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Effect of lead nitrate on histopathological changes in tissues of Freshwater fish *Labeo rohita* (Ham.)

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Abstract

Objective: To investigate lead nitrate toxicity with emphasis on the histopathological effects of fish *Labeorohita*.
Methods: The fishes were exposed to 31, 31.5, 32, 32.5 and 33ppm of lead nitrate (LN) solution at 24, 48, 72 and 96 hours of intervals. The lethal conc. (LC_{50}) value of lead nitrate was 33ppm for 96 h of exposure. Fishes were exposed to control and sub lethal conc. of lead nitrate (3.3ppm) over a period of 96 h.
Findings: Histopathology of fish organs, after 96 Hof LN exposure revealed the cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting. The vacuolation of hepatocytes and necrosis were observed in liver. The changes of the tissue cells were predominantly showed in 96hr.
Application/Improvements: LN is highly toxic to *Labeorohita* therefore its high conc. of LN in areas close to aquatic bodies should not be encouraged. Fishes from such water bodies is not suitable for human consumption because of the possibility of toxic material present in the fish. Fishes as well as water due to such pollution are toxic to human beings.
Keywords: Lead nitrate, Histopathology, *Labeorohita*, Hyperplasia, Water bodies, Heavy metal.

1. Introduction

Environmental quality of water bodies has deteriorated markedly over last two decades due to the continuous growth in population, rapid industrialization and the increased rate of discharge of pollutants into the environment. Water bodies especially rivers play a crucial role in assimilating or carrying industrial and municipal waste water, runoff from agricultural fields, roadways and other sources which are mainly responsible for water pollution. Among various organic and inorganic water pollutants, metal ions are toxic, dangerous and harmful because of their tissue degradation in nature.

Pollution of environment by heavy metals is of prime importance and unrestrained release of heavy metals into environment via discharge of industrial effluents, sewage and agrochemicals into the water resources has not only rendered it unusable but the same time have produced great harm to fishes. The toxicological studies of pollutants are gaining more significance in recent time and worldwide attempts have been made to identify a ‘‘Hazard’’ from toxic chemical present or released in aquatic environment [1]. The toxicity study is essential to find out toxicant limit and safe concentration so that there will be mining harm to aquatic fauna in the near future. Determination of toxic substances on commercially aquatic farms is essential to maintain the water quality for primary and secondary producers [2].

The admirable way to ensure minimal recurrence of such events is to understand heavy metal, physical, chemical and biological behaviors in aquatic systems and to utilize this knowledge to propose imitative measures when dealing with the problems of metal contamination [3]. Generally, there are two main reasons for studying the chemical behavior of metals in aquatic environments; to understand either the biological or the geo-chemical cycling of these elements. The biological cycling includes bioaccumulation, elimination, bio availability, toxicity and bio-transformation.

The geo-chemical cycling involves the transport, adsorption, desorption, precipitation, dissolution and complication of metals in seawater systems [4]. Amongst several aspects of toxicity studies of the bio assay constituents one of the most commonly used methods in aquatic environmental studies with suitable organisms [5]. Metals are natural components of freshwater systems and originate from natural processes such as the erosion of rock and volcanic activity [7]. Some of these metals are required as important nutrients in least conc. of copper, zinc, chromium and nickel [8]. The heavy metals cause the great threat to the Indian aquatic ecosystem. The discharge of heavy metals in water bodies has impacts health of aquatic systems. There may be an increase in residual level of water, sediment and biota, decreased productivity and an increase in exposure of humans to harmful substances [9]. Heavy metals primarily affect gill, liver, muscle and kidney tissues which are involved in the cleaning processes of body fluids. The danger of heavy metals is aggravated by their almost in definite persistence in the environment because they cannot be destroyed biologically but only transformed from oxidation state or by forming organic complex to another which bind to cell membranes affecting the inter cellular transport processes in the living forms [10]. The biological accumulation of metals by the aquatic organism poses serious problems to human population.

2. Materials and Methods

The 96hr static bioassay was conducted with fingerlings of *L. rohita*. There was done in order to study the toxic of LN (lead nitrate) on fishes to determine the LC_{50} (lethal concentration) for the short exposures.

1. Sources and collection

L. rohita was informed by its ability to withstand stress and its high commercial value in Tamil Nadu. *L. rohita* average 20±0.5 g in weight and 7.5± 0.125 cm size of fingerlings was used. They were brought from finfish hatchery in Vivekat, Maruthapattinam, Thiruvarur (Dt.), Tamil Nadu used.

2. Acclimatization of fish

The fishes were used in 90, 60 and 60cm size of aquarium tank with non-chlorinated water. The period of acclimatization was extended up to one week. The fish were fed during the acclimatization and the water was changed every four days in order to remove faecal and unconsumed feeds.

3. Histopathology

Fish organs were removed and prepared for histopathological observation with adapted [11-13].

3. Results

1. Histopathological effect

Histological structure of the tissues like gill, liver and kidney tissues of fish *L. rohita* exposed to LN showed marked variation in this structure.

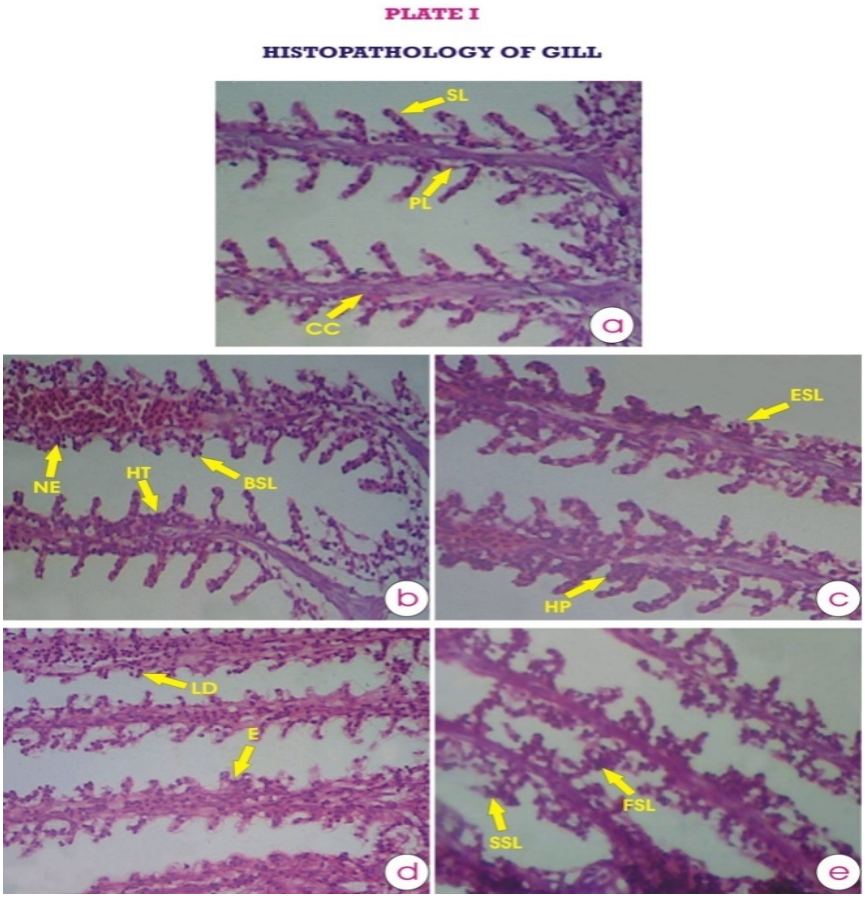
2. Gill

Histological structure of the gillin control fish was found to be similar to the teleostean. The gill was consisted with primary and secondary lamellae (Figure 1). The secondary lamellae were lined by pavement cells. Between secondary lamellae, primary lamellae is lined by stratified epithelium and mucous cells, while the chloride cells (CC) were scattered in the base of lamellae and the inter lamellar region. In control fish the gill tissues showed normal arrangement of filaments in double rows and the secondary lamellae arise from these filaments (Figure 1).

Gills of LN exposed fishes in LC_{50} showed degenerative change in epithelial cells of secondary gill filaments. In case of 24 and 48 hours exposure of concentration moderate necrotic changes in inter lamellar epithelial cells, twisting of gill filament tips, and infiltration of cells in primary axis were evident.

Swelling of the secondary gill lamellae are seen at the tips (Figure 1). In sub lethal concentration of 72 and 96hours exposed fish showed dilation and congestion of blood vessel in 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 were evident in gill of both exposures (Figure 1). Moreover, the degeneration of pillar and chloride cells, dilation of blood vessels, fusion, and shortening of secondary lamellae and loss of broken lamellar structure were import anted histopathological changes in exposure. The changes observed in the 96hr of concentration were more pronounced than those in the 24hr of concentration of lead nitrate (Figure 1).

Figure 1. Histological sections of gills of Labiorohita



3. Liver

Shows the marked variation in their histological structure. The liver tissues of control fish was covered by thin fibrous capsule and single layer of mesothelial cells. The hepatic parenchyma is not arranged into distinct lobules, the biliary channels and vascular elements did not exhibit the classical triads and theses structures seemed to be randomly dispersed throughout the parenchyma. In control fish is composed of polygonal hepatocytes, containing centrally placed spherical large prominent nucleus, the hepatocytes were normally arranged in cords of cells (Figure 2). Histopathology of liver after 72hr of exposure in concentration showed histological structure of the liver tissues in *L. rohita* affected severely. The hepatic cords show remarkable disorientation in structure. Some hepatocytes with disintegrated cell walls and, scanty cytoplasm were found (Figure 2). In addition, hemosiderosis and, intravascular haemolysis in hepatoportal blood vessels with dilation and congestion of sinusoids, venules and cellular degenerations were evident in the liver tissues (Figure 2). The changes in observed the 96hr concentration were more pronounced than that of 24hr LN exposure.

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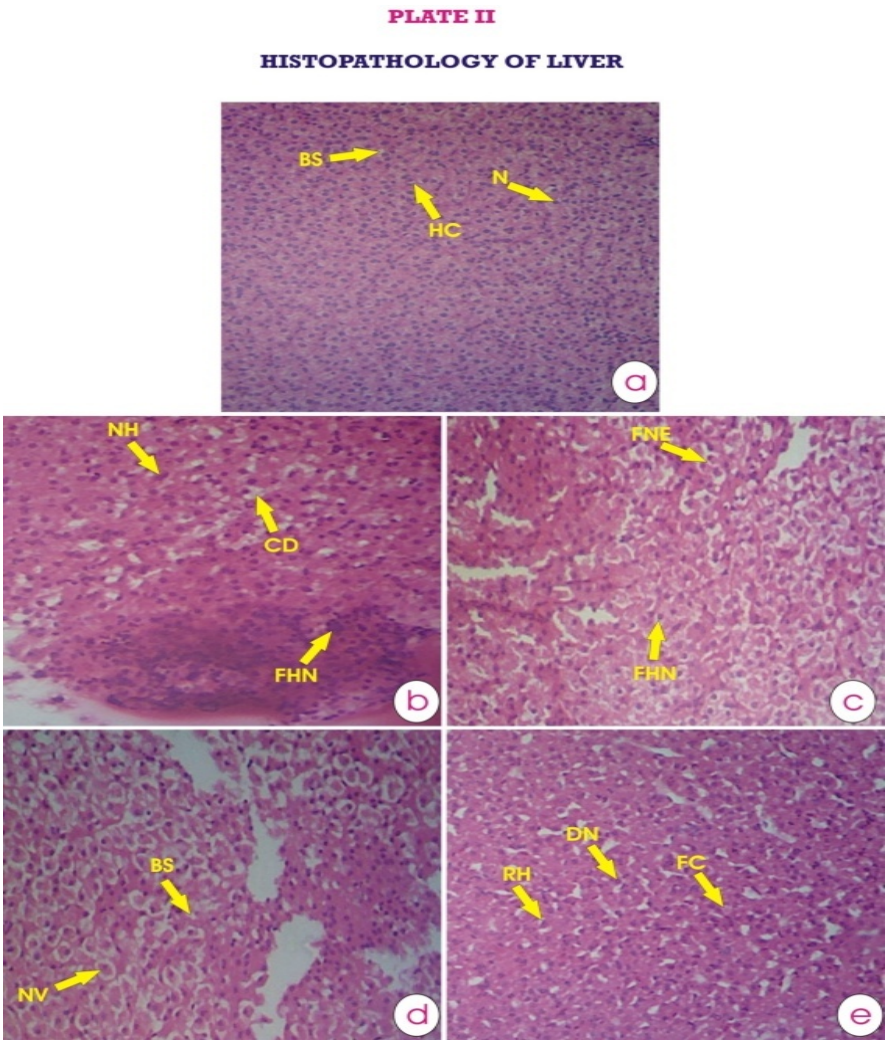
Figure 2. Histological sections of liver of Labiorohita



4. Kidney

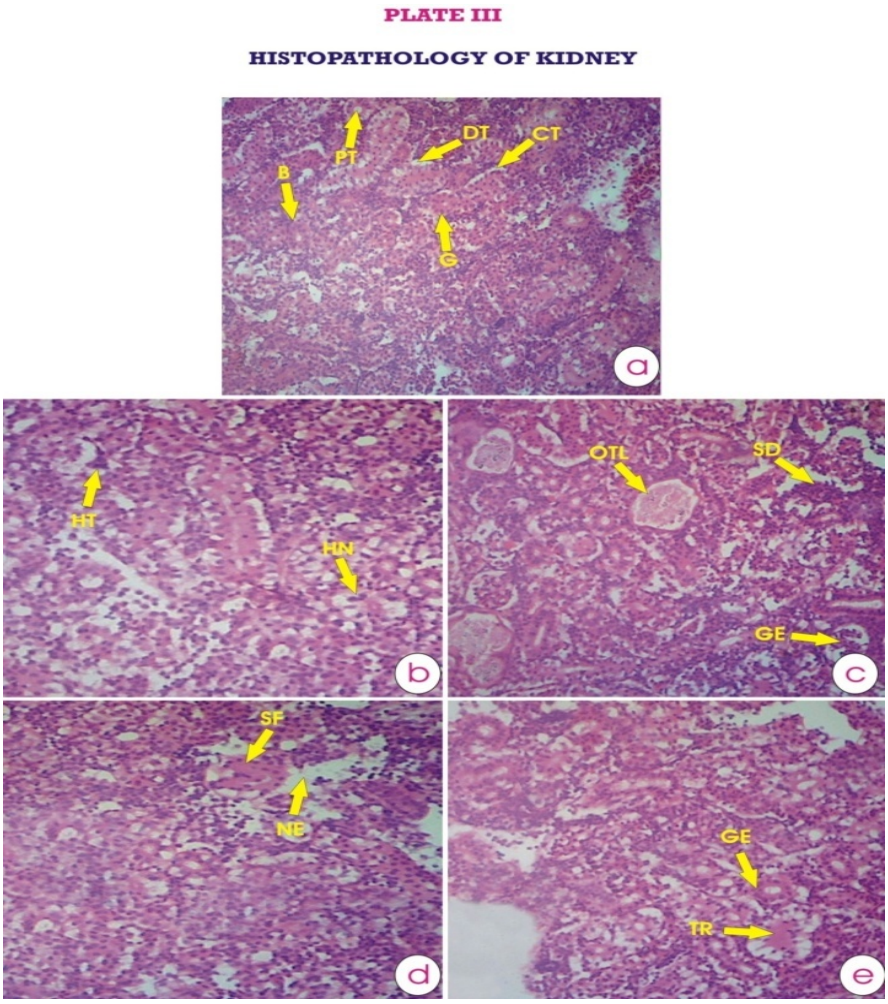
Kidney tissues showed normal glomerular histology with normal first proximal segment, second proximal segment and interstitial haematopoietic tissue in control (Figure 3). This tissue was composed of numerous renal corpuscles with fully developed glomeruli and tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices (Figure 3).

It was showed hydropic swelling and hypertrophy of tubules with dilated nuclei in to higher concentration of LN. Glomerular alteration was also observed. In some cases, the tubular cells showed hyaline droplet accumulation. Some tubules were dilated and necrotic. As the LN conc. and time increased the histoarchitecture was more pronounced than the 96hr LC_{50} LN exposure.

4. Discussion

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gill, liver and kidney [14]. Histological biomarkers of toxicity in fish organs are useful indicator of environmental pollution [15]. Heavy metals accumulation in fish tissues leading causes of structural wounds, toxic impacts on physiology and metabolic faults [16]. The heavy metal damage is an important factor in many pathological and toxicological processes [17].

Figure 3. Histological sections of kidney of Labio rohita



1. Gill

Histopathology of gill is the appropriate bio-indicator to pollution monitoring [18]. Histopathological alterations of gill tissues are linked with specific group of toxicants. Numbers of investigators have reported histopathological changes in the gills of different fish species exposed to heavy metals. The gills are important accessories for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion. It has been considered to be one of the most delicate structures [19]. It has appropriate indicators of water pollution [20].

In the present study the gill tissue of fish *L. rohita* exposed to LN in $LC_{50\,in}$ 24, 48, 72 and 96 hours. They were showed marked variation in their histological changes. There are several investigators have reported histopathological changes in gills of different fish species which were exposed to pesticides, petroleum hydrocarbon, PCB, PAH, and heavy metal. The Various histological and physiological changes in the gills *Tilapia mossambicus* exposed to sewage and industrial pollutants were also observed [21]. The morphological anomalies of the gills due to toxic exposure are similar to the gill structural damages reported for a variety of toxicants like detergents Pesticides and heavy metals [22]. Showed that experimental LN poisoning caused various microscopic and ultra structural abnormalities of gills in *L. Rohita* and pointed out that the histopathological alterations in the gills of fish coupled with reduction in the surface area of the respiratory barrier and inhibition of mitochondrial enzymes leads to inevitable death of the fish.

Histopathological changes in the gill tissues of *L. rohita* were reported by [23]. Which included epithelial proliferation, congestion of blood vessels and hyperplasia [24] subsequently reported dropsy, vascular degeneration, cloudy swelling and necrosis in epithelial and pillar cells of the gill tissues upon chlorpyrifos intoxication.

A gill tissue is an important because of its direct contact with water defect or agency has to go through it to come into the fish. The lamella epithelial lining reacts to dissolved lead creating tissue osmoregulatory imbalance [25]. The hyperactivity of fish when exposed to toxicants may be due to hypoxia faced by the fish due to gill damage by the irritant and the increase in haemoglobin level may be to compensate for impaired respiratory efficiency [26]. Degeneration of gill tissues may affect the ionic permeability and cause decreased ionic level in the blood.

2. Liver

Liver tissues of the fish is sensitive to environmental contaminants tend to accumulate the liver higher concentration than the other organs [27]. The toxicity effect of heavy metals and pesticides on liver histopathology has been studied by many workers. In [28] Reported that necrosis, hypertrophy and atrophy of the tissues, loss of polygonal shape of the cells, splitting of the cells and formation of spaces in the liver cells after exposure in lethal and sub-lethal conc. of copper and cadmium of *Cyprinuscarpio*. The current investigation intercellular gaps between the hepatocytes were observed in the liver tissue of *L. rohita*.

The hepatic cords show remarkable disorientation in structure. Some hepatocytes with disintegrated cell walls and scanty cytoplasm were found. Similar histopathological changes were also reported in *C. punctatus*, *Gambusia affin*is, *O.mykiss* and *Cyprinus carpio* after exposure to arsenic, deltamethrin, diazinon and heavy metal, respectively [29].

In support to present study changes in histological structure of fish liver have been reported in the response to agricultural, sewage and industrial pollutants [21]. Vacuolar degeneration and necrosis of hepatocytes was observed. Degeneration and necrosis of hepatocytes may be due to the cumulative effect of the heavy metals and increase in their concentration in liver tissues.

3. Kidney

The kidney degeneration has been reported by several workers with exposure of the fish to toxicants [30]. It exposed to higher conc. showed hydropic swelling and hypertrophy of tubules with dilated nuclei. Glomerular alteration was also observed. In some cases, the tubular cells showed hyaline droplet accumulation. Some tubules were dilated and necrotic. Histological changes observed in kidney tissues include shrinkage, destruction of tubular epithelium, vacuolarization and damage of glomerulus.

The renal corpuscles of the kidney tissues were scattered resulting in their disorganization and consequently obstruction to their physiological functions. Some of them were found clogging together while they were disintegrated in some tissues of the organ. The above result is corroborating with the present study. This also agreed with the findings [31]. It is a vital organ of fishes and it is maintaining the homeostasis. It is not only responsible for selective re-absorption, which also helps in maintaining pH of blood, body fluids and erythropoiesis [32]. It is one of the first organs to be affected by water contaminants.

They expressed the degree of distortion of their structure due to the exposure period and its toxic conc. of the metals. From the above observations it is clear that the heavy metals LN caused damage in organs like gill, liver and kidney in freshwater fish *L. rohita*. The present study point out about the toxins has been severely affected the aquatic life of the fishes. The problem can be more serious in the fish culture farms as the culture ponds are invariably located in or near the agricultural land, which were already loaded with toxic residues of all kinds. Added to this if the ground water used is also equally polluted with contaminants and pathogenic flora and fauna, the problem becomes compounded, with simulated experimental conditions as in the laboratory. Hence, a scientific method of detoxification is essential to improve the health of these economically important fish and reduce the losses caused by anthropogenic stress.

5. Conclusion

The present investigation concludes that the heavy metal, lead nitrate induced acute toxicity and histopathological alteration and caused significant metabolic effect on the physiological consequences.

These histopathological tissues were rapid and sensitive means of monitoring towards the impact of lead nitrate on *Labeorohita*. The lead nitrate generated from industries, agricultural runoff and domestic sewage pollute the freshwater ecosystem to an injuries level.

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