

# CHAPTER 50

## ZOOLOGY

### Doctoral Theses

01. DANGI (Kapil)  
**Role of Nitrosative Stress in Targeting Autophagy Elucidating Signaling Pathways Induced by Combinatorial Therapy of Gambogic Acid-Carboplatin in Breast Cancer.**  
Supervisors: Prof. Indrakant K. Singh and Prof. Anita Kamra Verma  
Th 27843

#### *Abstract*

Cancer continues to represent one of the most lethal diseases globally, accounting for an estimated 10 million fatalities annually. The therapeutic combination of Carboplatin and Gambogic acid has demonstrated enhanced chemosensitization capabilities, crucial for inhibiting mechanisms such as drug resistance, cellular proliferation, angiogenesis, and metastatic processes, thereby promoting the survival of healthy cells. The chemosensitivity and cytotoxic effects of both Carboplatin and Gambogic acid, whether applied singly or in combination, were evaluated on MCF-7 and MDA-MB-231 cancer cell lines in a manner dependent on both dosage and duration. In addition to empirical testing, in-silico analyses were conducted to predict and evaluate the binding affinities of Carboplatin and Gambogic acid with key apoptotic and autophagic proteins, aiming to elucidate the molecular targets involved in their action mechanisms. This computational approach aids in understanding how these compounds interact at the molecular level, potentially guiding targeted therapeutic strategies. Furthermore, the assessment of oxidative and nitrosative stress responses induced by the combined treatment with Gambogic acid and Carboplatin was performed. This aspect of the study investigates the biochemical pathways through which the drug combination exacerbates oxidative damage within cancer cells. The combination of Gambogic Acid (GA) and Carboplatin (CP) significantly augmented reactive oxygen species (ROS) accumulation and lactate dehydrogenase (LDH) release in both MCF-7 and MDA-MB-231 cell lines. Mitochondrial membrane potential (MMP) was notably reduced, while levels of glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GP) and glutathione reductase (GR) decreased, further enhancing ROS. Enhanced nitric oxide (NO) levels and reduced superoxide dismutase (SOD) activity contributed to increased nitrosative stress. Apoptosis was induced as evidenced by upregulated expression of Bax, p53, Caspase, and Caspase 3, alongside downregulated Bcl-2 expression. Furthermore, increased nitrosative stress promoted autophagy via upregulation of Beclin-1, ATG5, ATG7, and LC3. Collectively, these multidisciplinary approaches encompassing biochemical assays, molecular docking studies, and oxidative stress evaluations underscore the potential of combining Carboplatin and Gambogic acid as a more effective cancer therapy by addressing the complex pathways involved in cancer cell survival and resistance.

#### *Contents*

1. Review of literature
2. Materials and methods
3. To ascertain the therapeutic efficacy of ga and cp in breast cancer cells
4. Antineoplastic efficacy
5. Molecular

docking studies 6. In-vitro cell culture studies: assessment of oxidative and nitrosative stress 7. Induction of apoptosis and autophagy. Discussion. Summary. References. List of publications and posters presented.

02. GOSWAMI (Nidhi)

**Deciphering the Impact of Phytochemical in Inhibiting Neuroblastoma Progression Through a Targeted Approach.**

Supervisor: Prof. Indrakant Kumar Singh

Th 27845

*Abstract*

The purpose of this study was to identify an effective target and phytochemical for the inhibition of neuroblastoma tumour, and to assess the anti-proliferation, anti-migratory, and anti-cell death effects of these compounds on neuroblastoma cell line. The cell death pathway's molecular mechanism was deciphered using RT-qPCR and western blot. Databases and web servers were used to identify possible targets in neuroblastoma, and Cytoscape was used to create a molecular network. The majority of the genes that were found were connected to the cell cycle and apoptosis. On further assessing the common genes from the two pathways, aurora kinase B (AURKB) was selected. Nevertheless, the R2 platform's survival plot analysis revealed that AURKB's elevated expression lowers the survival rate of neuroblastoma patients. Twenty phytochemicals were selected after PubMed screening, and their ADMET evaluation was carried out using the predictive results obtained from several databases. Using Schrodinger's software suite, the top five phytochemicals selected based on the ADMET study results were subjected to docking, MD simulation, and binding free energy calculations. Subsequently, andrographolide was examined for its ability to inhibit cell death, migration, and proliferation. After a 24-hour incubation period, its cytotoxicity was less in the normal cell line NIH3T3 than it was in the mouse neuroblastoma cell line Neuro2a. Subsequent in vitro assay analysis revealed that andrographolide caused anti-migration, ROS production, cell cycle arrest, morphological changes, and enhanced cell death in Neuro2a cells via the intrinsic apoptotic mechanism. Finally, a functional analysis of the GeneMANIA webserver's Gene Interaction Map was conducted. Hub genes AURKB, ALYREF, CTNNB1, BCL2, BAX, CASP3, and MMP9 along with the target were chosen based on literature review on neuroblastoma. After Andrographolide treatment in Neuro2a cells, their expression analysis using RT-qPCR and western blot showed decreased expressions of pro-migratory and pro-survival genes/proteins and increased expressions of pro-apoptotic genes/proteins.

*Contents*

1. Introduction 2. Review of literature 3. Determination of potential target to inhibit neuroblastoma progression by in silico tools 4. Computational screening of phytochemical for inhibition of neuroblastoma progression 5. In vitro evaluation of screened phytochemical in neuroblastoma cell line 6. Molecular assay-based evaluation of target genes identified using gene interaction network 7. Conclusion and future perspective. List of publications. List of abstracts (Poster/oral) in conferences.

03. GUDDU KUMAR

**Formulation of Feeds for Snow Trout *Schizothorax Richardsonii* (Gray, 1832) to Increase its Omega-3 (Epa+Dha) Fatty Acid Content to Promote the Human Health.**

Supervisor: Prof. Rina Chakrabarti

Th 27846

*Abstract*

Snow trout, *Schizothorax richardsonii* Gray, 1832, (family Cyprinidae) is the most widely distributed and important fish of the Himalayan region. Snow trout, *S. richardsonii* is a bottom feeder and feeds on the attached material, specifically algae in the bottom stones and boulders. Major challenges for the culture of snow trout are poor growth rate and fish are more prone to fungal infections. Therefore, the selection of feed ingredients for snow trout and formulation of healthy diet are the focus of the present study. The herb *Achyranthes aspera* L. (family Amaranthaceae) seeds enhance the immunity of different carps. Biochemical composition of twelve freshwater macrophytes (*Azolla microphylla*, *A. pinnata*, *Enhydra fluctuans*, *Hydrilla verticillata*, *Ipomoea aquatica*, *Lemna minor*, *Marsilea quadrifolia*, *Pistia stratiotes*, *Salvinia molesta*, *S. natans*, *Spirodela polyrhiza* and *Wolffia globosa*) cultured in the outdoor facility were estimated. Sodium (Na) content was significantly ( $P<0.05$ ) higher in *P. stratiotes* compared to others. Significantly ( $P<0.05$ ) higher potassium (K) level was found in *W. globosa* compared to others. Polyunsaturated fatty acid  $\alpha$ -linolenic acid (C18:3 n-3) content was significantly ( $P<0.05$ ) higher in *I. aquatica* compared to others. This was followed by *L. minor* and *W. globosa*. Significantly ( $P<0.05$ ) higher final average weight was found in VC+AA diet fed snow trout compared to the control, VC and AA diets fed snow trout. Total n-3 PUFA content was significantly ( $P<0.05$ ) higher in VC+AA diet fed snow trout compared to other feeding regimes. Significantly ( $P<0.05$ ) higher eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) contents were found in LM-0 and LM-45 diets fed snow trout compared to other two treatments. EPA (C20:5 n-3), DHA (C22:6 n-3) and total n-3 PUFA contents were significantly ( $P<0.05$ ) higher (C22:6 n-6) in FLO-75 diet fed snow trout compared to other diets fed snow trout.

*Contents*

1. Introduction 2. Review of literatures 3. Materials and methods 4. Results 5. Discussion 6. Summary and conclusions 7. References 8. Publications.

04. JYOTI

**Taxonomic Studies on Sweat Bees (Hymenoptera: Apoidea: Halictidae) of North India.**

Supervisors: Prof. Sarita Kumar and Dr. Debjani Dey  
Th 27848

*Abstract*

Members of family Halictidae, commonly called as 'Sweat bees', are the most prevalent bees in many temperate regions of the world, after *Apis*. These form the second-largest group in terms of numbers within the Apoidea with more than 4700 species under 74 genera worldwide. The family comprises four subfamilies viz., Halictinae, Nomiinae, Nomoidinae, and Rophitinae. Present studies comprehensively analysed the distribution of Halictidae bees in different ecosystems of North India. Extensive explorations for collection of these bees were undertaken in different states of North India. The study also included old specimens of National PUSA Collection, Division of Entomology, ICAR-IARI, New Delhi; National Forest Insect Collection, Forest Research Institute; & specimens from Zoological Survey of India, North Regional Centre, Dehradun. An up to date annotated checklist of the Indian species compiled indicated a total of 243 species under 27 genera recorded from India. All synonyms and distribution records were included along with 39 new locality records in the checklist. The study included 50 species under the following 19 genera viz., *Halictus*, *Homalictus*, *Lasioglossum*, *Sphecodes*, *Austronomia*, *Crocisaspidia*, *Curvinomia*,

Gnathonomia, Hoplonomia, Leuconomia, Lipotriches, Macronomia, Maculonomia, Mayenomia, Nomia, Nomiapis, Pseudapis, Steganomus and Celalictus. Seven species new to science were described under genera viz., Homalictus (1), Lasioglossum (5) and Sphecodes (1). Several new characters including genitalia, >60 coloured plates with >350 photographs have been included in the study. Diagnostic keys for the genera and species studied have been compiled. The phylogenetic tree, constructed through maximum parsimony (MP) analysis based on 84 characters for genera and 145 characters for species with PAUP\*4.0b10, indicated that all the 19 genera and 50 species of family Halictidae formed different clades but shared a common ancestor and hence are paraphyletic. The present studies, thus have substantially augmented our knowledge of not only species diversity of Halictidae bees but also their phylogenetic relationships.

### Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Plates 6. Discussion 7. Summary and conclusion 8. Abstract 9. Literature cited 10. Annexures 11. List of publications.

05. KUMARI ILA

**Characterization and Expression of Genes Encoding Vitellogenin Receptor (VgR) and Aquaporin (Aqp), & their Localization in the Oocytes of the Murrel, Channa punctatus (Bloch).**

Supervisor: Prof. Neeta Sehgal

Th 27847

### Abstract

The eggs of all oviparous teleost are characterized by the presence of large amounts of nutrients for the developing embryo till it can feed on its own. These embryonic reserves are synthesized as vitellogenin(s) in liver, under the influence of estradiol-17 $\beta$  which is/are internalized via specific receptor-mediated endocytosis in oocytes. Thereafter, under favorable conditions, these oocytes undergo meiotic maturation and ovulation, accompanied with hydration process regulated by aquaporins. In the present study, vitellogenin receptor (VgR) and aquaporins (AQP) have been characterized using bioinformatic tools and localized in the oocytes. Although VgR protein has been localized in the oolemma of previtellogenic and vitellogenic oocytes, but the expression of vgr is upregulated in previtellogenic oocytes resulting in the pool of mRNA. These transcripts are translated and translocated to the oocyte membrane for active incorporation of vitellogenin during vitellogenesis. AQP1 has been localized in the post-vitellogenic oocyte, and the concentration of AQP1 increases with the progression of maturation and ovulation. Aquaporin plays a principal role in oocyte hydration, hence expressed maximally during spawning period. The annual profile of genes in the ovary (vgr, aqp1, and aqp3) and liver (vg, chg, and er) has been correlated with gonadosomatic index during the reproductive cycle. Three genes, (vgr, chg, and vgc) are expressed in the beginning of oogenesis followed by vgb and vga, and finally ovary attains maximum size. Thereafter expression of vg and chg is downregulated in the liver, oocytes resume meiotic maturation and simultaneously express maximum of aqp1 and aqp3. The study also indicates that murrel responds to long photoperiod and high temperature by accelerating the process of ovarian recrudescence. Coordinated expression of VgR and AQP along with Vg and Chg for specific functions highlights the complex interplay of molecular signals within oocyte during ovarian growth.

*Contents*

1. Localization and characterization of vitellogenin receptor (VgR) in the oocyte 2. Localization and characterization of aquaporins in the oocyte 3. Expression of genes in liver and ovary under natural as well as experimental conditions 4. Summary. References and list of publications.

06. MITTAL (Parul)

**Biological Evaluation of Hetero/Homodimeric Ligands Based on Small Biomolecules as Theranostics for Neurodegenerative Disorders.**

Supervisors: Prof. Namita Agrawal and Dr. Puja Panwar Hazari

Th 27901

*Abstract*

Neurodegenerative disorders (NDDs) are a group of neurological conditions marked by gradual loss of neurons in either the central nervous system (CNS) or peripheral nervous system (PNS). In neurodegenerative disorders, there is an alteration in the cholinergic system which leads to severe dysfunction of neuronal circuits. Cholinergic neurons have a pivotal role in both preserving the excitation-inhibition balance within neuronal circuits and fine-tuning brain function. Acetylcholine (ACh), is an important neurotransmitter released by Cholinergic neurons. ACh is essential for controlling associative learning, memory, sleep and sensory functions. Acetylcholinesterase (AChE) is responsible for the pathophysiology and pathogenesis of several neurological disorders. In certain neurological disorders such as Alzheimer's disease (AD) and Huntington's disease (HD), AChE is hyperactivated in the synapses due to which the levels of acetylcholine in the brain are significantly diminished, which leads to weakened neurotransmission and thereby memory loss and other adverse effects. AChE inhibitors have been reported for their potential role in the treatment of NDDs. The cholinesterase enzyme may also be, at least partly, responsible for the buildup of amyloid  $\beta$  plaques and the neurofibrillary tangles in AD brains. Even though there are several AChE inhibitors available they act only symptomatically and not causally thereby not exerting a significant treatment regimen. Thus, there is a need for the development of Multi-Target Directed Ligand (MTDL) based on AChE inhibitor which can target several therapeutic targets responsible for the occurrence and progression of neurodegenerative diseases. In the present study, we have screened several ligands based on AChE inhibitors and performed their docking with AChE protein of different species. Based on these insilico studies, we have selected one potent homodimeric ligand and synthesized it and further assessed its inhibitory potency and performed its in vivo evaluation in HD model of *Drosophila* to study its therapeutic effect.

*Contents*

1. Introduction 2. Objective Definement 3. Computational studies for evaluation and selection of hetero/homodimeric ligands as potent AChE inhibitor as theranostics for neurodegenerative disorders 4. Synthesis and inhibitory activity assessment of selected homodimeric quinoline ligand targeting AChE 5. In vivo evaluation of newly synthesized AChE inhibitor in transgenic HD drosophila model. Summary. References. List of publications and conferences.

07. MONIKA

**Carboplatin Encapsulated Hybrid Nanoparticles Mediated Alteration of EMT Dynamics: To Study the Interplay of Cytokines with Akt/NF-Kb/Snail Pathway in NSCLC (A549 & H1299) and ROS-Mediated autophagy in C57BL/6 Mice.**

Supervisor: Prof. Anita Kamra Verma

Th 27852

*Abstract*

Lung cancer is still the leading cause of cancer-related deaths. In 2020-2021, 1.8 million lung cancer related deaths were reported, out of 2.2 million new lung cancer cases reported worldwide. Lung cancer is categorized as small cell lung cancer (SCLC) accounts for 15% cases and non-small cell lung cancer (NSCLC) accounts for 80-85 % of cases. Carboplatin is second generation platinum drug commonly used in the treatment of lung cancer. While it can be efficient against lung cancer, it has some side-effects such as thrombocytopenia, kidney issues and drug resistance. Carboplatin encapsulated Lipid-polymer hybrid nanoparticles (CP-LPN) were synthesized to overcome the limitations and to evaluate its efficacy in both in-vitro and in-vivo studies. CP-LPN induces oxidative and nitrosative stress in both A549 and H1299 lung cancer cells. The reduced level of GSH, GST and GP causes less intracellular inactivation of carboplatin and increased ROS accumulation. Also, the expression of ABC transporters ABCB1, ABCC1, ATP7b and GST-pi were downregulated by CP-LPN, this results in reduced efflux of carboplatin. As a results, the intracellular accumulation of carboplatin then efficiently binds with DNA, thus triggering apoptotic cell death. CP-LPN inhibits Akt/NF- $\kappa$ B/Snail pathway which is a survival pathway responsible for the metastasis and invasion of cancer cells. The Akt/NF- $\kappa$ B/Snail pathway inhibition causes upregulation of E-cadherin and downregulation of Vimentin inhibiting the epithelial to mesenchymal transition necessary for metastasis and invasion of cancer cells. The induction of autophagy by CP-LPN which is negatively regulated by Akt pathway further inhibits migration via destabilization of transcription factor Snail. The CP-LPN significantly reduced the tumor burden, increased ROS accumulation and oxidative stress in tumor tissue via ROS-mediated autophagy in LLC-1 tumor bearing C57BL/6 mice model. Thus, activating intracellular ROS by suitable nano-drug delivery system in cancer cells holds a great potential in anticancer treatment strategies.

*Contents*

1. Review of literature 3. Materials and methods 3. Synthesis and characterization of lipid-polymer hybrid nanoparticles 4. In-vitro release kinetics pattern 5. In-vitro cell culture studies 6. THP-1 macrophages in NSCLC co-culture: Cytokines, EMT, drug resistance, migration and invasion dynamics 7. CP-LPN modulation of EMT, autophagy, apoptosis and drug resistance 8. Ex-vivo haemo-compatibility, pharmacokinetics & bio-distribution of LPN hybrid nanoparticles 9. in-vivo therapeutic efficacy of CP-LPN: Tumor Regression, oxidative stress, histology, and autophagy in C57BL/6 mice. Discussion. Summary References. List of publication and posters presented.

08. NARWAL (Ritu)

**Hypothalamic Neuropeptides (Gnih and Gnrh) Control the Synthesis and Release of Gonadotropin(S) from Pituitary to Regulate the Ovarian Development in the Murrel, Channa Punctatus (Bloch).**

Supervisor: Prof. Neeta Sehgal

Th 27853

*Abstract*

In teleosts, the neuroendocrine mechanism regulates reproduction through the Hypothalamus-Pituitary-Gonadal (HPG) axis. Although it is a well-established fact that Gonadotropin releasing hormone (GnRH) is the major factor stimulating the synthesis and release of pituitary gonadotropins, its functional antagonist is not known in many perciform fishes. The negative regulation of HPG axis through Gonadotropin inhibitory

hormone (GnIH) (RFamide peptide) has been well studied and reported in tetrapods, however, the physiological and functional significance of GnIH in teleosts is still being examined. This study has been planned to characterize GnIH and to comprehensively understand its physiological significance in the Indian freshwater murrel, *Channa punctatus*. The possible interaction of GnIH with GtHs has been explored through in-vivo, in-vitro and in-silico studies. The full-length cDNA encoding GnIH, its two receptors, and  $\beta$  subunit of GtHs (FSH and LH) have been identified. The circannual rhythm of all these factors has been studied. An inverse relationship of GnIH has been observed with GtHs and GSI during the annual reproductive cycle. Inhibitory action of GnIH on the transcription and translation of GtHs (FSH and LH) has been observed under in-vivo and in-vitro conditions. The study elucidates that critical environmental photothermal information is transduced in the brain to regulate the neuroendocrine factors. Photoperiod and temperature show negative regulation of GnIH but GtHs are being positively regulated. In addition, melatonin plays a cardinal role in regulating the expression of GnIH. Upregulation of *gnih* and *mellcr* genes has been observed in the presence of exogenous melatonin. To conclude, the existence of GnIH has been confirmed in murrel and GnIH has an inhibitory effect on pituitary GtHs. Hence, GnIH plays a pivotal role in the neuroendocrine regulatory mechanism of HPG axis in murrel.

#### Contents

1. GnIH, GnIHR and GtHs ( $\beta$ ): Molecular characterization and bioinformatics analysis
  2. Profiling of GnIH, GnIHR and GtHs ( $\beta$ ) under natural and experimental conditions
  3. Interaction between GnIH and GnRH fine-tunes the reproduction in murrel. Summary. References and list of publications.
09. NIVERIA (Karishma)  
**Redox-Responsive Mesoporous Silica Nanoparticles for Codelivery of Sirna and Paclitaxel Targeting Bony Metastasis in Breast Carcinoma: In Vitro and in Vivo Approach.**  
 Supervisor: Prof. Anita Kamra Verma  
Th 27854

#### Abstract

Metastatic Breast cancer (mBC) affecting bones is the most common consequence in women worldwide. The bone microenvironment, metastatic tumor cells, osteoclasts, and tumor-associated macrophages (TAMs) all play a crucial role in creating a favourable environment for the proliferation, progression, and survival of the metastatic tumor, which in turn induces osteoclast-mediated bone destruction. Understanding the influence of the microenvironment in driving this process will help elucidate the molecular mechanisms of bone metastasis. Sclerostin is the master regulator in bone remodelling and is reported in sequential metastasis cascade it was inevitable to exploit it as a diagnostic marker and a therapeutic target. Amine functionalized mesoporous silica nanoparticles (MSNs) were engineered for co-delivery of siRNA (Sost) and PAX (Paclitaxel) for bone targeting by surface modification with Taurine. The efficacy of functionalized MSN-NH<sub>2</sub>PAX/siRNA/Taurine NPs significantly inhibited the migration, invasion and growth of 4T1 cells. Further, the cross-talk in triple co-culture of breast cancer cells (4T1 cells), pre-osteoblast (MC3T3-E1) and pre-osteoclasts (RAW-264.7) was investigated. Results demonstrated the strong binding to in vitro triple co-culture model by inhibiting proliferation of 4T1 cells. Treatment with MSN-NH<sub>2</sub>-PAX/siRNA/Taurine not only inhibited the proliferation of 4T1 cells but also restored the normal functioning of bone formation (ALP and Runx2) and bone resorption (TRAP and RANK) by downregulating the SOST expression as validated by ELISA and RT-PCR. in-vivo murine model of mBC and bone metastasis was developed

and intraperitoneal injection of MSN-NH<sub>2</sub>-PAX/siRNA/Taurine NPs indicated significant suppression of osteoclast-mediated bone destruction and enhanced bone formation. Osteoimmunology studies indicated decreased levels of pro-inflammatory cytokines TNF- $\alpha$ , IL-11, IL-6 and enhanced IL-10 levels indicating restored bone homeostasis. MSN-NH<sub>2</sub>PAX/siRNA/Taurine showed anti-oxidant and anti-inflammatory potential in the in-vivo osteolytic lesion model. Our nano-dual delivery system may be able to address the research gap and help in treating the often-neglected osteolytic lesions in mBC.

### Contents

1. Review of literature 2. Materials and methods 3. Synthesis and characterization of MSNs 4. in vitro Release Kinetics 5. in-vitro Pharmacodynamics of MSN-NH<sub>2</sub>-PAX/siRNA/Taurine 6. Metastatic breast cancer disturbs bone remodelling 7. Triple co-culture osteolytic model simulating the vicious cycle 8. Ex-vivo biocompatibility, pharmacokinetics & tissue 9. Tumor regression by MSN-NH<sub>2</sub>-PAX/siRNA/Taurine 10. Molecular Mechanism Underlying siRNA Knockdown and its Effect on Bone Metastasis in Osteolytic Mice in Osteolytic Mice distribution of MSN-NH<sub>2</sub>-PAX/siRNA/Taurine-DiR 11. Osteoimmunology in osteolytic mice 12. Discussion. Summary. References. List of publications and poster presented.

10. P. LANBILIU  
**Formulation, Characterization and Bioefficacy Assessment of  $\beta$ -cyfluthrin Nanoemulsion against an Indian Strain of *Dysdercus koenigii* Fabricius (Hemiptera: Pyrrhocoridae).**  
 Supervisor: Prof. Sarita Kumar  
Th 27849

### Abstract

*Dysdercus koenigii*, a global cotton pest, inflicts significant crop losses via sucking moisture from the leaves and oil contents from the seeds. Since the growing use of conventional pesticides has caused environmental hazards, insecticide-nanoemulsions are being explored as suitable insecticide delivery vehicles for target pests. Current studies formulated, characterised and assessed the efficacy of  $\beta$ -cyfluthrin nanoemulsions against *D. koenigii*. Preliminary studies showed the significant impact of the technical  $\beta$ -cyfluthrin on the survival of the fifth instar nymphs of *D. koenigii* by topical application. The nanoemulsions were, then formulated combining  $\beta$ -cyfluthrin with DMSO (Dimethyl sulfoxide) in 1:2, 1:4, 1:6, 1:8 and 1:10 ratios. The  $\beta$ -cyfluthrin+DMSO (1:2) nanoemulsion (CYF-NE) was found as the most optimal formulation encompassing optimum average size (20.97 nm), good PDI (0.268), uniform morphology and causing 100% nymphal mortality. The efficacy assessment of  $\beta$ -cyfluthrin and CYF-NE on the growth and development of *D. koenigii* nymphs showed pronounced dose-dependent mortality, severe developmental anomalies, delayed growth, diminished adult emergence and reduced reproductive fitness with lower oviposition and egg hatch; higher effects imparted by CYF-NE. The nymphs also exhibited dose-dependent reduced lipid and carbohydrate contents while elevated protein levels indicating metabolic disturbances. The estimation of the detoxification enzymes' levels;  $\alpha$ -esterases,  $\beta$ -esterases, glutathione-S-transferase, cytochrome P450 monooxygenase and acetylcholinesterase; in *D. koenigii* nymphs also displayed their enhanced levels, except at the LD70 dose of treatment. In addition, haemocytes, which also play a critical role in the immune response, reduced in numbers significantly. The treatments dropped the count of various haemocytes, particularly granulocytes, higher impact triggered by CYF-NE. Moreover, the nymphal treatment with  $\beta$ -cyfluthrin and CYF-NE deleted 8 and 15 haemolymph proteins, respectively; while 5 proteins were differentially expressed in nymphs treated with CYF-NE. The study recommends the



use of eco-safe insecticide-nanoemulsions, with lower dose of active ingredient but higher efficacy, for pest control.

#### *Contents*

1. Review of literature 2. Materials and methods 3. Susceptibility status of  $\beta$ -cyfluthrin against *Dysdercus koenigii* and formulation of its nanoemulsion via high energy method 4. Comparative impact of  $\beta$ -cyfluthrin and  $\beta$ -cyfluthrin+DMSO (1:2) nanoemulsion (CYF-NE) on growth, development and physiological responses of *Dysdercus koenigii* nymphs 5. Effects of  $\beta$ -cyfluthrin and  $\beta$ -cyfluthrin+DMSO (1:2) nanoemulsion (CYF-NE) on the biochemical parameters, haemocytes count and haemolymph protein profile of *Dysdercus koenigii* 6. Discussion 7. Summary and Conclusion. References and List of publications.

11. PRIYANKA

#### **Elucidating the Role of PE\_PGRS1 and PPE15 Proteins of Mycobacterium tuberculosis in Modulation of Host Cell Death Pathway and Host Oxidative Stress.**

Supervisor: Dr. Monika Sharma and Prof. Sadhna Sharma

Th 27856

#### *Abstract*

*Mycobacterium tuberculosis* (Mtb) is an ancient bacterium that has evolved with the host and has adopted various strategies to escape host defence mechanisms to favour bacterial persistence. Mtb being a successful pathogen has effectors that target the host mitochondria for pathogenesis. In the present study, role of two uncharacterized Mtb proteins of a unique Proline-Glutamate/Proline-Proline-Glutamate (PE/PPE) family in host mitochondrial functions and ROS modulation have been investigated. In-silico analysis predicted mitochondria targeting sequences in Rv0109 (PE\_PGRS1) and R1039c (PPE15) proteins. Recombinant Rv0109 and Rv1039c were observed to localize to macrophage mitochondria and induced mitochondrial perturbations such as mitochondrial membrane depolarization, mitochondrial superoxides release, depleted ATP levels and release of Cytochrome-C in the cytoplasm. Also, increased expression of pro-apoptotic factors Bax, Bim, caspase activation and macrophage apoptosis was observed. Therefore, both Rv0109 and Rv1039c emerged as effectors that induce caspase-dependent macrophage apoptosis by targeting host mitochondria. The ROS levels in response to Rv0109 was observed to be upregulated whereas Rv1039c downregulated the ROS generation. Interestingly, Rv1039c possesses a C-terminal region with a eukaryote-like SH3-domain (SH3e-domain). The C-terminal region of Rv1039c was found to be conserved with that of mitochondria targeting pro-apoptotic host proteins and observed to be responsible for inducing host macrophage apoptosis. Expression of Rv0109 or Rv1039c in *M. smegmatis* enhanced intracellular survival of mycobacteria within macrophages, whereas *M. smegmatis* expressing C-terminal deleted Rv1039c had reduced survival. The SH3e-domain of Rv1039c interacted with p67phox and p47phox subunits of NADPH Oxidase Complex (NOX) in the cytosol which inhibited the assembly of NOX at the plasma membrane. The deletion of SH3e-domain from Rv1039c led to reduced interaction with p67phox and p47phox in the cytoplasm and ROS production remained unaffected. Therefore, both Rv0109 (PE\_PGRS1) and Rv1039c (PPE15) play a role in influencing host cellular processes and offer valuable insights into Mtb pathogenesis.

#### *Contents*

1. Cloning, expression and purification of Rv0109 (PE\_PGRS1) and Rv1039c (PPE15) proteins of mycobacterium tuberculosis 2. To study the mitochondria-mediated host

cell apoptosis-inducing potential of Rv0109 (PE\_PGRS1) 3. To study the role of C-terminal region of Rv1039c (PPE15) in inducing mitochondria-mediated macrophage apoptosis 4. To study the modulation of nadph-oxidase mediated ros generation by rv0109 (pe\_pgrs1) and rv1039c (ppe15) proteins. Summary and conclusion. List of publications. Conferences and awards.

12. RAWAT (Nitish)

**Assessment of Antibiotic Resistance in Commercial Broiler Chickens and 'Antibiotic-Free' Labelled Chicken Meat.**

Supervisor: Prof. Rajagopal Raman

Th 27857

*Abstract*

This thesis investigates the pervasive issue of antibiotic resistance in broiler chickens and retail meat in North India, with a specific focus on Escherichia coli, Salmonella enterica and Shigella sp. isolates. The research reveals concerning levels of resistance in broiler chickens, with 68.33% of E. coli isolates demonstrating extreme multi-drug resistance. Notably, resistance is observed against clinically important antibiotics, including quinolones, tetracycline, penicillins, and co-trimoxazole. The presence of antibiotic resistance genes (ARGs) associated with mobile genetic elements is also identified, emphasizing the potential for transmission to human gut microbiota. The study extends its analysis to antibiotic-free-labelled and conventional chicken meat in Delhi, finding no significant difference in resistance profiles among E. coli isolates associated between the two, raising questions about the efficacy of antibiotic-free labelling in reducing antibiotic resistance transmission. Alarming, antibiotic-free chicken meat E. coli isolates harbour extended-spectrum  $\beta$ -lactamase (ESBL)-encoding genes, posing significant clinical implications. The investigation further includes Salmonella enterica and Shigella sp., revealing high resistance levels in both antibiotic-free and conventional chicken meat. The study underscores the urgent need to reduce antibiotic use in food-producing animals, advocating for a ban on extra-therapeutic antibiotic use in the chicken industry. The findings caution consumers against misinterpreting labels, emphasizing the limited effectiveness of antibiotic-free-labelled products in minimizing antibiotic resistance transmission through the food chain. Overall, this thesis provides a comprehensive understanding of antibiotic resistance in medically relevant bacteria associated with broiler chickens and retail meat, emphasizing the global importance of addressing this critical issue from a one-health perspective.

*Contents*

1. Introduction 2. Review of literature 3. To evaluate antibiotic resistance to clinically relevant antibiotics in Escherichia coli isolates in commercial broiler chickens in North India 4. To perform a comparative analysis of antibiotic resistance in Escherichia coli isolates from antibiotic-free and conventional chicken meat retailing in Delhi, India 5. To perform a comparative analysis of antibiotic resistance in potential pathogens (Salmonella enterica and Shigella sp.) associated with antibiotic-free and conventional chicken meat retailing in Delhi, India 6. Bottomline. References. Appendices and list of publications.

13. RISHIKESH KRISHAN LAXMI

**Environmental DNA (eDNA) Analysis for Monitoring the Distribution Pattern of Fish and Macroinvertebrates in the Pranmati Stream, Uttarakhand, North-West Himalaya.**

Supervisor: Prof. Neeta Sehgal

Th 27850

*Abstract*

The assessment of aqua faunal diversity in Himalayan streams is an herculean task due to high flow conditions, rough terrain, flash flood etc. The current study has been undertaken to study the distribution pattern of fish and macroinvertebrates of Himalayan hill stream viz. Pranmati. Specimens of fish and macroinvertebrates have been collected and identified on the basis of morphology using traditional methods. The morphological identification of fish (genus) and macroinvertebrates (8 order, 25 families) validated by molecular approach. Protocol to extract eDNA from water samples has been standardized. Environmental DNA has been used to amplify the mt-co1 gene of the fish and macroinvertebrates. With the help of eDNA technique, presence of specific fish at those locations where they were neither caught nor seen in the visual survey has been shown. In addition, large number of taxa with finer taxonomic resolution of macroinvertebrates has been recovered using environmental DNA. Distribution of fish and macroinvertebrates in water shows discernible pattern, indicating that in the mid segment of stream environmental factors are unsuitable for the growth of macroinvertebrates. Correlation between concentration of eDNA and biomass; effect of temperature on release and degradation rate of eDNA; comparison between eDNA from live vs dead fish; and effect of microbial diversity on eDNA released in water has been investigated under controlled laboratory conditions using murrel as a source of eDNA. To summarize, environmental DNA is an additional tool for the assessment of distribution pattern of aquatic fauna in hill streams at high altitude. Environmental temperature plays a critical role in release and degradation of eDNA, hence must be considered in the eDNA assay. Finally, integration of bacterial diversity must be addressed on using eDNA in different habitat.

*Contents*

1. Identification and distribution pattern of fish and macroinvertebrates in Pranmati stream, North-West Himalaya 2. Effect of biomass and temperature on the eDNA release and degradation rate. Summary. References and List of publications.

14. SAHU (Uma Bharati)

**Insulin-like Growth Factors in the Indian Freshwater Catfish, *Heteropneustes fossilis* (Bloch): Characterization and Effect on HPG-L Axis.**

Supervisor: Prof. Neeta Sehgal

Th 27858

*Abstract*

Reproduction is predominantly governed by the hypothalamic-pituitary-gonadal (HPG) axis whereas somatic and gonadal growth are regulated by somatotropic (GH-IGF1) axis. Liver, a highly metabolic tissue, produces insulin-like growth factors (IGF1 and IGF2) under the influence of growth hormone (GH) to regulate growth. Therefore, hypothalamic-pituitary-gonadal-liver i.e., HPG-L axis, regulates the ovarian growth. The IGF system (igf1, igf2 and igf1r) has been characterized in the Indian catfish, *Heteropneustes fossilis* using bioinformatic tools. Protein models have been predicted and docked which have shown potential interactions between gonadal and somatic axes. Differential tissue expression shows maximum expression of igf in liver suggesting it to be a main source for energy. Presence of igf1r transcripts in ovary suggests a possible role of IGF in ovarian growth. The annual expression profile of igf1, igf2 and igf1r in pituitary, liver and ovary exhibits a direct correlation with gonadosomatic index that elucidates a pivotal role of IGF in the HPG-L axis. An inverse relationship between mRNA of igf in brain and liver tissues with GSI indicates a shift from gonadal to somatic growth in resting phase. Role of IGF1 in HPG axis

during breeding season of the catfish has been studied which illustrates that IGF1 influences the synthesis and release of gonadotropins (fsh $\beta$  and lh $\beta$ ) when administered with GnRH, whereas expression of gh is downregulated. A synergistic effect of both the hormones (GnRH and IGF1) on gonadotropins has been observed. GH stimulates synthesis and release of LH along with igf1. IGF1 also induces oocyte maturation indicating its potential effect on maturational events. To conclude, IGF1 plays a crucial role in regulating the hormones involved in reproduction and growth of the catfish.

#### Contents

1. Characterization and in silico analysis of genes involved in IGF system 2. Effect of IGF1 on gonadotropic and somatotropic axis. Summary. References and Publication.

15. SHAH (Vijay Kumar)  
**Growth Regulatory Activities of Ageratum Houstonianum on Dengue Vector Aedes Aegypti L. (Diptera: Culicidae).**  
 Supervisor: Prof. Kamal Kumar Gupta  
 Th 27859

#### Abstract

In the present work, *Ageratum houstonianum* leaf acetone extract (AhLAE) was tested for its growth regulatory activities on dengue vector *Aedes aegypti*. The extract showed larvicidal activity against third instar larvae (L3) and fourth instar larvae (L4) with LC50 value of 204.79 and 401.88 mg/L, respectively, after 24 h of exposure. Further, the larval mortality was increased during subsequent days of treatment. The AhLAE adversely affected larval development, causing a decrease in the growth indexes of treated L3 and L4 in a dose-dependent manner. Also, treatment with the extract increased the larval duration of both the L3 and L4. Larva-pupa intermediates were observed in AhLAE treated *Ae. aegypti* L4 at LC50. The GC-MS analysis of AhLAE showed presence of precocene I, precocene II, cubebol,  $\alpha$ -farnesene and  $\beta$ -caryophyllene; precocene I and II were most abundant. AhLAE caused reduction in JH III titre of the treated L4 of *Ae. aegypti* at LC50. Molecular docking studies revealed that both precocene I and II showed higher binding affinities than JH III towards juvenile hormone binding protein and, also had polar interaction. Treatment of *Ae. aegypti* L4 with AhLAE (LC10 and LC50) caused changes in protein profile. The number of proteins that are involved in proteolysis and protein phosphorylation (metabolic process) was increased at median-lethal treatment. At the same time, the number of proteins related to signal transduction (cellular process) was reduced. Precocene I and II showed higher binding affinities towards serine/threonine protein kinase (related to protein phosphorylation) than JH III and  $\alpha$ -farnesene. Precocene II showed at least one polar interaction to trypsin-like serine protease and aminopeptidase (related to proteolysis). Also, Precocene I & II have polar interaction with rho-GAP domain-containing protein and phosphodiesterase (related to signal transduction), respectively. This study explores the potential of *A. houstonianum* in the management of *Ae. aegypti*.

#### Contents

1. Review of literature 2. Materials and methods 3. Bioefficacy of the *ageratum houstonianum* leaf acetone extract on survival, growth and development of *aedes aegypti* 4. Phytoconstituents analysis of the *Ageratum houstonianum* leaf acetone extract and its impact on the juvenile hormone level of *Aedes aegypti* 5. Assessment of *Ageratum houstonianum* leaf acetone extract on the protein profile of *Aedes*

aegypti 6. In silico validation of the binding of bioactive molecules present in *Ageratum houstonianum* leaf acetone extract with the target proteins of *Aedes aegypti* using molecular docking. Summary. References. Publications and paper presentations.

16. SONAKSHI MODEEL

**DNA Barcoding of Fishes of River Beas (India) and Integration of Molecular Data to Resolve Taxonomical Ambiguities among Inter and Intra-specific Levels.**

Supervisor: Prof. Ram Krishan Negi

Th 27851

*Abstract*

The Beas River is one of the important rivers of the Indus River system located in Himachal Pradesh, India, that harbors a diverse range of freshwater fish species. The present study employed COI gene to investigate the ichthyofaunal diversity of river Beas. Through the sequencing of 203 specimens from Beas River, 43 species were identified belonging to 31 genera, 16 families, and 10 orders. To analyze the genetic divergence and phylogeny of identified species, 485 sequences of Indian origin were retrieved from BOLD, resulting in a dataset of 688 sequences. A consistent hierarchical increase in the genetic divergence and six species exhibiting high intra-specific divergence (>2%) was observed, suggesting the presence of cryptic species. *Labeo rohita* (Rohu), an economically important species of Asia represented a maximum number of sequences. To explore its genetic diversity, COI sequences from the BOLD and NCBI databases were retrieved to make an overall dataset of 289 sequences. The dataset displayed low nucleotide diversity, moderate haplotype diversity along with the presence of rare haplotypes with steady population growth and past decline. Further, the genetic diversity of genus *Channa* revealed a notable congeneric divergence of 12% in the dataset of River Beas. To delve into the genetic intricacies of the genus *Channa*, mitochondrial genetic diversity was analyzed using 1372 COI sequences mined from BOLD. The most prominently represented species were *C. striata* and *C. gachua*. The phylogenetic analysis revealed the presence of species complex within *C. gachua* and *C. marulius*, suggesting the potential existence of intra and inter-clades within one species group. The present study established the first DNA barcode inventory of freshwater fish species in the Beas River providing insights into economically exploited endangered and vulnerable species. The study also underscores the importance of investigating genetic diversity and cryptic species to better understand taxonomic identification and their evolution.

*Contents*

1. Review of literature 2. DNA barcoding of fishes of River Beas, India and characterization of genetic diversity among intra and inter-species levels 3. Investigating the genetic diversity, phylogeography, and population structure of economically important Cyprinid fish *Labeo rohita* (Hamilton, 1822) 4. Exploring the population genetic structure and phylogenetic challenges of genus *Channa*. Bibliography. Appendix. List of publications and conferences.