

Original Research Article

Effect of heavy metals on liver and gill of fish *Cirrhinus mrigala*

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A B S T R A C T

Keywords

Cirrhinus mrigala, mercuric chloride, lead acetate, histopathology, GOT, GPT

Samples of *Cirrhinus mrigala* were collected from Kalambe village reservoir near Kolhapur. The fishes were exposed to sublethal concentrations of mercuric chloride (0.0206 ppm and 0.0402ppm) and lead acetate (28.2 ppm and 14.1 ppm) for 30 days. Effect of these heavy metals on histology of gills and liver and transaminases activity of liver was studied. The results showed severe pathological alterations in gills and liver of *C. mrigala*. The major histopathological changes in liver include loss of cellular architecture, necrosis in hepatocytes and accumulation of fat in parenchymal cells. The gills showed lamellar degeneration, epithelial lifting and necrotic changes in intercellular epithelial cells. Increase in glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were noticed which indicate liver damage.

Introduction

In last few decades increase in population density, heavy industrialization and agricultural activities have resulted in more and more wastes entering in fresh water resources. Contamination of fresh water with a wide range of pollutants has become a matter of concern over last few decades (Vutukuru, 2005). Heavy metals released from domestic, industrial and other man made activities may contaminate the natural aquatic system extensively (Velez, 1998). Heavy metals have devastating effects on ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007). 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). Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro et al., 2005). In order to evaluate the adverse effects of the pollutants on aquatic organisms, there is a worldwide trend to complement physical and chemical parameters with biomarkers in aquatic pollution monitoring (Abdel et al., 2012). Study of histopathology is of prime importance in the diagnosis, etiology and prevention of disease. Data on tropical fish and effects on different fish tissues are still scarce (Mela et al., 2007). Moreover, there are no studies dealing with the steps associated with

metal toxicology including accumulation and damage in the different target organs. The fish liver is a vital organ concerned with basic metabolism and is the major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo et al., 2006). Impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of fish that respond specifically to the degree and type of contamination (Barhoumi et al., 2012). The liver is particularly susceptible to damage from a variety of toxicants. One of the most important functions of liver is to clean pollutants from the blood so it is considered as indicator of aquatic environmental pollution (Soufi, 2007).

There have been numerous reports of histopathological changes in liver of fish exposed to a wide range of organic compounds and heavy metals (Abdel, 2012; Au D.W.T, 2004). Changes in several haematological variables are recognized as indicators of metal exposure (Cyriac et al., 1989). GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) are important diagnostic tools in medicine and are used to detect adverse effects produced by various pollutants (Nelson, 2000).

Fish gills on the other hand are critical organs for respiration, osmoregulation and excretion. Gills serve as a good indicator of water quality. They are sensitive to any change of water components since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water (Serafy, 2009; Au. DWT, 2004). Gill histology is therefore extensively used as indication of environmental pollution.

C. mrigala is a major Indian carp used in pisciculture, highly esteemed as food and

is available throughout year and used as a model in various ecotoxicological studies. Hence it was considered as a model for bioassay.

The present study was aimed to evaluate the histopathological effects of heavy metals lead and mercury on gills and liver including liver enzyme assays (transaminase) of *C. mrigala* and to enhance the knowledge of tissue damage after exposure to heavy metals.

Materials and Methods

C. mrigala (70-72g weight and 19-20cm length) irrespective of sex were collected from reservoir at Kalambe village near Kolhapur, Maharashtra, India. Fishes were acclimatized for 15 days to laboratory conditions. During these days fish were fed with de-oiled groundnut oil cake. The laboratory water was analyzed for different physico-chemical parameters (APHA, 2010) and for mercury and lead. No fish mortality was recorded during acclimatization. LC₅₀ for mercuric chloride and lead acetate was statistically determined (Finney, 1971). Well acclimatized fishes showing no signs of stress were selected and divided into three groups of ten each for exposure to each toxicant. The first group served as a control and other two groups were exposed to sublethal concentrations of mercuric chloride and lead acetate. A dose of 0.0412 ppm (1/10th of LC₅₀) and 0.0206 ppm (1/20th of LC₅₀) of HgCl₂ and a dose of 28.2 ppm (1/10th of LC₅₀) and 14.1 ppm (1/20th of LC₅₀) of lead acetate was administered to experimental group daily for 30 days. The experiment was carried out in replicate. The fishes were kept in plastic tubs containing 20 l test medium without aeration. The test solution was renewed once after 24h replacing the test solution. Analytical grade mercuric

chloride and lead acetate (Qualigens) was used as a toxicant in this experiment.

After completion of experiment 5 fish from each group were sacrificed and liver and gill tissues were collected carefully from both treated and control group.

Both tissues were then fixed in aqueous Bouin's fixative for 48 h with a change after 24 hours. Fixed tissues were washed with 50% ethanol and dehydrated further through 70%, 90% and absolute ethanol and cleared in xylene. The tissues were then embedded in paraffin wax and sections of 5 microns were obtained on rotary microtome. The sections were then stained with Harry's haematoxylin and eosin to observe the architecture of gills and liver of both treated and control fish. Stained slides were observed under compound microscope, photographed and assessed.

Liver Enzymes: Blood samples were collected from the cardinal blood vessel of fish using the method of Kori-Siakpere and Egor (1997). EDTA coated tubes were used because unlike heparin it did not cause the blood cells to shrink. Blood samples were left to coagulate for 15 to 20 minutes at RT and then centrifuged at 3000 rpm for 10 min. to separate serum and the serum samples were stored in polyethylene Eppendorf test tubes at -20°C until serum analysis. Serum samples were used to estimate GOT and GPT. The quantitative determination was done by kinetics-based differential pulse volumetric method Merck kit by kinetic method (He and Chen, 1997).

Statistical Analysis- All statistical analysis was done, using the computer programme GRAPHPAD.

Results and Discussion

Physicochemical parameters – Water from where fish were collected and laboratory water was tested for heavy metals. Lead and mercury were below detectable level (BDL). The water parameters were temperature $20-22^{\circ}\text{C}$., pH 7.0-7.5, dissolved oxygen 6.8- 7.0 mg/l and total hardness 132-135 mg/l.

Liver enzymes: The mean values of liver enzymes of control and experimental fish are shown in table 1. The values of GOT and GPT of fish exposed to mercuric chloride and lead acetate were very high as compared to control.

Histopathological Findings: Liver – The untreated liver showed a typical compact architecture with characteristic distribution and morphology of cells. Histological examination of liver of fish treated with mercury showed loss of cellular architecture. Blood vessels seemed to be dilated. Haemolysis due to destruction of erythrocytes, inflammation of hepatic cells was also observed. Eccentric nuclei, vacuole appearance were common. Congestion in blood sinusoids appeared in liver tissue (Fig. 1). The most prominent alterations of liver after lead exposure were cytoplasmic vacuolation, intravascular haemolysis in blood vessels, dilation and congestion in sinusoids and venules and cellular degeneration. Focal necrosis was prominent. (Fig.3)

Gills: The untreated gills showed an arrangement of filaments in double rows and the secondary lamellae arise from these filaments. The gills of mercury exposed fishes showed degenerative changes in epithelial cells of secondary gill filaments, moderate necrotic changes in inter lamellar epithelial cells, twisting of

gill filament tips, infiltration of cells in primary axis. (Fig.2). The gills of lead exposed fish showed dilation and congestion in blood vessel of primary gill filament. Hyperplasia of epithelial cells between secondary lamellae led to fusion and separated from pillar system. Vacuolation and necrosis of lamellar epithelial cells. Congestion of central lamellar vein and hyperplasia of lamellar epithelial cells was evident in gills of fish exposed to lead (Fig.4). Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals (Mallatt, 1985). Edema with lifting of lamellar epithelium could be served as a mechanism of defense (Arellano et al.,1999).

The transaminases, serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are two enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases (Todd, 1964). The increased serum aminotransferases reflect myocardial and hepatic toxicity leading to extensive liberation of the enzymes into the blood (Heath, 1987, Abo Hegab et.al., 1993). Monitoring of liver enzymes leakage into the blood has proved to be a useful tool in liver toxicological studies (Osman et.al., 2010). In the present study, increase in GOT and GPT transaminases might be attributed to tissue damage particularly liver. GOT and GPT enzymes activity were found to increase in response to heavy metals in different fish species (Mekkawy, 2011). Zikie (2001) observed that chronic exposure of fish to metals like Zn, Cu and Cd elevates the levels of plasma GOT and GPT. Significant positive correlation between heavy metals concentration and GOT and GPT values in present studies confirm it.

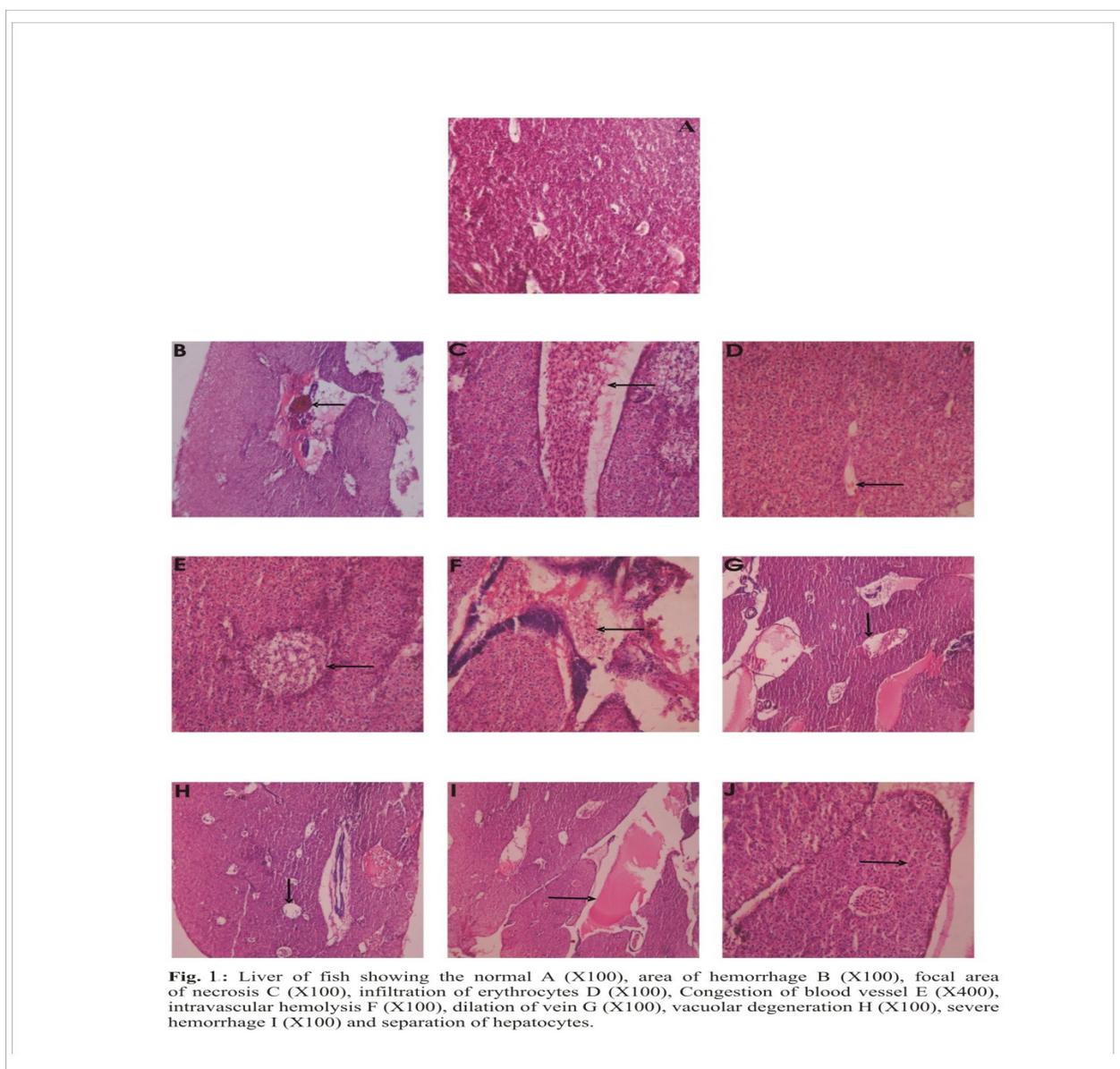
Present findings also coincide with reports of Aly et. al., (2003) stating histopathological lesions which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to liver injury.

The histological changes seen in the liver are not metal specific but are generally associated with the response of hepatocytes to toxicants (Hinton 1990). Histological biomarkers of toxicity in fish organs are useful indicator of environmental pollution (Peebua, 2008). Histopathological changes in gills, liver, kidneys and gonads of fish in response to agricultural, sewage and industrial pollutants have been reported by Mohamed (2003). Sorensen et al. (1980) derived a link between exposure to heavy metals and lesions in liver. Similar conclusions were described by Aly et al., (2003) in *Clarias gariepinus* after exposure to lead. Vacuolar degeneration and necrosis of hepatocytes was observed. Lesions like dilation of blood vessels, degeneration and necrosis in hepatocytes were observed in *Tilapia mossambicus*, *Clarias gariepinus* and *Mugil cephalus* from Nile river (Ibrahim, 2005). Various histological and physiological changes in a fresh water fish, *Tilapia mossambicus* exposed to hepatochlor were observed (Jayanth Rao, 1984). Vacuolar degeneration in the hepatocytes and necrosis was noted by Autman (2007). Degeneration and necrosis of hepatocytes may be due to the cumulative effect of the metals and increase in their concentration in liver. The cellular degeneration in the liver may be due to oxygen deficiency as a result gill degeneration and / or vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood. (Mohamed

Table.1 GOT and GPT of *C. mrigala* after chronic exposure to heavy metals.

Analysis	Mercuric Chloride			Lead Acetate		
	Control	Concentration in ppm 0.0412 ($1/10^{\text{th}}$)	Concentration in ppm 0.0206 ($1/20^{\text{th}}$)	Control	Concentration in ppm 28.2 ($1/10^{\text{th}}$)	Concentration in ppm 14.1 ($1/20^{\text{th}}$)
GOT (mIU/ml)	51.33±1.154	133.33±5.77	96.66±2.309	43.0±2.24	265.0±5	85.66±5.13
GPT (mIU/ml)	16.0±3.605	36.66±7.63	28.0±1.732	16.09±3.20	27.33±2.51	17.33±2.51

All results are the mean of three observations with ± standard deviation. The enzyme activity is measured in unit as mIU/ml.



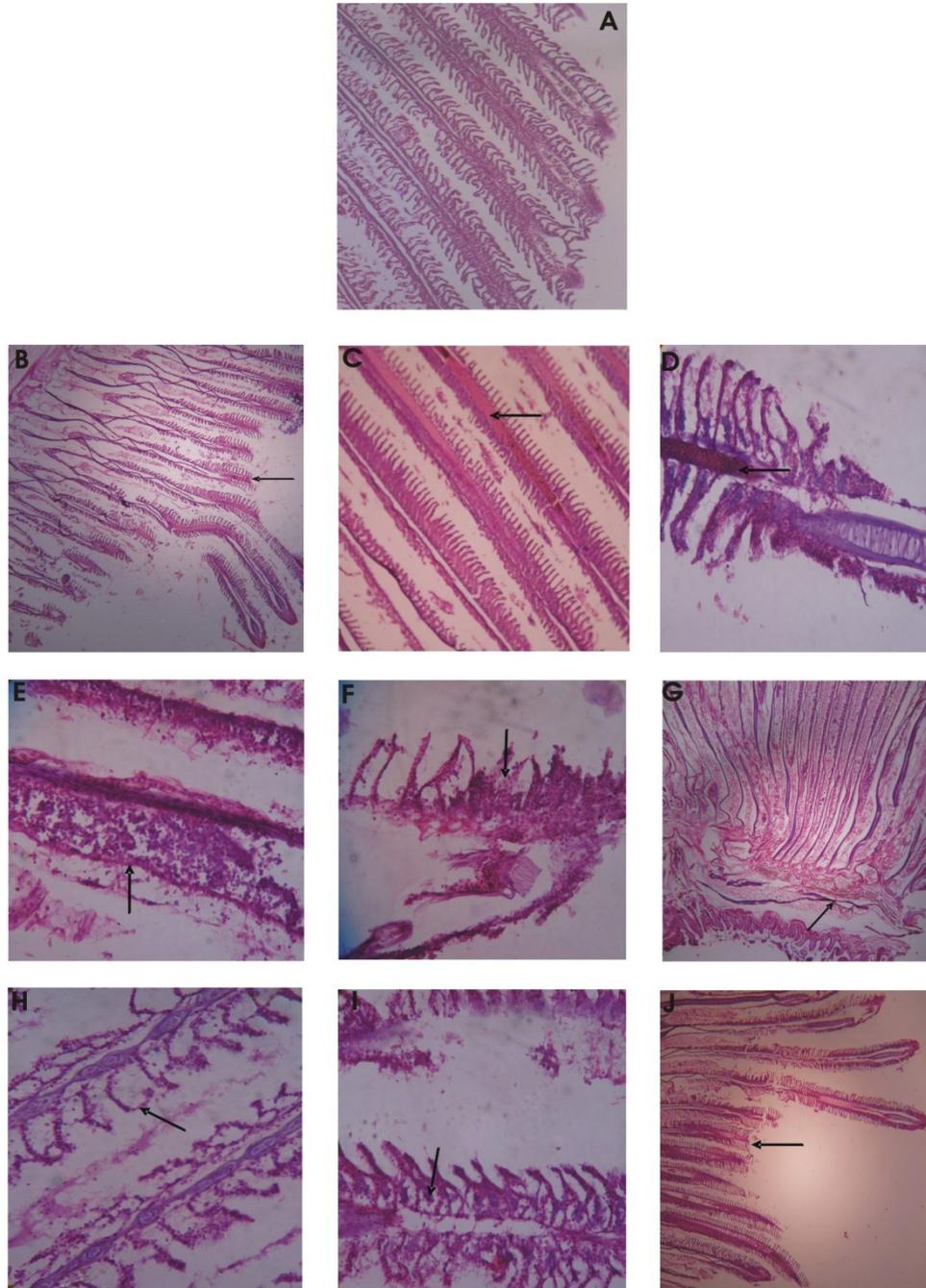


Fig. 2: Gill of fish showing the normal A (X100), arrow shows degenerative changes in gill filament B (X100), dilation and congestion of blood vessel in gill filament C (X400), degenerative and necrotic changes in epithelium of gill filament D (X400), severe degeneration in secondary gill lamellae E & F (X400), separation of gill filament from basement membrane G (X400), curling of secondary lamellae H (X400), proliferation of mucous cell I (X400) and atrophy of gill filament J (X100).

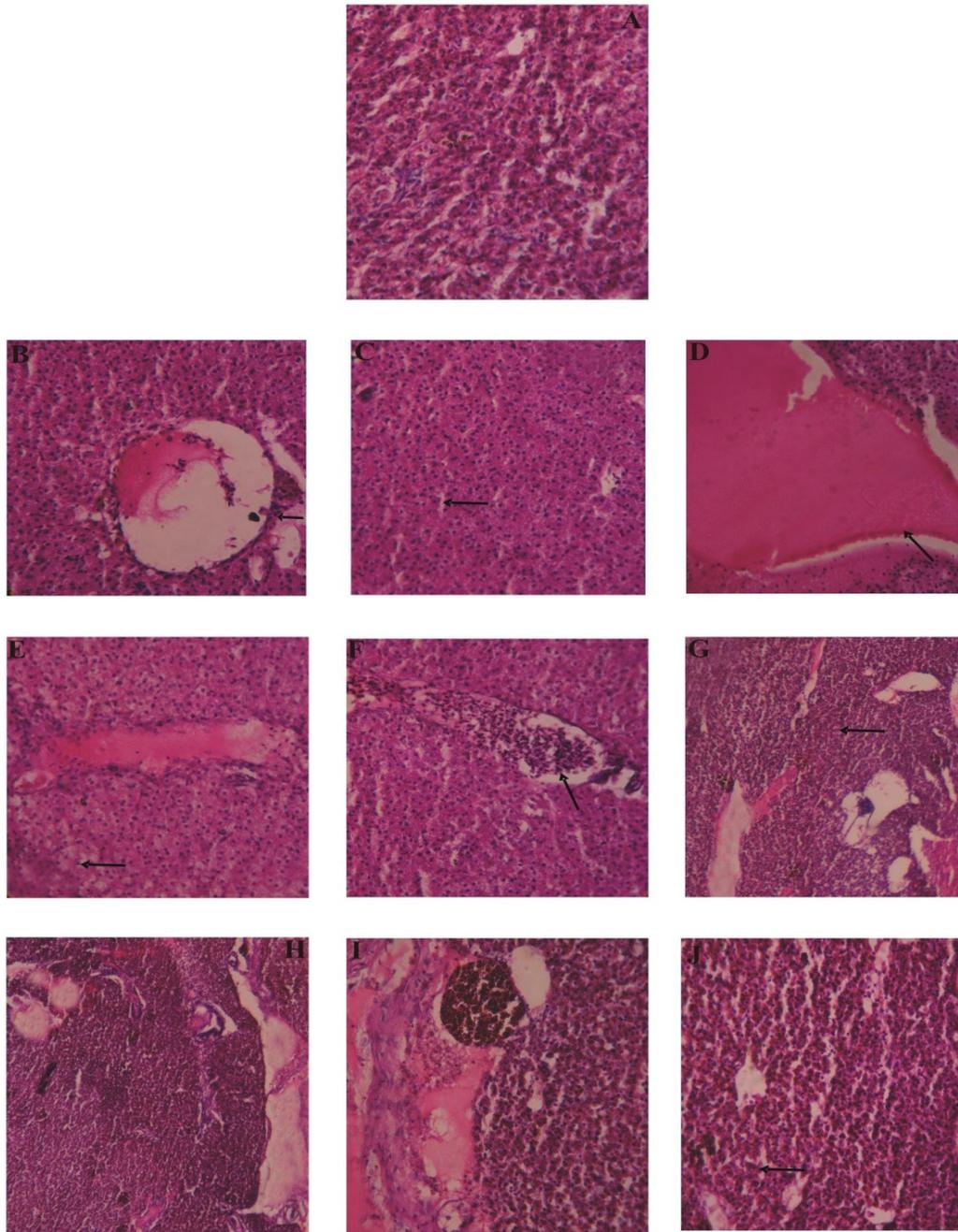


Fig. 3: Liver of fish showing the normal A (X400), arrow shows dilation of blood vessel and mild necrosis B (X400), disconnection between hepatocytes C (X100), hemorrhage D (X100), increased vacuolation and eccentric nuclei E (X400), hemolysis F (X100), hepatocyte degeneration and necrosis G (X100), mild hemosiderin H (X100), erythrocyte infiltration in blood sinusoids I (X400) and hepatocyte hypertrophy J (X400).

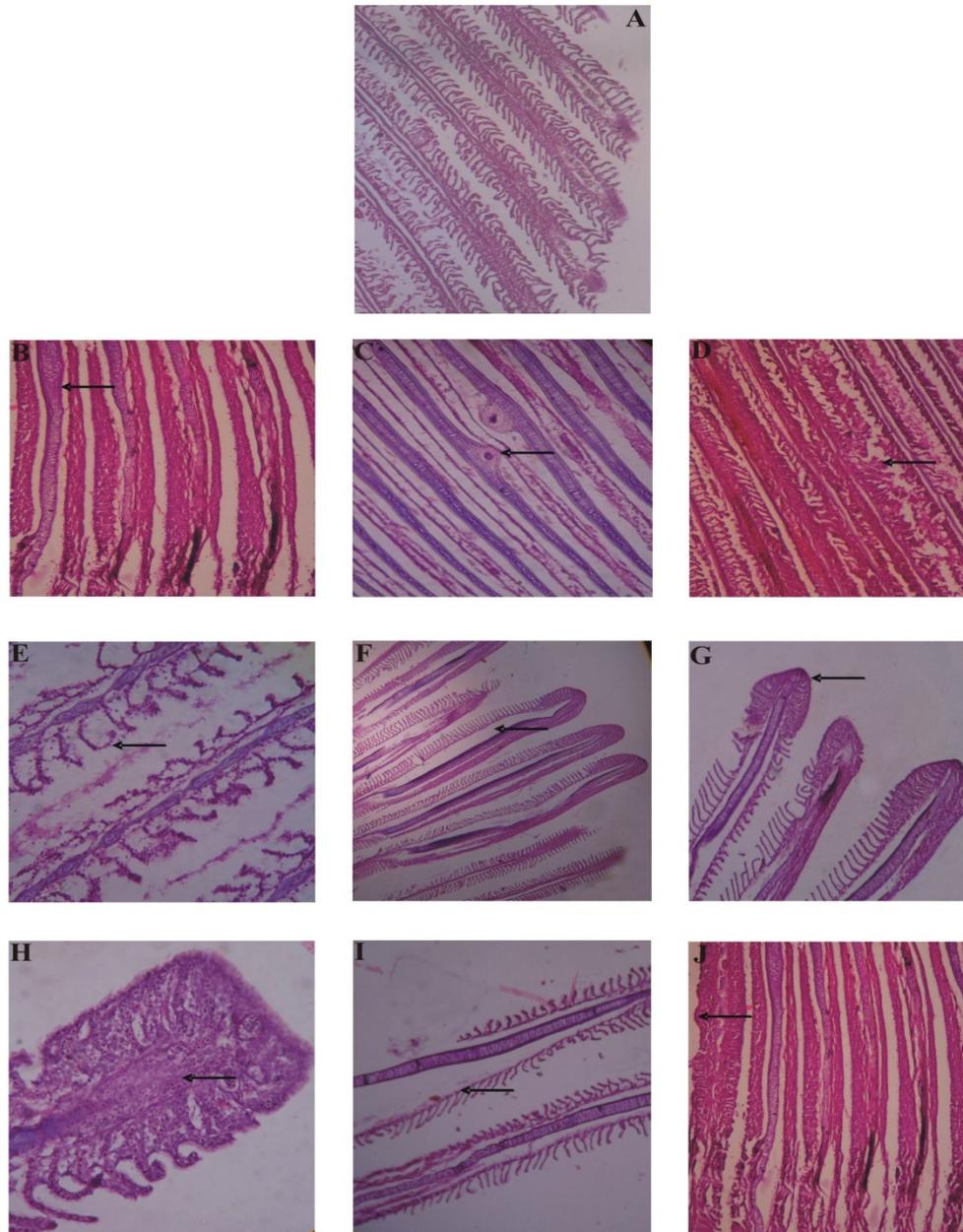


Fig. 4: Gill of fish showing the normal A (X100), arrow shows widening in gill filament B (X100), focal proliferation in epithelial cell C (X100), severe loss in histoarchitecture D (X100), curling of gill lamellae E (X400), separation of primary gill filament F (X100), fusion of secondary lamellae G (X100), hemorrhage in primary and secondary gill lamellae H (X400), loss of structural integrity of lamellae I (X100) and bulbing of epithelial cells J (X100).

2001). Significant increase in Kuffer cells in liver due to metal accumulation was sited (Koca, 2008). Biological alterations after exposure of the lake white fish *Coregonus clupeaformis* to nickel were cited by Ptashynski, et al., (2002). Histopathological lesions after exposure to lead for 9 days were noted (Olojo 2005).

Histopathological alterations observed in the gills may be a reaction to toxicant intake or an adaptive response to prevent entry of heavy metals and due to increased permeability of gills (Olurin, 2006). The respiratory system provides the most extensive interface of a fish with water and is frequently the first system to be affected by dissolved pollutants (Heath, 1995). Histopathological alterations in gills are linked with specific classes of toxicants. Number of investigators have reported histopathological changes in the gills of different fish species exposed to heavy metals.

Gills of *Labeo rohita* exposed to tannery effluent revealed fusion and clumping of primary lamellar epithelium (Fanta, 2003). Degenerative changes in lamellae and edema was observed in gills of fish exposed to heavy metals (Osman, 2009). *Channa punctatus* exposed to mercury showed contraction and sloughing of respiratory epithelium (Gupta, 2002). Nuclear degenerative changes in parenchyma cells with necrosis was reported in *Cyprinus carpio* due to heavy metals (Vinodini, 2008).

Since gills are the respiratory and osmoregulatory organ of fish, the histopathological changes of the gills might impair the respiratory function of the gills by reducing respiratory surface area resulted in hypoxia, respiratory failure problems (Alazemi, 1996; Yasser,

2011) and this badly affects the physiology and may lead to death of fish (Mohamed, 2003). Although, the present study did not include all these findings, yet we observed severe degenerative changes in liver and gill structure.

The use of enzymatic and histological parameters are very realistic approaches. The enzymatic and histopathological results from the present study indicate that the mercury and lead cause different degrees of injuries to the fish gill and liver. It is recommended to treat the effluent before discharging to the resources to avoid negative impact on aquatic biota.

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