Effects of lead nitrate on histo-cytological alterations of corpuscles of Stannius of stinging catfish, Heteropneustes fossilis

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ABSTRACT

Background: The present study investigated the effects of lead nitrate on the histo-cytological changes in the corpuscles of Stannius (CS) of *Heteropneustes fossilis.*

Methods: Catfish, *Heteropneustes fossilis* were subjected to 657.6 mg/L and 164.4 mg/L of lead nitrate for 96 h and 28 days, respectively. Blood from fish was collected on 24, 48, 72 and 96 h in short-term and after 7, 14, 21, and 28 days in long-term experiment. Blood was collected for analysis of calcium levels and CS were fixed for histological studies.

Results: Plasma calcium levels of the fish remain unaffected at 24 h. The levels decrease after 48 h which persists till 96 h. Plasma calcium levels of the fish exposed to lead for 7 days exhibit a decrease which persists progressively till 28 days.

After 96 h, AF-positive cells of CS exhibit increased granulation. No change in the nuclear volume of these cells has been noticed. An increased nuclear volume has been recorded in the AF-negative cells of CS of 96 h lead exposed fish. After 14 days, the nuclear volume of AF-positive cells decreases. Heavy accumulation of secretory granules and decrease in the nuclear volume of AF-positive cells have been recorded after 21 days which pronounced after 28 days. Moreover, few degenerating cells have also been encountered. AF-negative cells of CS exhibit an increase in the nuclear volume after 21 and 28 days lead treatment.

Conclusion: Present findings suggest that exposure of the lead to catfish *Heteropneustes fossilis* caused CS inactivity .

Keywords: Corpuscles Of Stannius, Heavy Metal, Lead, Plasma Calcium, Teleost.

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INTRODUCTION

Lead is a naturally occurring heavy metal which has been used in various ways including mining, smelting, refining, gasoline, battery manufacturing, electrical wiring, soldering, painting, ceramic glazing and the making of stained glass. Due to its non-degradable nature, it gets into our environment and eventually enters the human and animal's blood stream. There from, it is accumulated in soft tissues such as liver, kidneys, nervous system and the brain. In fishes, fish mortality (1) and accumulation of lead in various tissues (2, 3) and alterations in biochemical and hematological parameters (4) have been reported. Moreover, lead-induced changes in the histological structure of gills liver and kidneys have also been reported (5-9).

In fish inhabiting freshwater, blood ionic concentrations are maintained at much higher levels than those of the ambient water. Hence, they constantly face osmotic inflow of water and diffusional

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losses of ions across the body surface and gill epithelium. A disturbed hydromineral balance of the body fluids of fish is one of the most conspicuous phenomena observed during stress as there exists an intimate relationship between the surrounding water and the body fluids. There also exist few studies regarding the interaction of lead with calcium homeostasis in mammals and birds. However, there exists a single report from fish regarding the effects of lead on plasma calcium (10). To the best of our knowledge there exists no study regarding the effect of lead on the corpuscles of Stannius of fish. Hence, in this study an attempt has been made to investigate the impact of lead nitrate on the plasma calcium and corpuscles of Stannius of a catfish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Freshwater stinging catfish *Heteropneustes fossilis* (after two weeks acclimatization) were subjected to 657.6 mg/L (0.8 of 96 h LC_{50}) and 164.4 mg/L (0.2 of 96 h LC_{50}) of lead nitrate for short-term and long-term, respectively. Concurrently, a control group was also run. The media (both control and experimental) were changed every 24 h. The fish were sacrificed at 24, 48, 72 and 96 h in short-term experiment and at 7, 14, 21 and 28 days in long-term experiment. Blood was collected by sectioning of caudal peduncle and oozing blood was collected in heparinized eppendorf tubes. Plasma calcium levels were determined (from six samples from each group –control and experimental at each interval) by using Sigma kit (Sigma Chemical Co., kit # 587 A). After collection of blood samples the corpuscles of Stannius along with the adjoining portion of kidney were removed from the fish and fixed in aqueous Bouin's fluid. Tissues were routinely processed in graded series of alcohols, cleared in xylene and embedded in paraffin wax. Serial sections were cut at 6 μm. Nuclear indices (maximal length and maximal width) were taken with the aid of ocular micrometer and then the nuclear volume was calculated as –

$$\text{volume} = 4/3\pi\ ab^2$$

where 'a' is the major semiaxis and 'b' is the minor semiaxis. In the gland, when there are degenerating nuclei, only the indices of intact nuclei were measured.

All data were presented as the mean ± S.E. of six specimens and Student's t test was used to determine statistical significance. In all studies, the experimental group was compared to its specific time control group.

RESULTS

After short-term lead exposure, the plasma calcium levels of the fish were unaffected at 24 h. The levels decreased after 48 h and persisted till the end of the experiment (96 h) (Fig. 1). The plasma calcium levels of the fish exposed to lead for 7 days exhibited a decrease (Fig. 2). This decrease persisted progressively till the close of the experiment (28 days) (Fig. 2).

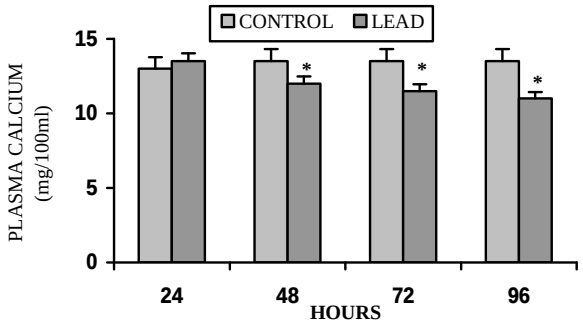

Figure 1. Plasma calcium levels of short-term lead nitrate treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.

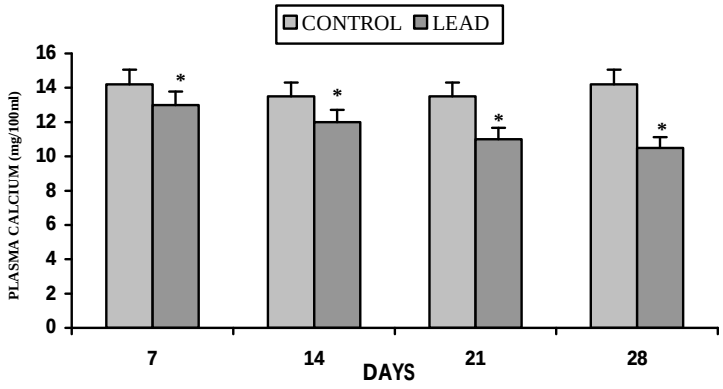

Figure 2. Plasma calcium levels of long-term lead nitrate treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P<0.05$) from control.

The corpuscles of Stannius of control *H. fossilis* exhibit two types of cells namely- AF-positive and AF-negative cells (Fig. 3). Up to 72 h after exposure of the fish to lead, the AF-positive cells of corpuscles of Stannius exhibit no histological change. After 96 h, these cells exhibit increased granulation (Fig. 4). No change in the nuclear volume of AF-positive and AF-negative cells has been noticed.

There is no change in the AF-positive cells up to 7 days following lead exposure. After 14 days, the nuclear volume of these cells decreases (Fig. 5). The heavy accumulation of secretory granules (Fig. 6) as well as a decrease in the nuclear volume of AF-positive cells (Fig. 5) have been recorded after 21 days of lead exposure. These changes are more pronounced after 28 days. Moreover, few degenerating cells have also been encountered (Fig. 7). No change has been noticed up to 14 days in the AF-negative cells of the fish exposed to lead. These cells exhibit an increase in the nuclear volume after 21 and 28 days lead treatment (Fig. 8).

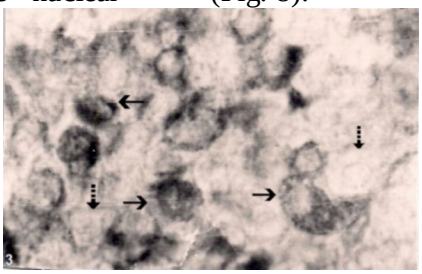

Figure 3. Corpuscles of Stannius of control *Heteropneustes fossilis* showing AF-positive (arrows) and AF-negative (broken arrows) cells. Aldehyde fuchsin X 800.

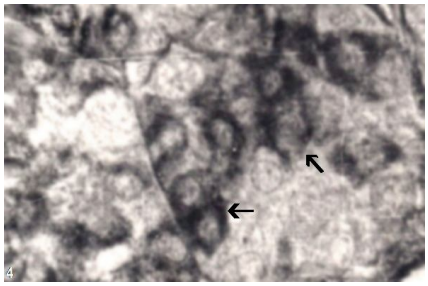

Figure 4. Corpuscles of Stannius of 96 h lead nitrate treated *Heteropneustes fossilis* exhibiting increased granulation (arrows) in AF-positive cells. Aldehyde fuchsin X 800.

DISCUSSION

Lead exposure to *H. fossilis* provoked hypocalcemia. This derives support from the studies of Rogers *et al.* (10) who have also reported hypocalcemia in lead-exposed rainbow trout. This is in agreement with the reports of other investigators who have also observed decreased blood/ plasma calcium content of fish after treatment with either aldrin (11), malachite green (12), cadmium (13,14,15), propoxur (16), formothion (16), chlorpyrifos (17), deltamethrin (18), cypermethrin (19) or botanical pesticides (20,21). On the contrary, elevation of plasma calcium concentrations has also been reported by other workers from the fish exposed to various toxicants (22-25). However, no effect on plasma calcium level has been noticed in methoxychlor exposed Northern puffer *Sphaeroides maculatus* (26), DDT treated flounders *Platichthys flesus* (27) and bifenthrin treated rainbow trout *O. mykiss* (28).

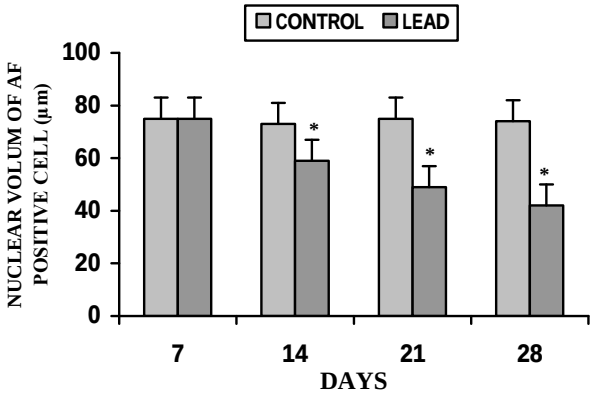

Figure 5. Nuclear volume of AF-positive cells of long-term lead nitrate exposed *Heteropneustes fossilis.* Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.

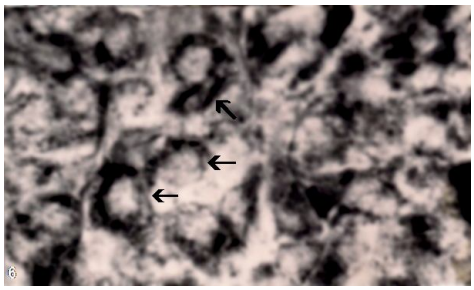

Figure 6. Corpuscles of Stannius of 21 days lead nitrate treated *Heteropneustes fossilis* exhibiting heavy accumulation of secretory granules (arrows) in AF-positive cells. Aldehyde fuchsin X 800.

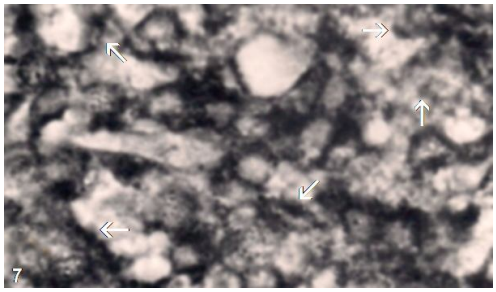

Figure 7. Corpuscles of Stannius of 28 days lead nitrate treated *Heteropneustes fossilis* exhibiting degeneration (arrows) of AF-positive cells. Aldehyde fuchsin X 800.

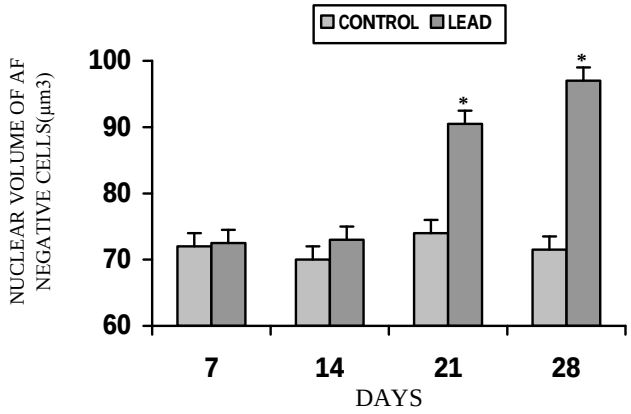

Figure 8. Nuclear volume of AF-negative cells of long-term lead nitrate exposed *Heteropneustes fossilis.* Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P<0.05$) from control.

The hypocalcemia observed in lead exposed *H. fossilis* may be attributed to the impairment of either net electrolyte influx at the gill or renal function. Rogers *et al.* (10) have reported reduced calcium uptake in lead-exposed rainbow trout. Several investigators have reported degenerative changes in the gills of fishes after exposure to various pesticides (5, 6). Degeneration of gills may affect the ionic permeability and cause decreased ionic levels in the blood. Tubular necrosis may be the other possible reason for the hypocalcemia observed in lead exposed *H. fossilis.* Kidney degeneration has been reported by several workers after exposure of the fish to toxicants (29, 30). The increased urine excretion rate of Ca^{2+} has been observed in lead treated rainbow trout (31).The degeneration of kidney may lead to decreased reabsorption thus causing increased urinary loss of these ions. This increased loss of ions through the kidney may be the possible reason for the decreased concentration of calcium in lead treated *H. fossilis.* In the past, Koyama and Itazawa (32), Roch and Maly (33), Larsson *et al.* (13) and Haux and Larsson (34) have also attributed degenerative changes in the renal tubules as one of the main causes of hypocalcemic responses in cadmium treated fishes. Patel *et al.* (31) have suggested that lead-induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca^{2+} homeostasis, is not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney.

The corpuscles of Stannius of lead treated fish exhibit increased granulation among the AF-positive cells and a decreased nuclear volume of these cells. The AF-positive cells have been associated with the release of stanniocalcin which functions as a hypocalcemic hormone in teleosts (35-39). There exists no study regarding the effect of lead on the corpuscles of Stannius of fish. In the present study the observed increased granulation in the AF-positive cells may be attributed to the perpetual hypocalcemia induced by lead challenge to the fish *H. fossilis.* The accumulation of secretory granules noticed in the AF-positive cells of lead exposed fish derives support from the studies of earlier investigators who have also noticed similar response in toxicant exposed fish (18, 40-42). Accumulation of

secretory granules among mammalian calcitonin cells (which secrete a hypocalcemic hormone) has been reported in response to induced hypocalcemia (43-46).

CONCLUSION

The present findings suggest that exposure of the lead to stinging catfish *Heteropneustes fossilis* caused disturbances in the blood calcium content as well as inactivity of the corpuscles of Stannius. The environmental contamination with this metal can pose hazard for the fish populations and a serious problem for the aquaculture due to the disturbances in calcium regulation which is important for several vital physiological processes and reproduction.

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