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Effect of Copper Sulphate and Lead Nitrate, Administered Alone or in Combination, on the Histology of Liver and Kidney of *Labeo rohita*

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Abstract.- The aim of this study was to assess the effect of acute doses of copper sulphate and lead nitrate, administered alone or in combination on the histology of liver and kidney of *Labeo rohita*. The 96h LC_{50} values for copper sulphate ($CuSO_4.5H_2O$) and lead nitrate [$Pb(NO_3)_2$] were found to be 3.15 mg L^{-1} and 6.80 mg L^{-1} respectively. In this study, four groups, one control and three experimentals, each of 40 fingerlings of *L. rohita* were used. One treatment group was exposed to 3.15 mg L^{-1} of $CuSO_4.5H_2O$, the second to 6.80 mg L^{-1} of $Pb(NO_3)_2$, the third was, exposed to mixture of 1.575 mg L^{-1} of $CuSO_4.5H_2O$. + 3.40 mg L^{-1} of $Pb(NO_3)_2$, whereas, the fourth group did not receive any metal treatment. Exposure of toxicants caused fatty change, karyopyknosis, nuclear vacuolization and cytoplasmic collapse in liver of all experimental groups. Congestion of blood vessel, liquefactive necrosis of first and second proximal segment, and irregularity in interstitial haematopoietic tissue were observed in kidney of all experimental animals. In addition to these, the extravasation of blood from blood vessel, coagulative necrosis of first and second proximal segments and irregular blood congestion, and tubular necrosis were also observed in E-3 group. These findings classify $CuSO_4.5H_2O$ and $Pb(NO_3)_2$ as strong toxic agents for *L. rohita*.

Key words: *Labeo rohita*, $CuSO_4$, $Pb(NO_3)_2$, LC_{50} values, liver, kidney, histopathology.

INTRODUCTION

The most common form of water pollution and practice in developing countries is that business and household activities that often discharge wastes directly into streams or ponds that are also used for water supplies and culturing of economically important fish frequently utilized as food by public (Stanitski, 2003). This waste contains most damaging forms of aquatic pollutants including sewage, which frequently contains infectious pathogenic organisms, oil and hydrocarbons, heavy metals, radioactive substances, pesticides, herbicides and corrosive substances such as acids and bases (Samantha *et al.,* 2005; Verma *et al.,* 2005).

Another prominent source of aquatic pollution is the frequent use of pesticides. At present, there are more than 200 types of organic pesticides which are available in thousands of different products. These pesticides contain various heavy metals such as iron, copper, chromium, cadmium, zinc, lead, nickel and manganese as active ingredients (Sharma and Agarwal, 2005). These heavy metals ultimately reaches the water bodies and adversely affect the growth, reproduction, physiology and survival of aquatic life, the non-target organisms, including major carps due to their stable and persistent existence in the environment (Hayat *et al.,* 2007).

Liver and kidneys are pivotal organs of the body responsible to maintain the homeostasis as liver is center of metabolism and detoxification, while kidneys are involved in elimination of the wasteful chemicals from body and selective re-absorption (Iqbal *et al.,* 2005; Palacios and Risbourg, 2006). Accumulations of the chemical pollutants are known to adversely affect the histology and functioning of liver, kidneys, muscles and other organs of fish (Iqbal *et al.,* 2004). Histopathology is considered an ultimate tool to find out the effect of pollutants like copper and lead on fish tissue, because the heavy metals are active toxicants for the normal physiology of the animal

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(Atamanalp *et al.,* 2008; Pathan *et al.,* 2009).

Keeping in view the current status of our natural aquatic environments and the use of pesticides and their leaching impacts on these environments the present study was conducted to sort out the after effects of the dissolved elements in water bodies on the fish. Following the aquatic life international criteria (Stephan *et al.,* 1985), based on 96h LC_{50} values protocol for acute toxicity test (Sprague, 1969), the trials in the experiments were conducted to investigate 96h LC_{50} values of copper sulphate and that of lead nitrate, the active ingredients of most pesticides frequently used by growers, further more to investigate the effect of 96h LC_{50} values of copper sulphate, lead nitrate, administered alone or in combination on histology of liver and kidney of economically important freshwater cyprinid fish (*Labeo rohita*) being frequently used as food by public in the area.

MATERIALS AND METHODS

Specimen collection

For stock, 165 fingerlings of freshwater cyprinid fish, *Labeo rohita*, of both sex having total body length ranging between 7.6-11.7 cm and body weight between 5.15-11.40 g were obtained from the Faheem Fish Farm, Mattital Road, Multan and were transported in plastic bags with oxygen to Fisheries Laboratory, Institute of Pure and Applied Biology, Zoology Department at Bahauddin Zakariya University, Multan, and they were acclimatized for 30 days to laboratory conditions. All the experimental procedure and fish handling protocols were approved by Ethical Committee of Zoology Department.

LC_{50} determination

For determination of 96h LC_{50} values, each group of 16 juveniles of *Labeo rohita* were exposed to one of the seven concentrations; 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 7.0 mg L^{-1} of copper sulphate ($CuSO_4.5H_2O$), separate groups of each with 15 fish were exposed to seven concentrations, 1.5, 3.0, 4.5, 6.0, 9.0, 12.0, 18.0 mg L^{-1} of lead nitrate [$Pb(NO_3)_2$]. Fish mortality rate was observed after 96 h. For calculation of LC_{50} values, regression line for copper sulphate and lead nitrate concentrations and fish mortality rate were prepared using Finney's Probit Analysis.

Experimental design

To determine toxicities of $CuSO_4.5H_2O$ and $Pb(NO_3)_2$ groups of 26 fish, in triplicates, in 300 L concrete tanks were exposed to one of the four following acute concentrations of heavy metals for 96 hours: Control group, exposed to heavy metal free lab water, designated as C; Experimental group-1, exposed to 3.15 mg L^{-1} of $CuSO_4.5H_2O$, designated as E-1; Experimental group-2, exposed to 6.80 mg L^{-1} of $Pb(NO_3)_2$, designated as E-2 and Experimental group-3, exposed to 1.575 mg L^{-1} of $CuSO_4.5H_2O$ + 3.40 mg L^{-1} of $Pb(NO_3)_2$, designated as E-3. All experiments were carried out in semi-static systems with water renewal after every 12h with the addition of fresh solution of toxicant with same concentration to sustain the nominal concentrations of copper sulphate and lead nitrate. Temperature, pH and oxygen concentrations of water were maintained throughout the experimental duration following Ali *et al.* (2006). The control mortality was corrected using Abbott formula (Abbott, 1925), where necessary. Alterations in swimming behaviors like motionless stay near the bank of concrete tank, reflexive and spontaneous cough and gill expulsion responses were also observed in study, similar to those described by Kwak *et al.* (2002) after pesticide exposure of fish.

Histology of liver and kidney

At the end of experiment, liver and kidneys were surgically removed from each treated and untreated fish, sliced and fixed in fixative solution (containing ethanol, formaldehyde, and glacial acetic acid: 1:3:7) followed by dehydration in ethanol, clearing in cedar wood oil and embedding in paraffin wax (Iqbal *et al.,* 2005). Sections (4-6 µm thick) were cut, stained with hematoxylin-eosin and observed under light microscope.

RESULTS AND DISCUSSION

LC_{50} Values of $CuSO_4.5H_2O$ and $Pb(NO_3)_2$

The 96h LC_{50} value for experimental group of *Labeo rohita* treated with $CuSO_4.5H_2O$ was found to be 3.15 mg L^{-1} as per regression line in Figure

1A. *L. rohita* could tolerate 0.5 mg L^{-1} $CuSO_4.5H_2O$ as no mortality was observed at this concentration while 100% mortality was observed at 7.00 mg L^{-1}. The 96h LC_{50} value for $Pb(NO_3)_2$ for *L. rohita* was found to be 6.80 mg L^{-1} as per regression line in Figure 1B. LC_0 value for 96h was 1.50 mg L^{-1} while 100% mortality after 96h of $Pb(NO_3)_2$ exposure, was observed at 18.0 mg L^{-1}. Since the LC_{50} values in E-1, E-2 and E-3 were the same and not significantly different from the control, the alive fingerlings were selected for histopathological examinations following the protocol used by Brungs and Mount (1978).

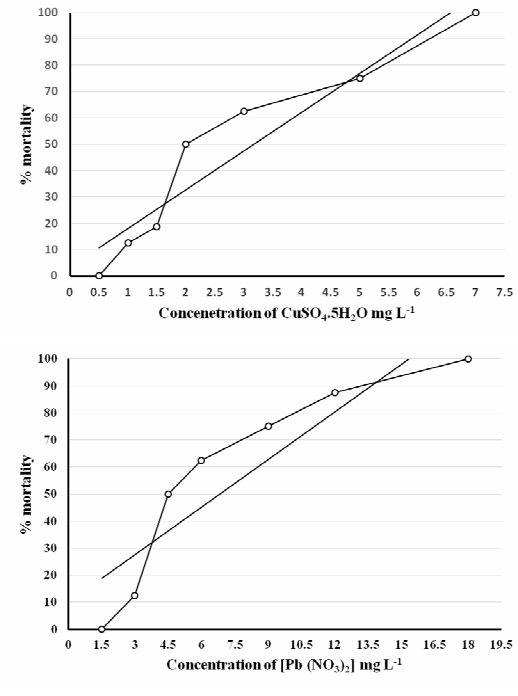

Fig. 1. The % mortality of freshwater cyprinid (*Labeo rohita)* individuals in 96h at various concentrations and calculation of 96h LC_{50} values for $CuSO_4.5H_2O$ (A) and Pb $(NO_3)_2$ (B) for *Labeo rohita* from regression line.

LC_{50} values of $CuSO_4.5H_2O$ (0.56 mg L^{-1}) and lead (27.2 mg L^{-1}) in *L. rohita* were reported by Adhikari (2003) and Abdullah *et al.* (2007) respectively which are not in agreement with our findings but several factors including composition of toxicant, experimental conditions and age of fish may affect the sensitivity of fish to heavy metal exposure (Abdullah *et al.,* 2007).

Histological changes in liver

Liver is composed of polygonal hepatocytes, containing centrally placed spherical large prominent nucleus (Fig. 2A). In liver histology of control group, the hepatocytes were normally arranged in cords of cells (Fig. 2A) with kupffer's cells (Fig. 2A).

In the present study, exposure of acute concentrations of copper sulphate (E-1), lead nitrate (E-2) and their combination (E-3) for 96 h caused karyopyknosis which is marked by irreversible condensation of chromatin in the karyopycknosis in E-3 group was followed by karyorrhexis marked by fragmentation of the nucleus in necrotic cells (Figs. 2D, 3D).

Liver of *L. rohita*, treated with copper sulphate exhibited accumulation of fat within the liver cells (Fig. 3C) displacing and compressing the nucleus karyorrhexis (Fig. 2B) whereby its chromatin is distributed irregularly throughout the cytoplasm), karyopyknosis, nuclear vacuolization (Fig. 2B), cytoplasmic degeneration or collapse leading to increase in size *i.e.* ballooning degeneration (Fig. 2B), and congestion of blood vessel with blood (Fig. 3C).

The liver of *L. rohita*, treated with lead nitrate showed congestion of blood vessel (Fig. 3B), fat deposition deposition (Fig. 2B,C) and karyopyknosis in hepatocytes (Fig. 2C).

The liver of *L. rohita*, treated with copper sulphate and lead nitrate together also exhibited fat deposition (Fig. 2D), karyorrhexis (Fig. 2D), karyopycknosis (Fig. 2D), congestion in blood vessel (Fig. 3 D), and nuclear vacuolization (Fig. 2D).

Similar types of histological changes have been reported by Abdel-Warith *et al.,* (2011), Bhatkar (2011), Gupta and Srivastava (2006), Mohamed (2009) and Das and Mukherjee (2000) in various fresh water fish associated with zinc and copper toxicity.

Our study revealed that copper sulphate and lead nitrate (Braunbeck *et al.,* 1990) caused pathological changes such as karyopycknosis in hepatocytes, followed by karyorrhexis are the cytologic conditions, indicating that hepatocytes are undergoing necrosis. These heavy metals can be taken up by the candidate fish through at least four

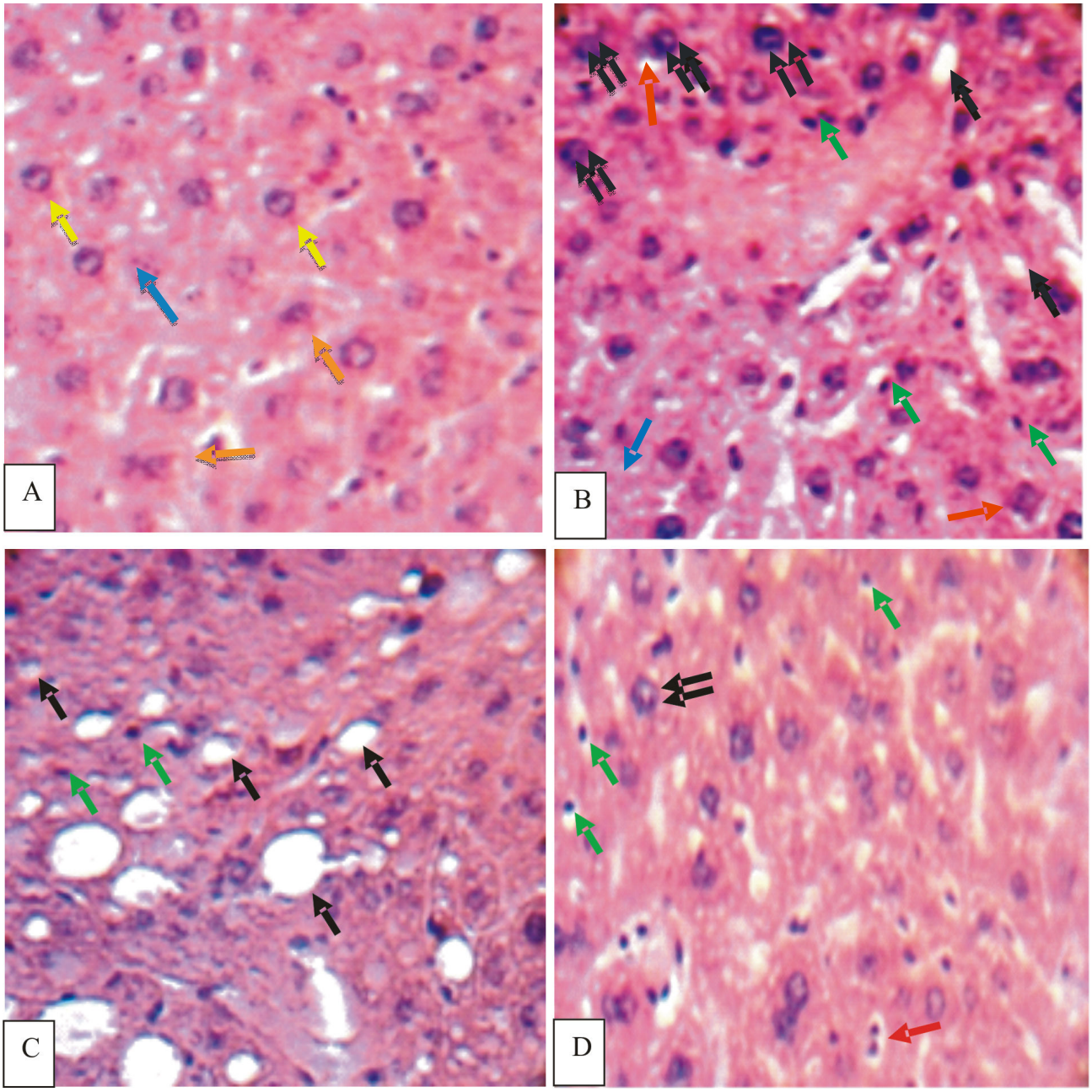

Fig. 2. **A**, Sections (4 µm thick) of liver of the fresh water Cyprinid, *L. rohita*, Control, indicating normal hepatocytes histology, brown arrow indicates Kupffer's cells, while yellow arrow indicates the polygonal hepatocytes with centrally placed nucleus. H & E staining 1000X; **B**, Sections (4 µm thick) of liver of the fresh water Cyprinid, *L. rohita*, treated with copper sulphate. Fatty change (single black arrow). Karyorrhexis (double black arrows); Karyopyknosis in hepatocytes (green arrow); nuclear vacuolization (red arrow) and cytoplasmic degeneration (blue arrows). H & E staining 1000X; **C**, Sections (4 µm thick) of liver of the fresh water Cyprinid, *L. rohita*, treated with Lead nitrate. Fatty change (black arrow). Karyopyknosis in hepatocytes (green arrow). H & E staining 1000X; **D**, Sections (4 µm thick) of liver of the fresh water Cyprinid, *L. rohita*, treated with copper sulphate and lead nitrate. Karyorrhexis (double black arrows); karyopyknosis in hepatocytes (Green arrow); and nuclear vacuolization (red arrows). H & E staining 1000X.

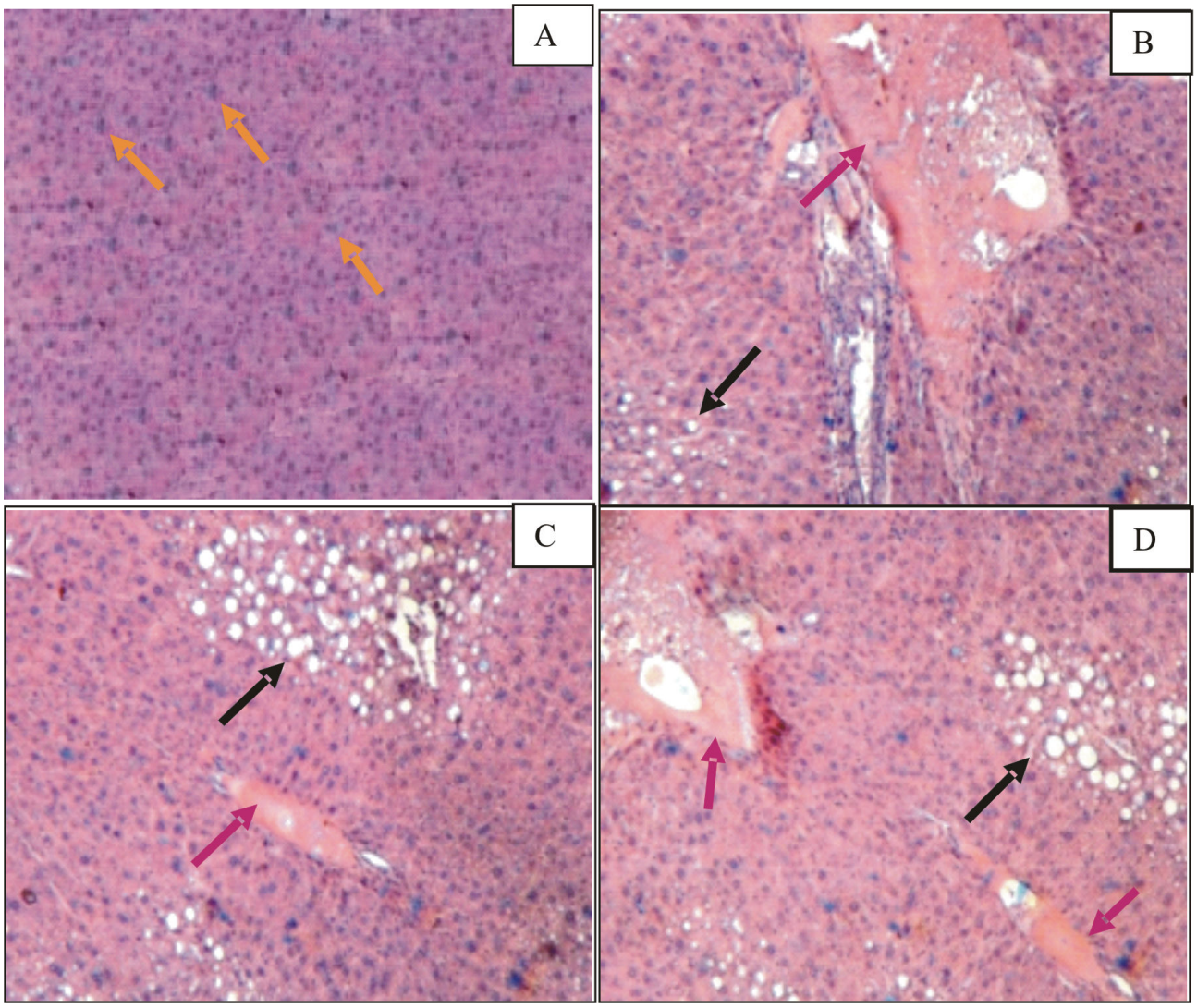

Fig. 3. **A**, Sections (4 µm thick) of liver of the fresh water Cyprinid, *L. rohita*, Control, indicating normal hepatocytes histology, brown arrow indicates for Kupffer's cells. H & E staining 400X; **B**, Sections (4 µm thick) of liver of the Lead nitrate treated fresh water Cyprinid, *L. rohita*, fatty change (Black arrow); blood vessel with blood congestion (pink arrow). H & E staining 400X; **C**, Sections (4 µm thick) of liver of the Copper sulphate treated fresh water Cyprinid, *L. rohita*, fatty change (black arrow); blood vessel with blood congestion (pink arrow). H & E staining 400X; **D**, Sections (4 µm thick) of liver of the copper sulphate and lead nitrate treated fresh water Cyprinid, *L. rohita*. Fatty change (black arrow); blood vessel with blood congestion (pink arrow). H & E staining 400X.

routes; the food ingestion, simple diffusion via gills pore, through drinking process and by intestinal or skin absorption (Fanta *et al.,* 2003).

Similar findings were reported by Das and Mukherjee (2000) and Bhatkar (2011) in *L. rohita* upon exposure to sub-lethal doses of hexachloro-cyclohexane and heavy metals, respectively.

Histological changes in kidneys

Kidney of control showed glomerular histology with normal first proximal segment (PI) second proximal segment (PII) and interstitial haematopoietic tissue (IH) (Fig. 4A).

The most prominent alterations investigated in the kidneys of *L. rohita* treated with copper sulphate were congestion of blood vessel; liquefactive

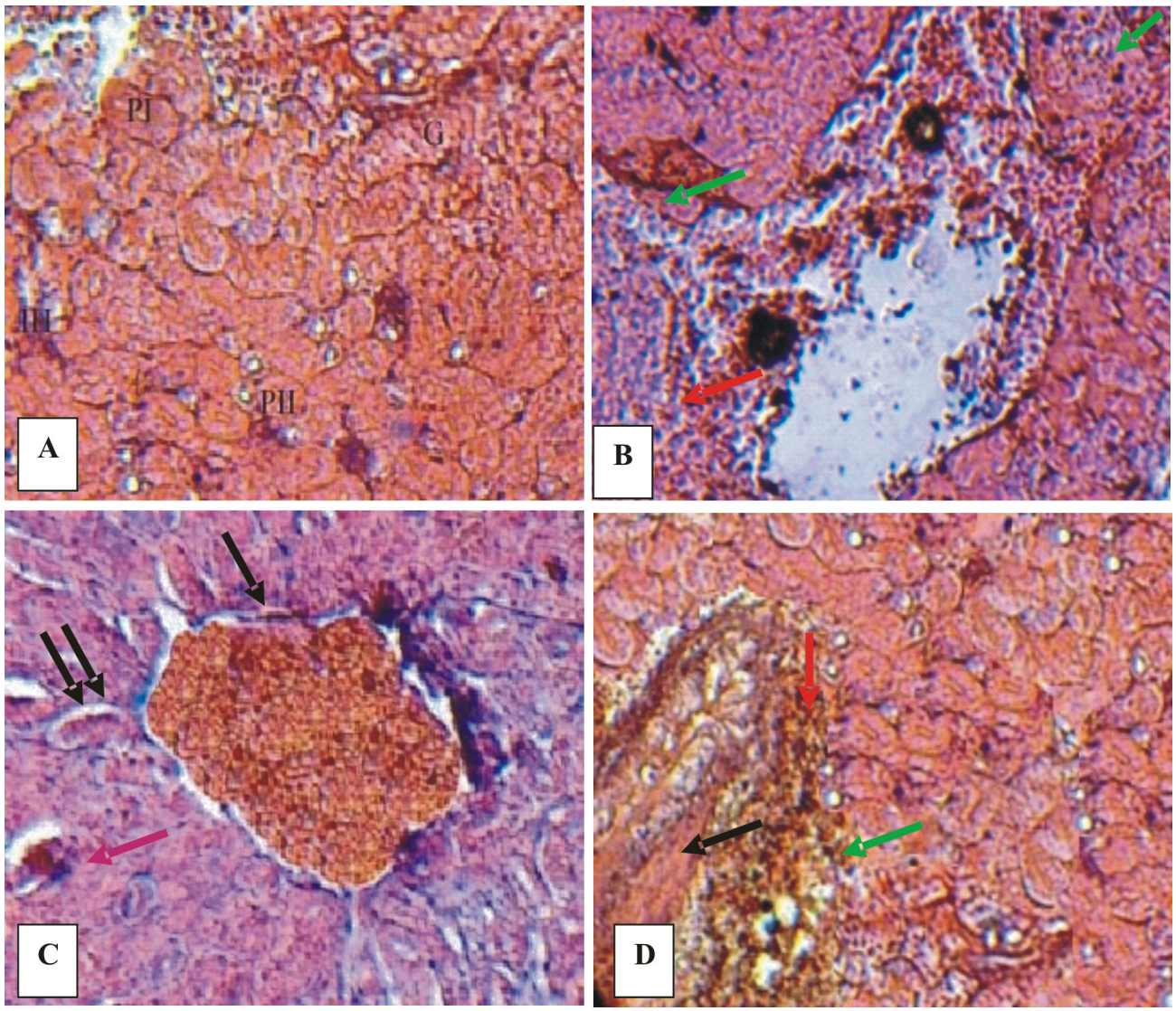

Fig. 4. **A**, Sections (6 µm thick) of kidney of the fresh water Cyprinid, *L. rohita*, Control. G indicating normal glomerulus histology, PI stands for first proximal segment; PII indicating second proximal segment and IH showing interstitial haematopoietic tissue. H & E staining 400X; **B**, Sections (6 µm thick) of kidney of the fresh water Cyprinid, *L. rohita*, treated with Ccpper sulphate. Green arrow indicating liquefactive necrosis of first and second proximal segments and red arrow showing irregularity in interstitial haematopoietic tissue due to tubular necrosis. H & E staining 400X; **C**, Sections (6 µm thick) of kidney of the fresh water Cyprinid, *L. rohita*, treated with Lead nitrate. Black arrow indicating heavy blood congestion in blood vessel; Pink arrow showing tubular necrosis. H & E staining 400X; **D**, Sections (6 µm thick) of kidney of the fresh water Cyprinid, *L. rohita*, treated with combination of copper sulphate and lead nitrate. Single green arrow indicating extravasation of blood from blood vessel, resulting in coagulative necrosis of first and second proximal segments and black arrow showing irregular blood congestion; red arrow indicate tubular necrosis. H & E staining 400X.

necrosis of first and second proximal segments and irregularity in interstitial haematopoietic tissue due to tubular necrosis (Fig. 4B).

The histopathological alterations in kidneys of *Labeo rohita* exposed to lead nitrate showed heavy congestion in blood vessels and tubular

necrosis of glomerulus (Fig. 4 C).

Similarly the histological abnormalities observed in kidneys of fish, treated with combination of copper sulphate and lead nitrate showed extravasation of blood from blood vessels; coagulative necrosis of first and second proximal segments, irregular blood congestion, and tubular necrosis (Fig. 4 D). These findings are in agreement with those reported by Das and Mukherjee (2000).

Heavy metals accumulation in fish organs is the leading cause of structural wounds, toxic impact on physiology and metabolic faults (Thophon *et al.,* 2003). The obvious histopathological alterations in the kidney of *L. rohita* in the present study revealed that the fish kidney may be a helpful tool in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment.

Our results demonstrated that copper sulfate is more toxic than lead nitrate for *L. rohita* as lower $CuSO_4.5H_2O$ concentrations caused higher mortality than $Pb(NO_3)_2$. The histopathological observation showed that exposure to acute concentrations of $CuSO_4.5H_2O$ and $Pb(NO_3)_2$ caused destructive impact in the liver and kidney tissues which may ultimately cause death of the fish and have indirect effect on humans as this fish is an important component of human food chain.

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