ejpmr, 2018,5(2), 416-420

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF LEAD NITRATE ON THE HISTOPATHOLOGY OF THE GILL, LIVER AND KIDNEY OF THE FRESH WATER FISH, *CIRRHINUS MRIGALA*

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Article Received on 11/12/2017 **Article Revised on 02/01/2018** **Article Accepted on 23/01/2018**

ABSTRACT

Background: The discharge of anthropogenic chemicals in the environment has resulted in long- term eco-toxicological implications all over the world which lead to an increase in the accumulation of toxic chemicals in soil and natural waters. **Objective:** The objective of the study is to evaluate the histopathological changes in the vital tissues like gills, liver and kidney exposed to sub chronic doses of lead nitrate. **Methods:** The experiment was designed to expose the fish to different concentrations of sub chronic doses of lead nitrate. One trough served as the control. Each trough contained ten fishes and the experiment was conducted in triplicate. The duration of the experiment was for 30 days. **Result:** The vital organs like gill, liver and kidney showed signs of degeneration. **Conclusion:** The pollution of waterways with anthropogenic activities is the major cause of aquatic loss and imbalanced food chain. Heavy metals like lead were tested in different organs like gills, liver, kidneys and flesh tissues of the fish enduring in natural water system. Most of the metals are present in the edible portion of fish. Humans are also affected by eating fish which can cause health problems.

KEYWORDS: *Cirrhinus mrigala*, Lead nitrate, histopathology, sub chronic doses, degeneration.

INTRODUCTION

The discharge of anthropogenic chemicals in the environment has resulted in long – term eco toxicological implications all over the world which lead to an increase in the accumulation of toxic chemicals in soil and natural waters. Most of the heavy metals are well-known toxic and carcinogenic agents and it represent a serious threat to the human population and the fauna and flora of the receiving water bodies. Heavy metals are major pollutants in the environment due to their toxicity and threat to creatures and human being at high concentrations. Heavy metals have a great tendency to bio-accumulate and end up as permanent additions to the environment. Their accumulation in the tissue is mainly dependent on water concentrations of metals and exposure period; although some other environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal's accumulation and toxicity to fish (Jitar *et al.*, 2014).

Lead is one of the most toxic heavy metals and its compounds are included in the grey list of international conventions (Taylor *et al.*, 1985). Mason (1991) categorized lead as one of the most toxic metals in fresh water. Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro *et al.*, 2005). Histopathological assessment is a sensitive biomonitoring mechanism indicating the impact of toxicants on fish health in polluted aquatic ecosystem. Histopathological examination of the tissue of fish exposed to toxin indicates signs of long – term injury in cells, tissues or organs. Changes in the tissue of test organisms exposed to sublethal concentration of a toxicant are a functional response of organism which provides visual information on the nature of the toxicant (Mathur and Gupta, 2008).

Heavy metals accumulated in the tissues of fish catalyze redox reactions that generate reactive oxygen species which may lead to environmental oxidative stress and, therefore, cause biochemical and morphological alterations in fish (Varanka *et al.*, 2001 and Monteiro *et al.*, 2005). The xenobiotics initiate a specific enzyme that alters metabolism by cellular intoxication at cellular level and necrosis on a tissue level. Heavy metals and chemicals are toxic to animals and many cause death or sublethal pathology of liver, kidneys, reproductive system, respiratory system or nervous system in both invertebrate and vertebrate aquatic animals (Wilbur, 1969).

The present work is to observe the histopathological changes in the vital tissues like gills, liver and kidney exposed to sub chronic doses of lead nitrate.

MATERIALS AND METHODS

Fresh water fish *Cirrhinus mirgala (*mirgal) were collected from Aliyar dam near Pollachi, Coimbatore district, Tamilnadu. These fishes were transported to the laboratory in oxygenated polythene bags. The healthy fingerlings of *Cirrhinus mirgala* ranging in length of 10-12 cm and weighing about 12- 14 g were used for the experiment. Fishes were acclimatized for 2-3 weeks in a large plastic trough containing plain tap water. The physico-chemical characteristics of water were analyzed.

The toxicant used in the static bioassay was lead nitrate in tap water. Fingerlings of cirrhinus mrigala were randomly distributed in plastic troughs of 20 liters capacity. One plastic trough served as the control and the other troughs were provided with different concentrations of lead nitrate namely 32mg/l, 34mg/l, 36mg/l, 38mg/l, 40mg/l and 42mg/l. Ten fishes were placed in each trough and mortality was recorded after 24 hrs, 48 hrs, 72 hrs, and 96 hrs. The LC_{50} at 96 hrs was determined by the Probit analysis method (Finney 1971).

To determine the sub lethal concentration of lead nitrate, $1/10^{th}$ of the concentration of LC_{50} value for 96 hours was taken. The experiment was designed to expose the fish to different sub chronic doses of lead nitrate. One trough served as the control. The experimental troughs were provided with 4mg/l, 6mg/l and 8mg/l of lead nitrate respectively. Each trough contained ten fishes and the experiment was conducted in triplicate. The duration of the experiment was for 30 days. The histopathology of the vital tissues was assessed after 30 days of treatment.

Gill, liver and kidney tissue excised from fishes of the control and experimental groups were fixed with 10% formalin solution. After proper dehydration by graded alcohols, paraffin blocks were prepared and 4-5µm thick ribbons were cut in a Rotator Microtome and were stained with Haematoxylin and Eosin. The histopathological changes observed were photographed.

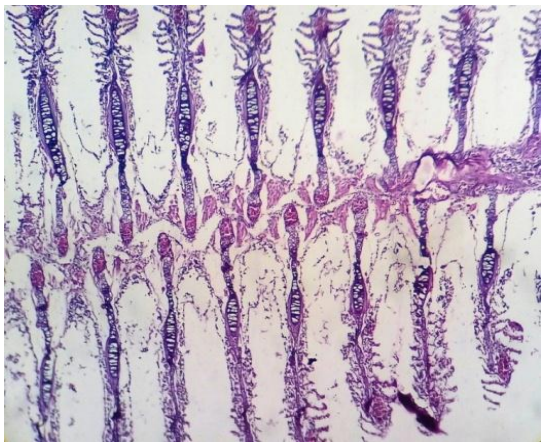

Fig. 1: Gill of control fish (HE x 400).

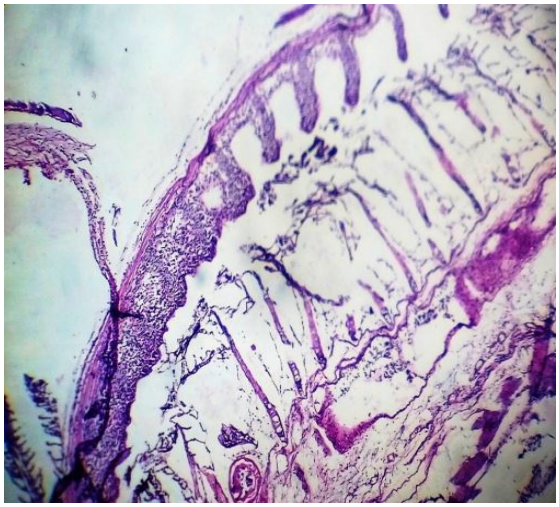

Fig. 2: Gill of fish treated with 4mg/l of lead nitrate (HE x 400).

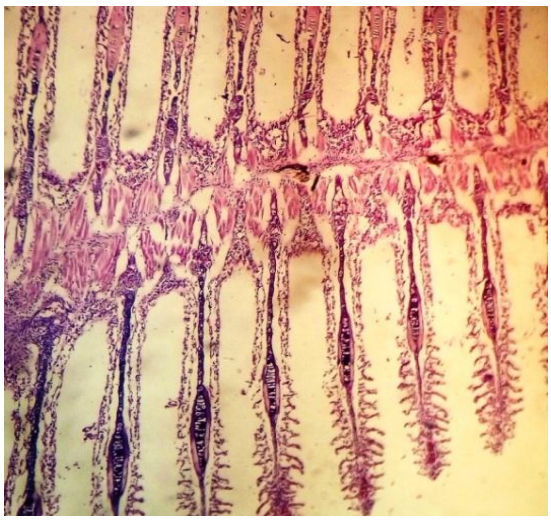

Fig. 3: Gill of fish treated with 6mg/l of Lead nitrate (HE x 400).

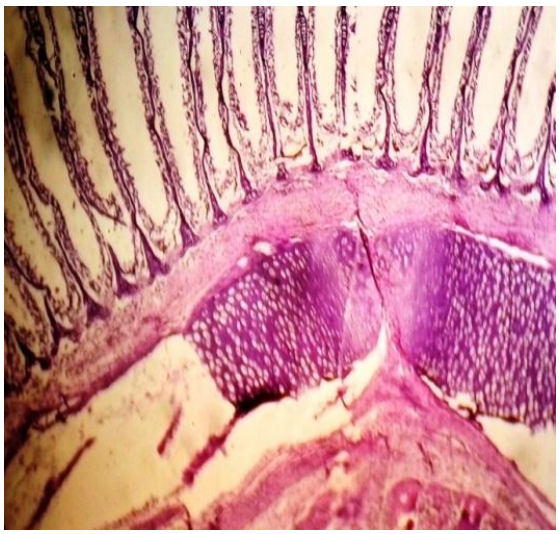

Fig. 4: Gill of fish treated with 8mg/l of lead nitrate (HE x 400).

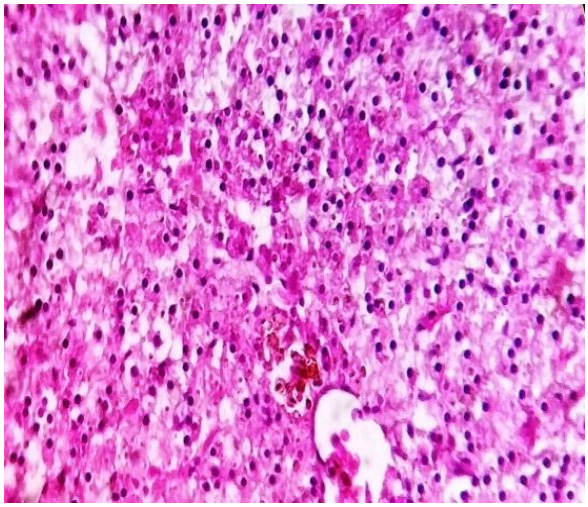

Fig. 5: Section of liver of control fish (HE x 100).

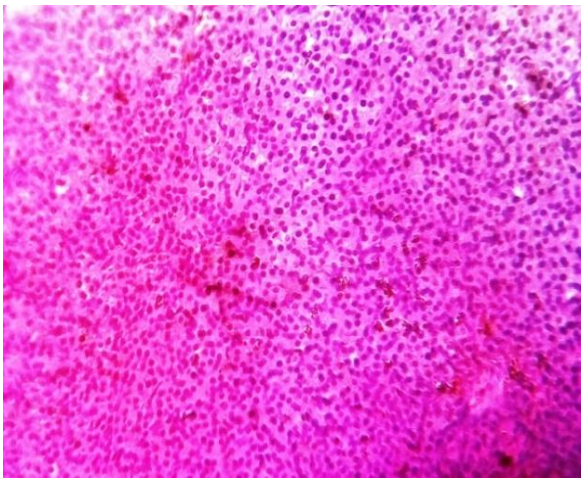

Fig. 6: Liver of fish treated with 4mg/l of lead nitrate (HE x 100).

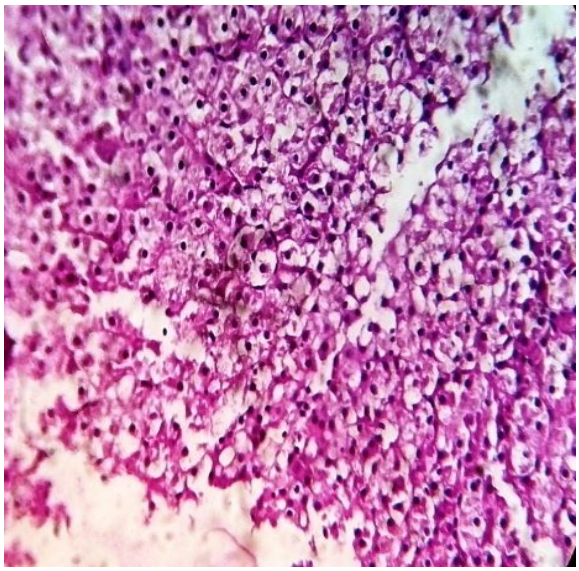

Fig. 7: Liver of fish treated with 6mg/l of lead nitrate (HE x 100).

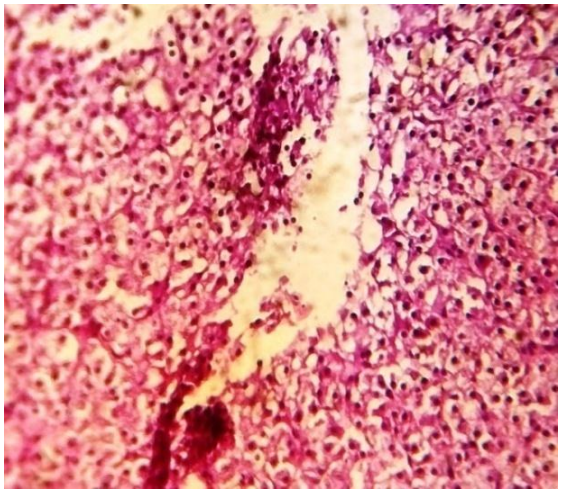

Fig. 8: Liver of fish treated with 8mg/l of lead nitrate (HE x 100).

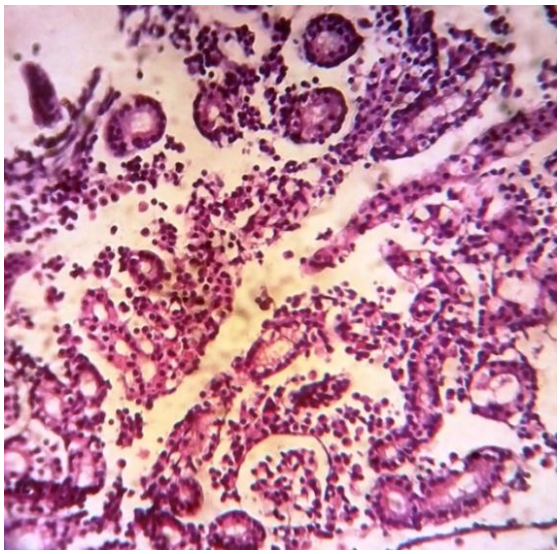

Fig. 9: Section of Kidney of control fish (HE x 400).

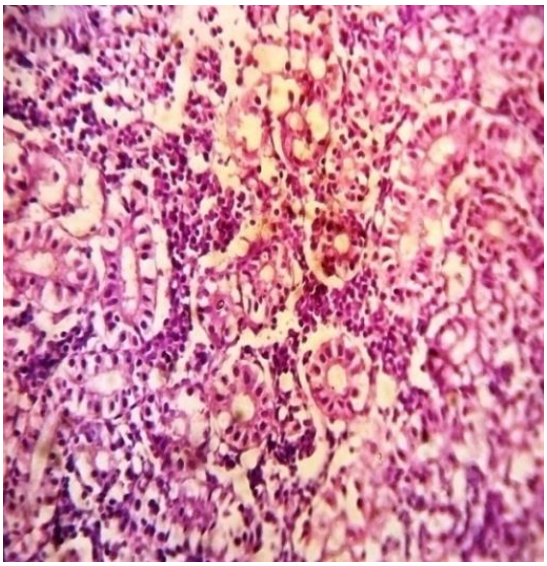

Fig. 10: Kidney of fish treated with 4 mg/l of lead nitrate (HE x 400).

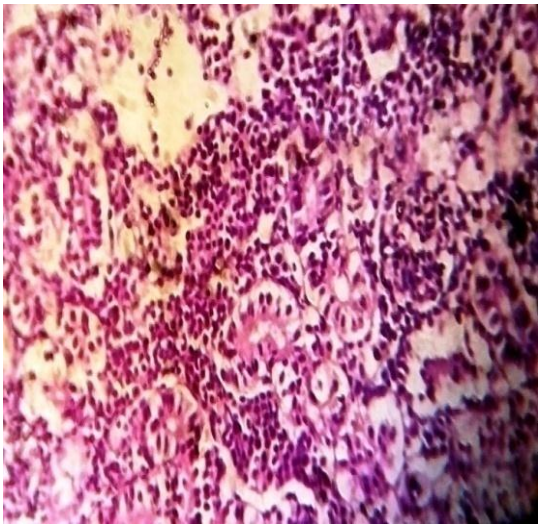

Fig. 11: Kidney of fish treated with 6 mg/l of of Lead nitrate (HE x 400).

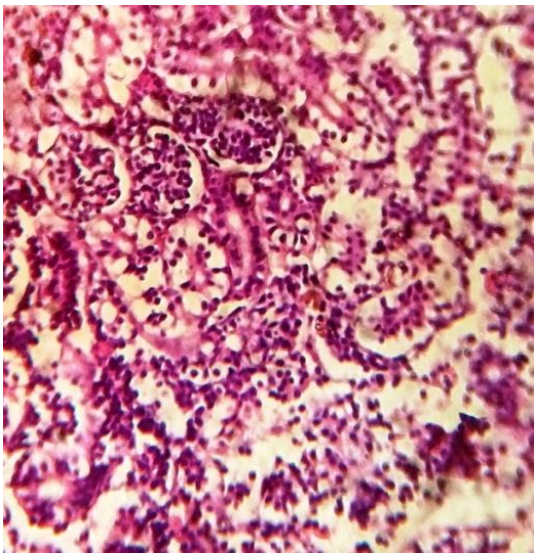

Fig. 12: Kidney of fish treated with 8 mg/l Lead nitrate (HE x 400).

RESULTS AND DISSCUSION

Gills

The histology of gill in control fish (Fig 1) measures 2.0cm. The structure of gills bear four pairs of gill lamellae and both the sides are supported by bony structure and primary lamellae. The secondary lamellae show numerous channels of blood capillaries, each separated by single layer of pillar cells when observed in vertical section. The laminar epithelium is thick followed by a basement membrane below where the pillar cells enclose blood spaces. Large number of mucous cells is present on the epithelial gill rakers, where as the primary lamellae had comparatively small and less number of mucous cells. In the 4mg/l treated fish measuring 2.0cm (fig 2), showed gills with loss of histoarchitecture of gill lamella and fusion of secondary lamella. Fishes exposed to 6mg/l of lead nitrate show loss of architecture and fusion of secondary lamellae (fig 3). Destruction of the gills is observed in certain areas. In fishes treated with 8mg/l, the gills showed degeneration of primary lamellae and fusion of secondary lamellae (fig 4).

High concentration of metals in the gill has often been used as an indication of acute exposure since the metals are fixed by absorption processes which occur very rapidly (Oladimeji and Offem, 1989; Noeqrohati, 2006). It has also been reported that much of lead can be bound externally (Hares *et al.*, 1991). The larger surface area of the gills in contact with the medium then could probably account for the higher concentration of lead in the gill. The presence of iron oxide in the gills is known to enhance lead disposition (Hare *et al.*, 1991).

Liver

The histology of liver tissue in the control group showed liver cells with normal exo- structure of hepatic cells (Fig 5). The connective tissue of liver expressed normal condition. Normal hepatic mass granulation was observed. A fragment of pale brown tissue measuring 1.2 cm was observed in the control liver. In 4mg/l treated fish, the liver specimen consisted of a fragment of pale yellow brown tissue measuring 1.0 cm (Fig 6). The liver parenchyma was observed with features of congestion and minimal degeneration of hepatocytes. In 6mg/l treated fish the hepatocytes were reduced in size (Fig 7). Liver parenchyma with feature of congestion and increased cytoplasmic vacuolation were observed. The histopathology of liver treated with

8mg/l lead nitrate showed severe damage and marked proliferation. Liver parenchyma showed cytoplasmic vacuolation of hepatocytes (Fig 8).

The high accumulation of metals in the liver could be related to the fact that the liver played an important role in accumulation and detoxification According to DeSmet and Blust, (2001) the liver showed degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. These findings were apparent as the liver is considered the organ of detoxification, excretion and binding proteins such as metallothionein. The metal-binding proteins were present in the nuclei of hepatocytes. Similar results were observed by Van-Dyk (2003) and Mela *et al., (*2007). Liver of the fishes is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver at much higher levels than in the other organs (Heath 1995).

Kidney

The control fish shows normal appearing renal parenchyma with intact tubules and glomeruli (Fig 9). In the 4mg/l treated fish, the kidney shows renal parenchyma with tubular necrosis and glomerular shrinkage (Fig 10). The 6mg/l treated fish show kidney having renal parenchyma with hydrophic degeneration of tubules and mild necrosis (Fig 11). In the 8mg/l treated fish renal parenchyma shows glomerular shrinkage and tubular necrosis (Fig 12).

The kidney is one of the first organs to be affected by contaminants in water (Thophon *et al.*, 2003). Ahmad *et al.*, (2011) also observed loosening of haemopoietic tissue, vacuolated cytoplasm, damaged uriniferous tubules, shrinkage in glomeruli and expansion of Bowman's space in the kidney of African catfish, Clarias batrachus exposed to cadmium. Iqbal *et al.*, (2004) studied the histopathological changes in the kidney of a common carp following lead nitrate exposure and observed increase in Bowman's space, degeneration of glomeruli, shrinkage of proximal tubule cells with pycnotic nuclei in the exposed fish.

CONCLUSION

Heavy metals like lead were tested in different organs like gills, liver, kidneys and flesh tissues of the fish enduring in natural water system. Most of the metals are present in the edible portion of fish. Humans are also affected by eating fish which can cause health problems. The level of toxic elements in different fishes depends on the fish sex, age, season and place. The pollution of waterways with anthropogenic activities is the major cause of aquatic loss and imbalanced food chain.

ACKNOWLEDGEMENT

With gratitude and respect we record our indebtedness to the Department of Zoology, Nirmala College for women, Coimbatore, for providing all resources to carry out this research work.

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