CHAPTER 3

AIMS AND OBJECTIVES

- The marine catfish, *A. arius* was used as an experimental animal in the present study.

- To determine the LC$_{50}$ value of Cd in marine catfish, *A. arius*.

- To quantify Cd accumulation levels in liver, kidney and gill tissues on exposure to 5, 10 and 20 ppm of Cd for a period of 24, 48 and 72 h.

- To study the micro-architectural and histological alterations in the liver and kidney tissues on exposure to 20 ppm of Cd for a period of 24, 48 and 72 h.

- To study apoptosis (DNA fragmentation, Caspase-3 activity and DAPI Nuclear staining) in the liver and kidney tissues on exposure to 20 ppm of Cd for a period of 24, 48 and 72 h.

- To quantify MT induction levels in liver, kidney and gill tissues on exposure to 5, 10 and 20 ppm of Cd for a period of 24, 48 and 72 h.

- To find the correlation between Cd accumulation and MT induction in liver, kidney and gill tissues.

- To confirm the MT protein, expression and quantification in liver and kidney tissues by Western blot.

- To find the localization of MT in liver and kidney tissues by Immunohistochemical techniques.

- To purify MT protein (from Liver tissue) by Affinity Chromatography.
To find the molecular weight of purified MT of *A. arius* in liver tissue using MALDI-TOF MS.

To find the amino acid sequence of MT of *A. arius* in liver using MALDI-TOF MS Peptide Mass Fingerprinting.

To design the primary, secondary and 3D structure of MT-1 from the amino acid sequence obtained, using bioinformatics software’s.
DESIGN OF THE STUDY

Experimental Animal - Marine Catfish, *Arius arius*

Treatment with Cadmium Chloride

**LC$_{50}$** = 56.4 ppm of Cd

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**BIOLOGICAL STUDY**

**Cd Accumulation and Quantification:**
- Tissues: liver, kidney and gill,
- Cd treatment: 5, 10 and 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**Histological Alterations Analysis:**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**Histomorphometric and Stereological Analysis:**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**DNA Fragmentation:**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**Nuclear Morphology (DAPI Staining):**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**Estimation of Caspase-3 Activity:**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

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**BIOCHEMICAL STUDY**

**MT Induction and Quantification:**
- Tissues: liver, kidney and gill,
- Cd treatment: 5, 10 and 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**MT Confirmation and Expression (WB):**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control), 24, 48 and 72 h

**MT Localization (Immunohistochemistry):**
- Tissues: liver and kidney; Cd treatment: 20 ppm,
- Duration of exposure: 0(Control) and 72 h

**MT Purification by Affinity Chromatography:**
- Tissue: liver; Cd treatment: 20 ppm,
- Duration of exposure: 72 h

**Purified MT – Molecular Weight Analysis (MALDI-TOF MS):**
- Purified MT-1 Amino Acid Sequence (MALDI-TOF MS and PMF) using Mascot software search with SwissProt and NCBI nr databases

**MT Structure Designing (Bioinformatics Softwares and Homology Modelling):**