Histopathological Alterations in *Clarias gariepinus* Exposed to Lead Nitrate

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Abstract: The heavy metals which are essential for normal life processes become toxic to the aquatic organisms when released continuously into the water bodies. They exert synergistic and antagonistic effects on each other which change the water quality and thereby affect the primary producers of aquatic communities. Heavy metals by direct contact with microorganisms and fish, enter into human population and accumulate producing deleterious effects. Toxic levels of heavy metals not only change the physico-chemical properties of water, but also cause various severe pathological lesions in the structure and functions of cells including enzymatic and metabolic pathways of both flora and fauna. In the present study, effect of sublethal concentration of lead nitrate on the liver tissues of *Clarias gariepinus* was studied. The result indicates that Lead nitrate is toxic to the fish. The changes include degeneration of the liver cells. The hepatocytes were found with hypertrophy of hepatic nuclei and clumping was evident in many places.

Keywords: Lead nitrate, *Clarias gariepinus*, hepatocytes, histopathology, liver tissue.

I. INTRODUCTION

Ever since man embarked on the path of civilization, he began to exploit and pollute air, water and land indiscriminately without realizing or caring for the long range and long term effects of his action in the form of environmental pollution. This has resulted in imbalance of the ecosystem, which has now reached a state of environmental crisis (Gimeno *et al.*, 1995). The aquatic environments covered about 71% of the earth surface and in this environment about 28,000 fish species inhabited along with other innumerable aquatic organisms. Heavy metal in water quality and their effect on aquatic environmental have received much attention. Metals like Cu, Cd, Zn frequently found in natural water in parts per billion. In trace quantity heavy metal serve as essential micronutrient for enzymatic transformation. Anthropogenic activities have resulted in an increase in pollution of many heavy metals. The metals are of special concern, because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms (Coombs, 1980).

Lead(II) nitrate is an inorganic compound with the chemical formula $Pb(NO_3)_2$. It commonly occurs as a colourless crystal or white powder and unlike most other lead(II) salts, is soluble in water. Lead(II) nitrate does not occur naturally. Lead and lead compounds are generally toxic pollutants. Lead(II)salts and organic lead compounds are most harmful ecotoxicologically. Lead salts are attributed to water hazard class II, and consequently are harmful. Through plant uptake, lead enters food chains. Consequently, lead based pesticide application is prohibited in most countries. Lead accumulates in organisms, sediments and sludge. Lead in wastewater mostly stems from streets and roofs. Lead exists as four stable isotopes, and no less than 26 instable isotopes (Christensen *et.al*. 1977).

The micro and macro fauna in aquatic environment are hence in threat of lead pollution. As fish is the major diverse group of animals that live and breathe in water, hence are best model to study pollution index. The increased levels of Pb in the water can increase mortality rate and may cause many biochemical and histopathological alterations in survived fish. Studies of histopathology have been recognized to be reliable bio-indicators of compression in fish. The histological changes on fish is a noteworthy and promising field to understand the extent to which changes in the structural organization are occurring in the organs due to environmental pollution (Moitra *et al.* 2012).

Singh *et al* (1990) studied environmental pollution and its effects on aquatic animals. Heavy metal toxicant leads to many pathological changes in different tissues of fish and has been reported for *Labeo rohita* exposed to mercuric chloride and *Chana punctatus* exposed to phenyl mercuric acetase (Karuppasamy, 2000). Srivastava *et al*. (1982) have observed the hisotopathological changes and accumulation potential in the fish tissues under chromium stress.

The liver tissues of *Heteropneustes fossils* exposed to a sublethal concentration of malathion showed hypertrophy of hepatic cells and liver cord disarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cell membrane and necrotic centrolobular area (Dutta *et.al.*, 1993). *L. rohita* showed histological changes due to aflatoxin given to fish intraperitoneally (Sahoo *et al*., 2001).

The aim of the current study was to evaluate the damage impacts on the hepatic tissues of *C. gariepinus* exposed to sublethal concentrations of lead nitrate for 96 hrs. Liver is the most important organ of detoxification for breakdown of toxic substances. Hence, liver was selected to study the histopathological alterations.

II. METHODOLOGY

2.1. Experimental Fish:

Healthy adult male fishes, *C. gariepinus* of size (15-20 cm) and weight (50-60 gm) were collected from local market. They were brought to the laboratory and disinfected by the treatment with 0.01% potassium permanganate solution for 1-2 min. The fishes were then acclimated in the laboratory conditions (26 ± 2 ºC) for 15 days in glass aquaria. The fishes were exposed to natural photoperiodism i.e., 12L:12D photoperiod. The pH and dissolve oxygen content of the water were 7.0 ± 1 and 7.5 ± 0.5 ppm respectively. The water used was tap water filtered by Dee Bee Filters (India) to remove excess of iron. The entire experiment was conducted at a controlled room temperature of 25 ± 2 ºC. During acclimation, water was changed every day to discard the metabolic waste products. Fishes were fed with *Tubifex tubifex* and were starved for one day prior to transfer into the toxic environment for test (Doudoroff *et al* 1951).

2.2. Experimental Design and Treatment of Fish:

After acclimation, fishes were divided into two groups, of 10 fishes each. One of the groups represented the control, while the other as experimental group. They were transferred to respective aquaria for the exposure period of 96 hrs. The control group was kept in normal water. The experimental groups were kept in (5mg/L).

2.3. Fixation and Preparation of Liver for Histological Studies:

Both the experimental and control fish were sacrificed every 24 hrs. Immediately after decapitation, the liver tissues were removed and dropped into aqueous Bouin's fluid. After fixation for 24 hours, tissues were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in the paraffin. 6 µm thick sections were cut on microtome and stained in Hematoxylin-Eosin. Pathological lesions were examined under optical microscope.

III. RESULT AND DISCUSSION

The normal structure of the hepatic tissue from the control fish group is shown in Fig. 1. The liver cells are large in size, polygonal in shape with homogenous eosinophilic cytoplasm and centrally located nuclei. A large number of blood sinusoids were observed which separate the hepatic cords from one another.

The histopathological examination of the hepatic sections from the treated fish groups revealed an obvious damage to the hepatic tissue ranged from mild to severe alterations. In fish at 24hrs of treatment, swollen hepatocytes were noticed with thickening in walls of veins. Degeneration of hepatocytes, vacuolation or space formation was evident, throughout the tissue. At 48 hrs of exposure, accumulation of blood vessels, congestion of hepatic tissue were also observed.

After 72hrs of treatment, the liver was highly damaged subcapsular vacuolization, dilated central vein, necrosis, indistinct cell boundaries in many places and pyknotic nuclei were also observed. As the duration increased, severe degradation of the liver cells or hepatocytes and hypertrophy of hepatic nuclei and clumping was evident in many places. After 96 hrs of treatment, the liver show degeneration of hepatocytes showing distinct vacuoles, necrosis with sinusoidal lesions and fibrosis as the maximum alterations observed.

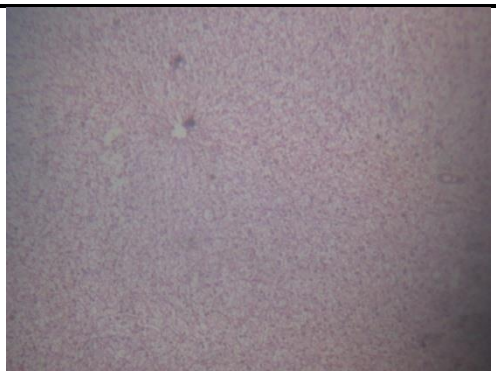

Fig.1: Control liver

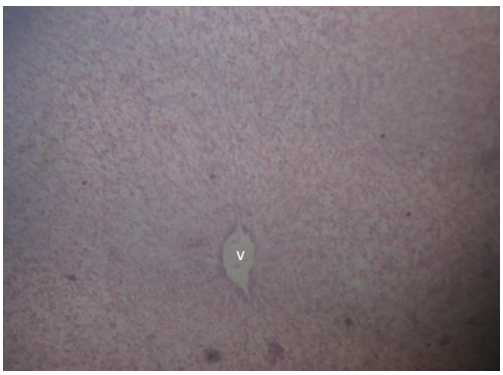

Fig. 2: 24 hrs treated liver

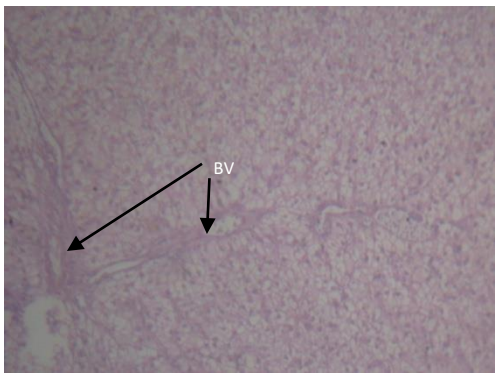

Fig. 3: 48hrs treated liver

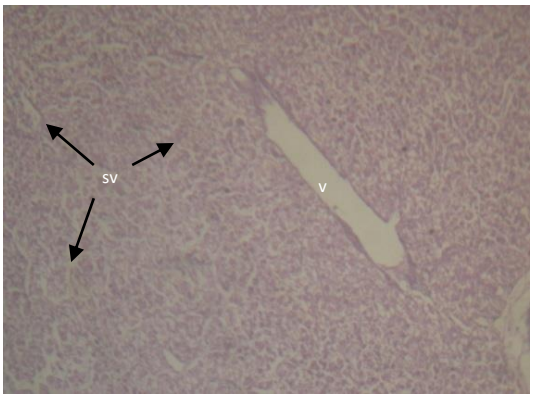

Fig. 4: 72 hrs treated liver

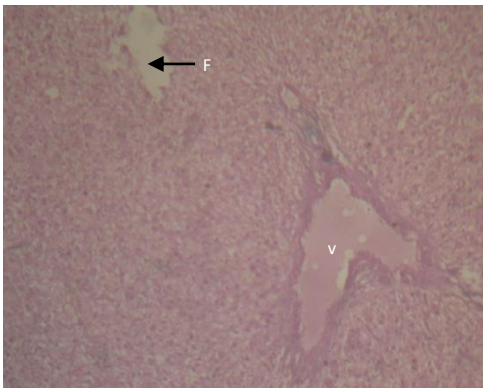

Fig. 5: 96 hrs treated liver

Fig. 1-5: photos of the hepatic sections (100X; H & E): Control fish (Fig.1) observed the normal hepatic tissue structure; 24h treated section (Fig.2) showing the thickenings in the wall of the central vein (v); 48h treated section (Fig.3) reveals accumulation of blood vessels (BV); 72h treated hepatic section (Fig.4) shows subcapsular vacuolization (SV) and dilated central vein; 96h treated section (Fig.5) showing the fibrosis (F) and distorted and dilated veins with much thickening of wall.

The histological changes on fish is a noteworthy and promising field to understand the extent to which changes in the structural organization are occurring in the organs due to environmental pollution. In the present study, the African catfish *Clarias gariepinus* were exposed to sublethal concentration of lead nitrate for 96h period. The result indicates that lead nitrate is toxic to *Clarias gariepinus*. Different histological alterations were found in tissues of liver. The liver was highly damaged with subcapsular vacuolization, necrosis, indistinct cell boundaries in many places and pyknotic nuclei were also observed. As the duration increased, severe degradation of the liver cells or hepatocytes were found with hypertrophy of hepatic nuclei and clumping was evident in many places. The hepatocytes were found with hypertrophy of hepatic nuclei and clumping was evident in many places.

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REFERENCES:

[1] Coombs, T. L. (1980). Heavy metal pollution in the aquatic environment. In: *Animals and environment fitness* (ed. R. Gills). Pergamon Press, New York. pp. 283-302.

[2] Christensen, G., Hunt, E. and Fiandt, J. (1977). The effect of methyl mercuric chloride, cadmium chloride and lead nitrate on six biochemical factors of the brook trout, *Salvelinus fontinalis. Toxicol. Appl. Pharmacol.* **42:** 520-523.

[3] Doudoroff, P., Anderson, B.G., Burdick, D.E., Galtsof, P.S., Hart, W.B., Patrick, R., Strong, E.R., Suber, E.W. and Van Horn, W.M. (1951). Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. *Sewage Ind. Wastes*, **23:** 447 - 452.

[4] Singh NK, Verma MC, Munshi JSD (1990). Accumulation of copper, zinc, lead, iron and cadmium in certain freshwater fishes of river Subarnarekha. *J. Fresh Wat. Biol.,* **2:** 189-193.

[5] Dutta, H.M., Adhikari, S., Singh, N.K., Roy, R.K. and Munsi, J.S.D. (1993). Histopathological changes induced by malathion in the liver of a freshwater catfish, *Heteropneustes fossilis* (Bloch). *Bull. Environ. Contam. Toxicol.* **51:** 859 - 900.

[6] Gimeno, L., Ferrando, M.D., Sanchez, S., Gimeno, L.O., Andreu, E. (1995). Pesticide effects on eel metabolism pesticide; levels did not decline at any time when animals were exposed to 4.1 mg/liter. *Ecotoxicol. Environ. Saf.* **31 (2):** 153 - 157.

[7] Moitra S., Bhattacharjee R. and Sen N.S. (2012). Histopathological Changes in the gills of air breathing teleost *Clarias batrachus* Linn. Exposed to endosulfan**.** *Asian J. Exp. Sci.,* **26 (1):** 23-26**.**

[8] Karuppasamy R (2000). Tissue Histopathology of *Channa punctafus* (Bloch) under Phenyl Mercuric Acetate Toxicity. *Bulletin of pure and applied science.* **19**: 109-116.

[9] Srivastava VMS, Tripathi RS, Saxena AK (1982). Changes in Gill of *Punctius Sophore* under Chromium Stress. *J. Biol. Res.*, **2:** 85.

[10] Sahoo PD, Mukherjee SC, Nayak SK, Dey S (2001). Acute and Subchronic Toxicity of Aflatoxin *B1* to Rohu, *Lobeo Rohita* (Hamilton). *Ina. J. Expt. Tool.,* **39:** 453-458.