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Acute Cadmium Toxicity Induced Impairments in the Liver and Kidney of Freshwater Catfish, *Heteropneustes fossilis* (Bloch)

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Abstract

Background/objective: The heavy metal, Cadmium enters aquatic environment through natural / anthropogenic sources and exerts deleterious effects in fish. Hence, the present study is aimed at investigating the acute Cd toxicity induced histological alterations in the liver and kidney of catfish, *Heteropneustes fossilis*. **Methods:** The bioassay was performed in a static renewal regime with *H. fossilis* exposing to varying acute toxicity concentrations *viz.,* 18.45, 36.9, 73.7, 147.4 and 294.8 mg/l for 96 h. The LC_{50} was determined to be 44.13 mg/l. Since 100 % mortality occurred in the highest concentrations after 96 h, liver and kidney tissues were collected from fish exposed to the first three lower concentrations. Standard histology protocol was followed to study the histological alterations. **Findings:** The histological alterations like increased kupffer cell and pycnotic nucleus, ruptured hepatic tissue and nucleus, cellular necrosis and focal necrosis were observed in the liver of fish exposed to Cd. Similarly, vacuolation, increased periglomerular and peritubular space, shrunken glomerulus, melanomacrophages and loss of cytoplasm were observed in the kidney of fish treated with Cd. The intensity of the histological alterations in both liver and kidney was found to be concentration and duration dependent. Hepatic tissues were found to be ruptured in the liver of fish exposed to all the three concentrations viz., 18.45, 36.9 and 73.7 mg Cd/l. The appearance of melanomacrophage aggregates is a generalized non-specific marker, indicating environmental stress. In the present study, melanomacrophages were observed in kidney of fish exposed to 36.9 and 73.7 mg Cd/l. Our present observations on histological alterations in the liver and kidney of *H. fossilis* were in conformity to the histological observations made in the liver and kidney of various fish species exposed to different toxicants. **Applications:** The histological alterations in liver and kidney *H. fossilis* induced by Cd suggest that Cd can be a potential toxicant and hence these alterations can be used as biomarkers to monitor aquatic pollution.

Keywords: Acute Toxicity, Cadmium, Histology, *Heteropneustes fossilis*, Kidney, Liver

1. Introduction

The natural aquatic system is extensively contaminated with heavy metals that are released from domestic, industrial and several other anthropogenic activities[1,2]. Meanwhile, research is also underway for the expulsion of heavy metals from effluents[3]. Heavy metals are harmful pollutants for the aquatic organisms by themselves or through their toxic salts that show high salinity[4]. Cadmium, a toxic heavy metal is increasingly important as an environmental hazard to both humans and wildlife[5-8]. It is a ubiquitous contaminant in the aquatic environment.

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There are several studies on the acute toxicity of Cd in fish[9]. Its exposure adversely affects fish morphology and physiology: morphological changes in gill[10], kidney[11], liver[10,12,13], gonads[14] and stomach and intestine[15]. It causes tissue damage, notably in the kidney, by inducing cell death which results in renal dysfunction[16].

Histopathological technique is a sensitive tool that helps to detect direct effects of xenobiotics and toxic chemicals within target organs of fish[17]. The histological studies provide direct evidence of any adverse effect on fish. Liver is a principal organ of detoxification in vertebrates in general and fish in particular. In addition, it is the potential site for lipid deposition in these animals[18]. Furthermore, fish liver is a good indicator of aquatic environmental pollution, as the liver is able to clean of any poisons or pollutants from the blood coming from the intestine [19].

The kidney is a vital organ of body and helps to maintain the homeostasis. It is responsible for selective reabsorption. Thus, it helps in maintaining volume and pH of blood and body fluids and erythropoiesis[20]. In fish, the kidney aids in electrolyte and water balance and maintenance of the stable environment. It is also an important indicator of possible pollution. Several workers studied the impacts of various toxicants on the histology of liver and kidney of the various fish species. There are very few studies on the effects of chemicals on the air breathing fish, *H. fossilis*[21]. Nevertheless, there is only little experimental data on the histopathological impacts of Cadmium on kidney and liver of the freshwater Asian stinging catfish, *Heteropneustes fossilis*. Hence, in the present study, an attempt has been made to study the acute toxic effects of Cadmium on the histoarchitecture of liver and kidney of *H. fossilis*.

2. Materials and Methods

The catfish, *Heteropneustes fossilis* with an average length of 18.36 ± 0.248 cm and weight of 38.86 ± 1.413 gm were procured from a local fish market in Tirunelveli, Tamil Nadu. They were acclimatized to laboratory conditions in Fiberglass Reinforced Plastic (FRP) Tanks of 500 L capacity for one month prior to exposure to Cadmium. The water was changed every day. Fishes were fed with chicken liver *ad libitum* daily.

The experimental design was based on Static Renewal Test (SRT), Range Finding and Definitive Test (Acute Toxicity Test) described by Sprague[22] and USEPA[23]. For each bioassay test, a series of five test concentrations of Cadmium and a control were used. The acute toxicity test concentrations were selected based on the range finding test. The concentrations selected were 18.45, 36.9, 73.7, 147.4 and 294.8 mg/l. After the acclimatization period, adult stinging catfishes were randomly selected and stocked at the rate of 10 fish per FRP tank with 140 liter water for the five experimental runs and a control. A duplicate set was also maintained simultaneously. Exposure medium was changed every 24th hrs to maintain the desired concentration of Cadmium. Mortality of fishes was recorded. Then, LC_{50} of Cd was determined following the procedure of Finney,[24] and it was found to be 44.13 mg/l. All the fishes in the last two concentrations died during the test period. Hence, tissue samples were taken from the fishes exposed to the first three concentrations only. At the end of the experiment (96 hrs), live fish samples were collected from the above-mentioned three concentrations, sacrificed and their liver and kidney were excised out and fixed in Bouins fixative for 24 hrs. Later, the tissue samples were processed adopting the usual histological procedure by Humason,[25] and thin sections of 5 µm was stained with haemotoxylin and eosin for microscopic observation. Also, light photomicrographs were taken. The morphological changes of the liver and kidney sections noted in the experimental fish were compared with those of control fish.

3. Results and Discussion

The histopathological changes in the liver and kidney of the control fishes and fishes exposed to Cadmium were observed. Histological changes were observed in the liver of the fishes exposed to Cadmium at 18.45 mg/l. The changes included ruptured nucleus, increased kupffer cell, ruptured hepatic tissue, cellular necrosis and increased pycnotic nucleus (Figure 3). Very distinct marked changes such as cellular necrosis, ruptured hepatic tissue, ruptured nucleus and focal necrosis were observed in the liver of fishes exposed to Cadmium at 36.90 mg/l (Figure 4). Some distinct changes were observed in the liver of the fishes exposed to Cadmium at 73.70 mg/l. The changes included focal necrosis, increased pycnotic nucleus, cellular necrosis and ruptured hepatic tissue (Figure 5). The above cells were normal in the case of liver collected from control fishes. In control fish liver, normal hepatic tissue showing hepatocytes with granular cytoplasm with round nucleus hepatocytes cells was observed (Figure 1). The toxicity effect of heavy metals on liver bas been studied

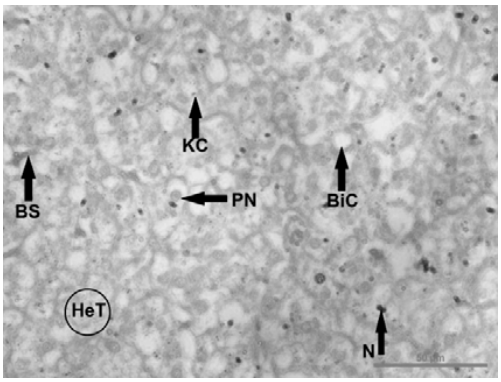

Figure 1. Photomicrograph of liver of control fish at 96 hrs. BS: Blood Sinusoid, HeT: Hepatic Tissue, PN: Pycnotic Nucleus, KC: Kupffer Cell, BiC: Bile Canaliculi and Nu: Nucleus. (5 µm thick; H&E staining; 400X

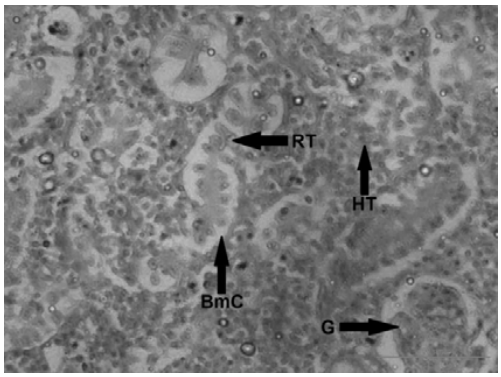

Figure 2. Photomicrograph of kidney of control fish at 96 hrs. BmC: Bowman's Capsule, RT: Renal Tubules, HT: Hematopoietic Tissue and G: Glomerulus.(5 µm thick; H&E staining; 400X).

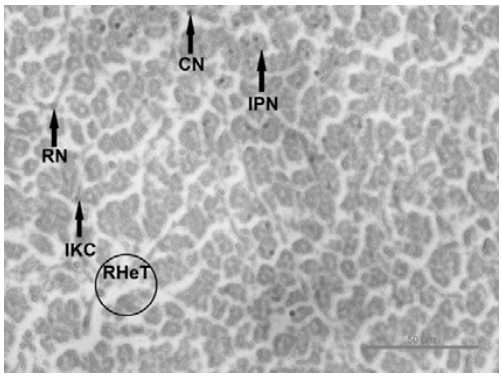

Figure 3. Photomicrograph of liver of fish exposed to Cd at 18.45 mg/l after 96 hrs. RN: Ruptured Nucleus, IKC: Increased Kupffer Cell, RHeT: Ruptured Hepatic Tissue, CN: Cellular Necrosis and IPN: Increased Pycnotic Nucleus (5 µm thick; H&E staining; 400X).

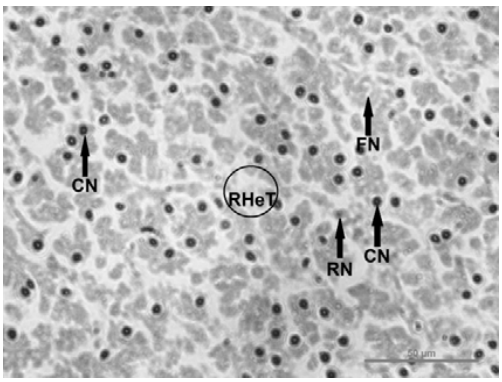

Figure 4. Photomicrograph of liver of fish exposed to Cd at 36.90 mg/l after 96 hrs. CN: Cellular Necrosis, RHeT: Ruptured Hepatic Tissue, RN: Ruptured Nucleus and FN: Focal Necrosis (5 µm thick; H&E staining; 400X).

by several workers. The effects of acute Cadmium on the liver of *Heteropneustes fossilis* is in conformity to other similar kind of studies.

Histological alterations like degeneration of hepatocytes, vacuolization, congestion of hepatic tissues, subcapsular vacuolization, necrosis, indistinct cell boundaries and pyknotic nuclei were observed in the liver of the catfish, *Clarias batrachus* exposed to Cadmium[26]. Degenerative changes like hepatocellualar dissociation, necrosis and hypertrophy were observed in the freshwater fish, *Ophiocephalus striatus* exposed to Cadmium Chloride[15]. Almost similar histopathological changes were observed in the liver of *H. fossilis* treated with dry leaf extract, dry bark extract and dry seed extract of the plant *Madhuca indica*[27]. Naigaga[28] observed lesions in the liver of *O. mossambicus* exposed to Cu. In his study, the sequential appearance of lesion in the order of hepatic vacuolar degeneration, fatty degeneration and necrosis indicated a gradual increase in damage with duration and Cu concentration. The author attributed liver hyperfunction to initial liver lesion formed due to vacuolar degeneration and attributed liver hypofunction to fatty degeneration and early stages of necrosis which could be related to the damage to cellular organelles like mitochondria. The presence of macrophage aggregates in the liver is a generalized non-specific marker of environmental stress[29]. Initial lesion in the liver during the present study might be due to physiological changes that took place in the liver tissue in the process of trying to homeostatistically regulate and detoxify the metal during continuous exposure as suggested by Naigaga[28]. Our present observation on histological alterations on liver is in conformity

with observations made in similar work carried out with different toxicants on various fish species[12, 30, 31].

Several studies used histological characteristic of kidney as an indicator of pollution. Histology of kidney of control fishes showed normal type of cells. Histological studies revealed that the kidney sections from control fishes showed normal histoarchitecture. Kidney is characterized by well-built haemopoietic tissue, uriniferous tubule and glomerulus with clear Bowman's capsule (Figure 2).

In the present work, histological changes in the kidney after exposure to Cadmium at 18.45 mg/l (96 hrs) were vacuolation, increased periglomerular space, shrunken glomerulus (Figure 6). Some distinct changes like melanomacrophages, increased periglomerular space, vacuolation, increased peritubular space and shrunken glomerulus were observed in the kidney of the fishes treated with Cadmium at 36.90 mg/l (Figure 7). The changes like melanomacrophages, increased peritubular space and periglomerular space and loss of cytoplasm were observed in fishes exposed to 73.70 mg/l (Figure 8). Almost similar kind of observations was made in *Channa punctatus*[6], *Cirrhinus mrigala*[32], *Labeo rohita*[12], Hybrid walking catfish (*Clarias macrocephalus x C. gariepinus*)[30] and *Lates calcarifer*[31].

Chronic Cadmium exposure in the freshwater fish *Colosoma macropomum* produced an anomalous head kidney structure and induced an inflammatory process in this organ, affecting haematopoietic cell differentia-

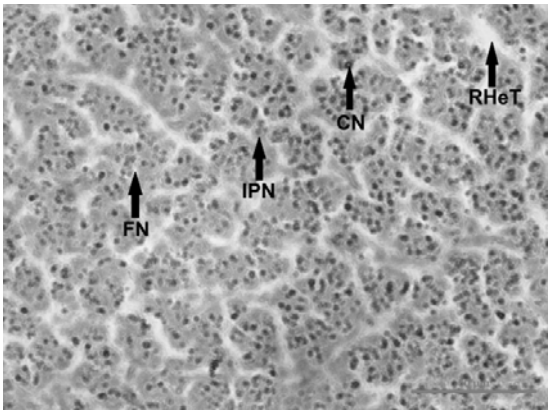

Figure 5. Photomicrograph of liver of fish exposed to Cd at 73.70 mg/l after 96 hrs. FN: Focal Necrosis, IPN: Increased Pycnotic Nucleus, CN: Cellular Necrosis and RHeT: Ruptured Hepatic Tissue (5 µm thick; H&E staining; 400X)

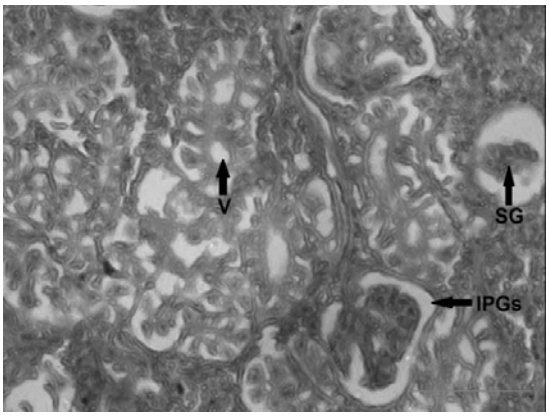

Figure 6. Photomicrograph of kidney of fish exposed to Cd at 18.45 mg/l after 96 hrs. V: Vacuolation, IPGs: Increased Periglomerular Space and SG: Shrunken Glomerulus. (5 µm thick; H&E staining; 400X).

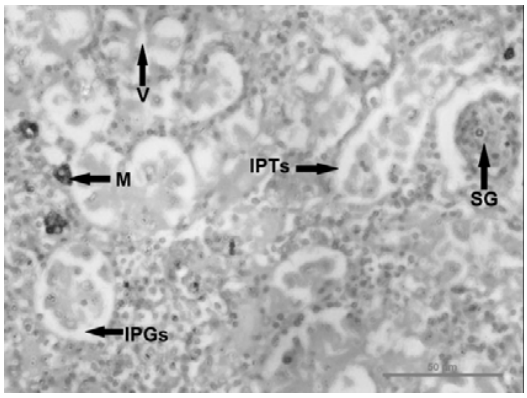

Figure 7. Photomicrograph of kidney of fish exposed to Cd at 36.90 mg/l after 96 hrs. M: Melanomacrophages, IPGs: Increased Periglomerular Space, V: Vacuolation, IPTs: Increased Peritubular Space and SG: Shrunken Glomerulus. (5 µm thick; H&E staining; 400X).

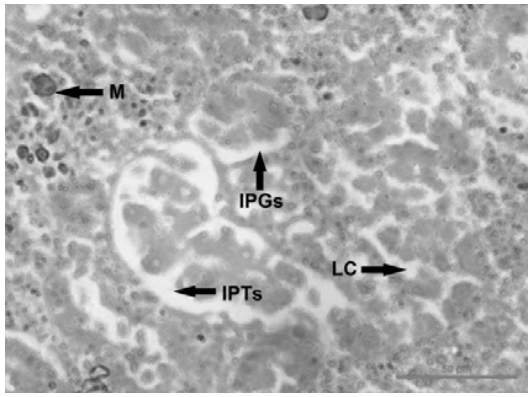

Figure 8. Photomicrograph of kidney of fish exposed to Cd at 73.70 mg/l after 96 hrs. M: Melanomacrophages, IPTs: Increased Peritubular Space, IPGs: Increased Periglomerular Space and LC: Loss of Cytoplasm. (5 µm thick; H&E staining; 400X).

tion, especially with regard to granulocytes and perhaps affecting its functions[33].

Amin et al.[11] reported histological alterations like loosening, formation of cluster and lumps in haemopoietic tissue, deshaping of uriniferous tubules narrowing of tubular lumen, vacuolization and degeneration of the cells of increase of space in renal corpuscles and shrinkage in glomeruli in the fish *Channa punctatus* exposed to Cadmium Chloride. Rupture of tubule boundary cells formation of melanomacrophage, congregation of nuclei, damage of epithelial cells and coagulated mass of blood cells were observed in freshwater fish, *Cirrhinus mrigala* exposed to Cadmium Sulphate[32].

4. Conclusion

Histological alterations in the catfish, *Heteropneustes fossilis* under the influence of Cd can be used a sensitive model to monitor the aquatic pollution. The present result suggested that acute toxic exposure to Cadmium leads to damages in the tissues of liver and kidney of stinging catfish, *Heteropneustes fossilis*, confirming the possibility of Cadmium to be a toxicant.

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