International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 2, Issue 5, pp: 250-257, 2017 **http://www.ijzab.com**
https://doi.org/10.5281/zenodo.1312117
Research Article

SUBLETHAL EFFECTS OF LEAD ARSENATE ON HISTOLOGY OF SELECTED ORGANS OF FRESHWATER FISH *CIRRHINUS MRIGALA*

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Article History: Received 18th September 2017; Accepted 24th October 2017; Published 27th October 2017

ABSTRACT

Acute toxicity (96 h LC_{50}) of lead arsenate was evaluated in the *Cirrhinus mrigala* in static bioassay over a 96 –h exposure period using probit method. The 96 h LC_{50} values (with 95% confidence limits) of lead arsenate for fingerling fish were estimated as 7.21 mg/l respectively. Histopathological investigations revealed various degrees of pathological lesions in different organs like gill, liver, intestine and kidney. The gill showed the fusion, malformation of secondary lamellae at the tips, vaculation, hyperplasia and disintegration of epithelium. The liver of fish exposed to phenol showed cytoplasmic vaculation, necrosis and loss of hepatic cell wall. In the intestine, necrotic lesions in the epithelial layer, swellings and fusion with adjacent villi, fusion and integration of columnar epithelial layer. The kidney showed shrinkage of glomeruli, breakdown of Bowman's capsule, vacuolization and disintegration of renal epithelium. The histological alterations in the fish could be used as an important tool for assessment of aquatic pollution.

Keywords: *Cirrhinus mrigala*, Acute toxicity, Lead arsenate, Histology.

INTRODUCTION

The histological methods have been in use for assessing the effects of pollutants on aquatic organisms, since such studies bear a direct testimony to the deleterious effects of toxicants (Hinton *et al.,* 1973). Due to rapid industrialization, modern agriculture (application *of* synthetic fertilizers and various insecticides) and domestic sewage many aquatic environment in India are experiencing complicated problems of pollution (Reddy and Rawat, 2013). These pollutants have devasting effects on ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.,* 2007).

A number of pathological changes have been reported in fishes exposed to different heave metals, pesticides, herbicides, biopesticides and industrial effluents. Bakar (1969), Wobeser (1975) and Sinovcic *et al.* (1980) noted copper and mercury induced pathological lesions in *Pseudopleuronectes americans*, *Salmo gairdneri* and *Halobatrachrus didactyls* respectively. Patel and Bahadur (2010) have described various abnormalities in gill and kidney of *Catla catla* during exposure to copper ions. Gupta and Srivastava (2006) demonstrated alternations in the kidney histology of fish, *Channa puncatus* exposed to zinc. Marked structural alterations in the gill and liver tissues are evident in the *Clarias gariepinus* fingerlings following exposure to the herbicide glyphosate (Olurin *et al.,* 2006). Adhikari *et al.* (1998) demonstrated alterations in the gill ultrastructure of *Heteropnuestes fossilis* (Blouch) exposed to malathion. Haniffa and Sundaravadhanan (1984) have observed severe histopathological changes in the tissues of *Barbus stigma*

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exposed distillery effluents. Olojo *et al*. (2005) reported severe histopathological changes in the gill and liver tissues of African catfish, *Clarias gariepinus* exposed to heavy metal lead.

Nickel induced changes in the histology of gill structure in the histology of gill structure in the teleost fish, *Oreochromis niloticus* has been reported by Al-Attar (2007). Abbas and Ali (2007) studied in detail the morphological changes in the gill of *Oreochromis spp*., induced by hexavalent chromium.

The objective of the present study is to high-light various histopathological changes in gills, liver, intestine and kidney of *Cirrhinus mrigala* exposed to sublethal concentration of lead arsenate for 10, 20, and 30 days. The aim of histopathological studies was mainly to assess the extent of internal pathological lesions caused due to excess accumulation of lead arsenate compounds. Pathological studies bear direct testimony to the toxic nature of lead arsenate on the tissues.

MATERIALS AND METHODS

Animal maintenance

The healthy fingerlings *C. mrigala* of the weight (6±05g) and length (7±0.5 cm) were selected for the experiment and were collected in aqua farm near Pattukkottai, Tamil Nadu, India. The collected fishes were safely brought to the laboratory and acclimatized for one month in a large cement tank (1000 L capacity). During the acclimatization period, the fish ad libitum with rice bran and groundnut oil cake. Food was provided once a day. The water was renewed daily to avoid accumulation and contamination of excretory materials and feeding was withheld 24 h before the commencement of the experiment. Fish showing and abnormal behavior was removed as soon as possible. In the present study tap water free from chlorine was used which had the following physic- chemical characteristics; temperature 28±0.13 pH 7.6±0.04, salinity 1.2±0.13 ppt, D.O.5.6±0.2 mg / l and total hardness 35±0.5 mg / L. Before the start of the experiment suitable numbers of fish were transferred into two glass aquaria which were continuously aerated.

Preparation of stock solution and determination of 96 h LC_{50} value of lead arsenate

Stock solution of lead arsenate was prepared by dissolving 1 g of lead arsenate in an appropriate amount of water. For the determination of median tolerance limits or LC_{50} different concentrations of lead arsenate (5, 6,7,8,9 and 10 mg/L) were prepared from the stock and added in separate glass aquaria containing 50 L of water. Three replicates were maintained for each concentration and 10 fishes of equal size and weight were introduced. The test water was renewed at the end 24 h and freshly prepared phenol was added to maintain the concentration of lead arsenate at a constant level. A concurrent control of 30 fish in three different glass aquaria was maintained under identical conditions. The mortality was recorded after 24, 48, 72 and 96 h, and median lethal concentration (LC_{50}) values were calculated by the Finney method (1971). 1/10th value of the LC_{50} value for 96 h was taken as the sub-lethal concentration (Sprague, 1973).

Sub-lethal studies

For sub-lethal toxicity tests 100 fingerlings were selected and divided into four groups (one control and three experimental) with 25 fish in each aquarium filled with water. The desired concentration (1/10 of 96 h LC_{50}) of the toxicant was added directly in order to maintain constant concentration of the toxicant. The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. At the end of the stipulated periods (10^{th}, 20^{th} and 30^{th} day) of exposures fish were randomly selected and sacrificed for histological studies.

Histology

On 10, 20 and 30th day fish were taken out, sacrificed and the gill, liver, kidney and intestine were excised out. The tissues were fixed in Bouin's fluid and then they were processed (Gurr**,** 1950) and embedded in paraffin wax (58 – 60^{o}C). Serial sections of 8 µm thickness were cut and deparafinshed sections were stained in haematoxylin and counterstained with aqueous eosin.

RESULTS

LC_{50} value for 96 hrs

The LC_{50} value based on probit analysis was found to be 7.21 mg/l for 96 h of exposure to lead arsenate. During this study the behaviour of the control fish was normal, while the fish introduced into the sublethal concentration of the lead arsenate showed different abnormal behaviour. Abnormal behaviour such as erratic swimming, increase in surface activity, spreading of excess of mucus of the body and restlessness were observed in fish exposed to the lead arsenate.

Histological observation of the control fish gill

The structure of primary gill lamellae of the control fish are laterally compressed leaf like structures (Figure 1. Each primary gill lamella bears finger – like structures called secondary gill lamellae and were thin, slender and attached on either side of the primary lamellae. The lamellae consisted a numerous vascular cells with blood lacunae covered by thin epithelial layer.

Histological alterations in lead arsenate treated fish gill

Several pathological lesions appeared in the gills of lead arsenate treated fishes. Gills were characterised by the fusion, malformation of secondary lamellae at the tips, vaculation, hyperplasia and disintegration of epithelium. At the end of the test exposure periods (10, 20, 30 days), severe pathological lesions were seen in both 20 and 30 days of 10% sublethal concentrations treated fishes (Figure 2-4).

Histological observation of the control fish liver

The liver of the normal fish comprised continuous mass of hepatic cells with cord like formation. They were large in size and hexagonal in shape with centrally placed nucleus (Figure 5).

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Histological alterations in lead arsenate treated fish liver

Fish exposed to sublethal concentrations of lead arsenate several pathological changes were noticed in liver tissue. In 10, 20, 30 days treated fishes, the liver tissue revealed many necrotic cells. In most of the hepatic cells the integrity of the cell wall was completely lost. Intracellular vaculation was also apparent. The liver tissue was found severely injured in 10% sublethal concentration of lead arsenate 30 days treated fishes compared to 10 and 20 days treated fishes (Figure 6-8). In most of the places cytoplasmic vaculation, necrosis was common.

Histological observation of the control fish intestine

The intestine of control fish consists of simple columnar epitheliums which were thin and slender (Figure 9). The columnar epithelial cells arranged uniformly in the mucusa layer had a distinct double layered tunica propria.

Histological alterations in lead arsenate treated fish intestine

In fish treated for 10, 20, 30 days the intestine showed several necrotic lesions in the epithelial layer, swellings and fusion with adjacent villi, fusion and integration of columnar epithelial layer (Figure 10-12). On 30 days exposure of 10% sublethal concentration these lesions were prominent.

Histological observation of the control fish kidney

In control fish, the kidney involved in excretory functions is formed by a large number of nephrons. Each nephron consists of a renal corpuscle and a coiled uriniferous tubule (Figure 13). The corpuscle was made up of glomerulus and Bowman's capsule.

Histological alterations in lead arsenate treated fish kidney

The fish exposed to the subthal concentrations of lead arsenate showed shrinkage of glomeruli, breakdown of Bowman's capsule, vacuolization and disintegration of renal epithelium. Thereafter, the degeneration changes were further exaggerated 30 days of 10% sublethal concentration of lead arsenate treatment (Figure 14-16).

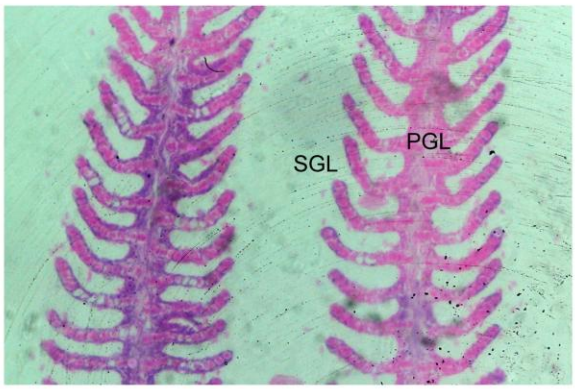

Figure 1. Normal gill (400x) primary gill lamellae econdary gill slamellae.

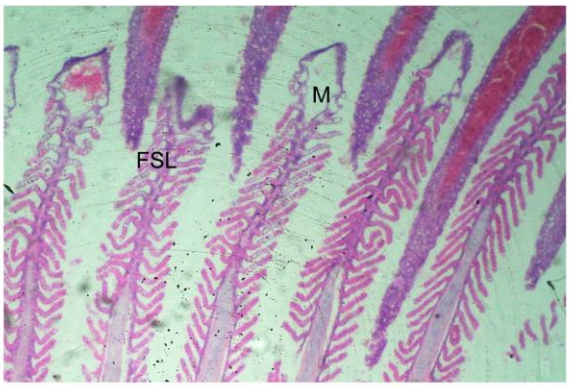

Figure 2. 10 Days treated gill (100x) primary gill lamellae fusion and malformation of secondary gill lamellae.

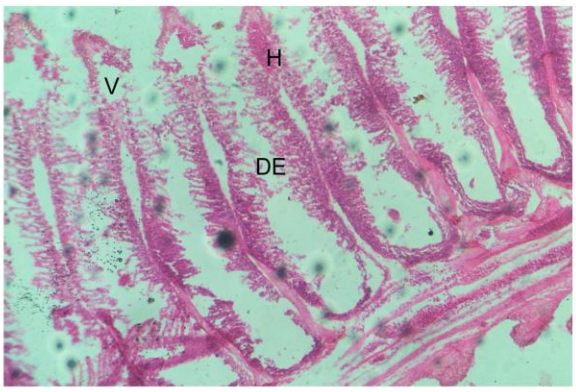

Figure 3. 20 Days treated gill (100x) dasintegration of gill epithelium, Vaculation, hyperplasia

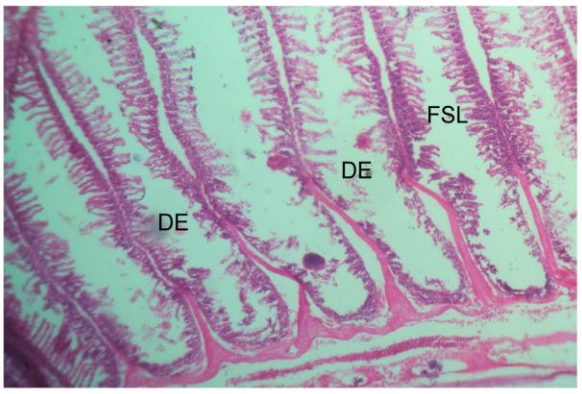

Figure 4. 30 Days treated gill (100x) dasintegration of gill epithelium, Vaculation, hyperplasia, fusion of secondary gill lamellae.

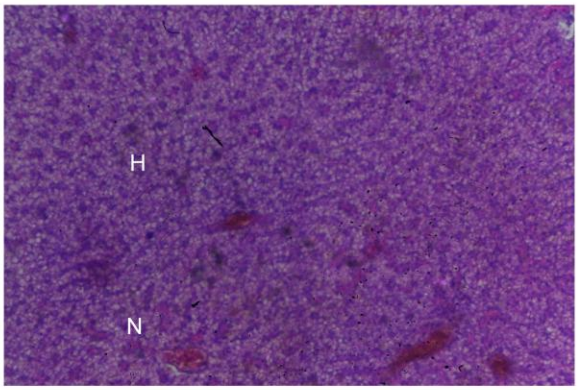

Figure 5. Normal liver (100x) hepatic cells, nucleus.

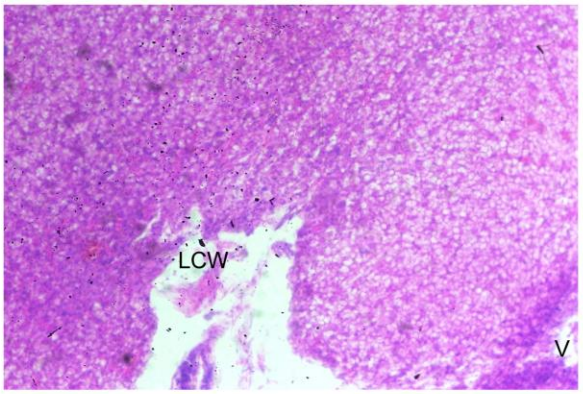

Figure 6. 10 Days treated liver (100x) loss ofcell wall, vaculation.

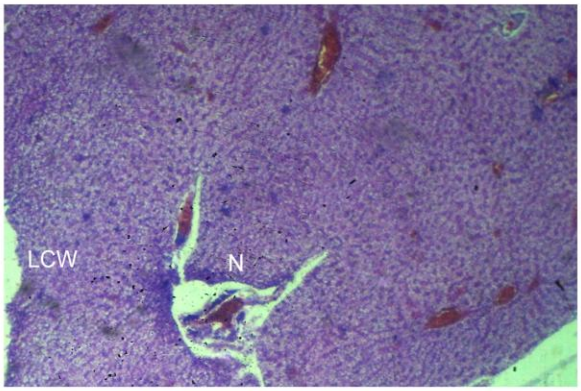

Figure 7. 20 Days treated liver (100x) necrosis, loss of cell wall.

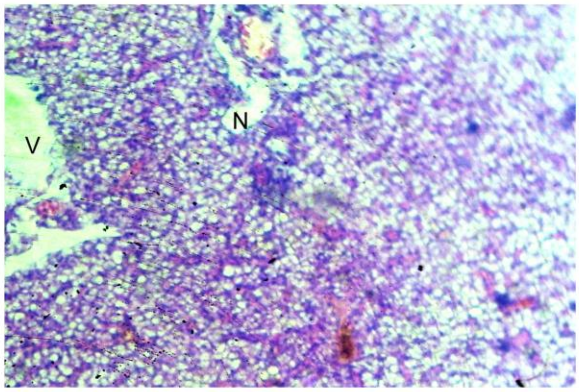

Figure 8. 30 Days treated liver (400x) necrosis, vaculation.

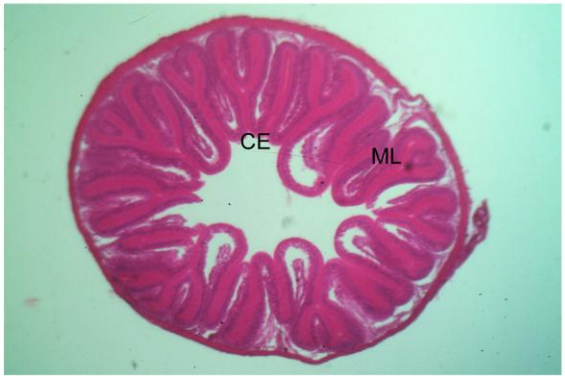

Figure 9. Normal intestine (400x) mucusa layer, columnar epithelium.

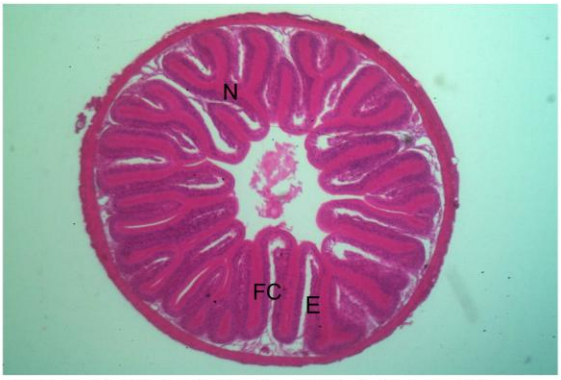

Figure 10. 10 Days treated intestine (400x) necrosis usion of , fcolumnar epithelial layer.

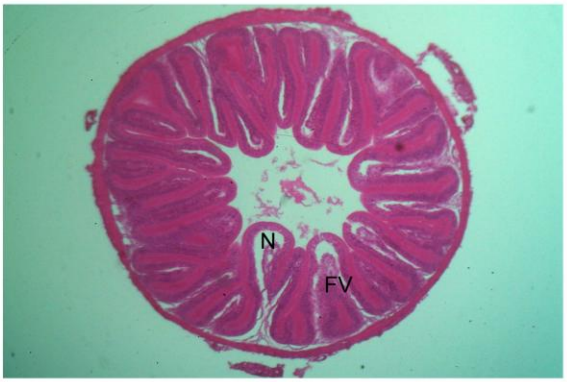

Figure 11. 20 Days treated intestine (400x) necrosis, fusion of villi.

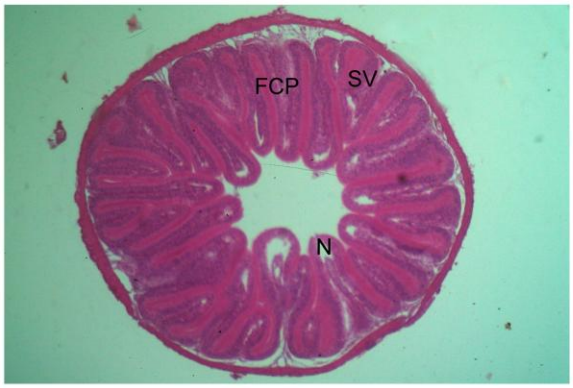

Figure 12. 30 Days treated intestine (400x) necrosis, fusion of columnar epithelial layer, swellings of villi.

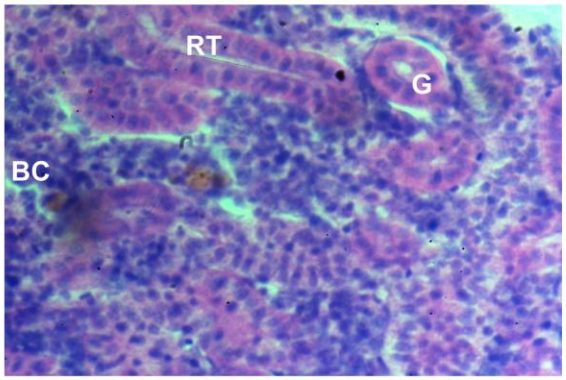

Figure 13. Normal kidney (400x) renal tubule, glomerulus, Bowman's capsule.

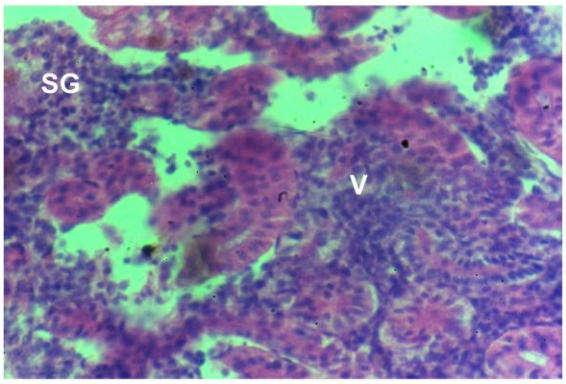

Figure 14. 10 Days treated kidney (400x) shrinkage of glomeruli, vacuolization.

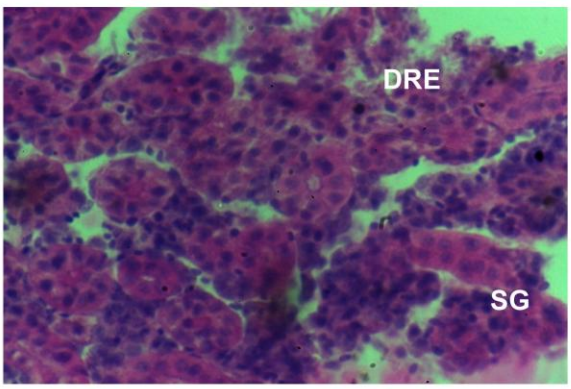

Figure 15. 20 Days treated kidney (400x) shrinkage of glomeruli, disintegration of renal epithelium.

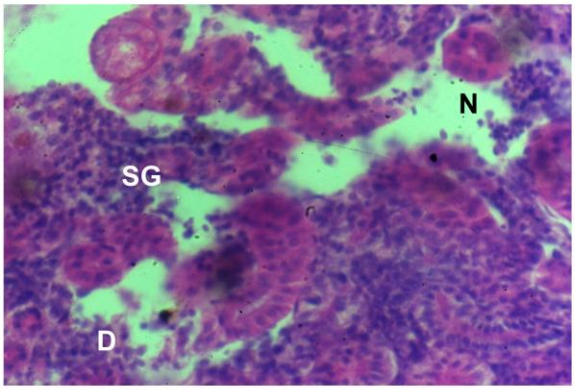

Figure 16. 30 Days treated kidney (400x) shrinkage of glomeruli, disintegration of renal epithelium, necrosis.

DISCUSSION

The gills which participate in many important functions, in the fish, such as respiration, osmoregulation and remain in close contact with the external environment and particularly sensitive to changes in the quality of water are considered the primary target of the contaminants (Camargo and Martinez, 2007; Fernandas and Mazon, 2003). Pathological changes observed in the gills of fish exposed lead arsenate were characterized by the fusion of secondary lamellae degeneration of epithelium, hypertrophy, erosion of secondary lamellae and malformation at the tips of the gill lamellae**.**

The histological lesions of the gills of fish, *Oreochromis niloticus* exposed to heavy metals were reported in detail by Kaoud and El-Dahshan (2010). They observed histological changes like edema, hyperplasia fusion and focal desquamation of the epithelial lining of the secondary lamellae. Georgieva *et al.* (2010) have also reported the histological alternations of gills due to heavy metal copper toxicity in Crucian carp, *Carassius gibelio* and attributed them to the congestion of blood vessels in the gills. Vinodhini and Narayanan (2009) observed proliferation of epithelial cells fusion and degenerative changes in the lamellae and oedematous separation in epithelial cells in heavy metals (Cd, Pd, Cr and Ni) *Cyprinus carpio*. These observations are in agreement with the results reported by Gaafar *et al*. (2010) in Nile Tilapia treated with pesticide edifenphos. Natarajan and Aruna Devi (2006) recorded histological changes in the gills of distillery effluent treated carp, *Labeo rohita*. Further, Sravanan *et al*. (2010) also observed such gill damages in endosulfan treated *Labeo rohita*.

Histological alterations of the gill result in the failure in absorption of the O_2 from the aquatic medium .The decreased oxygen uptake by the damaged gill tissue results in histotoxic anoxia in which the gill tissue not only suffers from oxygen debt but also losses the capacity to remove CO_2 from blood, and consequently greater accumulation of the byproducts of glycolysis (Sathivel *et al.*, 1991).

The fish exposed to sublethal concentrations of lead arsenate revealed the loose arrangement of hepatic cells with vacuolation, dilation of blood sinusoids, cytolysis and necrosis)**.** Histopathological changes were pronounced on the 30% sublethal concentration of 30^{th} day of exposure to lead arsenate. Lead arsenate induced histopathological changes in the liver of fishes have been reported by many authors. Butchiram *et al.* (2013) in *Labeo rohita*. They observed enlargement of nuclei in liver cells, atrophic cells, enlarged sinusoids in liver due to lead arsenate toxicity in addition to formation of number of vacuoles and loose arrangement of cells, similar to the observations in the present study. El-Serafy *et al*. (2009) suggested that the extent of liver damage due to phenol treatment was dose and duration dependent. In the present investigation also, with highest sublethal concentration of the lead arsenate at 30 days of exposure, histopathological changes were more pronounced. Similar observations were also reported by Abdel Hameid (2007) in the fish, *Oreochromis* aureus exposed phenol. Radha Krishnan and Hemalatha (2010) observed in cadmium chloride treated *Channa striatus*, the presence of vacuolation blood vessel congestion and necrosis were observed to the occasionally present. Narayan and Singh (1991) also reported that *Heteropneustes fossils* when exposed to Thiodon showed degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in the liver. The present observations in *C. mrigala* is highly comparable with the previous reports on the histopathology of fishes.

The fish exposed to the sublethal concentration of lead nitrate showed the occurrence of inflamed lamina propria, fusion of columnar epithelium and loss of brush border. These observed histological deformities in the intestine are in agreement with the previous report on the histopathology of in other species of fishes.

Chronic exposure to fluoride produced irritation and destruction of the mucosa membrane in the intestine of *L. rohita* (Bhatnagar *et al*., 2007) reported the broken villi in the intestine . The pathological alterations in the intestine of the studied fish are in good agreement with those observed by the many investigators about the effects of different toxicants of fish intestine (Hanna *et al*., 2005; Cengiz and Unlu, 2006).

Fish exposed to the sublethal concentration of lead arsenate showed shrinkage of glomeruli, vacuolation, enlargement of renal tubules, necrosis and hyperplasia. These changes were the same as that reported by Persis and Kalaiarasi (2003) who worked on pathological changes of the kidney in the fresh water catfish, *Mystus vittatus* exposed to pesticide dimethoate. Rashatwar and Ilyas (1984) have reported the histopathological changes in kidney to lead and noted swelling of renal tubules in *Nemacheilus denisonii* acutely exposed to phosphamidon. Mohapatra and Noble (1992) have observed vacuolation of epithelial cells of renal tubules enlargement of renal tubules and extensive desquamation in nuvan treated mullet *Liza partsia*. Necrosis and vaculation in kidney were observed by Iqbal *et al*. (2004) in *Cyprinus carpio* exposed to nitrate. Sukumar and Karapagaganapathy (1986) observed a number of striking changes in the histological structure of the kidney of *Colisa lalia* exposed to carbofuran for a period of 30 days. Prashanth (2011) studied histopathological changes in the kidney of *C. mrigala*, exposed to cypermethrin. The results of the present observations in *C. mrigala* exposed to sublethal concentrations of lead arsenate were in agreement with those of the earlier workers, especially in the degeneration and shrinkage of glomeruli, vacuolation and necrosis.

CONCLUSIONS

Under the light of this study, it is concluded that lead arsenate is moderately toxic to fingerlings catfish, *C. mrigala.* Histopathological studies revealed changes in the structural integrity of the cells of gill, liver, intestine and kidney. These results of the present study indicate that

C. mrigala is a potential biological indicator of environmental pollution and might be suitable species for the evaluation of water quality.

ACKNOWLEDGMENTS

Authors would like to thank the authorities of Khadir Mohideen College, Adirampattinam for providing necessary facilities to carry out the experimental part of this work.

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