAT activity in Liver, Gill and Muscle tissues, while higher activity was noted in the control group. The decrease in the CAT activity may be the metallic nature of nanoparticles and the presence of transition metals encourages the production of reactive oxygen species (ROS) leading to oxidative stress. Tissue specific responses in the activities of antioxidant enzymes such as SOD and catalase were observed during sublethal AgNPs exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues. Significant increase in the activity of glutatione peroxidase (GPx) in the liver, gills and muscle tissues of Zebrafish, *Danio rerio* exposed to AgNPs. Toxicity of AgNPs in zebrafish increase oxidative substances in cells, which may cause an elevation of antioxidant enzymes as a defence mechanism.

Key words: Nanotoxicology, Silver nanoparticles (AgNPs), Antioxidant enzymes, *Danio rerio*.

BIOCHEMICAL AND HEMATOLOGICAL PROFILES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) UNDER SUBLETHAL EFFECTS OF HEXAVALENT CHROMIUM

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Objective: In natural waters and/or aquaculture medium, fish are often exposed to chromium waste and demonstrate accumulative deleterious effects. Hexavalent chromium is considered as more toxic in the environment due to its higher solubility and mobility. To our knowledge, there are no studies concerning the effects of hexavalent chromium on *Oreochromis niloticus* hematology. This study presents hematological and biochemical parameters of Nile Tilapia (*Oreochromis niloticus*), affected by sublethal concentration of hexavalent chromium.

Methods: The fish in the experimental aquaria (three replicates each) were exposed to a sublethal potassium dichromate concentration of 2 mg L^{-1}, which was prepared as stock solution and added depending on the volume of the aquaria to obtain the required concentration. After a period of 28 days, parameters such as hemoglobin (Hb), hematocrit (Hct), neutrophils (Neu), lymphocytes (Lym), total protein (TP), albumin, immunoglobulin M (IgM), glucose, red and white blood cells (RBC and WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were studied.

Results: Chromium exposure for 28 days significantly ($P<0.05$) between the Cr -exposed fish and the control. The amounts of Hb, Hct, RBC, WBC, MCHC and MCH significantly decreased ($P<0.05$) as a result of chronic hexavalent chromium exposure, whereas blood glucose and albumin significantly ($P< 0.05$) increased in the experimental fish as opposed to the control.

Conclusion: Water born metals may alter the haematological and biochemical parameters of fish. Hematological indices of fish, caused by hexavalent chromium toxicity to *Oreochromis niloticus*, can be secondary responses to toxicants, including exposure to low concentrations of heavy metals, which reflect the launch of stress reaction in the affected fish.

Keywords: Biochemistry**,** Hexavalent chromium, Hematology, Nile Tilapia.

EVALUATION OF TERATOGENIC EFFECTS OF *ANACARDIUM OCCIDENTALE* L. NUTSHELL EXTRACTS ON ZEBRAFISH (*DANIO RERIO*) EMBRYOS

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Objective: Herbal extracts of therapeutic relevance are of great importance as they have the capability of being used as lifesaving medicines. While conventional medicines have well-defined chemical composition and are supported by evidence-based published studies in terms of their efficacy and toxicity, this has not been the case with herbal medicines. One such herb which is being employed largely in various pharmacological formulations is *Anacardium occidentale* L. commonly known as Cashew nut plant. The nutshells of this plant have a variety of therapeutic applications against cancer, aging, urinary and metabolic disorders. However, in order to avoid the health concerns that might result due to its excessive usage, there is a need to investigate its teratogenic risks in early phases of development. Our work, therefore, aims at evaluating the developmental toxicity of cashew nutshell extracts on Zebrafish (*Danio rerio*) embryos.

Methods: The four extracts of cashew nutshells were obtained by Soxhlet extraction method. Out of these, three extracts were shortlisted for further teratogenicity assays on the basis of their solubilities. The embryotoxicity of these extracts was determined on zebrafish embryos as per the OECD guidelines. The teratogenic effects like heart rate, scoliosis, functional area change were observed in treated embryos. The extract showing no observable teratogenic effects was then selected for further biochemical analysis. The total protein content, lipid content, cholesterol content, aspartate transaminase and alanine transaminase activities were determined from whole embryo homogenate and compared with control set.

Results: Among the four extracts obtained, the aqueous, chloroform and organic extracts showed higher yield and solubility and thus selected for embryotoxicity assays. The aqueous extract showed the highest LC50 with no observable teratogenic defects in treated embryos. However, it was observed that the treatment with higher concentration of aqueous extract has resulted in disturbed heart systole, decreased protein content, increased lipid and cholesterol with elevation in AST and ALT activities in treated embryos. All of these teratogenic effects were reported at the concentrations above 62.5 µg/ml.

Conclusion: From the study, it was concluded that aqueous extract of cashew nutshell is nonteratogenic when used below the concentration of 62.5 µg/ml.

Keywords: Cashew nuts, embryotoxicity, developmental defects, Biochemical analysis

IN SILICO TOXICITY ANALYSIS AND ANTI-TRYPANOSOMAL ACTIVITY OF BUFADIENOLIDES FROM TOAD TOXIN

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Objective: Toxins can also comprise of components that are beneficial in small doses, but poisonous in large amounts. Therefore, they serve as excellent blueprints for drug design and therapeutics. Until the last decade, an increasing number of studies have shown that toad toxin is a source of lead compounds for the development of potential drugs. Bufadienolides, found primarily in toad toxin have been documented to be used in therapeutic applications against variety of infectious diseases. One of such disease is Trypanosomiasis also known as sleeping sickness that have caused devastating epidemics during the 20th century and is targeted for elimination by WHO. The disease is caused by the infection of *Trypanosoma brucei* and is transmitted to humans by bites of infected tsetse flies. In the absence of a vaccine, control of this disease relies only on case detection and treatment. The present study therefore is undertaken to provide comprehensive details on targeting bufadienolides against the hydrolase of *Trypanosoma brucei* using molecular docking-based virtual screening, binding mode analyses and free energy calculations.

Methods: Bufadienolides structures were downloaded from PubChem in sdf format and screened for their potential toxicity using protox-ii software. Each bufadienolides were then ranked on the basis of their potential toxicity on variety of organs, cell types and receptors. The bufadienolides showing minimum toxicity were shortlisted and molecular docking was performed using Autodock software. The complexes of bufadienolides with hydrolase of *Trypanosoma brucei* were simulated for 50 runs. On the basis of RMSD plots, the best-ranked simulation showing maximum binding energy score was determined.

Results: The toxicity evaluation of bufadienolides have shown moderate to mild toxicity in-silico with acute toxicity score ranging between 2 to 6. The two bufadienolides namely resibufogenin and marinobufogenin have shown minimum toxicity and therefore selected for further study. These toxins displayed desirable molecular interactions and excellent binding affinity towards the *Trypanosoma brucei* hydrolase when compared with standard drugs.

Conclusion: From the study, it was concluded that Resibufogenin and marinobufogenin have the potential to be employed as anti-trypanosomal drugs. Further in-vitro studies are required to authenticate the effectiveness of these bufadienolides against *Trypanosoma brucei* parasites.

Keywords: Bufotoxins, Autodock, Hydrolase, Protox-ii, Trypanosomiasis.

IN VITRO TOXICITY OF ESSENTIAL HEAVY METALS ON THE RED BLOOD CELLS OF *CAPRA HIRCUS*

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Objective: Essential heavy metals play an indispensable role in various physiological processes and metabolic activities of organisms in trace quantities. However, excessive exposure to higher concentrations of these elements is linked with cellular and systemic disorders in animals. Owing to their stable and non-destructive properties, they are widespread and fairly persistent in the ecosystems. Heavy metals accumulate in animal blood and tissues as they consume the plants and water with higher heavy metal load. Additionally, farmers across the world are known to administer essential heavy metals to animal feed in order to meet their nutritional requirements. The present research was therefore undertaken to study the in-vitro effect of essential heavy metals namely Cobalt, Copper, Zinc, Iron, Manganese and Molybdenum on the red blood cells of ruminants.

Methodology: The venous blood of *Capra hircus* was collected in CPDA buffer, washed with phosphate buffered saline and red blood cell suspension of 50% haematocrit was prepared. Five concentrations for each of the heavy metals below their saturation points were selected and haemolysis was calculated by INVITTOX Protocol No. 9. Thin blood smears of the treated suspension were prepared and observations regarding the area and shape of the RBCs were noted down.

Result: After the treatment of RBCs with the selected heavy metals, no significant haemolysis was observed. However, poikilocytic cells were seen in large numbers in all treated concentrations. In the suspension treated with cobalt, the number of teardrop cells increases with the concentration while in copper treated cells, presence of HbC crystals were reported. Cells exposed to iron showed increase in agglutination with increasing concentration while in cells mediated with manganese, elevation in macrocyte count was observed. Derpanocytes and keratocytes were reported at highest concentrations in molybdenum treated cells. In case of zinc, increase in microcytes and agglutination was documented with rising concentration.

Conclusion: From this study, it can be concluded that the treatment with essential heavy metals induces notable changes in shape of red blood cells. These imperceptible effects may cause reduction in oxygen carrying capacity of RBCs and thus possibly affect the overall health of an animal.

Keywords: Poikilocytosis, HbC crystals, Agglutination, Haematology

THE TOXIC EFFECT OF PESTICIDE DICLOROVOS ON THE PROTEIN METABOLISM OF THE FRESH WATER FISH, *CHANNA PUNCTATUS*

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Objectives: A list of water sources has been compiled, and the overall state of water quality in the rivers, lakes and groundwater has been examined.
Risks of polluted water to health of living organisms and the impact on environment have been adequately assessed. For the prevention, treatment, and restoration of polluted water in rivers, lakes, and ground water, appropriate policies and programmes have been developed.

Material and Methods: The fresh water resources are very precious for the life on our planet. The number of dams, reservoirs, tanks, etc. has significantly increased in last few years. The aquatic ecosystem is important and it has large number of economically important animals especially fish which is an important source of food. The fresh water fish, *Channa punctatus* was collected from local fish market and washed with 0.5% KMnO4 Solution to prevent it from infection and kept in an aquarium of capacity 50 litres, After One weeks of acclimatization fishes were exposed to different concentrations of Dichlorvos. Stock solution of 1mg/ml Dichlorvos was prepared in absolute alcohol. Fishes of similar sizes (15 ±5 cm) and weight (± 160.25 gm.) were sorted out and separated into 2 groups of 20 fish each. They were exposed to 0.625 ppm of Dichlorvos in 50 litre glass aquaria. One set of fishes are maintained as control in tap water. Fishes were exposed in 0.625ppm concentration of the pesticide Diclorovos for 24, 48 72 and 96 hours respectively Changes in protein content (mg/g) in the liver, Kidney, Muscle and gills of *Channa puntatus* exposed to pesticide Diclorovos for different periods**.**
Result and Conclusion: The Protein content in liver, Kidney, Muscle and gills tissues exposed to pesticide Diclorovos for 24, 48 72 and 96 hours showed decreasing trend when compare to control.

Key words: Protein, Dichlorvos, *Channa punctatus*

OXIDATIVE STRESS RESPONSE OF ADULT ZEBRAFISH (*DANIO SP.*) TO CLOTHIANIDIN

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Objective: In India, pesticides are widely used in agriculture in order to increase the yield and to improve the quality and storage life of the products. Use of pesticides is increasing in residential areas as well for controlling pathogenic and non-pathogenic insect pests. Synthetic organic insecticides like neonicotinoids and other types, can potentially alter the ecosystems and can be also harmful to humans. Neonicotinoids account for 26% of the global insecticide market. Clothianidin is one of the neonicotinoid insecticides. Being a neonicotinoid insecticide, Clothianidin has comparatively higher LC50 values for vertebrates and it is considered as a safe insecticide. However, data regarding effects of sublethal concentrations of Clothianidin on vertebrate model organisms is missing in the current literature. Therefore, the objective of the present study was to determine the effect of chronic treatment of Clothianidin in fresh water systems on the antioxidant enzyme system using adult zebrafish (*Danio rerio*) as a model organism. Zebrafish is one of the popular model organisms which is approved by NIH and has many advantages in carrying out various experimental studies.

Methodology: Adult zebrafish were exposed to sublethal concentrations ranging between 30 to 110ppm of commercial formulation of Clothianidin for 21 days in a semi static system. A control and vehicle control group was simultaneously maintained. All the animals were maintained as per the OECD guidelines at the CPCSEA registered zebrafish housing facility of Sophia College. After the treatment period, animals were sacrificed ethically and specific activities of oxidative stress enzymes such as Catalase (CAT), Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Peroxidase were measured in the liver tissue. Specific activity of enzyme Acetylcholinesterase (AChE) was measured in the brain tissue.

Results: Results obtained in this study showed that the specific activity of GST, AChE and Peroxidase decreased for all the treated groups. However, specific activity of SOD increased in all the treatment group as compared to control. Specific activity of Catalase increased in treated groups of lower concentrations whereas it decreased for those exposed to higher concentrations.

Conclusion: This study showed that the neonicotinoid insecticide - Clothianidin may induce oxidative stress response in adult zebrafish on chronic exposure. Further investigations through other biochemical parameters as well as histopathological studies would help to understand the mechanism of action of Clothianidin on the oxidative stress enzyme system in adult zebrafish.

Key words: Clothianidin, Pesticide toxicity, Zebrafish, Oxidative stress enzyme system

ECOTOXICITY OF PLASTICIZER DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) IN EMBRYOS OF ZEBRAFISH (*DANIO SP.*)

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Objective: Plasticizers have been used for centuries to improve the processability and flexibility of a variety of materials. Today the most common plasticizers are esters of phthalates, adipates, citrates etc., the majority of which are used to produce flexible polyvinyl chloride (PVC) products. Exposure of the human fetus and infants to phthalates via maternal exposure is a rising matter of concern. DEHP enters and pollute the environment through release from industrial settings and plastic waste disposal site and therefore poses a threat to aquatic fauna. The current study was undertaken to understand the impact of DEHP on embryos and larvae of zebrafish. Nearly transparent and fast growing embryos of zebrafish are ideal models for this study as they have 70% genome similarity with humans. The research study will not only help to know the toxic effects of DEHP in developmental stages of vertebrates but also to know the adverse effects on environment.

Methodology: Five pairs of adult zebrafish were kept for breeding (mass spawning) in an aquarium with mesh at the bottom to prevent cannibalization of the eggs. Six 24-well plates were filled with 2 ml of test solution, one for control and five for different concentrations of DEHP – 0.2, 20, 80, 140 and 200 µg/L. The fertilized eggs at 1 hpf (hour post fertilization) were put into the plates, one egg in each well. Each of five plates of the treatment groups had 20 embryos in DEHP solution and 4 embryos as internal control. The embryos were incubated at 28±1°C and observed every 24h under compound microscope upto 96 hpf. The study was carried out according to the guidelines of OECD Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

Result: The results exhibited significant increase in cumulative mortality of the treated groups after 48 hpf compared to the control. Developmental and morphological abnormalities like yolk sac edema, pericardial edema, cerebral edema, delayed hatching, altered body curvature, bent spine were seen in treated embryos.

Conclusion: From this study it could be concluded that DEHP exposure even for short period leads to developmental defects in early life stages of zebrafish. Widespread use of DEHP could lead to potential harm to nontarget species like fishes and aquatic vertebrates in fresh water ecosystem.

Keywords: Phthalate, DEHP, Zebrafish embryo, Developmental toxicity

PROPHYLACTIC AND CURATIVE ROLE OF L-ASCORBATE ON CADMIUM ACCUMULATION IN THE HEPATOPANCREAS OF THE FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS* (LAMARK)

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Objective: To investigate the prophylactic and curative role of L-ascorbate on Cadmium accumulation in the hepatopancreas of the freshwater bivalve, *Lamellidens marginalis*

Methods: 1. Acclimatization: Bivalves were collected from Titwala river which is nearly 15 Kms away from Kalyan city Dist: Thane (M.S.). After Collection, bivalves were acclimatized in laboratory conditions They were cleaned and acclimatized in laboratory condtions at room temperature for 2-3 days. The active acclimatized bivalves approximately of same size were selected for experimentation.

2. Treatment: Bivalves were divided into three groups such as A, B and C

i) 'A' Group Bivalves were maintained as Control

ii) 'B' Group Bivalves were exposed to chronic dose ($LC_{50/10}$) of heavy metal salt $CdCl_2$ (0.0252ppm Cd^{++} for 21 days

iii) 'C' Group Bivalves were exposed to chronic dose ($LC_{50/10}$) of heavy metal salt $CdCl_2$ (0.0252ppm Cd^{++} with ascorbic acid (50mg/L)

Bivalves from 'B' group were divided into D and E group after 21 days chronic exposure.

iv) 'D' group bivalves pre-exposed to chronic dose ($LC_{50/10}$) of heavy metal salt $CdCl_2$ were allowed for self cure in normal water while,

v) 'E' group bivalves pre-exposed to Chronic dose ($LC_{50/10}$) of heavy metal salt $CdCl_2$ were exposed to ascorbic acid (50mg/L) from rapid recovery from tissue damage.

The experimental bivalves of A, B and C group were dissected after 7days and 21 days and from D and E group of recovery after 3 days and 6 days. Hepatopancreas from all 5 groups were dried at 80 ^{o} C in an oven still constant weight was obtained. These powders obtained were stored in airtight specimen bottles by waxing the cork outside. These powders were used to estimate bioaccumulated cadmium by Atomic Absorption Spectrophotometer.

Results: Cadmium Content (ppb) in hepatopancreas of *L.marginalis* after heavy metalsalt $CdCl_2$ with and without ascorbic acid and during recovery.

Treatment		7 days	21 days	Recovery	
				3days	6days
Control (A)		00	00	-	-
B Gr.		5396.2	6535.0	-	-
C Gr.		2503.6	3920.15		
After 21 days	D Gr. Normal water	-	-	6239.3	5338.4
	E Gr. Normal water with Ascorbic Acid	-	-	5139.4	3259.2

Conclusion: L-ascorbic acid is an excellent antioxidant to prevent and cure heavy metal accumulation in the hepatopancreas of *Lamellidens marginalis*

Keywords: Cadmium Chloride, Bioaccumulation and *Lamellidens marginalis*

IN VITRO CYTOTOXICITY AND ANTI-AGEING ACTIVITY OF HYDROLYZED COLLAGEN FROM SHRIMP SHELLS FOR ITS COSMETIC APPLICATION

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Objective: Collagen is an essential protein that gives our skin youthful appearance. The primary cause of early wrinkling is ultraviolet radiation, which accelerates the degradation of collagen fibres present in our skin. Thus, there is a need to explore the possibilities for external application of collagen through cosmetic formulations. Collagen can be obtained from variety of animal sources such as rat tail, duck feet, equine tendons, frog skin, etc. Even though these sources are cheap and easy to attain, the process is inhuman and unethical. As a solution to this problem, the feasible alternative source is marine collagen. The remains of shrimp waste can act as an excellent source of collagen that can be incorporated into novel products with a high functional value. In this paper, collagen was extracted from indigenous shrimp shell, with the goal of including it as a component in cosmetic formulations.

Method: The hydrolysed collagen was obtained from four species of dried shrimp shells by complete and minimal hydrolysis. The collagen content from hydrolysate was determined by hydroxyproline assay and the species with highest collagen content was shortlisted for anti-ageing and toxicity evaluation. The assessment of anti-ageing activity was performed by anti-elastase assay kit (MERCK). The cytotoxicity of hydrolysed collagen on 3T3 fibroblast cells was determined using MTT assay. It was then incorporated in oil-in-water form of emulsion and various parameters such as pH, homogeneity, appearance, stability and after feel were observed.

Results: Out of the four species of shrimps, the red tail shrimp yielded highest amount of hydrolysate with maximum collagen content. The hydrolysate also showed low cytotoxicity on 3T3 fibroblast cells with IC50 value 31.28 mg/ml. The anti-elastase activity of hydrolysate was found to be excellent with IC50 value 268.52 µg/ml. The oil-in-water emulsion containing hydrolysed collagen have shown good spreadability, humectant property and was stable for more than 6 months.

Conclusion: The shells of red tail shrimp can be used to obtain collagen rich hydrolysate which can be utilized for formulating anti-aging cream. The further clinical studies to evaluate the efficacy of anti-aging formulation is needed to be conducted.

Keywords: Anti-elastase, MTT, Hydroxyproline, Oil in water emulsion

IN VITRO RBC TOXICITY AND ANTIPLASMODIAL ACTIVITY OF *ANACARDIUM OCCIDENTALE* L. NUTSHELL EXTRACTS

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Objective: Malaria is a life-threatening disease causing major public health concern in many countries. Emergence of new *Plasmodium* strains resistant to conventional antimalarial drugs are acting as barriers in combating malarial infections. Therefore, there is a need to explore novel antimalarial compounds with higher potency and efficacy. While synthetic pharmaceutical agents continue to dominate antimalarial research, attention has increasingly been directed to natural products. Several plant species with plausible antimalarial effects are currently being explored by the scientific community. *Anacardium occidentale* L. is one such plant which is believed to have unique phytocompounds with potential therapeutic activity against malaria. Our work therefore aims at investigating toxicity of *A. occidentale* nutshell extracts on human red blood cells and assessing their antimalarial activity.

Methods: The four extracts of *A. occidentale* nutshells were obtained by Soxhlet extraction. Out of these, three extracts were shortlisted for further assays. The toxicity of these extracts on red blood cells were evaluated by assessing their hemolytic potential and hemoglobin denaturation index by INVITTOX Protocol number 9. The shortlisted extract was then subjected to antimalarial assay against *Plasmodium falciparum* 3D7 cells *in vitro*. After 72 hours of exposure, reduction in parasitemia in culture treated with extract was calculated and IC_{50} was determined using regression analysis.

Results: From the four extracts obtained, three extracts namely aqueous, chloroform and organic were selected because of their higher yield and solubility. The toxicity studies on red blood cells showed that the aqueous extract exhibited the least toxicity with highest HC_{50} (Hemolytic concentration 50) value 29.643 mg/ml. The same extract has also not shown any hemoglobin denaturation till its saturation point. In antimalarial assays, the aqueous extract showed maximum inhibition of parasites followed by organic and chloroform extracts. After evaluating the data obtained from toxicity and antimalarial studies, the therapeutic index of aqueous extract was found to be higher when compared with other extracts.

Conclusion: From the study, it was concluded that the aqueous extract is best suited to be used as an antimalarial agent because it showed least RBC toxicity along with highest antimalarial activity.

Keywords: Malaria, Cashew nuts, Hemolysis, Therapeutic index.

SOIL CONTAMINATION BY HEAVY METALS: A RISING ENVIRONMENTAL PROBLEM

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Objectives: Bhandup Pumping Station (BPS) is a pumping station where the sewage and waste water from Mumbai suburbs are received, treated and recycled. BPS is located along the Eastern Express Highway, in the proximity of the thane creek. The mangroves and mudflats here serve as an ideal habitat for different species of birds and other organisms. However, several anthropogenic activities have caused serious damage to this ecosystem; including heavy metal pollution. Heavy metals have potential toxicity and can transport through the roots of the plants into the fruits and leaves on which the birds feed. Thus, there would be bioaccumulation and biomagnification of the heavy metal pollutants. The heavy metal pollution is of great threat to the flora and fauna in any ecosystem. Therefore, the objective of the study is to determine the levels of heavy metals (Pb, Cu and Zn) in soil sediment samples at BPS to evaluate its toxicity and to take further measures.

Methods: Soil sediment samples were randomly collected from 10 different sampling stations at BPS. It was processed for further analysis. Heavy metal Lead was estimated by sodium sulphide method. Copper was detected by the carbamate method. Zinc was determined by dithiozone method.

Results: In the present findings, the lowest concentrations of lead detected in soil sediments was 0.01$\pm$ 0 mg/g while the highest value was found to be 1.61$\pm$ 0.01 mg/g. The minimum amount of copper was found to be 0.90$\pm$ 0.01 µg/g and maximum level recorded was 7.57$\pm$ 0.01 µg/g. Zinc estimated was found to be at a least value of 0.5$\pm$ 0.02 µg/g and highest value noted was 2.32 $\pm$ 0.04 µg/g in the soil sediments.

Conclusion: Our study determines the levels of heavy metal pollution in soil sediment samples. The results obtained will help to monitor and control the heavy metal pollution at BPS.

Keywords: Heavy metal, Environment, Bhandup pumping station.

EVALUATION OF ALCOHOLIC BARK EXTRACT OF *GARUGA PINNATA* ROXB. ON REPRODUCTIVE FUNCTION IN FEMALE SWISS ALBINO MICE

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Objective: The use of herbal medicines has become a popular alternative to the synthetic drugs. There is common belief that the natural drugs do not have any side effects. The herbal extract exhibiting a functional efficacy may have a systemic effect on another organ. The common target of organ toxicity is skin, liver, lungs, and kidney. However, there is need to evaluate the mechanistic effect of the drug interaction with the reproductive function. The present study evaluated the effect of alcoholic bark extract of *Garuga pinnata* Roxb. on the reproductive function in female Swiss albino mice. The bark of this tree from Burseceae family is traditionally used to treat various aliments like eye infections, inflammation, wounds, liver disorders, diabetes to enlist a few.

Method: The experimental animals were divided into two groups with eight females and two males. Both the groups received respective doses of test extract 7 days prior to mating. Control group received 0.5% carboxymethyl cellulose and experimental group received a daily dose of 250 mg/kg body weight of reconstituted extract by gastric intubation. The observations were recorded pertaining to maternal behaviour, food intake, survival rate of the females and duration of pregnancy. The litter size, viability index, gross malformations, general development of the newborn pups was also recorded.

Results: All the parameters under study were normal except the litter size. The viability index was found to be 91% in control animals and 86% in test animals.

Conclusion: The data obtained were analysed using Single factor ANOVA. It was found that the P-values for both, litter size and viability index were greater than (>) 0.05 level of significance thus leading to the conclusion that the differences between the control and test group were statistically non-significant.

Keywords: Female reproductive toxicity, *Garuga pinnata* Roxb., Swiss albino mice.

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