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# Ultra Structural Studies of Liver Damage by Lead in Fresh Water Fish Cyprinus Carpio

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**Abstract:** The lethal action and liver damage of heavy metal lead on fish Cyprinus carpio has been investigated. Lead nitrate were exposed of different concentration in adult fishes (0.0, 20, 40, 80 and 160mg/l) respectively. Fishes were observed for any symptoms every 6 h interval after exposure of lead nitrate until 6 days. Electron micrograph revealed that lead nitrate exposed adult fish liver hepatocytes showed damaged compartmentation, increased myelinated bodies, damaged glycogen and few lipid droplets. 60mg/l of lead concentration produced 50% of lethal rate in fish ($LD_{50}$) after 120h exposure.

**Key words:** Fish · Lead · Liver damage · Electron microscopy

## INTRODUCTION

Metallic elements from natural and anthropogenic sources are environmentally ubiquitous, readily dissolved in and are transported by water. Lead exposure due to rapid industrialization continues to be a worldwide problem. They are taken up by aquatic organisms due to bioaccumulation and biomagnifications in the food chain as elements or their metabolites, thus causing a concern for the potential concentrations in animals at the top of the food chain [1]. The accumulation of metals in fish depends on equilibrium between absorption and depuration rates [2].

The gills and liver are the main target organs of heavy metals [3-4]). Fish gills are responsible for pH and osmotic regulation, gas exchange and for the excretion of nitrogenous wastes. Because of its fundamental role in metabolism and sensitivity to pollutants, the liver is one of the most studied organs in toxicological studies [5-6]. Liver specimens treated with toxic levels of copper and cadmium showed an increase in fat vacuolization, erythrocyte-filled sinusoids and venules, hepatocyte necrosis, condensation, swelling and lysis in mitochondria, dilation and fragmentation and vacuolization in rough endoplasmic reticulum of hepatocytes [6]. [7] also reported that muscles of all fish were contaminated by Cr and Pb, which seem to be the most concern on metal fish pollution in the Paraiba do Sul River. According to [8] metal accumulation was higher in *Chondrostoma* nasus than in *Barbus capito pectoralis*. Moreover heavy metal concentrations in *C. nasus* were found to be highest in the liver and lowest in muscle tissues. Therefore in this study efforts have been made to examine the toxicity of lead nitrate on the liver of common carp *Cyprinus carpio*.

## MATERIALS AND METHODS

**Fish:** Fresh water fish *Cyprinus carpio* (200n) were collected from the ponds situated nearly Thiruvarur District, Tamil Nadu, India. The average weight of adult fish were 30-40 g and held in individual closed water circulation systems (200 L) for at least 15 days to acclimate to laboratory conditions prior to experiments.

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During the acclimation period, about 40% of the water in system was replaced daily. Throughout the acclimation period and subsequent periods of lead nitrate exposure, fish were held under a photoperiod of 12 hours of light and 12 hours of darkness. fish were fed 5% and 2.5% body weight twice a day with commercial pellets from AQUAFINE Company Mumbai, India respectively.

**Water Quality:** During the experimental period, physicochemical characteristics were maintained optimally Temperature: 26±2 °C; pH: 7.0 ± 0.2; Dissolved oxygen: 6.8 ± 0.5 mg/L, Total hardness: 20.4 ± 2.6 mg. Total hardness and total alkalinity were measured by the titration method and dissolved oxygen concentration was measured by the Winkler method. Water temperature and pH were determined with a glass electrode.

**Laboratory Experiment:** Stock solution of anhydrous lead nitrate [Pb $(NO_3)2$] was made by dissolving 1.0 g of lead to 1 liter of distilled water (100mg/1). one set of experiments (adult )in triplicates were examined. About 10 days of acute toxicity was conducted following the methods described by [9]. Fish in each set were randomly allotted at ten (10) fish per group (A, B, C, D and E) based on the lead nitrate concentrations 0.0, 20, 40, 80 and 160 mg/l respectively for the adult fish. Fish allotted to group A served as the control for the one sets of experiment conducted adult). Fish were observed at 2-hour intervals for the first day and at after 6-hour intervals for 6 days. Dead fish were immediately removed from the experimental set-up. Based on the observation of [10], $LD_{50}$ of Pb exposure were calculated after 120 h, the individuals from each control and treated groups were killed in a 0.02% MS222 solution. Liver samples of control and test fish were dissected and processed for light microscopy and electron microscopy.

**Light Microscopy:** For histopathological studies control and treated samples of liver were fixed in ALFAC (alcohol 80%, formaldehyde 40% and acetic acid 5%), dehydrated in a graded series of ethanol and embedded in Paraplast Plus resin. Seven-micron sections were stained with hematoxylin and eosin and images were obtained and recorded by Olympus Microscope.

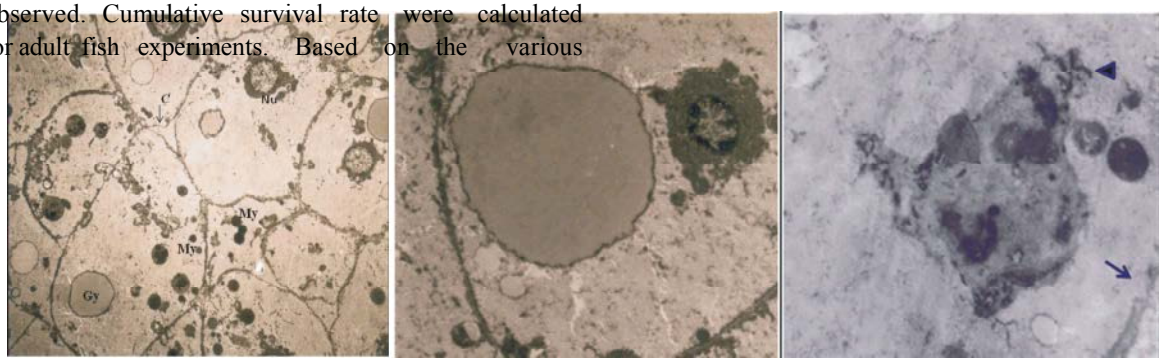
**Electron Microscopy:** Samples of liver for transmission electron microscopy were fixed in glutaraldehyde 1%, paraformaldehyde 2%, cacodilate buffer 0.1 M, CaCl2 5 mM and pH 7.2 fixative solution. The samples were immersed in fixative solution, exposed to high microwaves for 2-3 s and maintained in cacodilate buffer 0.1 M, post fixed in 1% osmium tetroxide, pre-contrasted with uranil acetate (2%), dehydrated in a graded series of ethanol, propylene oxide and embedded in PoliEmbed 812 DER 736 resin. Semi thin and ultrathin sections were obtained by using a Leica Ultramicrotome. The ultrathin sections were contrasted by uranil acetate (5%) and lead citrate and were analyzed in a JEOL JEM 100SX Transmission Electron Microscope (TEM) (80 Kv) with 132 CCD camera in the department of Microscopy Unit, Cancer Institute, Chennai, INDIA.

## RESULTS

During the experimental period loss of balance, skin bleaching and weakness were observed in animals of both sizes in high concentration. Thick layer of mucus on the skin covered the dead fish and the percentage and number of survivors decreased with increasing concentrations of lead in the water. In control adult fish, hepatocytes are characterized by a system of parallelly stacked cisternae of rough ER in the vicinity of the centrally located round nucleus, associated by a few mitochondria and a few other myelinated bodies. The cellular compartmentation is clearly separated into a central, organelle-rich area and a peripheral cell area with storage material, consisting large glycogen storage sacs (Figure 1A,B). After exposure of lead nitrate, fish liver hepatocytes were disorganized and damaged compartmentation (Figure 1C).

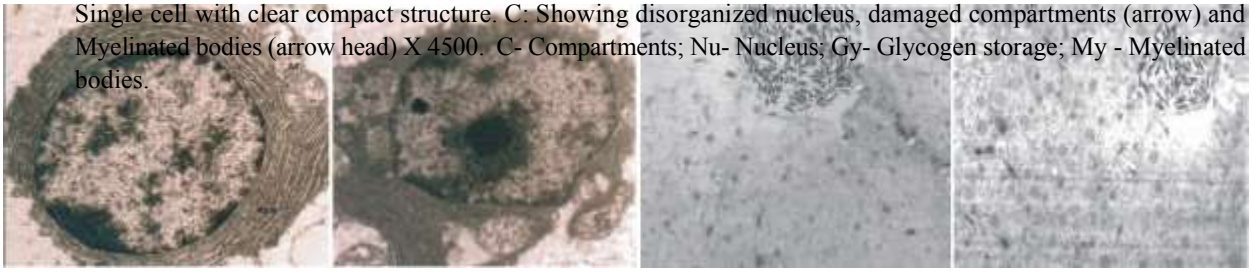
Increased myelinated bodies were observed near the damaged glycogen and few lipid droplets. When compared with control(Figure 2A), deformations of nucleus were also observed (Figure 2B). Long parallel Endoplasmatic reticulums with stacked cisternae surrounding the nucleus were observed in control liver. In lead treated liver showed short and degranulated cisternae and increased volume of mitochondria also observed near the nucleus. Mitochondrial swelling and broken cristae were seen. Increased perivasular connective tissue around blood vessels, darkly clumped cytoplasm, mitotic structures and loss cord structure was observed. The rough ER showed numerous structural alterations including proliferation, fragmentation and vesiculation of cisternae and little dilation and degranulation. In light micrograph, experimental fish liver showed increased number of external cells when compared with control in bile canaliculi (Figure 2C & D). Cytoplasmic compartmentations were completely damaged and increased numbers of lipid droplets were

observed. Cumulative survival rate were calculated for adult fish experiments. Based on the various




(a) (b) (c)

Fig. 1: Electron micrograph of liver of *Cyprinus carpio*. A: showing normal liver cells and histoarchitecture. X 1500. B: Single cell with clear compact structure. C: Showing disorganized nucleus, damaged compartments (arrow) and Myelinated bodies (arrow head) X 4500. C- Compartments; Nu- Nucleus; Gy- Glycogen storage; My - Myelinated bodies.



(a) (b) © (d)

Fig. 2: A: Electron micrograph of control liver of *Cyprinus carpio* showing the nucleus enlarged and endoplasmic reticulum. B: Experimental liver of *Cyprinus carpio* showing increased mitochondria, inclusions in nucleoplasm (myelin biodies), slight RER-alterations and perinuclear cisternae enlargement. X 20000. C: Light micrograph of control liver of *Cyprinus carpio* showing only mononucleated cells without pars fibrousa and strongly developed cytoplasmic compartmentation. D: Experimental liver of *Cyprinus carpio* showing fractionation of nuclear membrane fenestration of cisternae and no cytoplasmic compartmentation. X 400.

concentration of lead exposure in adult fishes 60% and 50% were died after 120h of exposure in 40mg/l and 80mg/l concentration respectively. However around 60mg/l of lead concentration produce 50% of lethal rate in fish ($LD_{50}$) after 120h exposure.

## DISCUSSION

Carp *Cyprinus carpio*, is one of the commercial freshwater and cultivable fish of south east part of India. The most commonly occurring metallic polluting elements in fresh waters are lead, zinc and copper from the industrial effluents. The fish species showed a great capacity to accumulate metals, with highest bioaccumulation for the essential element iron and lowest bioaccumulation for the non-essential element lead. And we observed high level of lead accumulation in their tissues in both sizes when exposed to high dose. The result of this study agreed with that of [11] who observed that fish exposed to sub lethal level of lead produced dose-dependent significant increases in the concentration of lead in the liver and muscle of *cyprinus corpio*. Moreover [12] reported that Pb accumulation was similar in all the organs of fish *Cyprinus carpio* than other heavy metals.

After exposure of lead, fish liver hepatocytes were disorganized and cell compartments were damaged. Increased myelinated bodies were observed near the damaged glycogen and few lipid droplets. Mitochondrial swelling and broken cristae were also seen. Liver

alterations have been shown in other studies to be suitable markers which indicate prior exposure to environmental stressors [13-14]. A major cytopathological response in the hepatocytes of field exposed fish in the present study was the loss of cellular compartmentation, which has been shown to occur in response to a wide variety of environmental changes [14] and which might be due to chemical attack on the cytoskeleton [15]. In addition, it has been assumed that a poorly developed cellular compartmentation indicates a severe disturbance of cellular metabolism [14]. The rough ER showed numerous structural alterations including proliferation, fragmentation and vesiculation of cisternae and little dilation and degranulation. Similarly other reports also stated that alterations of the rough ER, including proliferation, fragmentation and vesiculation, are common reactions to xenobiotic stress [16-18] correlated alterations of the rough ER with a higher biotransformation capacity of hepatocytes and [19] interpreted the dilation of ER cisternae as a result of enhanced storage of proteins due to a reduced secretory activity.

$LD_{50}$ for adult fish of *C.carpio* were calculated as 60 mg/l of lead nitrate exposed up to120 h respectively. Death rate of fish was drastically increased in high dose of lead treatment. Moreover [20-23] also reported that mortality occurred predominantly during the first 4-6 days of Pb exposure and chronic exposure resulted in a general ion-regulatory disturbance affecting K+, Na+ and Ca2+ homeostasis. Most of the reports demonstrated that Pb readily accumulates in the gill tissue. However our findings suggest that liver is one of the important organs to accumulate Pb and disturb its regular function leads to death of fish. High concentration of Pb exposure in fishes will results in higher accumulation.

## CONCLUSION

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