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Short term effect of Zinc chloride in gills Histomorphology of Snake head, *Ophiocephalus punctatus*(Bloch)

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Abstract :

The study was carried out to find out lethal and sub-lethal effect of Zinc on the histomorphology of gill in *Ophiocephalus punctatus*. Zinc chloride at lethal and sub-lethal concentration caused deleterious effect on the gills. The apical region of the primary gills lamellae became herptrophied, bent and fused. The secondary gill lamellae became hypertrophied, fused and vacuolated and branchial blood vessels got damage. The pillar cells 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 zinc 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:

Zinc is an essential trace element for all living organisms. As a constituent of more than 200 metalloenzymes and other metabolic compounds, zinc assures stability of biological molecules such as DNA and of biological structures such as membranes and ribosomes (Vallee 1959; National Academy of Sciences [NAS] 1979; Casey and Hambidge 1980; Mason *et al*. 1988; Llobet *et al.* 1988b; Leonard and Gerber 1989). Clinical manifestations of zinc deficiency in animals include growth retardation, testicular atrophy, skin changes, and poor appetite (Prasad 1979). Zinc poisoning has been documented in some mammals and fishes, usually as a result of ingesting galvanized metal objects, certain paints and fertilizers, zinc-containing coins, and skin and sun block preparations containing zinc oxide (Wentink *et al.* 1985; Ogden *et al.* 1988; Lu and Combs 1988a; Binnerts 1989; Robinette 1990). Aquatic populations are frequently decimated in zinc-polluted waters (Solbe and Flook 1975; Everall *et al.,* 1989b). Zinc in the aquatic environment is of particular importance because the gills of fish are physically damaged by high concentrations of zinc (NAS, 1979).

Zinc concentrations in fish and other aquatic vertebrates are modified by diet, age of the organism, reproductive state, and other variables. In fish, diet is the major route of zinc uptake. In juveniles, accumulation of zinc from the aquatic medium takes place more rapidly than in embryos or larvae (Cutshall *et al*., 1977; Eisler 1981). A reduction in the level of zinc in serum during egg formation in a flatfish (*Pleuronectes platessa*) may represent a transfer of zinc to eggs (Overnell *et al.,* 1987b). High zinc concentrations in eggs of Atlantic salmon are sometimes associated with increasing mortality, although low concentrations seem to have no adverse effect on survival (Craik and Harvey 1988).

Material and Methods:

Ophiocephalus punctatus is a bottom dwelling fish. All the experimental fingerlings *Ophiocephalus punctatus* selected for present study were purchased from local fish market of Mulchera, District-Gadchiroli (M.S.), India. Fish were brought 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 adlibitum. Fish were separated according their size 12-20 cm and weight 80±5 gm.

For static bioassay heavy metal like zinc chloride ($ZnCl_2$) was selected and solution was prepared in tap water. The test organisms (fingerling of *O. punctatus)* were randomly distributed in small aquaria (20 liter capacity) filled with different concentrations of zinc chloride solution i.e. 10, 20, 30, 40 and 50 mg/l and mortality was recorded at 24, 48, 72 and 96 h. Ten fishes were used per concentration and the experiment was conducted in triplicate. The aquaria were not aerated during experimentation. For calculating the exact death rate on exposure to zinc chloride, the comparison was made with death of fish occurred in controlled aquarium since the beginning of exposure. All experiments were carried out for a period of 96 h. The number of dead fish was counted every 12 h and removed immediately from the aquaria.

For the study of histomorphological changes the fish were exposed to Zinc chloride at lethal concen. 50 for four day(short term exposure). On aquarium containing ten fish in 10 liter of tap water was used as controlled. Other aquarium containing 10 liter of Zinc chloride solution was used for experimentation . Temperature of controlled aquarium throughout the experimental period was estimated 22.8 ± 0.394 ºC. and of the experimental aquarium was ranging from estimated 22.8 ± 0.394 ºC to 26.8 ± 0.264 ºC. 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. Sagittal sections of gill lamellae were cut at 5-6 um with rocking microtome. Sections were stained with haematoxylene and eosin. Photograph were taken with Coslabtrinocular microscope attached with CCD camera.

Result and discussion:

Histopatholoigcal changes in the Gills:

It was observed that, on the first day of exposure (after 24 hrs) the gill underwent quite minor change that included epithelial edema and breakdown of the pillar cells. Gills showed hypertrophy and secondary gill lamellae showed curling in some part of the gill (Fig.75 and 76).

On the second day of exposure (after 48 hrs) as compared to above observation intensity of lamellar destruction was increased. Secondary gill lamellae showed the epithelial damaged. Epithelial cells underwent hypertrophy and some cell detached from the stratified epithelial layer (Fig.77 and 78). Cytoplasmic vacuolation and vacuolation in the gill was observed. In some region secondary gill lamellae got fused. Blood got engorged in branchial blood vessel and showed congestion. There was no fusion of primary gill lamellae anywhere in the gills.

Most prominent change in the gill was observed on 3rd and 4th day of exposure (i.e. after 72 and 96 hrs), epithelial cells of stratified epithelial layer shrinked and became detached from the primary gill shaft and pillar cells supporting the secondary gill lamellae (Fig.79). Hypertrophy and hyperplasia, nuclear degeneration and vacuolization in the pillar cells were observed (Fig.80). The secondary gill lamellae showed fusion in some region. Vacuolization was observed in the epithelial cells along with nuclear degeneration. Cellular dystrophy was also seen in the gills. Blood congestion was also seen at 96 hrs of exposure.

Zinc at LC-50 for short duration altered the normal behaviour of fish that was implicated by continuous fin movement, intermittent twitch and rapid opening and closing of operculum and mouth. All these activities were much more frequent in initial time of exposure, later fish became somewhat normal. It was also observed

that, all these behaviour concomitant with excess secretion of mucous and shading of scales. However at 96 hrs of exposure the caudal region became blood red coloured. Similarly, at low and high dose of Zinc same behaviour was observed in fish as at LC-50 of Zinc in early period of exposure. However no redness in the caudal region at low dose was observed. The secretion of mucus was the common phenomenon for short as well as long duration of exposure.

Zinc is an essential element but at lethal concentration and sub-lethal concentration it deleteriously affected the gills of *O. punctatus*. The results obtained in the present investigation showed that probably the zinc influence an initial oxidative stress in fish. Verification for this are the results from some past investigations of Dobreva *et al*., (2008) where they found a decrease of breathing intensity in *Carassius gibelio*, decrease of its sustainability towards Oxygen deficit, as well as changes in the hematological indicators (Arnaudov *et al*., 2009). These processes lead to disorders of blood circulation and a full or big foreclosure of the gas diffusion between gills and water. Skidmore and Tovell (1972) showed that the initial changes in the gill tissue under the influence of zinc are typical for an acute inflammatory infection accompanied by blood circulation disorder and a death possibility at a longer exploitation.

The damage of epithelial gills led to impeding of other vital processes–- the maintenance of the alkaline acid balance, ion regulation and the excretion Nitrogen metabolites. This, combined with the caused hypoxia, is probable reason for the high mortality which the zinc ions caused, which had been found by investigations of Dobreva *et al.,* (2008). Unlike their results, the investigations of Fernandes *et al.,* (2007) showed changes in the gills of the leaping grey mullet (*Liza saliens*) that are mostly of the circulation– aneurysms, hyperplasia, lifting and dilatation of the vessels but degenerative and hyperplastic changes are missing in the secondary lamellas. Changes in gill histology of different fish species under the influence of zinc are reported in the works of Cerqueira and Fernandes (2002), Tkatcheva *et al*., (2004), Fernandes and Perna-Martins (2001) as well but they do not track the relation between the metal content and the degree of the changes found. Velcheva *at al*., (2010) reported desctrctive changes in gills of *Carassius gibelio* at lower concentration of zinc chloride. By increasing the zinc concentration they were observed mainly hyperplastic changes that were reaching the final phase of adhesion of the gill plates. According to them this changes could be due to a compensatory reaction towards the Oxygen deficit related to formation of new epithelia cells in the gills aiming to improve the gas exchange. They had come to the conclusion that the zinc influenced on the tissue structure of Gibelio carp gills by causing the degenerative, circulation and hyperplastic changes. By increasing the zinc concentration the hyperplastic processes predominated over the degenerative and circulation ones. The probable reason for this could be the capillaries compensation of the enlarged epithelial tissue of gills by high zinc concentrations.

In the present study, gills reveal responsive changes on exposure to lethal and sub-lethal concentration of zinc chloride.

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Short term effect of. $ZnCl_2$ LC-50 on Gills histomorphology

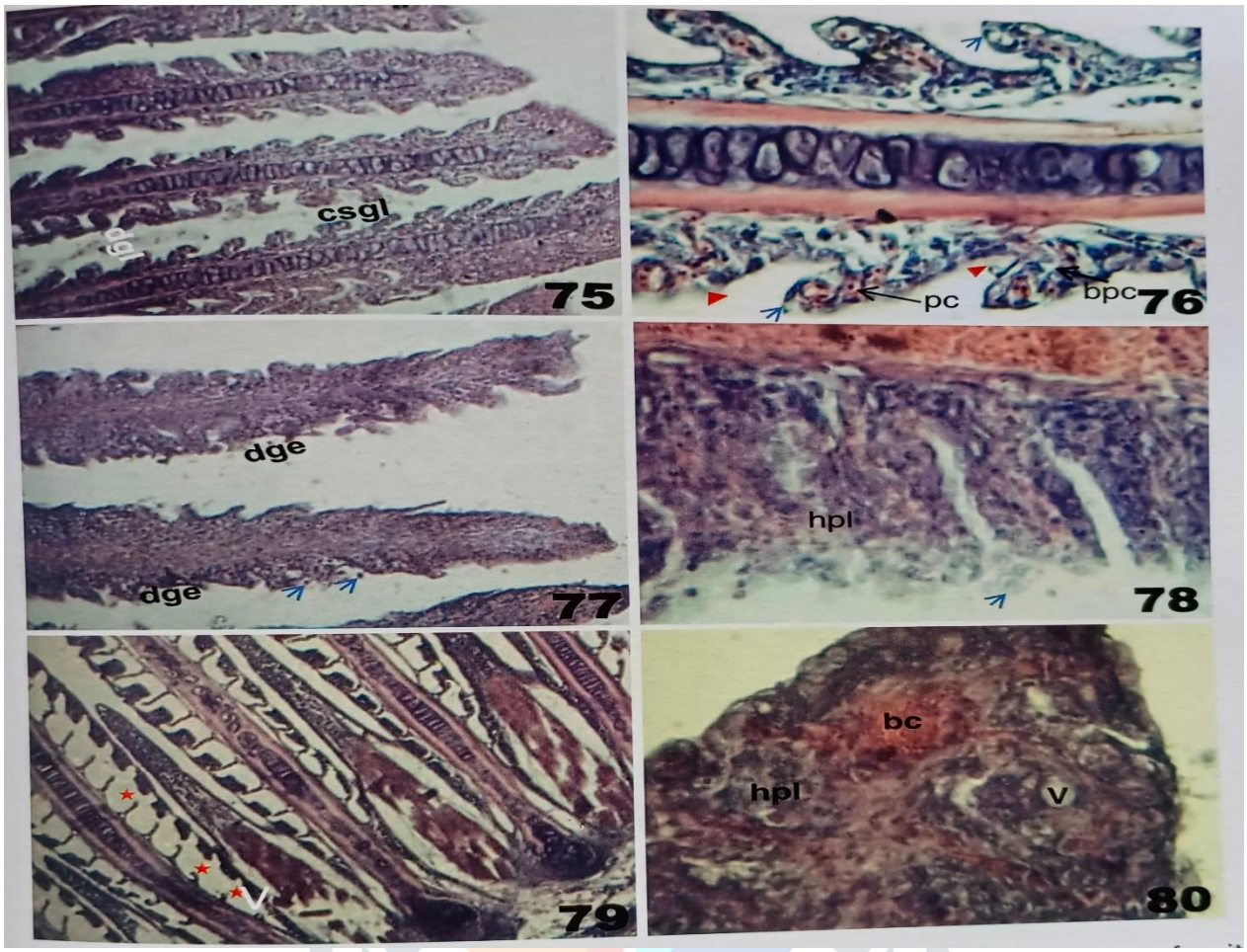

Fig.75-S.S. of gills, 24 hrs after exposure $ZnCl_2$ LC-50 showing destruction of epithelium of gill lamellae(dge) and curling of secondary gill lamellae(sgl), 100x

Fig.76- S.S. of gills, 24 hrs after exposure shows odema (▶), hypertrophy (□), breaking of pillar cell(bpc), 100x

Fig.77- S.S. of gills, 48 hrs after exposure shows damaging of epithelium of gill lamellae (dge), hypertrophy(□) and detached epithelium cells from the gill epithelium lining, 100x

Fig.78-- S.S. of gills, 48 hrs after exposure showing hyperplasia(hpl), hypertrophy(□) and detached epithelium cells from the gill epithelium lining, 400x

Fig.79- - S.S. of gills, 72 hrs after exposure showing lifting of epithelium from pillar cells(*) and gills shaft, 100x

Fig.80-- S.S. of gills, 96 hrs after exposure showing blood congestion(bc), hyperplasia(hpl) and vacuole(v), 400x

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