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EFFECT OF SUBLETHAL TOXICITY OF ZINC CHLORIDE ON LIVER HISTOLOGY OF ESTUARINE EDIBLE FISH, *LIZA MACROLEPIS* (SMITH, 1846)

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ABSTRACT

In the present study, estuarine edible teleost, *Liza macrolepis* of size 9.6 ± 1.2 cm length were exposed to sublethal concentration ($^1/_{10}$ of LT_{50}/96 h) of heavy metal, Zinc chloride for a period of 15 days. The treated fish groups were compared with the control group for histopathological changes in the liver tissue. The histopathological changes occurred in the liver of L. *Macrolepis* suggest that histopathological studies are considered as direct evidence referring to any adverse effect on fish health. Moreover, the liver is considered as the principal target organ of detoxification in vertebrates and particularly in fish.

Keywords: *Liza macrolepis*, Zinc chloride, Liver tissue, Sublethal toxicity, Histopathology.

INTRODUCTION

The contamination of aquatic bodies with a range of heavy metal pollutants has become a global concern over the past few decades (Fostner and Wittman, 1979; Meyers and Hendricks, 1985; Wickland–Glynn, 1991; Bryan and Langston, 1992; Bucke, 1995; De Forest, *et al.*, 2007; Kaoud and El-Dahshan, 2010). Heavy metals contribute to anthropogenic contamination of aquatic resources such as rivers, estuaries and seas (Bryan and Langston, 1992; Moore *et al.*, 1999). Some of them are essential to the normal fish metabolism and others are toxic even at low concentrations (Pillai, 1983; Kalay and Canli, 2000; Sen *et al.*, 2011 Chavan and Muley, 2014).

Fish, having great economic importance are affected immensely by varied metals directly or indirectly in many ways (Rashed, 2001; Gaber, 2007; Al-Weher, 2008; Pourmoghaddas and Shahryari, 2010). The toxic effects of heavy metals have been studied by several workers in freshwater species of fishes and some of these studies indicate high mortality of juvenile fish and reduced breeding potentially of adults after acute and chronic exposures to heavy metals (Besirovic *et al.*, 2010; Rambhare and Bakare 2012).

Lead (Pb) is one of the most toxic heavy metals in aquatic environs and its toxicity and accumulation have been reported on fish metabolism and histological responses. Absorption of lead occurs by different ways through the gills and skin or by ingestion of contaminated water and food (Aruldoss and Indra, 2005; De Forest *et al.*, 2007; Authman and Abbas, 2007; Khan *et al.*, 2011 Javeed and Usman 2011; Fatima and Usmani, 2013).

Histopathology of fish is not used as a standard tool for monitoring metal pollution but rather it is considered for inclusion as a viable tool for determining fish health in the laboratory and field studies (Weber and Gingerich, 1982; Bucke, 1993; Van Dyk *et al.*, 2007; Bhatkar, 2010; Deore and Wagh, 2012; Mokhtar *et al.*, 2013; Chavan and Muley, 2014). Perusal of the literature reveals that histopathological alterations in body tissues of freshwater fishes have been reported by metal toxicity (Ajmal *et al.*, 1985; Javeed, 2005; Akan *et al.*, 2009; Ambedkar and Muniyan, 2011). Very few studies are available on the

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effects of metals on the histological responses of estuarine edible fishes (Avenimo *et al.*, 2005; Zhao *et al.*, 2012). With this view in mind, an attempt has been made to observe the acute effect of sublethal toxicity of lead nitrate on the liver histology of an edible teleost fish, *Liza macrolepis* of Thengaithittu estuary, Puducherry, South East Coast of India.

MATERIALS AND METHODS

Collection and maintenance of test animal

Liza macrolepis (n = 250) juveniles were collected from the estuary during low tide regime and brought to the laboratory. Fishes were acclimatized in fish tanks (50L cap) for one week. Fish were fed with fish food (goat meat) and the water in the tanks was renewed by freshly collected estuarine water at every 24h.

Test chemical: preparation of test concentrations

Zinc chloride (Qualigens AR Grade Mumbai) was procured and 10% stock solution using DDW. Varied concentrations viz. 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90 (mg/L) were prepared by v/v method.

L_{T50} determination

Healthy juveniles of *L. macrolepis* with (9.6 ± 1.2 cm length; 10.3 ± 0.78 g weight) were chosen from the mass rearing to determine L_{T50} value. Three replicates of groups of 5 fishes were exposed to each one of the test concentrations of Zinc chloride. Dead fish were removed from the tanks and counted at 24, 48, 72 and 96h of exposures. Death was presumed when fishes were immobile and showed no response to touch with a glass rod. From the mortality values obtained to different concentrations of $Zncl_2$, a concentration rendering 50 percent population dead i.e., LT_{50} / 96h was calculated adopting Spearman–Karber Airithmetic mean method (Hamilton *et al.*, 1977).

Bioassay studies

Acclimated juvenile fish, *L. macrolepis* with uniform body length (9.6 ± 1.2 cm) and mass weight (10.3 ± 0.78 g) were chosen for bioassays. Two groups were formed, each consisting of five animals. One group was considered as the experimental and exposed to (10% of LT_{50} / 96 h) sublethal toxicity of $Zncl_2$ for 15 days. The other groups was treated with $Zncl_2$ concentration and kept as control. Both controls and exposed groups were maintained simultaneously.

Histological analysis

Liver samples from control and sublethal treated fishes were carefully removed at intervals of 5 days and fixed in 10% Neutral buffered formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into 5 to 7 micrometers thick (Steedman, 1960) and then stained with Haemotoxylin Eosin method according to Bucke (1972). Then the sections were examined on light microscope (Krempt–Wetzler) under 100x magnification and photographed by using a microscopic camera.

RESULTS

The liver of control fish is covered by a thin layer of fibrous layer of mesothelial cells. The parenchyma is dispersed randomly throughout the liver tissue. Within the parenchyma, the hepatocytes (H) were spread out as irregular cords.

The histopathological changes observed in the present study after chronic exposure to (10% of LT_{50}/96 h) sublethal concentration of zinc chloride in the liver of the estuarine fish, *L. macrolepis* have been depicted in Photoplate I (A to C). The liver of the fish exposed to $ZnCl_2$ metal sublethal toxicity exhibited marked histopathological alterations. The most alterations induced by $Zncl_2$ after 5 d sublethal exposure were the damage of lead characterized by cloudy swelling with large vacuoles (CSV) and cytoplasmic vacuolation (CV).

In addition, Degeneration of Pancreocytes (DP) and Necrosis (N) were observed in the liver tissue of the fish *L. macrolepis* after 10 d sublethal exposure (Plate. B). Hypertrophy of hepatocytes (HH) and psycnotic nuclei(PN) were the histopathological lesions also noticed in the liver tissue of the fish exposed to 15 days of sublethal toxicity of $ZnCl_2$.

Microphotograph showing transverse sections of liver tissue of the fish, *Liza macrolepis* exposed to sublethal (10% of LT50/96 h) concentration of the metal, Zinc chloride for 5(A), 10 and 15 days (CSV = Cloudy Swelling with large Vacuoles, CV = Cytoplasmic Vacuolation, DP = Degeneration of Pancreocytes, H = Hepatocytes, HH = Hypertrophy of Hepatocytes, N = Necrosis, PN = Psycnotic Nuclei).

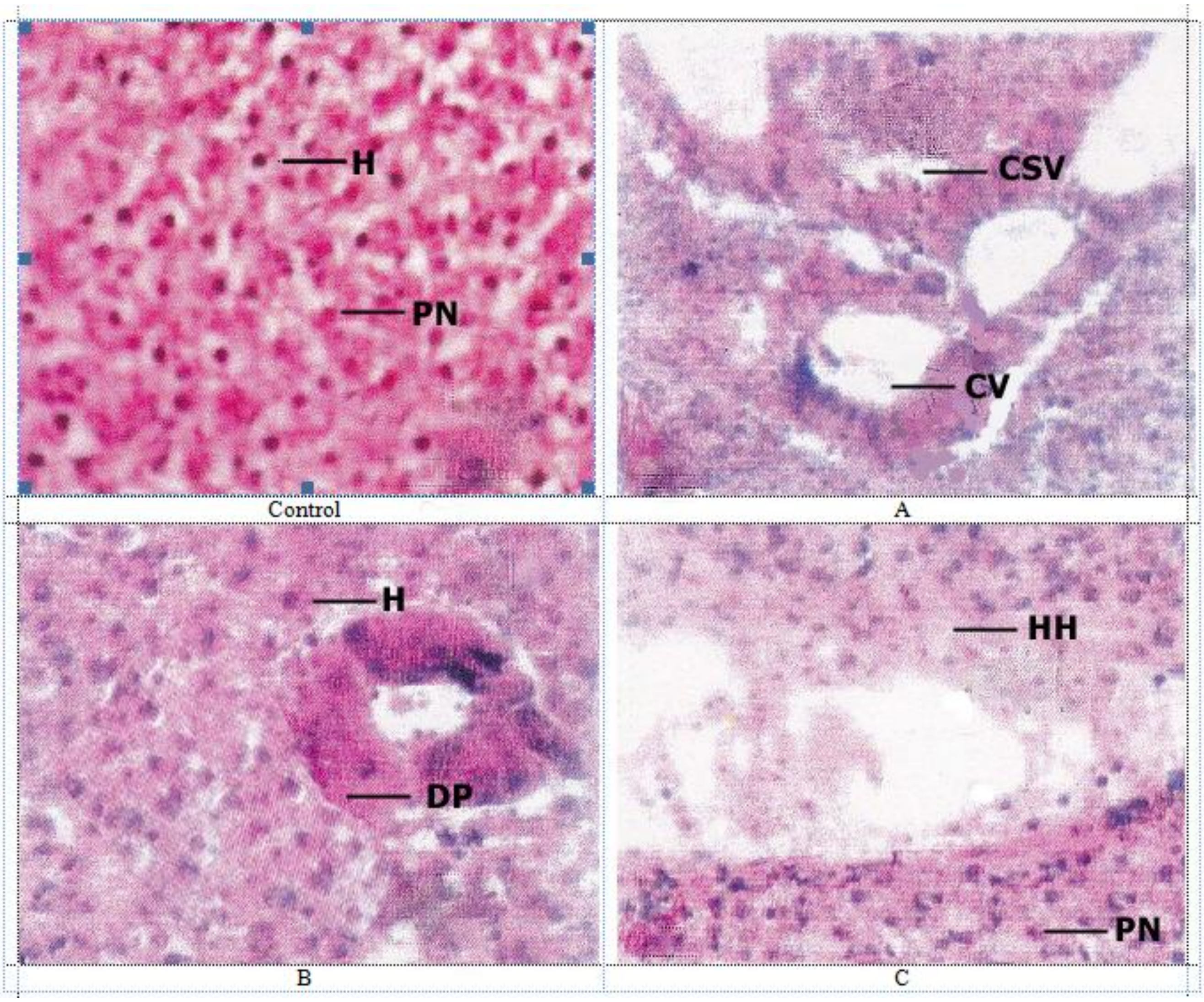

Plate I. Microphotograph showing transverse sections *of* liver tissue of the fish, *Liza macrolepis* exposed to sublethal (10% of LT50/96 h) concentration of the metal, Zinc chloride for 5(A), 10 and 15 days (CSV = Cloudy Swelling with large Vacuoles, CV = Cytoplasmic Vacuolation, DP = Degeneration of Pancreocytes, H = Hepatocytes, HH = Hypertrophy of Hepatocytes, N = Necrosis, PN = Psycnotic Nuclei).

DISCUSSION

Fishes have the ability to accumulate toxicants such as heavy metals in their tissues viz. gills and liver (Mobarak and Sharaf, 2011). The present study revealed that there is a strong link between liver damage and sublethal toxicity of heavy metals (Weber and Gingerich, 1982; Radaiah and Jayantha Rao, 1992). Pathological changes in the liver of the fish, *L. macrolepis* exposed to $^{1}/_{10}{}^{th}$ of LT_{50}/ 96 of $Znll_2$ were depicted in the photoplate (A–C). The liver of control fish is covered by a thin layer of mesothelial cells. Hepatocytes and pancreocytes were randomly dispersed throughout the liver tissue Marked variations in the liver architecture were observed by the 5^{th} days, 10^{th} days and 15^{th} days (Photoplate: A, B and C).

The most alterations of the liver exposed to Zinc sublethal 5d exposure were cloudy swelling of cells with large vacuoles (CSV) and cytoplasmic vacuolation (VC). These observation were in agreement with the reports of Aruldoss and Indra (2005) in tilapia, *Oreochromis mossambicus* Gaber (2007) in nile tilapia, *Oreochromis niloticus*; Deore and Singh (2012) in the carp, *Channa gaticua* (Photoplate–A). In addition marked variations in liver tissue histology were noticed after 10d exposure and they were degeneration of pancreocytes (DP) and hepatocyte hypertrophy (HH). Similar alterations in the liver tissue were observed in several species exposed to heavy metals and these alterations were described by Kaoud and El- Dahshan (2010) in nile tilapia, *O. niloticus*; Pourmoghaddas and Shahryari (2011) in three carnivorous fishes; Mokhtar *et al.*, (2013) in *O. niloticus*; Histopathological lesions such as Necrosis (N) degeneration of Pancreocytes (DP) and Pycnotic Nuclei (PN) were recorded in the liver tissue of the fish exposed for 15 days. Such findings on liver pathological lesions were studied by Van Dyk *et al*.(2007) in *O. mossoambicus;*

El-Naggar, *et al.* (2009) in the liver of *O. niloticus* due to the toxicity of Fe, Mn, Cu, Zn, Cd and Pb and Gaber *et al.*, (2014) in two fishes, *Sparus aurata* and *Dicentrarockus labrax*.

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