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A STUDY ON HISTOPATHOLOGICAL ALTERATIONS IN TISSUES OF FRESHWATER FISH, *CLARIAS BATRACHUS* (LINN.) DUE TO LEAD CONTAMINATION FROM PARICHHA DAM, JHANSI

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The present study was designed to investigate the histopathological effect of lead on liver and kidney of air breathing freshwater fish, *Clarias batrachus* of Parichha dam during 2010. The fish samples were exposed to 10% sub lethal concentration at 7ppm (96 h LC50) of Lead for a period of 30 days. Several histopathological alterations including vacuolar degeneration of hepatocytes, profuse heamorrhage and haemolysis, between hepatocytes, hepatocytes with broken plasma membrance,nuclear degeneration, engorgement of blood cells or hyperaemia of hepatocytes; interstitial leucocyte infilteration, endothelial wall thickening, mild degenerative, changes of tubular epithelium, vacuolar degeneration of tubular epithelium in kidney were observed.

Heavy metal contamination of aquatic ecosystem has long been recognized as a serious problem[1-4]. Heavy metal contamination may have devasting effect on ecological balance of recipient environment and diversity of aquatic organism[5]. These Heavy metal pollution posses a great threat to fishes. When fishes are exposed to great elevate level of metal in polluted aquatic ecosystem, they tends to take these metals up from their direct environment[8]. The fish constitutes a valuable commodity from the stand point of human consumption. So heavy mental contamination of freshwater bodies and aquatic biota becoming a serious concern from human health point of view. Heavy metal pollution of aquatic ecosystem posses a serious environmental hazard because of their persistence and toxicity.

These heavy metals are available in the water and are further added into aquatic ecosystem as result of direct input of atmospheric deposition, leaching of mineral and soil erosion due to rain water which causes the hazardous effects on aquatic biota specially fishes[10]. These heavy metal toxicants are accumulated in the fish through general body surface which affect severally their life support system at molecular biochemical levels. Once these toxic substance enter into body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders[2].

Essential metals such as Cu, Zn and Fe have normal physiological regulatory functions[7], but may bioaccumulate and reach toxic levels. Non-essential metals are usually potent toxins and their bioaccumulation in tissues lead to intoxication, decreased fertility, tissue damage and dysfunction of variety of organs. Heavy metals are non-biodegradable and once discharged into water bodies, they can either be adsorbed on sediment particles or accumulated in aquatic organisms. Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts and are electing toxicological effects at critical targets[4]. Fish may also accumulate significant concentrations of metals even in water in which those metals are below the limit of detection in routine water samples, therefore, fish might prove a better material for detecting metals contaminating the freshwater ecosystems. Intensive studies were conducted on the levels of heavy metals in different water bodies. The bioaccumulation of heavy metals in the different fish tissues has been studied by several investigators[1,6].

Among heavy metals, lead is one of the major contaminants found in soil, sediments, air and water. Total annual emissions of lead by motor vehicles and industrial plants alone throughout more than half a million tone. Lead can persist in the environment for 150-5000 years,Parichha is main thermal power plant owned by UPRVUNL (Uttar Pradesh Rajya Vidyut Nigam Limited), having total capacity of 220 MW / day and coal used as fuel.

The main objective of the present work was to study the effect of Lead (Pb^{++}) on the different organs of *Clarias batrachus* of

Parichha Dam. The Parichha dam is recieving heavy metals through fly-ash, release from Parichha Thermal Power Station, Jhansi with emphasis on histopathological alterations.

MATERIALS AND METHODS

Study Area : Parichha dam is built on the bank of Betwa river, is about 25 Km away from Jhansi, Uttar Pradesh on the national highway no. 25 in India, extending about 15 km in length. It has 4 Zones (Parichha head to Ujyan ghat, Ujyan ghat to Tilaitha ghat, Tilaitha ghat to Kolba ghat, and Kolba ghat to Not ghat)

Sampling: During 2010, water samples (1L) were collected from Zone-I, Zone-II, Zone III and Zone-IV of Parichha dam, in polyethylene bottles, separately. The samples were acidified by nitric acid and transferred to the laboratory in an ice- box to be analyzed.

At the same time, samples of *Clarias batrachus* were collected from the same zones. The fish measured about 30.0 to 55.2 cm in total length and 550 to 750 g in weight and acclimatized to laboratory conditions for 30 days. Fish were fed with minced goat liver and water was renewed every 24 h, with routine cleaning of the aquaria, leaving no faecal matter, unconsumed food or dead fish. Five groups of ten fish each were exposed to 20 L of 46.25 mg /L lead (E. Merck, India). Each of 7 ppm (10% of 96 h LC50) Lead solution prepared in well water having dissolved oxygen 6.54 mg/L, PH=7.2, water hardness 23.2 mg/L and water temperature 28±2oC. Parallel control groups of fish were kept in 20 L of plain tap water (without addition lead). Five fish, each from the experimental as well as control groups were sacrificed to analyze the lead accumulation, remaining fish were sacrificed for histopathological analyses.

STUDY AREA (PARICHHA DAM)

Estimation of lead in water sample and fish tissues: Analysis of lead in the water was carried out using semi-micro analytical method (SMAM) and atomic absorption spectrophotometers. Liver and kidney were dried in an oven at 80°C for 12h. Tissues were digested for overnight in concentrated nitric acid and perchloric acid (3:1) at 100°C for overnight. The digested samples were diluted in de-ionized water and lead concentrations were determined using atomic absorption spectrophotometery.

Histopathological analysis: Liver and kidney were fixed in Bruin's fluid and 10% neutral buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 5-6µm then stained with haematoxylin and eosin. The sections were examined microscopically.

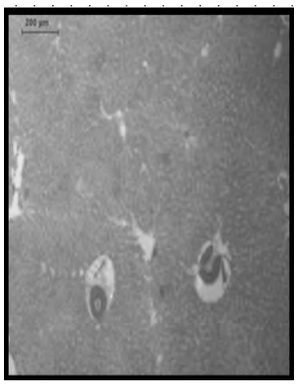
RESULTS AND DISCUSSIONS

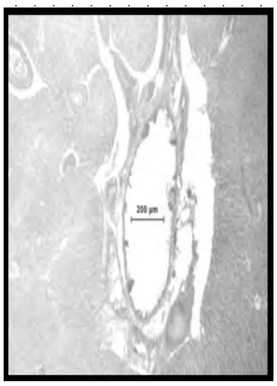
After exposure of lead, the tissue of liver and kidney of the fish, *Clarias batrachus* showed various degenerative changes after 30 days . The degenerative histopathological changes observed in liver and kidney were indicative of toxic nature of lead.

Histopathological alterations on liver

Control: In the control fish liver, the cords of hepatocytes arranged in a radial net like pattern around the central vein which make liver lobules. Each lobules are connected with each other a connective tissues, Gilsson's capsules. The hepatocytes are polyhedral and have distinct central nucleus. Few sinusoids are distributed irregularly between the hepatocytes. The hepatocytes have homogenous cytoplasm. In connective tissues, interlobular veins, interlobular arteries, bile ducts are present. The biliary system occurs as intercellular bile canaliculi which eventually anastomose to form typical bile ducts. Haemopoetic tissues are found around the large vessels of the liver(Fig.1)

Exposed : The liver of *Clarias batrachus* after lead exposure for 30 days showing disorganization of hepatocytes. Their nuclei were displaced to the periphery. The plasma membrane of some hepatocytes were ruptured (Fig. 4). Nuclear degeneration of hepatocytes, engorgement of blood cells or hyperaemia and eccentric displacement of nuclei in hepatocytes were observed (Fig. 4). The hepatocytes became degenerated and vacuolated. Profuse haemorrhage and haemolysis between hepatocytes and in blood capillaries were also found (Figs. 2,3).





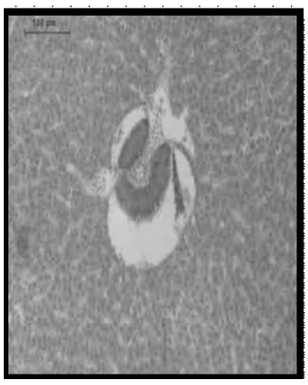


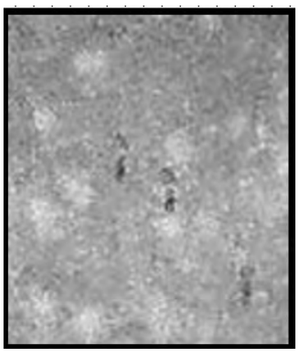
Fig. 1 **Fig. 2** **Fig. 3** **Fig. 4**

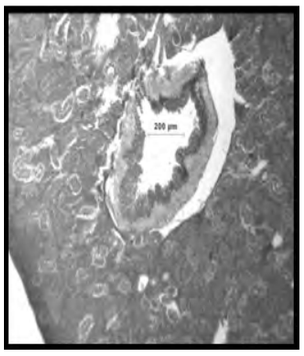
Fig.1. T. S. of liver of controlled fish illustrating normal structure showing hepatocytes with granular cytoplasm (Haematoxylene-Eosin stain 10X)

Fig.2. T.S. of liver of *Clarias batrachus* showing profuse hemorrhage in central vein and displacement of nuclei of hepatocytes (4x).

Fig.3. T.S. of liver of *Clarias batrachus* showing profuse hemorrhage in central vein and displacement of nuclei of hepatocytes (10x).

Fig.4. T.S. of liver of *Clarias batrachus* showing engorgement of blood cells necrosis and vacuolar degeneration of hepatocytes,ruptured plasma membrane (10x).





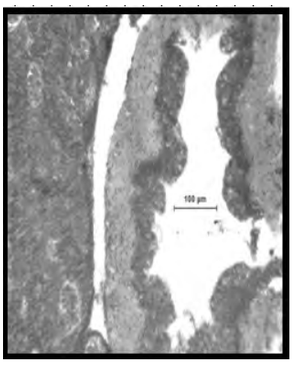


Fig. 5 **Fig. 6** **Fig. 7** **Fig. 8**

Fig. 5. T. S. of trunk kidney of controlled fish illustrating normal structure showing section of uriniferous tubules with epithelial lining with distinct nucleoli and interstitial tissues. (Haematoxylene-Eosin stain10 X)

Fig. 6. T.S. of trunk Kidney of *Clarias batrachus* showing endothelial wall thickening of tubule necrosis hyperplasia of epithelial cells interstitial leucocyte infilteration. (4x).

Fig. 7. T.S. of trunk Kidney of *Clarias batrachus* showing hypertrophy of epithelial cells of convoluted tubules and profuse hemorrhage (10x).

Fig. 8. T.S. of liver of *Clarias batrachus* showing necrosis and displacement of nuclei of hepatocytes (10x).

Histopathological alteration in Kidney

Control : In normal fish, kidney is functionally as well as structurally differentiated into head kidney and trunk kidney. Histologically trunk kidney is rich in uriniferous tubule and it takes the excretory function. The tubules are lined with columnar epithelial cell with distinct nuclei. The interstitial tissues in the intertubular spaces have parenchymatous cells with distinct nuclei (Fig.5).

Exposed : After thirty days exposure to lead the interstitial leucocyte infiltrations and endothelial wall thickening were observed at the glomerular capillaries level (Fig. 6). Mild degenerative changes of tubular epithelium was noticed. Tubular epithelium showed necrosis characterized by karyorrhexis and karyolysis. Vacuolization of epithelial cells, necrosis of hematopoietic tissue were highly noticed (Figs. 6,8). Hypertrophy of epithelial cells of convoluted tubule and profuse haemorrhage were observed (Fig. 7).

The damage caused in liver might be due to the cumulative action toxicant on blood and ultimately to other cellular structures. There seems to be a definite correlation between tissue damage and certain physiological alterations. Liver is the major metabolic center and any damage to this organ would do so many physiological disturbances leading to subsequent mortality of fish. The hepatic damage observed in the present investigation are in accordance to the observations made by different researchers[11-12].

Damage of blood capillaries in the liver along with disarray of hepatic cords, supports the view of previous workers that metal cause hamorrhage in the internal organs[11,13]. Severe damage in liver of *Clarias batrachus* exposed to 10 mg/l of zinc sulphate. Degeneration of epithelium, necrosis of haemopoetic tissues, shrinkage and degeneration glomeruli and haemorrhages from thin blood capillaries in the kidney of *Channa striatus* after heavy metal poisoning have been reported[9]. In fishes, kidney is main organ of excretion therefore the toxicant has to pass through it in its degraded from which may be more toxic and as the histological unit of kidney are more delicate. Kidney is highly susceptible to toxic injury because of its high blood supply.

In conclusion,the present study proved that the heavy metal,Lead causes the several histopathological alterations in liver and kidney of fresh water fish, *Clarias batrachus* after thirty days exposure.

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