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HISTOPATHOLOGICAL CHANGES IN LIVER OF INDIAN FLYING BARB, *ESOMUS DANRICUS* (HAMILTON-BUCHANAN), EXPOSED TO COPPER

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ABSTRACT
Indian flying barb, *Esomus danricus,* a teleost fish commonly inhabiting fresh waters of northern India, was exposed to three sublethal doses of 0.005, 0.0025 and 0.001 μgl^{-1} Cu for 28 days and the histological sections of liver, stained by haematoxylin-eosin, were observed under light microscope. Liver showed several reactions including necrosis, fat filled cytoplasm/ foamy hepatocyte cytoplasm, lymphocytic infiltration, portal triaditis and extensive degeneration of cytoplasm. Higher doses of exposure had more severe effects on the liver.

KEY WORDS: Copper, sublethal, teleost, hepatocyte.

INTRODUCTION

Copper (Cu) is recognized as one of the major environmental pollutants and produces toxic effects in living organisms (Solomon and Lowery, 1993). It is a trace metal essential for the human body and for many enzyme systems. However, excessive exposure to high concentrations of Cu can result in adverse health effects. The maximum contaminant level of Cu in drinking water is 1.3 mgL^{-1} (USEPA, 2002). Its persistence in the environment and rapid uptake and accumulation in the food chain contributes to its potential hazards. The risk of Cu in the environment, especially in fish was observed by Vutukuru *et al.,* 2005. Cu is used in various industries like textile, tanneries, paints, battery, laundry, copper ware and piping for water distribution systems and find their way into water bodies. The bottom sediments of aquatic ecosystems often become reservoirs of such xenobiotics. 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 and economically important both as ornamental and food fish is used for histopathological studies. Exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Bucke *et al.,* 1996) including 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 *et al.,* 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 and Monteiro, 1999).

MATERIALS AND METHODS

Fish and experimental system

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 and Gupta, 2009). They were acclimatized under laboratory conditions seven days prior to experimentation. Temperature, pH, hardness and dissolved oxygen under laboratory condition were 29°C, 6.8, 30 mg l^{-1} and 5.5 mg l^{-1} respectively. Stock solution of Cu was prepared from $CuCl_2.2H_2O$ (Merck, Germany) and serial dilutions were prepared using chlorine free tap water as per dilution techniques (APHA, 2005). Static-with-renewal acute toxicity tests were conducted with ten fish in each graded concentration and 96 hours LC_{50} value was found to be 0.01 μgl^{-1} in a prior study (Das and Gupta, 2010). Three sub lethal test concentrations *viz*., 0.005, 0.0025 and 0.001 μgl^{-1} Cu were selected for inducing histological changes in fish liver.

Histology

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 at 10X eye piece magnification and 40X objective magnification {Olympus (model U-CMAD3) with Camera attachment of Samsung (model SDC-313B)}.

RESULTS

On analysis of control liver (Fig. 1) of *Esomus* at 40X magnification, 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 expose to 0.005µg l^{-1} of Cu for 28 days, liver showed widespread 'piecemeal' necrosis. Portal triaditis with inflammatory portal tract infiltration could be also be seen. Hepatic cells showed fatty accumulation and central nucleus with fat filled cytoplasm/ foamy cytoplasm were observed (Fig. 2). In fish exposed to 0.0025µgl^{-1} Cu for 28 days, showed focal lymphocytic infiltration, that is, chronic inflammatory lymphocyte cells in portal triad. Extensive portal triaditis with fibrosis was also well marked (Fig. 3). Liver of fish exposed to 0.001µgl^{-1} of Copper for 28 days showed focal fatty changes. The effect observed is less severe compared to the higher doses of Copper (Fig. 4).In the liver, histological changes observed were more pronounced in fish exposed to higher Cu concentrations.

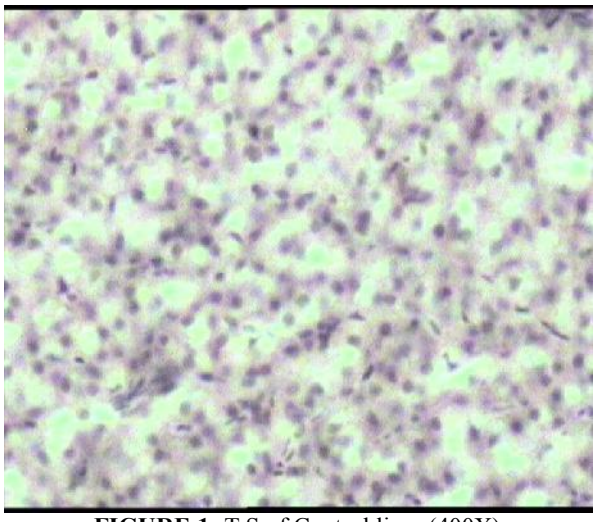

FIGURE 1: T.S of Control liver (400X)

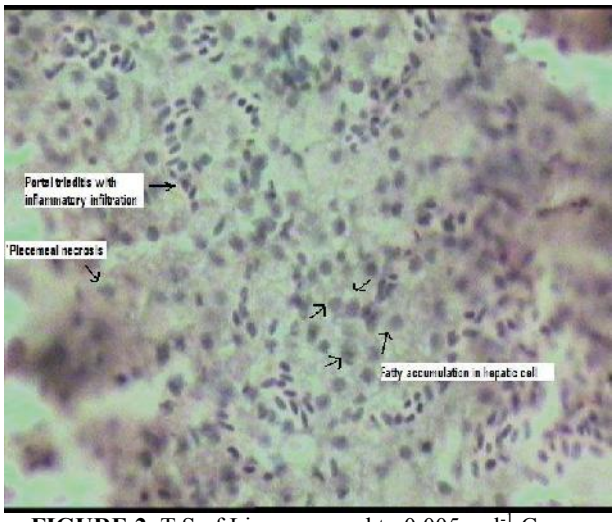

FIGURE 2: T.S of Liver exposed to 0.005µg l^{-1} Copper (400X)

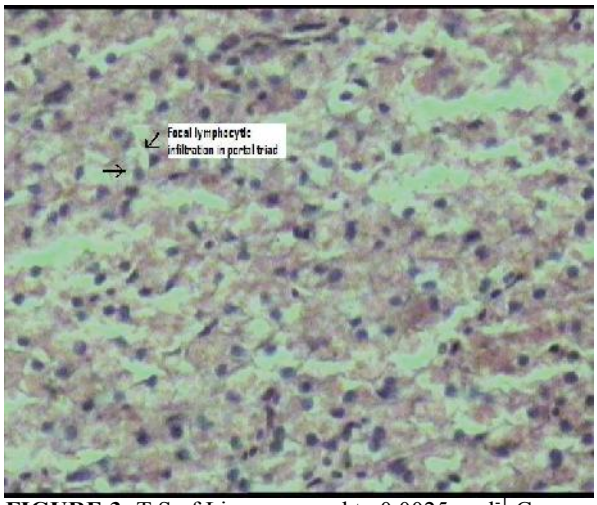

FIGURE 3: T.S of Liver exposed to 0.0025 µg l^{-1} Copper (400X)

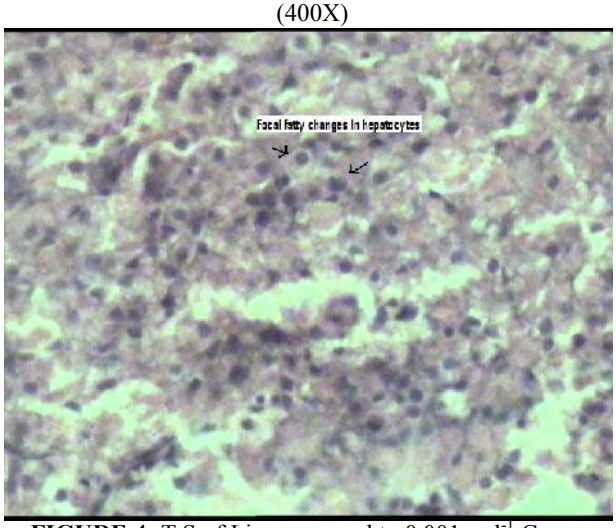

FIGURE 4: T.S of Liver exposed to 0.001µg l^{-1} Copper (400X)

DISCUSSION

Histopathological investigations are a sensitive tool to detect direct effects of xenobiotics and toxic chemicals within target organs of fish (Schwaiger *et al.,* 1996). Liver of Indian flying barb is a relatively large organ. The colour of liver is usually reddish brown. But at certain time of the year it may be yellow colored or even off white. The liver is located in the anterior abdomen. The liver being an organ for metabolizing and eliminating toxicants shows visible abnormalities in the treated organism. Functional changes are known to be reflected in structural changes of hepatocytes Schwaiger *et al.,* 1996; Couch, 1975; Meyers and Hendricks, 1982) which in turn can be used as biomarkers to trace environmental pollution caused by chemicals (Arias *et al.,* 1988). In the present study, liver of *Esomus* exposed to sublethal doses of Cu 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 many workers(McCarthy and Shugart,1990; Pourahamad and O'Brien, 2000) for metals, who found that chronic metal accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and ultimately death. Studies in *Cyprinus carpio* also showed a variety of changes in the liver, resulting from exposure to copper sulphate (Varanka

et al., 2001). Studies have shown that long-time ingestion of water containing Cu greater than the maximum contaminant level can lead to liver damage (Zietz *et al.*, 2003). Cu is also known to impair glycolysis in freshwater fish, *Labeo rohita* (Radhakrishnaiah *et al.*, 1992). Copper also acts as an enzyme activator as it is incorporated into enzymes like cytochrome oxidase, superoxide dismutase (USEPA, 1987) The activity of these enzymes is dependent on the adequate supply of metal but excess copper can also inhibit the activity of enzymes. Copper due to its redox potential generates oxidative stress causing free radicals, which damages cellular components like lipids by causing lipid peroxidation, DNA and proteins (Vutukuru *et al*., 2005). Liver, along with gill and kidney, are the main sites of metallothionein (MT) production and metal retention (Gupta *et al.,* 1991). One of the main reasons for the increased presence of Cu 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 Cu by binding to it (Klavercamp *et al.,* 1984). The present study, thus, establishes the tissue damaging effects of Cu.

CONCLUSION

Thus, although copper is an essential metal, it is extremely harmful to fish even at low doses.

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