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Histopathological anomalies in liver of freshwater teleost under arsenic stress: LM study

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Abstract- Arsenic (As) is an important metalloid. It is most noxious heavy metal pollutant. It has the ability to accumulate in the several vital organs of the fish body. Heavy metals are most noxious pollutants owing to their diverse effects. Some metals are soluble in water and readily absorbed into the living organisms. Heavy metals have been shown to be that accumulated in many important organs of fish. In the experimental protocol a fresh water air breathing fish *Clarias batrachus* (Linn.) was selected to study the histo-pathological impact of As_2O_3. The LC_{50} of As_2O_3 for fish was calculated as per standard method. The fish were exposed to 1/6, 1/3 and 1/2 concentration of LC_{50} for 1 week and 4 week respectively. At the termination of the exposure, the fish was anaesthetized and the autopsy was done. The fish liver was extracted and doble stained paraffin spread sections were prepared for light microscopy. The major histopathological anomalies incurred in the liver of arsenic treated fish were: infiltration of fibrous tissues and eosinophil cells in the central vein, accumulation of hemorrhagic clots in the central vein and sinusoids, enucleation and degeneration of hepatocytes, formation of pycnotic clumps, pointing towards hepatoblastoma. All these major histopathological anomalies seriously alter the fish hepatic metabolism leading of complete hepatic failure at high dose of arsenic. The finding of the present study provides an insight to the deleterious impact of As_2O_3 on fish liver. There is an emergent need for the bio-conservation of these affected groups of teleost.

Keywords : Arsenic, Histopathology, Hepatic tissues, *Clarias batrachus*

INTRODUCTION

Fishes are largely being used for the assessment of the quality of aquatic environment and as such can serve as bio-indicators of environmental pollution. Heavy metals and pesticides contamination of the aquatic system has attracted the attention of researchers all over the world. The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other human activities. They may have devastating impact on the ecological balance of the recipient environment and a diversity of aquatic organisms including fish.

Severe alterations in the physiological activities and biochemical parameters both in tissues and blood of fish due to heavy metal toxicity have been reported.[1] Vutukuru *et al.*[2] have reported a significant increase in the activity of ALT and AST, due to arsenic exposure, pointing towards severe hepatic damage and distress condition in fish. Amali

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et al.[3] have reported thioactamide induced liver damage in zebra fish embryo, as a disease model for steatohepatitis.

The characteristic appearance of liver fibrosis in the heavy metal exposed fish was supported by report of sunfish in Texas reservoir contaminated with selenium enriched power plant.[4] Bihar is emerging as "hotspot" for arsenic contamination of groundwater next to West Bengal in India. Fishes being at the top of aquatic food chain get easily victimized to the arsenic toxicity. Through food chain these arsenic contaminated fish may be consumed by human, posing serious health hazards. *Clarias batrachus* has been declared as 'fish of the state' by Animal Husbandry and Fisheries Dept. of Bihar (2008). The arsenic contaminationof ground water as well as that of shallow water in wetland of Gangetic plain has increased tremendously in last few decades and posing a serious threat to the survival of fishes of worth importance.

The present study has been designed to elucidate a systematic deleterious impact of arsenic on the histopathology of liver and kidney of fresh water air breathing fish *Clarias batrachus*.

MATERIALS AND METHODS

In the experimental protocol *Clarias batrachus* has been considered as experimental model" popularly known as "Mangur".

Different age group of *Clarias batrahcus*, of mean body length18±2 cm & mean body weight 74±6 gm was collected from Phulwarisharif Fish Farm, Patna, Bihar.

The LC_{50} of As_2O_3 for *Clarias batrachus* was determined by standard APHA[5] method and calculated as 15.0 mg/l (Arsenic as 11.4 mg/l).

The three doses considered in the experimental protocol were 2.5 mg/l, 5.0 mg/l and 7.5 mg/l and accordingly stock solutions were prepared by dissolving appropriate amount of As_2O_3 in the deionized water. At the termination of each exposure the different groups of anaesthetized fish were sacrificed and 10 pieces of the liver samples of each group were collected and paraffin spread slides were prepared using double stains as per standard method for light microscopic study.

RESULTS

It was observed that during acclimatization period fish showed normal swimming behaviour but immediately after administration of arsenic in the aquaria the fish became restless which was evident from their motion with a burst speed, more frequent visit on the top of water surface, swirling movement & smashing at the corner of the aquaria.

At higher doses of arsenic exposure for longer duration the colour of the fish became faint yellowish black from deep silver black. The snout portion of the fish showed a series of remarkable wound in the form of bloody scar surrounded by white patches. The scars were also very prominent in the caudal peduncle region of fish which in some cases, led to amputation of tail and ultimately death of fish.

The most striking behavioral change in arsenic treated fish was excess mucus secretion by fish which make the water slimy. Such fish showed loss of appetite as evidenced by their non-responsiveness to the feed pellet when compared with the control fish.

Light Microscopic Observation

Control Liver: In teleost, the liver is a large bilobed organ, consisting of right and left lobe. Left lobe is further divided into two lobes- anterior and posterior. Each lobe is further divided into several lobules. Liver lobule was the classical structure and fundamental unit of liver. The liver in teleost primarily consists of two or three (in some cases) tissues i.e. parenchymatous hepatic tissue and the biliary drainage tree. It is roughly, irregular or polyhedral prism like structure. Each lobule consists of centrally placed central vein and peripheral portal triads (Plate I, Fig. 1 & 3). The central vein is a thin walled vessel receiving blood from the hepatic sinusoids. The endothelial lining of central vein is surrounded by small amounts of spirally arranged connective tissue fibre having endothelial cell lining and few fibroblast (Plate I, Fig. 1 & 3). The sinusoids are irregularly dilated vessels and are lined by fat cell similar to endothelial cells and few macrophages (Plate I, Fig. 1, 2, 3 & 4; Plate II, Fig. 1, 2, 3 & 4). FewKupffer cells were marked lining the sinusoids (Plate I, Fig. 3). Hepatocytes constitute about 80% of the total cell population in the liver. They are binucleated and having granulated cytoplasm.

Arsenic Treated Liver

2.5 ppm arsenic treated

The transverse sections of liver of 2.5 ppm arsenic treated fish showed prominent dilation in central vein with

edematous fluid (Plate II, Fig. 2 & 3). At few places, central vein showed constriction (Plate II, Fig. 1) and heavy deposition of fibrous tissue around it (Plate II, Fig. 1 & 3). Hepatocytes exhibited mass enucleation (Plate II, Fig. 2), prominent bridging necrosis and their fusion (Plate II, Fig. 2 & 4). Hepatocellular necroses were marked at few places (Plate II, Fig. 3). Degenerated necrotic hepatocytes bridging necrosis of hepatocytes and formation of massive pycnotic clumps pointing towards hepatoblastoma were also marked (Plate II, Fig. 3). At few places rosette of necrotic hepatocytes appeared and marked the condition of peliosis hepatitis (Plate II, Fig. 3). Excessive fibrous tissue deposition around portal vein marked the condition of serum hepatitis (Plate II, Fig. 3 & 4).

5.0 ppm arsenic treated

The transverse sections of liver of 5.0 ppm arsenic treated fish showed dilation & widening of sinusoids and constriction in hepatic chords. Central vein showed a great degeneration in its cyto-architecture in comparison to control section as evidenced by cross linking of two central veins, filled with lymphocytes and hemorrhagic clot (Plate-III, Fig. 1, 2, 3 & 4).

7.5 ppm arsenic treated

The transverse sections of liver after one-week treatment of 7.5 ppm arsenic showed constriction of central vein and massive infiltration of hemorrhagic clots and eosinophils into it. The usual endothelial cells were replaced at few places by fibrous tissues (Plate IV, Fig. 1 & 2). The sinusoids got dilated and filled with blood cells and pycnotic nuclei (Plate IV, Fig. 1 & 3). A few degenerated nodule and neoplastic infestations were also prominent (Plate IV, Fig. 4).

DISCUSSION

Histopathology provides a rapid method to detect the effect of irritants in various organs. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Liver is most suitable organs for histological examinations in order to determine the effects of xenobiotics.[6, 7]

Researchers all around the world are now seriously concerned with heavy metals and pesticide contamination of the aquatic ecosystem.[8] Even at lower sub lethal concentration of 2.5 ppm of arsenic (As_2O_3) central vein showed marked constriction and a heavy deposition of fibrous tissue around it. It showed massive infiltrations of eosinophilic inclusions & hemorrhagic clots. Similar observations have been reported in the liver of *Clarias batrachus* exposed to endosulfan.[9] Besides, mass enucleation of hepatocytes, bridging necrosis of hepatocytes and clumps of pycnotic nuclei marks a case of hepatoblastoma. Inflamed and dilated portal vein showed massive deposition of fibrous tissue and edematous fluid within it. Such types of histopathological anomalies are usually marked in the case of perivenular fibrosis and serum hepatitis.

At higher doses of 7.5 ppm of arsenic central vein is reported to be plugged completely by hemorrhagic clots and eosinophilic inclusions. Functional degenerated enucleated hepatocytes arranged themselves in the form of rosette clumps. Plugging of portal vein by hemorrhagic clots & eosinophils, formation of degenerated nodules and few neoplastic infestations were prominent anomalies incurred in hepatic tissues.

Shrinkage of central vein is primarily due to the deposition of fibrous tissue around the endothelial wall. Widening of sinusoids can be correlated with simultaneous shrinkage of hepatocytes of a particular region due to arsenic toxicity.

Arsenic toxicity affects tubulin assembly and then probably stops synthesis of α and β dimers. It also brings about asymmetry in cytoskeletal structure of hepatocytes leading to elliptical, cylindrical or irregular shape of hepatocytes.

Hepatic fibrosis is a common feature in which a major amount of liver parenchymal cells are replaced by fibrous connective tissues. Experimentally hepatic fibrosis has been shown to be induced by the administration of CCl_4, thioacetamides, paracetamol, etc. ROS have been shown to produce tissue injury through covalent binding and lipid peroxidation. Later has been shown to augment fibrosis as seen from increased collagen synthesis.[10] It has been reported that the lipid peroxidation could stimulate collagen synthesis by fibroblast.[11]

CONCLUSION

It can be convincingly stated that arsenic (III) generates histopathological alterations in the hepatic cells of the teleost leading to hepatic failure. It may be considered as bioindicator of arsenic toxicity in fish.

PLATE - I

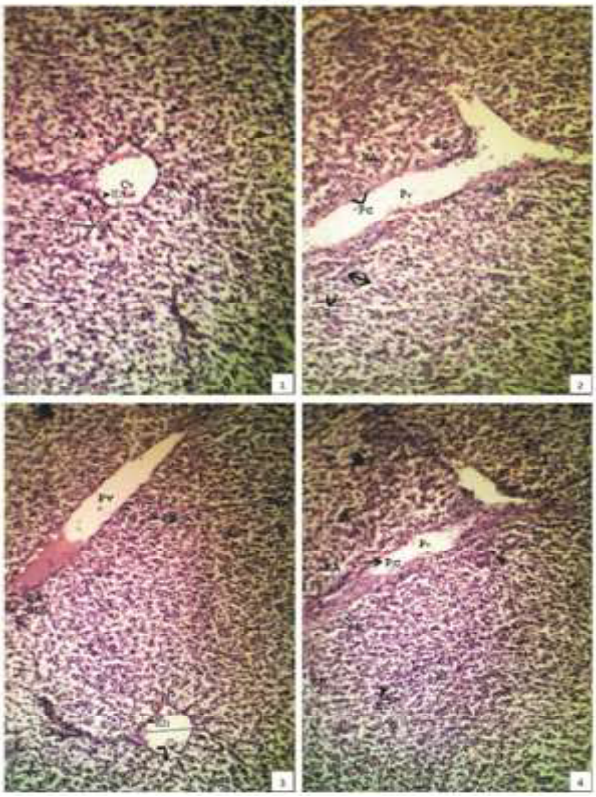

PLATE -II

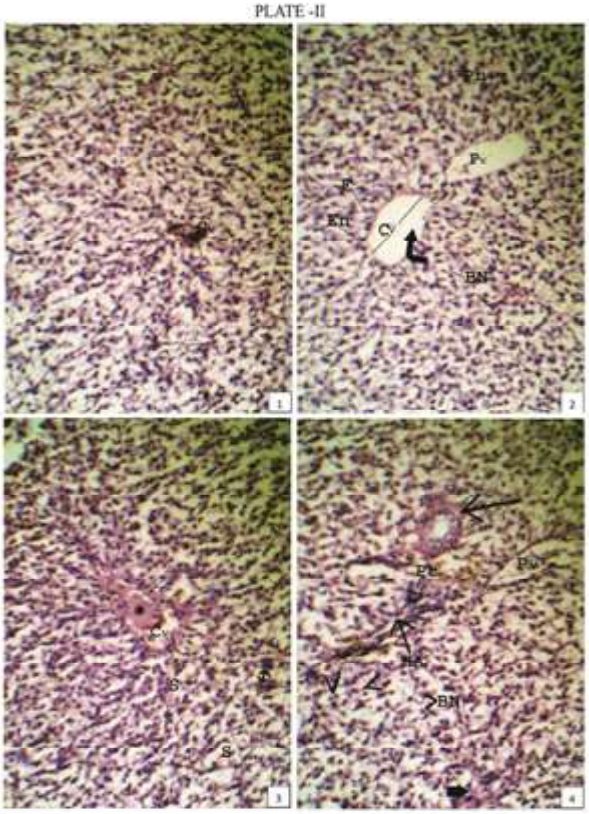

PLATE - III

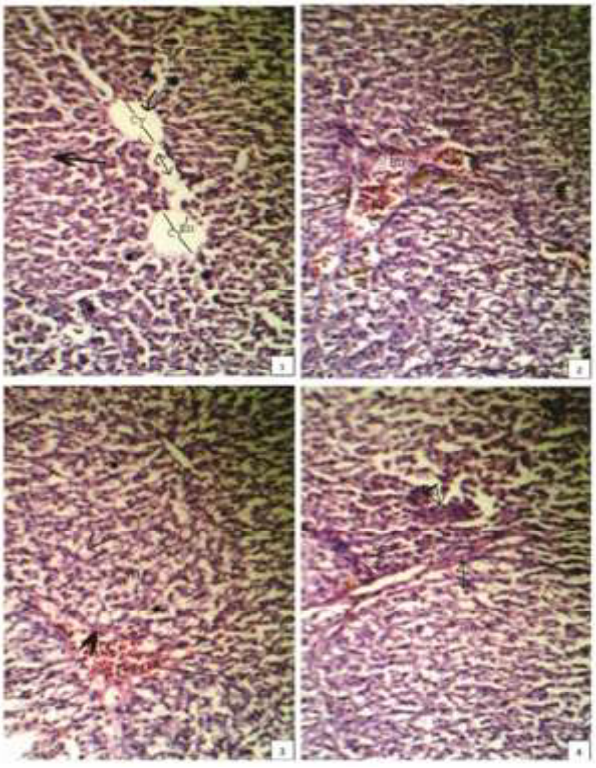

PLATE - IV

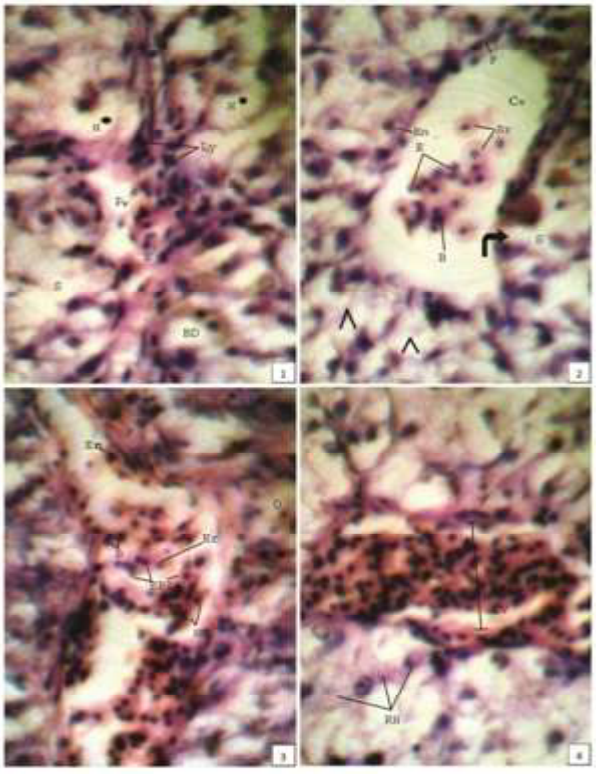

Plate- I: **Photomicrographs of transverse sections of normal liver of *Clarias batrachus* stained with Haemotoxyline and Eosin**

Fig.1. Section of liver showing cords of hepatocytes (double arrow) separated by sinusoid (s), draining into central vein (Cv), which is lined by endothelial cell (En) and fibroblast (F). Note the presence of bile canaliculus (BC) in between two hepatocytes (H).X100

Fig.2. Section of liver showing portal vein (Pv) lined by parenchymal cell (Pc), bile duct (BD) and hepatic artery (HA). Hepatic cords (double arrow) are separated by uniform sinusoids (S). X100

Fig.3. Section of liver showing central vein (Cv) lined by endothelial cell (En). Note the presence of clearly visible opening of sinusoids (S) into the central vein (curved arrow) normal portal vein (Pv), hepatic artery (HA) and bile duct (BD). The nuclei of hepatocytes clump at few places to form pycnotic nuclei (Pn). X100

Fig.4. Section of liver showing portal vein (Pv) lined by parenchymal cell (Pc), hepatic artery (HA), bile duct (BD). The sinusoid (S) separates the cords of hepatocytes (H) and contains kupffer cell (Kc). X100

Plate – II:Photomicrographs of transverse sections of liver of one week 2.5 ppm arsenic treated *Clarias batrachus* stained with Haemotoxyline & Eosin.

Fig.1. Section of liver showing constriction in central vein (double arrow), heavy deposition of fibrous tissue (arrow) around it. Note the mass enucleation of hepatocytes (arrow head). X100

Fig.2. Section of liver showing dilation of central vein (Cv) and clumping of nuclei of hepatocyte as pycnotic nuclei (Pn). Note bridging necrosis (BN) among nuclei of hepatocytes. Besides portal vein (Pv) and sinusoidal opening (curved arrow) are almost normal. X100

Fig.3. Section of liver showing dilation of central vein filled with edematous fluid (asterisk) and deposition of fibrous tissue around the central vein (arrow). Hepatocellular necrosis (double arrow) is marked in central right side of photomicrograph. X100

Fig.4. Section of liver showing deposition of fibrous tissue around dilated bile duct (arrow). Bridging necrosis (BN) and fusion of hepatocytes (arrow head) are well marked. A deposition of edematous fluid (deep arrow) is also seen. X100

Plate-III: Photomicrographs of transverse sections of liver of one week 50 ppm arsenic treated *Clarias batrachus* stained with Haemotoxyline &Eosin.

Fig.1. Section of liver showing widening of sinusoids (S), constriction in hepatocyte cords (arrow), infiltration of lymphocytes (arrow head) and few unhealthy degenerated hepatic cells (asterisk). Cross linking of two central veins (double arrow) is also marked. X100

Fig.2. Section of liver showing complete disorganization in architecture of bile duct & central vein (Cv) with marked hemorrhagic clots (Hc) and eosinophilic inclusion (EPI), plasma cell (PL) and lymphocytes (Ly). X100

Fig.3. Section of liver showing enucleation (arrow) in degenerated hepatocytes, a few multinucleate hepatocytes (MH) and dilated sinusoids (S*).Abundance of fibrous tissue (arrow head) and mass eosinophillic inclusions (EPI) in portal vein marks the sign of portal cirrhosis. X100

Fig.4. Section of liver showing abundant necrotic parenchymal cell (arrow), bile laden macrophages (Kc) on the fringe of edematous portal tract (double arrow) marks degenerated liver with hepatic cholestasis. X100

Plate – IV: **Photomicrographs of transverse sections of liver of one week 7.5 ppm arsenic treated *Clarias batrachus* stained with Haemotoxyline & Eosin.**

Fig.1. Section of liver showing magnified view of enucleated hepatocytes (H*), dilation of bile duct (BD). Portal vein (Pv) is infiltered with different types of blood cells predominated by erythrocytes (Er) and lymphocytes (Ly). X400

Fig.2. Section of liver showing dilation of central vein (CV) lined by endothelial cell (En) & fibroblast (F). Its lumen is filled with scanty eosinophils (E), basophils(B), erythrocytes (Er) and few lymphocytes (Ly). Hepatocytes show degenerative condition (arrow head).X400

Fig.3. Section of liver showing extended portal vein surrounded by fibrous tissue (F) with edematous fluid (O). Besides hemorrhagic clots (Hc) and eosinophilic inclusions (EPI) are prominent in the area of portal vein (Pv). X400

Fig.4. Section of liver showing constricted central vein (Cv) surrounded by thick fibrous tissue (F) containing clots of different kinds of blood cells. Besides, rosettes hepatocytes (RH) mark the condition of peliosis hepatitis. X400

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