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ORIGINAL ARTICLE

TOXICOLOGICAL IMPACTS OF ZINC SULPHATE ON HISTOPATHOLOGY OF GILL AND SKIN OF THE FRESHWATER CATFISH *Clarias batrachus* (Linn.)

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ABSTRACT

Heavy metals enter into an organism and reach the target organ by passing through living membranes. In the present study efforts have been made to understand the sublethal toxic effects of zinc oxide on the histopathological alterations in gill and skin of *Clarias batrachus* for a period of 28 days. On exposure to sublethal concentration of zinc sulphate solution the gills showed extensive damages in their lamellar configuration even though the gill continue to regenerate repeatedly after every wear and tear, showed fusion of SL with the neighbouring gill filament and form undifferentiated mass of cells. The blood vessels running through PL show congestion of blood material and appeared red due to engorgement of RBCs after 14d of exposure period. After 21d of exposure the respiratory epithelium of the SL frequently became lifted from the pillar cell system and the PL became highly vacuolated. Large scale of wear and tear in the PL as well as SL took place after 28d of exposure in the form of extensive fusion and vacuolization which induced severe haemorrhage. After 7d of exposure the epidermis showed hyperplasia of the MCs as well as CCs. The epidermis in general showed sloughing of PCs. Due to continued exposure of the fish upto 14d the rupturing of MCs were noted and some MCs fused together and were hyperactive. In some places the mucus layer gives a vertically split appearance. Apart from the MCs some sloughed PCs were also noted. The continuous exposure of vanadium upto 21d, large scale erosion of the epidermis also began at the surface. Extensive damage of the OL caused dumping of destroyed MCs and ECs over the skin surface after 28d of exposure were observed.

Keywords: Zinc sulphate, freshwater fish, histopathology, *Clarias batrachus*

1.INTRODUCTION

The heavy metals cause the greatest threat to the health of aquatic ecosystem (Joshi *et al.*, 2002) and the danger of heavy metals is aggravated by their almost indefinite 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 intracellular transport processes in the living forms. These compounds enter into an organism and reach the target organ by passing through living membranes. Zinc has been chosen for the present study because it is a wide spread trace metal pollutant of high toxicity not only to warm blooded vertebrates but also to aquatic animals including fishes (OSPAR, 2002). Zinc, in its elemental form occurs naturally in the earths' crust. Zinc is a trace metal, nutritionally essential and helps for the normal functioning of several enzymes. It is used in batteries, construction materials, pigments and printing process. It is also used as a protective coating over iron, steel, brass, etc. The major sources of environmental zinc include the smelting of ores, municipal refuses, automobile exhausts etc. In fishes, high levels of Zn decrease oxygen consumption, damage gills, retard growth and reduce reproductive potential. The histopathological technique provides a real picture of the detrimental effects and the involvement of the heavy metal toxicants in the major vital functions such as respiration, metabolism and reproduction in aquatic animals. It is generally evident that changes in microscopic structure are more serious than functional abnormalities. In fishes, the gill is the multifunctional organ involved in respiration, osmoregulation and ion balance as well as acting as a barrier between the internal and external environment. As the gills are the major routes for the entry of heavy metals (Hemalatha and Banerjee, 1997; Aruljothi *et al.*, 2008), its accumulation is significantly more amount than the other tissues (Walczak

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et al., 1986; Cuvin-Arular and Furness, 1990; Radhakrishnan, 2002). Several studies showed that various heavy metals caused the histopathological changes in gills of fishes (Mazon *et al.,* 2002;Figueiredo-Fernandes *et al.,* 2007; Olojo *et al.,* 2005). Skin the outermost covering of fish, any unwanted change in the medium reflected in the skin in the form of physiological and histopathological changes (Radhakrishnan, 2002). Information reveals that various pollutants cause histhopathological changes on different tissues of fish. Therefore, the present study is proposed to assess the zinc sulphate e toxicity on the histopathological changes of vital organs like gill and skin of the catfish, *Clarias batrachus*.

2.MATERIALS AND METHODS

The fresh water fish *Clarias batrachus* (17 ± 2 cm length and 38 ± 2 g weight) were collected locally from Annamalainagar, Cuddalore district, Tamilnadu, India, were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate days. Water in the tank was renewed, three or four times in a daily and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank.

Prior to the commencement of the experiment, the median lethal concentration (LC_{50}) for 96 hrs was calculated using graphic monogram method of Litchfield and Wilcoxon (1949) and was found to be 29.0 ppm at 95% confidence limit. For the present study 2.9 ppm (10% of 96h LC_{50}) was selected as sublethal concentration. Four groups of 10 fish each were exposed to 2. 9 ppm of zinc sulphate prepared in tap water for a period of 7, 14, 21 and 28 days. In parallel, in control groups, zinc salt was not added. Feeding was allowed for a period of 3h every day, just before the renewal of the media. After the expiry of 7d, 14d, 21d and 28 days of exposure five fish each from the respective experimental as well as control groups were sacrificed. Gills and skin were excised and fixed in 10% neutral formaldehyde, aqueous Bouins fluid and Helley's fluid. Tissue samples were dehydrated in graded ethanol, cleared in xylene and stored in cedar wood oil before paraffin embedding. 5 µm sections were stained in Ehrlich's Haematoxylin/ Eosin (H/E) for routine histopathological observations.

3.RESULTS

Control:

C. batrachus possesses four pairs of gills, each bearing two rows of primary gill lamellae (PL) (gill filaments) which in turn bear series of secondary lamellae (SL) (respiratory lamellae) on both sides arranged alternatively. The lining of the SL on each side consists of single layered epithelium which rests on a basement membrane covering serially arranged row of pillar cells. These pillar cells alternate with blood channels to form the vascular component of the SL. Mucous cells (MCs) are mostly observed in between the two SL and also at the distal tip of PL (Fig 1).

Experimental:

On exposure to sublethal concentration of zinc sulphate solution the gills showed extensive damages in their lamellar configuration even though the gill continue to regenerate repeatedly after every wear and tear, especially after 7d of exposure (Fig 2). The gill showed fusion of SL with the neighbouring gill filament and form undifferentiated mass of cells after 7d of exposure (Fig 2). The blood vessels running through PL show congestion of blood material and appeared red due to engorgement of RBCs after 14d of exposure period (Fig 3). After 21d of exposure the respiratory epithelium of the SL frequently became lifted from the pillar cell system and the PL became highly vacuolated (Fig 4). Large scale of wear and tear in the PL as well as SL took place after 28d of exposure in the form of extensive fusion and vacuolization which induced severe haemorrhage (Fig 5). To compensate the loss, blood rushes into the vascular elements of the SL and PL. The intact blood vessels thus appear completely engorged with RBCs (28d). After 28d of exposure the accumulation of blood material is more pronounced and the ECs of the RE of the SL were fused to be seen due to the uncontrolled hyperplasia of ECs. The fusion of SL is seen on one side and at the same time large scale wear and tear is also noted at this stage. Some of the SL was also seen engorged with blood materials and consequently the thickness of PL were increased at this stage of exposure. The pillar cell blood channel system become haphazardly arranged.

SKIN

Control:

The epidermis of *C. batrachus* consists of polygonal epithelial cells (PCs), large sized club cells (CCs) and goblet mucous cells (MCs). For convenience of description the epidermis may be divided into three different layers: the basal layer (BL) consisting of a single row of columnar epithelial cells (ECs); the middle layer (ML) consisting mainly of large sized club cells (CCs) and a free medium sized round MCs with laterally compressed ECs filling the interstices; the outermost layer (OL), consisting mainly of PCs and small sac like MCs (Fig 6).

Experimental:

After 7d the epidermis showed hyperplasia of the MCs as well as CCs. The epidermis in general showed sloughing of PCs (Fig 7). Due to continued exposure of the fish upto 14d the rupturing of MCs were noted and some MCs fused together and were hyperactive. In some places the mucus layer gave a vertically split appearance. Apart from the MCs some sloughed PCs were also noted (Fig 8). The continuous exposure of zinc sulphate upto 21d, large scale erosion of the epidermis also began at the surface (Fig 9). Extensive damage of the OL caused dumping of destroyed MCs and ECs over the skin surface after 28d of exposure. Abnormal and unusual hyperplasia of CCs was noted in this stage of exposure. At many places the epithelium was richly supplied with lymphocytes and large scale wear noted in the skin as inter as well as intracellular vacuolization after 28 days (Fig 10). Eventhough the epidermis showed signs of regeneration, the MCs and CCs still remained hyperplastic. The epithelium showed prominent non-tissue spaces.

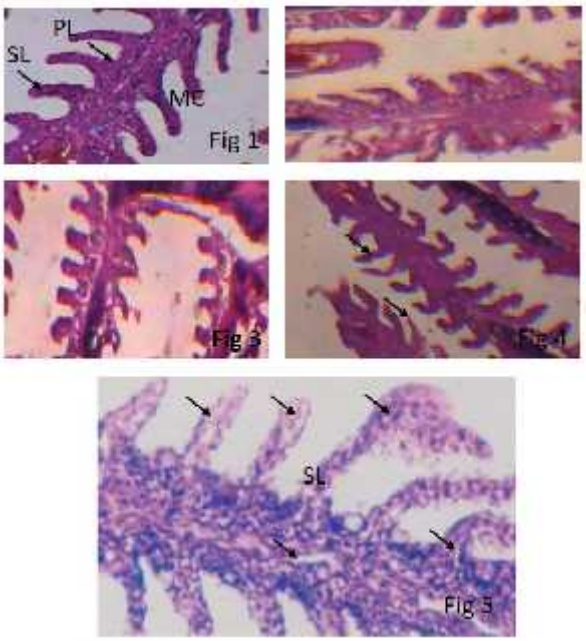

Fig 1. L.S. of control gill of *C. batrachus* showing blood capillary (BC), mucous cells (MC), pillar cell (PC), primary lamellar epithelium (PLE), pavement cells (PV) and secondary lamellae (SL) (H/E X 400).

Fig 2. Showing extensive damages in their lamellar configuration, fusion of SL (F) with the neighbouring gill filament after 7d of exposure (H/E X 400).

Fig 3. Showing the blood vessels running through PL show congestion of blood material and appeared red due to engorgement of RBCs after 14d of exposure period (H/E X 400).

Fig 4.Showing the respiratory epithelium of the SL frequently became lifted from the pillar cell system and the PL became highly vacuolated after 21d of exposure (H/E X 400).

Fig. 5 Showing large scale of wear and tear in the PL as well as SL took place after 28d of exposure in the form of extensive fusion and vacuolization which induced severe haemorrhage (H/E X 400).

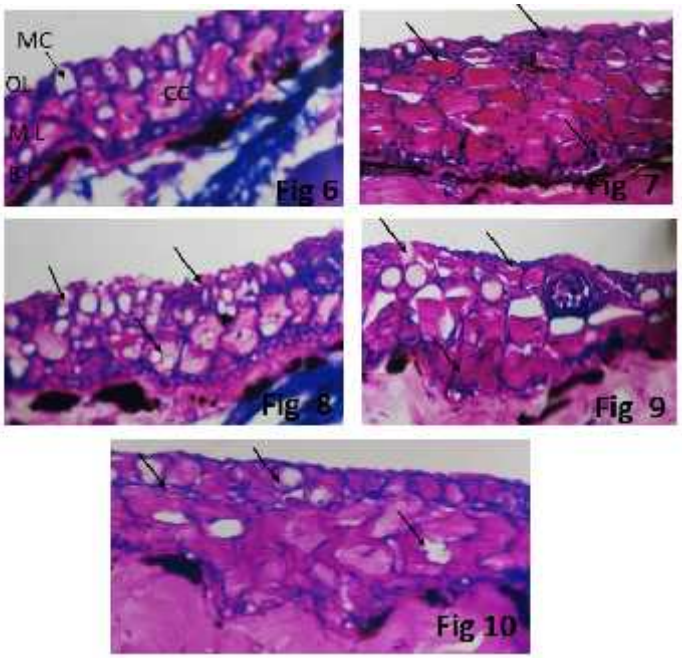

Fig 6 Shows the T.S. of the normal architecture of the body skin of *C. batrachus* (H/E × 450). (ECs = epithelial cell(s), CCs = club cell(s), MCs = mucous cell(s), OL = outer layer, ML = middle layer, BL = basal layer).

Fig 7 Shows the hyperplasia of the MCs as well as CCs after 7d of exposure (arrows) (H/E × 450).

Fig 8 Shows the rupturing of MCs and some MCs fused together and hyperactive. The mucus layer gives a vertically split appearance after 14d of exposure (arrows). (H/E × 450).

Fig 9 Shows large scale erosion of the epidermis lead to extensive damage of the OL after 21d of exposure (H/E × 450).

Fig 10 Shows dumping of destroyed MCs and ECs over the skin surface after 28d of exposure. (H/E × 450).

4.DISCUSSION

In fish, the gills are not only the prime organs of gaseous exchange, they also perform several physiological functions including osmoregulation and excretion. Changes in environmental parameters often damage this vital organ because of its delicate structure. Recent review articles (Dutta *et al.,*1996; Wendelaar Bonga, 1997) on ambient toxicants in fish have clearly demonstrated that increased concentrations of several heavy metals seriously damage the gills of the teleostean fish. Gills have an extensive surface area and minimal diffusion distance between dissolved oxygen and blood capillaries for efficient gaseous exchange. This organ system remains protected by a thin layer of mucous coating (Hughes *et al.,*1979; Powell *et al.,*1992). Electron microscopic studies have shown that the surface of the epithelia is provided with numerous microridges which anchor the mucus film (Hughes and Wright, 1970). Fish gills represent a multifunctional organ which carries out ion transport activities, gaseous exchange, acid-base regulation and waste excretion (via) branchial epithelium. Mucus provides the epithelium with a protective and lubricating cover that also provides an unstirred layer. At the ultrastructural level damages in the gill could also be observed. The elevated but low concentrations of water borne metals have ionoregulatory effects at the gills of fresh water fish whereas high concentrations cause severe gill damages and mucus accumulation that affect respiration. Fish gills have sensitive respiratory ionoregulatry membranes and they are the first point of contact between water borne metals of a fish (Wood, 1992; Wood *et al.,*1997). In the present study, the gills showed oedemateous swelling of SL, extensive fusion of gill epithelium, hyperplasia and hypertrophy of various cell types, extensive vacuolization, disintegration of various cell types and invasion of epithelia by phagocytic cells. All these alterations may be the attempts by the fish to prevent the entry of zinc salt. According to (Chavin. 1973) histopathological characteristics of special organs express the health conditions and represent the time integrated endogenous and exogenous impacts on the organisms. According to (Skidmore and Tovell 1972) in the gills the toxicants appear to breakdown the adhesion between epithelial branchial cells and the underlying pillar cells which is accompanied by a collapse of structural integrity of the SL and subsequent failure of the respiratory functioning of the gills. The several damages in terms of necrosis and rupture of the gill epithelium resulted in hypoxia and respiratory failure which may be the direct response to the action of zinc sulphate.

The extensive fusion of the SL leads to a drastic reduction in the respiratory surface area of the gills and several other xenobiotics are also known to induce fusion of the SL of gills (Leino *et al.,*1987). According to (Daoust *et al.* (1984) exposure to heavy metals very often alters the chemical composition on thickness of mucus layer that may disturb the normal ability to recognize different cell types. They concluded that this is due to contact stress and may also be the transformation of electric charge properties of the ECs which favour adhesion between the cells of two neighbouring SL which has been a very common manifestation of the toxic impact of large number of xenobiotics including zinc salt. In constant contact with the water, the gill is a sensitive primary target for a variety of insults including heavy metals (Hinton *et al.,*1992). Gills are the major sites of osmotic and ionic regulation in fish and any change in gill morphology may result in perturbed osmotic and ionic status. Changes in water pH in the gill microenvironment of the fish may need to be considered for some metals such as copper and aluminum which have relatively large changes in speciation or solubility as water pH changes rather than reflecting direct toxic action, lifting and hyperplasia of lamellar epithelium could be interpreted as defense responses of the fish as these alterations increase the distance across which water borne irritants must diffuse to reach the blood-stream (Mallat, 1985). The heavy metals were least often associated with lamellar aneurism, a lesion that seems to involve pillar cell disruption. Mallat (1985) suggested that pillar cells may be more resistant to metals than to most other kinds of irritants. However

(Mazon .2002) and Martinez *et al.* (2004) showed that lead and copper can cause lamellar aneurism in *Prochilodus* gills. These branchial responses that would serve to show the entry of lead have the undesirable side effect of reducing oxygen diffusion, since they increase water-blood distance for gas diffusion (Mallat, 1985).

The pollutants have the potential to affect the uptake of oxygen by damaging the gills (Wilson and Taylor, 1993) and perhaps to affect oxygen transfer to the exercising tissues through secondary effects such as haemo concentration (Butler *et al.*,1992). The gillsof fish are particularly vulnerable to many water-borne pollutants including heavy metals at low pH. A major toxic effect of lead is the disruption of the ability of the gill to act effectively in its capacity to exchange oxygen either for respiratory gases or ions. Zinc can induce gill damages like hyperplasia, increased mucus secretion, ECs thickening and vacuolization which would result in greater diffusion distance and hence the less exchange of O_2 and CO_2 (Hemalatha and Banerjee, 1997). These effects of metals are particularly important in ion-poor and poorly buffered acidic water where waterborne metals are especially toxic to fish (Spry and Wiener, 1991). Metal species may change with water pH, dissolved organic matters along with Ca^{2+} and even H^{+} reduce the toxic effects of metals at fish gills. Binding of metals at negatively-charged fish gills, competition for metal binding sites on gills by cations such as Ca^{2+} and H^{+} and complexation of metals by inorganic ligands such as hydroxide and inorganic carbon may reduce the availability of metals to bind at the gills (Pagenkopf, 1983). The gill surface interaction model applies to metals which disrupt physiological functions of the gills and it is assumed in the model that the rates of metal-gill interactions are fast in relation to the time needed for a bioassay test and it is also assumed that gill surface have a finite number of metal binding sites. Competition at the biological membrane and metal complexation in the water itself reduces metal binding to the membrane and therefore reduces metal toxicity (Moore and Ramamoorthy, 1984).

Copious secretion of mucous is one of the probable reasons against the heavy metal toxicity. To minimise the toxicity of the heavy metal salt in the skin form a thick layer of slime laid by the MCs onto the surface as a protective barrier of glyco-proteins which has the capacity to trap heavy metals (McKone*et al.*,1971; Coombs *et al.*,1972; Lock and Van Overbeeke, 1981). The mucous coating of the fish body may act as a metal binding resin due to the capacity of the metal to form a strong covalent bond with –SH group of proteins, containing amino acids and wide range of biological molecules (Friberg *et al.*,1974; Webb, 1979). This capacity of mucous may cause elimination of part of zinc thereby preventing the entry through the skin. Increased mucogenesis by fishes under the intoxication of a number of pollutants including heavy metals (Arillo and Melodia, 1990; Rajan and Banerjee, 1991; Hemalatha and Banerjee, 1997a, b) and detergents (Roy, 1988) have also been reported. Moreover, the heavy metals have the property of coagulating fish mucous and form a slimy coat over the body which offers a protective barrier (Rajan and Banerjee, 1991). In the present investigation the increased mucogenesis by the fish maybe regarded as an effort to resist the penetration of zinc through the skin. In addition to MCs, the ECs of the OL also secrete some amount of mucous at various stages of exposure and the active synthetic process of EC shave also been reported by Rajan and Banerjee (1991), Hemalatha and Banerjee, (1997a; 1997b). This alteration may help to control the local pH of the epithelia to decrease the acidity. Due to continuous low level impact of zinc emptying, shedding and slower rate of regeneration of the MCs and washing away of the mucous coat as a scab into the media were noted. Consequently the scab may get sloughed and destroyed. This may be one of the probable reasons to delay the entry of zinc temporarily. As a result of sloughing and destruction of the OL the CCs showed abnormal hyperplasia and hyper activity. The contents of the CCs are discharged over the body surface and along with the debris of the cell types form a thick crust which being proteinaceous in nature, is not easily dissolved and hence continue for a longtime as a scab. This can act as a second line of defense due to the failure of the mucous coating because mucous coat is neither a permanent barrier nor

sufficient enough to withstand the continuous deleterious effect of zinc.

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