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ORIGINAL ARTICLES

Lead Nitrate Induced Histopathological Changes in the Gills of the African catfish *Clarias batrachus*

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ABSTRACT

Investigations were carried out on the gill tissue of African catfish (*Clarias batrachus*) after exposed to sublethal concentrations (10 and 15mg/l) of lead nitrate for the period of 30 days under laboratory conditions. The resultant histopathological changes in the gills were recorded by light microscope. The 96hrs LC50 value of lead nitrate was found to be 300.45 mg/l in *Clarias batrachus*. Gill histopathological analysis revealed lesions such as lifting of the epithelial linings from the surfaces of secondary lamellae, curling and fusion of secondary lamellae, infiltration of erythrocytes, and at few places, degeneration of lamellar epithelium were recorded. Simultaneously, uncontrolled regeneration of the primary and secondary lamellae occurred, leading to extensive hyperplasia of the epithelial cell lining in the primary and secondary lamellae. This led to extensive hemorrhage from the gills, arrangement of the pillar cells, blood capillaries of the gills collapsed and chloride cells also exhibited periodic fluctuations in their density. The present investigation illustrates that the presence of high concentration of lead nitrate in water are stressful to fishes.

Key words: *Clarias batrachus*, histopathology, gills, lead nitrate toxicity

Introduction

Metal contamination in the environment is an ongoing problem, particularly in aquatic environments, and there has been extensive investigation of metal effects on aquatic organisms (Niyogi and Wood, 2004). A great variety of pollutants affect the majority of water course which receive domestic, industrial and agricultural effluents. The complexity of this situation becomes apparent when toxicity is keenly considered in terms of its ramifications and environmental consequence. The contamination of freshwater with heavy metals such as lead has become a matter of great concern over the past decades not only because of their threat to public water supplies but also because of the damage caused to aquatic life especially fishes. Lead (Pb) is an immunotoxicant which through human exposure results in immune function changes and has the potential to adversely affect human health. It has many uses in industry including pipes, paints, enamels, glazes, motor industry and others. The major hazard in industry arises from the inhalation of dust and fume but the organic compounds may also be absorbed through the skin. The natural waters are continuously being contaminated by lead due to increased anthropogenic activities and industrial exploitation of this metal (Chandravathy and Reddy, 1996). Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb (Reglero, *et al* 2009, Abdallah *et al* 2010, and Mirhashemi, *et al* 2010)

The gills are not only the prime organs for gaseous exchange; they also perform several other physiological functions including osmoregulation and excretion. Recent review articles on ambient toxicants in fish have clearly demonstrated that increased concentrations of several heavy metals seriously damage the gills of teleostean fish (Wenderlaa, 1997). Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal (Nowak, 1992).

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Effects of glyphosate herbicide on Tilapia was investigated by (Ayoola 2008) filament cell proliferation, lamellar fusion, lamellar cell hyperplasia and epithelial lifting were observed. The major effects observed on the gills were Oedema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae. The data concerning lead toxicity are mainly related to studies with mammalian subjects and to air-borne pollutants. Therefore in this study efforts have been made to examine the toxicity of lead nitrate on the gills of African catfish *Clarias batrachus.*

Materials and Methods

Apparently healthy live specimens of *Clarias batrachus* having mean weight 100g and length 20cm were purchased from local fish market, Bhopal and brought to the laboratory conditions. The fish were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing in the aquarium fish were treated with 0.1% of $KMnO_4$ solution to serve any dermal infection. The fish were acclimatizing to laboratory condition for at least one week prior to the start of experiment. The fish were fed with chopped meat daily. The 96hrs LC50 value of lead nitrate was found to be 300.45mg/l in *Clarias batrachus* using the Probit analysis method (Finney, 1971). After determining LC50 96hrs value, they were divided in three groups have 10 fish in each aquarium. The first group of aquarium was kept as control with plain fresh water while the second contained lead nitrate at 10mg/l concentration and third contained 15mg/l of lead nitrate concentration. Water of each aquarium was changed on every 5^{th} day and lead nitrate was maintained through out the experiment duration of 30 days.

Histopathological procedure:

On the 15^{th} and 30^{th} day of the exposure of different concentrations of lead nitrate, both treated and untreated fish were sacrificed by giving a sharp blow on the head and dissect out gill were removed and washed in saline water to remove blood and fixed aqueous fixative for 24hrs. The fixed samples were dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Sections of 6 microns thickness were cut out, mounted and stained with Hematoxylin and Eosin for examination by light microscope.

Result and Discussion

Control group:

The lamellae are lined by a squamous epithelium composed by pavement and non-differentiated cells. Below that epithelium are lamellar blood sinuses separated by pillar cells. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride, mucous and pavement cells (Fig.1).

Treated groups:
10mg/l of $Pb(No_3)_2$ After 15 and 30 days:

After 15 days significant histological changes included fusion and curling of secondary lamellae, hyperplasia in the interlamellar region of secondary lamellae, hypertrophy of chloride cells and epithelial lifting of secondary lamellae (Fig.2). After 30 days gill section were revealed the hypertrophy of epithelial cells, fusion of secondary lamellae, degeneration of epithelial lining of secondary lamellae, and pillar system displayed hemorrhagic foci and the infiltration of erythrocytes in central venous of gill (Fig 3).

Treated groups:
15mg/l of $Pb(No_3)_2$ After 15 and 30 days:

After 15 days exposure gill section were showed dilation of central venous with marked cellular hyperplasia, shortening of secondary lamellae and epithelial lifting at the base of secondary lamellae (Fig. 4). After 30 days fish shows, lamellar fusion, hypertrophy and hyperplasia of epithelial cells that produced the thickening of the gill epithelium, fusion of the secondary lamellae and a consequent reduction of the distance between adjacent lamellae with shortening of secondary lamellae and dilation of venous central sinus (Fig. 5).

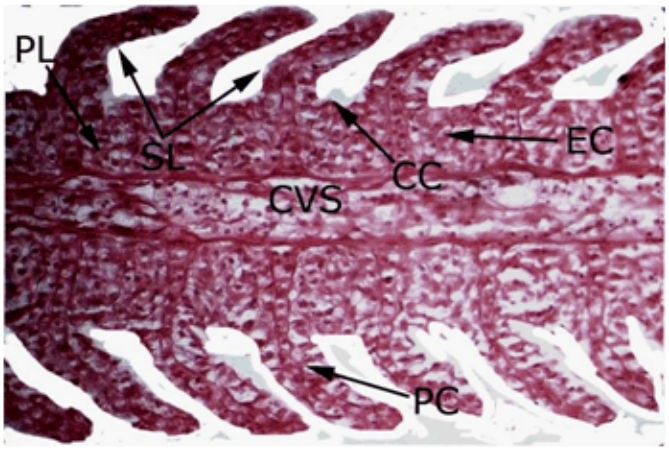

Fig. 1: Photomicrograph of the Gill of *Clarias batrachus* of control group shows normal gill architecture. (H&E) (x100).

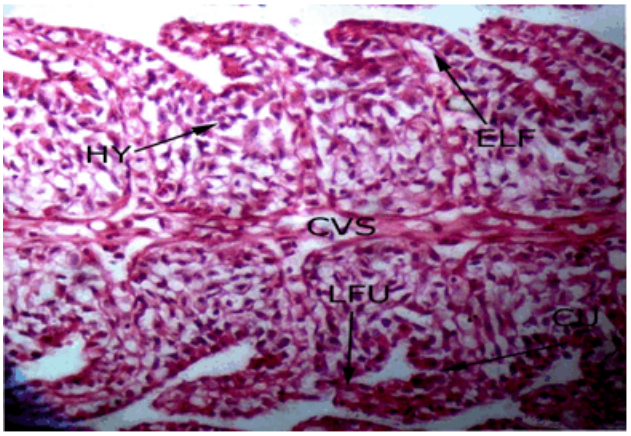

Fig. 2: Photomicrograph of the Gill of 10mg/l of lead nitrate intoxication after 15 days, showing epithelial lift- ing, hyperplasia of epithelial cells and lamellar fusion. (H&E) (x100).

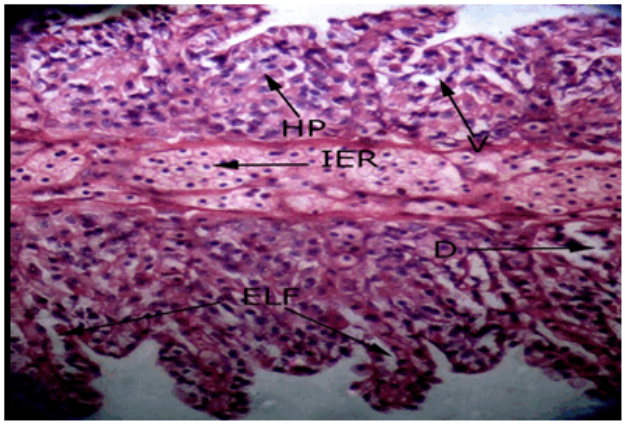

Fig. 3: Photomicrograph of the Gill of 10mg/l of lead nitrate intoxication after 30 days, showing degeneration of epithelial cells, epithelial lifting, infiltrattion of erythro-cytes in central venous and hyperplasia of epithelial cells. (H&E) (x100)

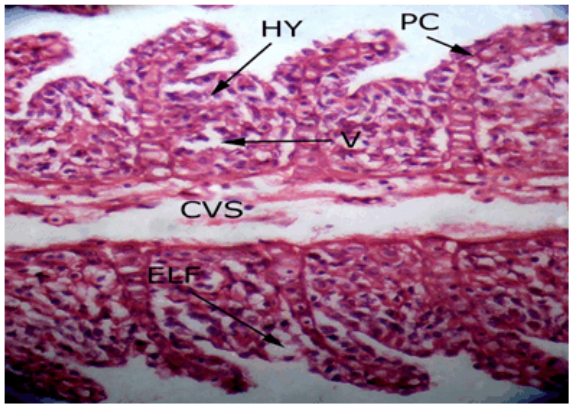

Fig. 2: Photomicrograph of the Gill of 15mg/l of lead nitrate intoxication after 15 days, showing dilation of central venous, hyperplasia of epithelial cells, vacuola- tion, and epithelial lifting. (H&E) (x100)

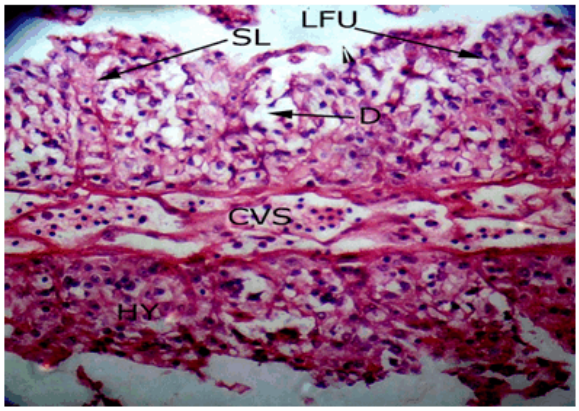

Fig. 3: Photomicrograph of the Gill of 15mg/l of lead nitrate intoxication after 30 days, showing degeneration lamellar epithelium, infiltration erythrcytes, lamellar fus-ion. (H&E) (x100)

Abbreviations:

SL- Secondary lamellae; PL- Primary lamellae; HY- Hyperplasia; PC- pillar Cells; CVS-Central venous sinus; ELF- Epithelial lifting; D- Degeneration; LFU- Lamellar fusion; EC- Epithelial cells; IER- Infiltration of erythrocytes; CC- Chloride cells

Discussion:

The results presented here revealed that lead nitrate exposure resulted in histopathological changes in the gills of *Clarias batrachus* and no one of the exposed fish could escape the toxic effect of lead nitrate**.** Histological study of the gills shows a typical structural organization of the lamella in the untreated fish. However, fish exposed to lead nitrate shows several histological alterations, included hypertrophy, lifting of the epithelial linings from the surfaces of secondary lamellae, and at few places, degeneration of lamellar epithelium. Hyperplasia of epithelial cells that resulted in the fusion of many lamellae and curling at the tips of gill lamellae. Similar observations were reported due to exposure of other freshwater fish to lead (Abd El-Gawad, 1999; Martinez, 2004; Palaniappan, 2008) and copper (Parkand. Heo, 2009, Kosai 2009). These structural changes increase the diffusion distance between the respiratory blood and xenobiotics. As a result, they also increase the oxygen distance for gaseous exchange due to the decreased surface of the secondary lamellae. The lifting of lamellar epithelium is other histological change observed, probably induced by the incidence of severe edema (Pane, 2004, Schwaiger 2004). The fusion of gill lamellae and lifting of lamellar epithelium, disintegration of laminar axis, and disruption of gill tips of air-breathing catfish *Heteropneustes fossilis* exposed to 6.2 mg/l lead nitrate for different durations (Parashar and T.K. Banerjee, 2002). Disruption of gill tips and fusion of gill lamellae in *Oreochromis niloticus* exposed to 2.7 mg/l lead nitrate for 28 days

(Mallatt,1985) Disintegration of laminar axis and lamellar aneurism was also found in the gills of *Cyprinus carpio* exposed to 2.5, 3.5 and 4.5 mg/l lead nitrate for 90 days (Wilson,2002). Edema with lifting of lamellar epithelium could be serve as a mechanism of defense, because separation epithelial of the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream (Arrelano, *et al*, 1999). These gill histological alterations has been observed by several authors in fish submitted to (Karan, 1998, Chen, 2001, DeBoeck 2001). However, these changes also can be due to the exposition to different kinds of pollutants, such as endosulfan and arsenic (Hwang, 1993). Epithelial hyperplasia, aneurism, curling and fusion of secondary lamellae were noticed in *Cirrhinus mrigala* after exposure to monocrotofos (Vermurugan, 2007) in *Gambusia affinis*, after 30 days of exposure to deltametrin (Cengiz and Unlu, 2006). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since; in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Poleksic, and. Mitrovic-Tutundzic, 1994, Fernandes, and Mazon, 2003). Toxicants induced alterations in gill histology are mostly non-specific in nature, which partially represent the damage and partially the compensatory response of the fish. Examples are lamellar epithelial lifting and lamellar aneurysm as observed during the present study.

The present investigation on gills indicates that due to continued toxic impact of lead nitrate, the protective role of the thin layer of slime collapses and fails to prevent the penetration of lead salt, subjecting the cellular constituents lining the extensive surface area of the gills to the toxicity of the heavy metal. This leads to various degrees of wear and tear, which causes damage to the delicate protective device of the gill epithelia of *Clarias batrachus.*

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