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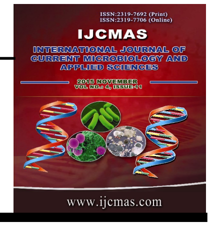

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Original Research Article

Short Term Effects of Cadmium Chloride on Branchial Histomorphology of Snake Head, *Ophiocephalus punctatus* (Bloch)

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A B S T R A C T

The study was carried out to find out lethal and sub-lethal effect of cadmium on the histomorphology of gillsin *Ophiocephalus punctatus*. Cadmium chloride at lethal and sub-lethal concentrations caused deleterious effect on the gills. The apical region of the primary gill lamellae became hypertrophied, bent and fused. The secondary gill lamellae became hypertrophied, fused and vacuolated, and branchial blood vessels got damaged. The pillarcells and epithelial layer of the secondary gill lamellae got damaged. Epithelial nuclei became hyper-pycnotic and degenerated. The changes were also accompanied by hyperplasia of chloride and mucous cells of secondary gill lamellae. It is concluded that cadmium at lethal as well as sub-lethal concentrations affect the histomorphology of gills and may severely affect the general metabolism of the body due to reduction of oxygen supply.

Introduction

Heavy metals pose a great impact on both the aquatic and terrestrial animals through air and aquatic pollution. These days heavy metals pollution has become a common threat to all the living beings due to its immense use in the ever fast growing industrialization. Heavy metal contamination of the aquatic environment has drawn increasing attention because of its devastating effect on the ecological balance and food chain of the recipient environment and aquatic organisms. Amongst animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Vosyliene and Jankaite, 2006; Farombi *et al.*, 2007). Fish readily absorb dissolved metals and may serve as indicators of pollution (Adham *et al.*, 2002; Farkas *et al.*, 2002; Shukla *et al.*, 2007).

Cadmium is a non-essential heavy metal but it has accumulative polluting effects, and causes toxicity to aquatic organism even at minute concentrations. Hence, it is regarded as one of the most toxic elements in the environment. The occurrence of cadmium in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems (Arno *et al.*, 2002; Audry*set al.*, 2004; Kiran *et al.*, 2006).

Fish gills are important target organs for heavy metals because of their large surface area in contact with external environment and their thin membrane separating the internal medium from the external medium (Ferrari *et al.*, 2009). Thus, gills come immediately in contact with heavy metals dissolved in water provoking morphological and functional disturbances. Alterations in gill structure affect the normal function of vital physiological processes such as gas and ion exchange, osmoregulation, excretion of nitrogenous wastes and acid-base equilibrium (Bonga *et al.*, 2009). On this basis, gills arise as an appropriate model for studying the effects of environment stressors on fish (Vigliano *et al.*, 2006).

Materials and Methods

Healthy fingerlings of *Ophiocephalus punctatus* measuring the size 12–15 cm were purchased from fisherman of Mulchera (District-Gadchiroli) of Maharashtra State (India). They were carried to the laboratory in hygienic condition and acclimatized for fifteen days. During the period of acclimatization fish were fed with rice bran, dried minced prawn and boiled egg *ad libitum*. Six aquariums (20 liter water holding capacity) were cleaned with detergent and rinsed with distilled water. First aquaria containing 10 fishes/10 liter of tap water was used as a control. Other five aquariums were filled with 10 liters of tap water containing different concentration of Cadmium chloride solutions i.e. 10, 20, 30, 40 and 50 mg/l and mortality was recorded at 24, 48, 72 and 96 hrs. The aquariums were not aerated during experimentation. For calculating the exact death rate, comparison was made with death of fish occurred in controlled aquarium and experimental groups. All experiments were carried out for the period of 96 hrs. The number of dead fish was counted every 12 h and removed immediately from the aquaria.

Percent mortality was calculated and the values were transformed into probit scale and analyzed as per Finney, 1971. Regression lines of probit against logarithmic transformation of concentrations were obtained. Slope function (S) was calculated.

For the study of histomorphological changes the fish were exposed to Cadmium chloride at lethal concentration-50 for four days (short term exposure). One aquarium containing ten fish in 10 liter of tap water was used as a controlled. Other aquarium containing 10 liter of Cadmium chloride solution (LC-50) was used for experimentation. Temperature of the controlled aquarium throughout the experimental period was 24.05 ± 0.660ºC and of the experimental aquarium was ranging from ranging from 24.05 ± 0.660ºC to 26.08 ± 0.287ºC. The water was not aerated during the entire period of experimentation. Fish after every 24 hours were removed from the aquarium and gills were dissected out and fixed in alcoholic Bouins fixative for 24 hrs. Dehydration was carried out and blocks were prepared in paraffin wax. Sagital sections of gill lamellae were cut at 5-6 µm rocking microtome. Sections were stained with hematoxylene and eosin. Photographs were taken with Coslabtrinocular microscope attached with CCD camera.

Results and Discussion

The section of gills of *Ophiocephalus punctatus* showed dramatic histological changes after exposure to Cadmium chloride LC-50 for the period of four days are summarized in detail as follows.

Histology of gills

Gill of the controlled fish exhibited no pathological lesions (Fig. 1a); both the primary and secondary gill lamellae were intact. However, the primary gill lamellae are flattened structures with a central rod like supporting axis and a row of leaflet like secondary gill lamellae on each side of it. The secondary gill lamellae were equally spaced along the columnar structures with intact cellular layer attached at their bases with the primary lamellae and free at their bases with the primary lamellae and free at their bases with the primary lamellae and free at their distal ends. The normal secondary lamellar epithelium was simple, consisting of a thin single or double sheet of epithelial cells, blood vessels and a row of pillar cells. The region between the two adjacent secondary gill lamellae is known as inter-lamellar region. The epithelial cells were undifferentiated and chloride cells were normal.

Histopathological changes in the Gills

On the first and second day of exposure gill exhibited hyperplasia and fusion of some secondary gill lamellae. Epithelial cells became hypertrophied. Lamellae in some area of the gills got disorganized followed by lamellar aneurysm and hemorrhage with rupture of the lamellar epithelium (Fig. 1b). The lifting of shrinked epithelial cells along with severe epithelial disintegration was observed (Fig. 1c). However, gills showed an extensive hypertrophy and the curling of secondary gill lamellae was not evident on the first day of exposure but it was observed on the second day of exposure.

Epithelial cells became hypertrophied and fusions of the primary and the secondary gill lamellae (Fig. 1d) were seen on the 3rd day of exposure. Severity of epithelial disintegration and lamellar disorganization was also extensive at this time. Pillar cells underwent bending and breakdown. In the blood vessels of primary gill lamella mild congestion was noticed.

On the fourth day of exposure the severity in the shrinkage and hypertrophy of epithelial cells, damaging of pillar cells, lamellar disorganization and disintegration was increased (Fig. 2b). Gills showed extensive hypertrophy. The fusion of adjacently located primary gill lamellae and secondary gill lamellae was more along with the cellular hyperplasia. In primary gill lamellae blood engorged and coagulated (Fig. 2a). The epithelial cells detached off from the pillar cells and vacuolation in the fused secondary lamellae were prominently seen. Also, the secondary and primary gill lamellae became curled. The epithelium of gill filament and secondary gill lamellae proliferated.

Water is the universal solvent and therefore many things get dissolve in it. The propagation of industries occurs near water resources like rivers, dams, sea etc. which releases several kinds of pollutants in the water body that directly or indirectly affects the aquatic life. The possible effects may range from impairment of growth, reproduction and metabolic functions in organisms or changes in the physical and chemical properties of the ambient medium that indirectly affect the resident biota in water.

In the present investigation, gills of untreated *Ophiocephalus punctatus* showed a normal structural organization of the lamellae. However the treated one showed progressive architectural distortion at the end of the exposure period. This corroborates with the observation of Jana and Bandopadhyaya (1987) who reported that gills are important tissues because of its direct contact with water and any effect or agent has to go through it to come into the fish body. The lamella epithelial lining reacts to dissolved lead creating tissue osmoregulatory imbalance.

Fig.1 **a.** L.S. of gill in controlled *O. punctatus* shows normal gill histology. 40X; **b.** L.S. of gill, 24hr after exposure to $CdCl_2$ shows hemorrhage (h), rupture of gill epithelium (★), 100X; **c.** L.S. of gill, 48hr after exposure to $CdCl_2$ shows shrinkage of epithelial cells (▲), 100X; **d.** L.S. of gill, 72hr after exposure to $CdCl_2$ shows fusion of primary (pgl) and secondary gill lamellae, hyperplasia (hpa), 100X

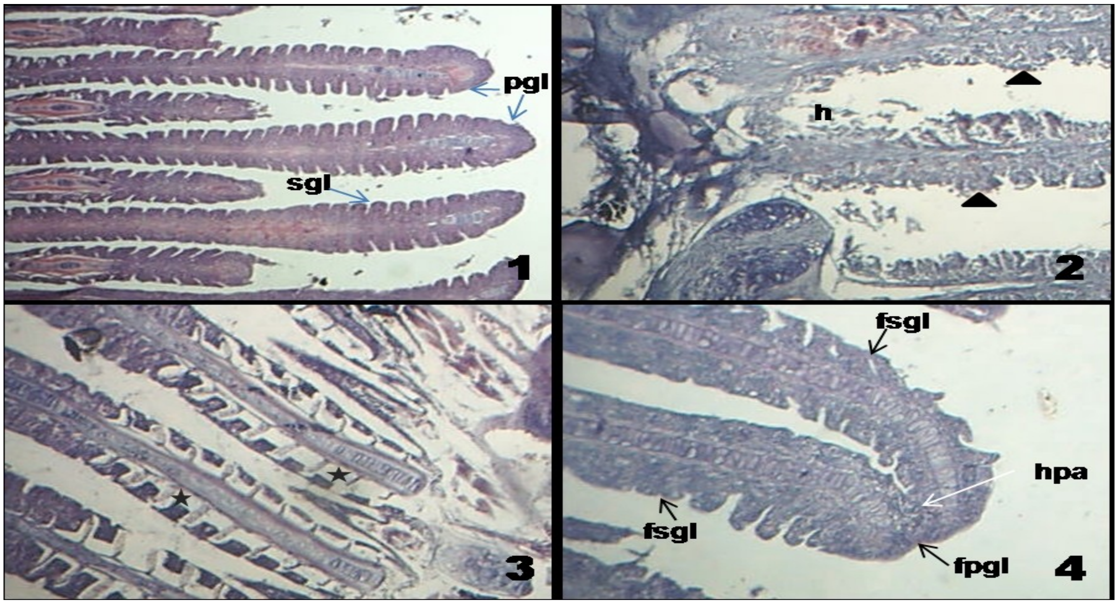
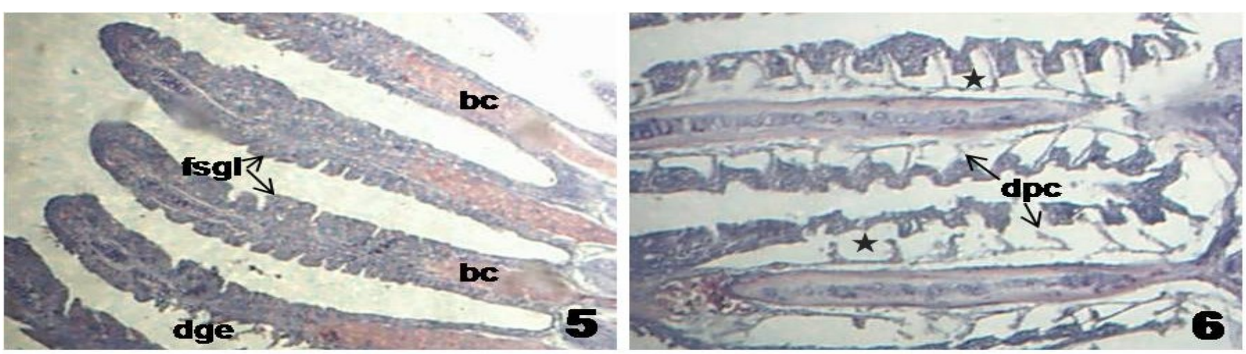


Fig.2 **a**. L.S. of gill, 96hr after exposure to $CdCl_2$ shows blood congestion (Bc), fusion of secondary gill lamellae (fsgl) and damage of gill epithelium (dge), 100X; **b.** L.S. of gill, 96hr after exposure to $CdCl_2$ shows shrinkage of epithelial cells (★),100X



According to Cladwell (1997), the end result would be reduced flow of oxygen-enriched water to lamellar tissues and ultimately, a reduction in the fish's performance capacity. The change in physiological properties was evident in the shrinkage and fusion observed at the lamella thus, suggesting that lead intake mostly occurred via the gills. In cadmium treated gills, marked hyperplasia of branchial arch, pilaster cell vacuolization and congestion of blood vessels were well marked. The results are in parallel with the works of Kapila and Ragothaman (1999) who reported for *Boleopthalmus dessumieri* exposed to sublethal concentrations of cadmium. However Pantung *et al.* (2008)

have reported some breakdown of pillar cells during the entire period of cadmium exposure in hybrid walking catfish (*Clariasmacro cephalus*x *Clarias gariepinus*). The epithelial cells displayed considerable edema and some breakdown from the third day and onwards. Histopathological changes in the gill of *Labeorohita* were reported by Vijayalakshmi and Tilak (1996) which included epithelial proliferation, congestion of blood vessels and hyperplasia. Tilak *et al.* (2005) subsequently reported dropsy, vascular degeneration, cloudy swelling and necrosis in epithelial and pillar cells of the gills upon chlorpyrifos intoxication.

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