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# Histopathological Changes in Liver of Indian Flying Barb, *Esomus Danricus* (Hamilton-Buchanan), Exposed to Cadmium

KEYWORDS: Cadmium, sublethal, hepatocyte, pycnosis

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**ABSTRACT** *The liver of Indian flying barb, Esomus danricus, exposed to sublethal doses of 636.3, 63.6 and 6.3 µgl-1 Cd showed several reactions including fatty changes in hepatocytes, liver cord disarrangement, nuclear pycnosis and extensive degeneration of cytoplasm, which are characteristic abnormalities in fishes exposed to toxicants. Higher doses of exposure had more severe effects on the liver.*

## Introduction

Cadmium (Cd) is recognised as one of the major environmental pollutants and produces toxic effects in living organisms (De Conto Cinier et al.1998). It is persistent in the environment and rapidly accumulates in the food chain. Cd is used in rechargeable nickel-cadmium batteries, electroplating, pigments and plastic production and find their way into water bodies. The bottom sediments of aquatic ecosystems often become reservoirs of such persistent chemicals. It is, therefore, very likely to affect the biota in general and fishes in particular. In the present study, the Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a common teleost fish species of North India is used for histopathological studies. Exposure of fish to chemical contaminants is likely to induce a number of lesions in liver (ICES 1997). The monitoring of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies. Heavy metals can either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on type and concentration, fish species, length of exposure and other factors (Paris-Palacios, Biagianti-Risbourg & Vernet 2000). In the present study, the liver of Indian flying barb is examined because it plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring due to toxicants (Rocha & Monteiro 1999).

## Material and methods

Fishes of similar length (46.77 ± 4.30 mm) and weight (0.86 ± 0.16 g) were collected from unpolluted, freshwater ponds near Assam University campus, Barak valley, South Assam, India (Das & Gupta 2009). They were acclimatized under laboratory conditions seven days prior to experimentation and fed twice daily. Temperature, dissolved oxygen, hardness and pH under laboratory condition were 29 ± 0.13°C, 5.5 ± 0.24 $mgl^{-1}$, 30 ± 0.5 $mgl^{-1}$ and 6.8 ± 0.03 respectively (all the values are in mean ± SE). Test media were renewed every 24 hours and commercially available fish food was given *ad libitum* twice daily. A stock solution of actual concentration of Cadmium was prepared from compounds ($CdCl_2$. $H_2O$) obtained from Merck, Germany. A stock solution of 1000 mg $l^{-1}$ (1.0 g $l^{-1}$) of Cd was prepared by adding 1.0 g of metal to 1 litre of double distilled and deionized water. The amount of metal salt which contained 1.0 g of metal was determined from the molecular and atomic weights as: Molecular weight of compound ($CdCl_2$. $H_2O$)/Atomic weight of Cd. Serial dilutions of stock solutions were prepared using tap water as per standard dilution techniques (APHA 2005). 96-hours $LC_{50}$ value for Cd in *Esomus danricus* was found to be 6363.0 µgl-1 in a previous study (Das & Gupta, 2010) and calculated by Probit method (Finney 1971). Three sub lethal test concentrations viz., 636.3 µgl-1, 63.6 µgl-1 and 6.3 µgl-1 were selected for inducing histological changes in fish liver. Ten fish for each concentration of test chemical were kept separately in three litres of toxicant treated media for 28 days. Food was given during the study period. Test water was renewed every 24 hrs. After 28 days of exposure, fish were sacrificed and liver were removed immediately and kept in 10% Formalin, as fixative, for 24 h, dehydrated, embedded in paraffin and sections cut at 5 µm thickness and stained with Harris Haematoxylin and Eosin. Changes induced by treatment in the liver tissues were photographed and analyzed by light microscope (Olympus: model U-CMAD3) with Camera attachment of Samsung (model: SDC-313B).

## Results

On analysis of control liver (Fig. 1a) of *Esomus,* a normal architecture was observed and there were no pathological abnormalities. The hepatocytes present a homogenous cytoplasm and a large central or subcentral spherical nucleus. The qualitative liver histology in fish exposed to 636.3 $µgl^{-1}$ of Cd for 28 days showed focal inflammatory cell collection. Infiltration in portal triad was also observed along with widespread hepatocellular necrosis. Large numbers of inflammatory cell infiltration were well marked in exposed tissue (Fig. 1b). In fish exposed to 63.6 $µgl^{-1}$ of Cd, liver after 28 days of exposure showed hepatocellular necrosis. Hepatocytes showed nuclear pycnosis. Degeneration of nucleus was also well marked (Fig. 1c). In fish exposed to 6.36 $µgl^{-1}$ of Cd for the same duration, the liver histology showed focal inflammatory cell collection. Though infiltration in portal triad was also observed, infiltrations were much less compared to higher doses (Fig. 1d). In the liver, histological changes observed were more pronounced in fish exposed to higher Cd concentrations.

**Fig1: (a)T.S of Control liver (400×)**

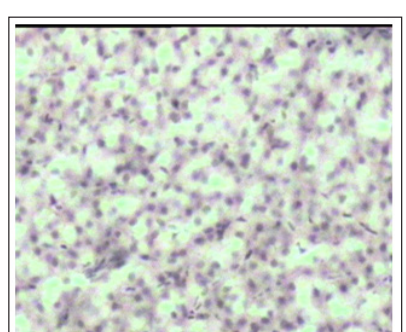

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**Fig1: (b)T.S of Liver exposed to 636.3 µg $l^{-1}$ Cadmium (400×)**

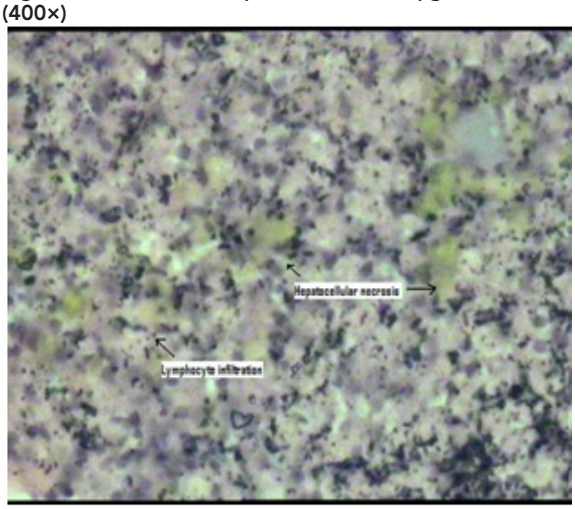



**Fig1: (c) T.S of Liver exposed to 63.63 µg $l^{-1}$ Cadmium**

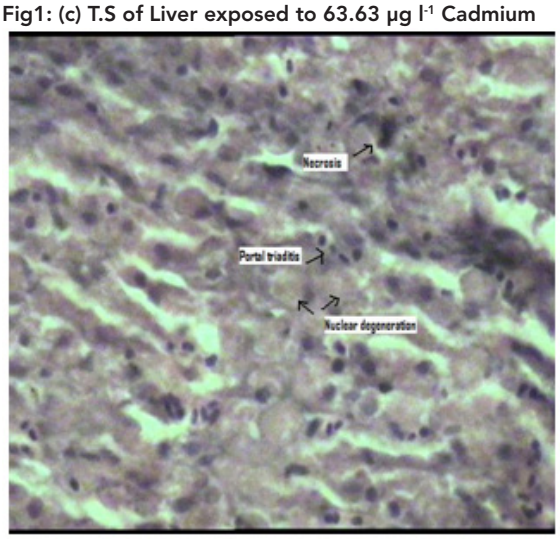
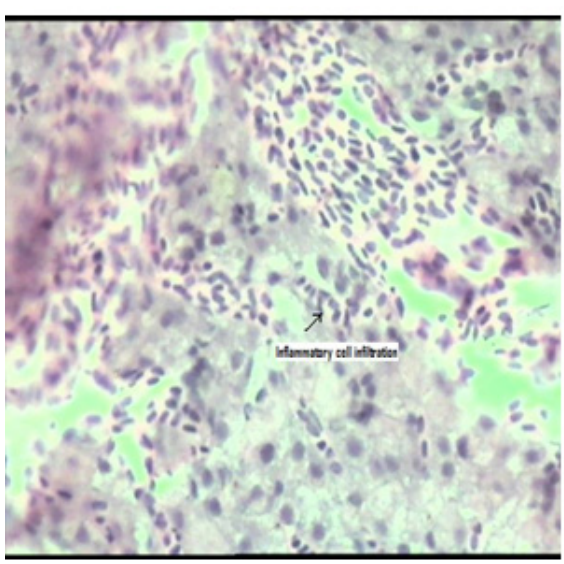



**Fig1: (d) T.S of Liver exposed to 6.3 µg $l^{-1}$ Cadmium (400×) (400×)**




**Discussion**

Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Schwaiger et al. 1996). Liver of Indian flying barb is a relatively large organ and is located in the anterior abdomen. Functional changes are known to be reflected in structural changes of hepatocytes (Arias et al. 1988), which in turn can be used as biomarkers to trace environmental pollution caused by chemicals (McCarthy & Shugart 1990). In the present study, liver of *Esomus* exposed to sublethal doses of Cd showed several reactions including fatty changes in hepatocytes, liver cord disarrangement, nuclear pycnosis and extensive degeneration of cytoplasm, which are characteristic abnormalities in fishes exposed to toxicants and well supported by Pourahamad & O'Brien (2000), who found that chronic metal accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and ultimately death. Studies in *Oreochromis niloticus* also showed a variety of changes in the liver, resulting from exposure to different toxic chemicals (Visoottiviseth et al. 1999, Figueiredo-Fernandes et al. 2006a, b). Moreover, evidence provided by studies in rodents show that there is inhibition of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in Cd toxicity (Gupta et al. 1991). Liver is one of the main sites of metallothionein (MT) production and metal retention (Klavercamp et al. 1984). One of the main reasons for the increased presence of Cd in these organs is their capacity to accumulate this metal by induction of the metal binding protein, MT, which is believed to influence the uptake, distribution and toxicity of Cd by binding to it (Wimmer et al. 2005). Thus, sublethal doses of cadmium have adverse effects on the liver of Indian flying barb. However, the severity increases with dose of exposure.

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