Zinc Induced Histopathological Alternations in Gill, Liver, and Kidney of Freshwater Fish *Gonoproktopterus kolus* (Sykes)

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Abstract The present study aims to investigate the histopathological changes in gill, liver, and kidney of freshwater fish *Gonoproktopterus kolus* (Sykes). The fingerlings of fish were exposed to Zinc (Zinc sulphate) in acute toxicity experiment (96 hrs.). The LC₀ and LC₅₀ concentrations were 2.430 ppm and 4.860 ppm respectively. After acute exposure, fish were sacrificed and tissues were obtained separately from control and LC₀ and LC₅₀ groups. These tissues were fixed in Bouin's fluid and standard protocol was followed to study histopathological alterations in gill, liver and kidney of experimental fish. In gills, fusion and degenerative changes were observed in secondary gill lamellae. In liver, hepatocytes showed hypertrophy due to degenerative changes in cellular structure. Empty hepatocytes showed shifting of nucleus. Kidney showed degenerative nuclei in the epithelial lining of the tubule. Haemopoietic tissue showed hypertrophy and disarray. Distended glomerulus was observed. Renal tubules showed disarrangement as compared to control. Thus, in present investigation, it was observed that heavy metal zinc is affecting the fishes at tissue level, which may interfere in physiological processes.

Keywords *Gonoproktopterus kolus*, Gill, Liver, Kidney, Acute Toxicity, Zinc Sulphate, Histopathological Changes

1. Introduction

Heavy metal pollution is a serious problem in the aquatic ecosystem due to their ability to interfere in the food chain and accumulate in the aquatic organisms Berman [3]. Amongst the aquatic fauna, fishes are the important creatures in the study of heavy metal pollution, because they move freely within diverse trophic levels in an aquatic environment Chopra [6], Palaniappan, [25]. Study of histology is sensitive parameter and is very critical in detecting cellular alterations that may occur in target organs, such as the gill, liver and kidney.

Even though zinc, is an essential trace metal, it is toxic when the dietary supply becomes excessive Ho [12]. The main uses of zinc are in the manufacture of galvanized iron, bronze, paint (white), rubber, glazes, enamel glass, paper, as a wood preservative (ZnCl₂, fungicidal action), petrochemicals, and fertilizers, and steam generation power plants, etc. Nriagu [23]. Some zinc is released into the environment by natural processes, but most comes from the activities of people like mining, steel production, coal burning, and burning of waste. It attaches to a soil, sediments, and dust particles in the air. Zinc compounds can pass into the groundwater, lakes, streams, and rivers. Generally, zinc bound to soil particles. Zinc concentration moderately increases in water due to corrosion of drainage pipes, which are made of zinc. Thus, it enters in fish and other organisms Joshi [15]. Zinc (Zn) is one of the most

significant traces elements in the body, which influences the biological function of some proteins and enzymes Maity [22].

Fish gill on the other hand is critical organs for respiration, osmoregulation, and excretion. Gills serve as a good indicator of water quality. They are sensitive to any change of water components since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water Au [2]. The fish liver is a vital organ concerned with basic metabolism, accumulation, biotransformation, and excretion of contaminants in fish Figueiredo [9]. One of the most important functions of the liver is to clean pollutants from the blood so it is considered as an indicator of aquatic environmental pollution Soufy [30]. The kidney is an important organ for the maintenance of homeostasis concerning water and salts, metabolic waste excretion from blood, and partially for the metabolism of xenobiotics Thophon [32]; Mabrouk [21]. Liver and kidney are considered as the crucial organs suitable for histopathological examination to study the damage to cells and tissues Rabitto [28]; Oliveira [24].

2. Materials and Methods

Live fingerlings of 7-8 cm in length and 10–12 gm in weight were collected from the unpolluted Krishna River near Satara. Fishes were taken to the laboratory. Rectangular glass aquaria of dimension 45 cm x 22 cm x 30 cm and capacity of 25 liters were used for stocking. Ordinary chlorine free tap water was used during acclimatization. Fishes were acclimatized for 15 days in the laboratory condition. Water was changed twice a day and fishes were fed with groundnut cake once in a day during acclimatization. PH, dissolved oxygen and hardness of the water used for acclimatization was recorded. Before exposure of the fish to the metals, feeding was stopped for 24 hrs. The well-acclimatized fishes to the laboratory condition were used for experimentation. Group of 10 fingerlings was held separately in a plastic container of 20-liter capacity containing metals of different concentrations making a total volume of 10 liters. Experiment for zinc sulfate (ZnSO_4) was carried out and the test concentrations used were 1.620, 2.430, 3.240, 4.050, 4.860, 5.670, and 6.480 ppm. (Pilot experiments were carried out to find out the toxic range of zinc sulphate in milliliters. Then the established concentrations were converted into ppm.). The experiments were carried for 96 hr for the toxicity of the metals. The control group without toxicant was run simultaneously for every experiment. All the experiments were carried out on the natural day- light rhythm. Test concentrations from the experimental containers were renewed every 24 hours. The death rate and behavior of fishes were noted before each change of

the water from the plastic container. The toxicity tests were repeated thrice, and for 96 hr LC_0 and LC_{50} values 2.430 ppm and 4.860 ppm. Respectively, were recorded. After studying 96 hrs. acute toxicity of Zinc sulfate, selected body parts like gill, liver, and kidney belonging to LC_0 , LC_{50} , concentrations along with control were removed and fixed in aqueous Bouin's fluid for 48 hrs. The tissues were then washed under tap water, dehydrated in ethyl alcohol grades, cleared in xylol, and embedded in wax. They were sectioned at 5-6 micron. Sectioned tissues were stained with Harris hematoxylin and alcoholic eosin and mounted in DPX. All the observations for microphotography were made under microscope Dewinter DEW/106.

3. Results and Discussion

Histopathology of Gills:

The normal histological structure and changes induced by the effects of heavy metal at acute concentration are shown in **Figure No. I (Figure A to C)**.

Control of Acute Test-

The histological structure of gills of control fishes was normal having complete skeletal and lamellar parts. The ceratobranchial and epibranchial of each gill arch showed two rows of primary gill lamellae. It was seen that they were supported by bony elements. Primary gill lamellae gave rise to secondary gill lamellae from dorsal and ventral sides. Primary gill lamellae were lined by mucoidal epithelium. The secondary gill lamellae consist of a vascular network of capillaries reinforced by pillar cells (**Fig.-1 A**).

Zinc Sulphate: Concentration 2.430 ppm:-

Swelling of primary gill lamellae. Degenerative changes were seen in secondary gill lamellae, in some places they were completely damaged. There were breaks and cuts in the skeletal elements. Blood clots were found in some parts of the lamellae. The epithelium was also found detached from the skeletal elements (**Fig -1 B**).

Zinc Sulphate: Concentration 4.860 ppm:-

Due to exposure to this concentration, the changes were more drastic. Hyperplasia and hypertrophy were observed in the mucoidal epithelium. At many places, the fusion of secondary lamellae was seen. There was a reduction and loss of secondary gill lamellae. Blood clots were also seen in some places. Skeletal elements showed cuts, cracks and vacuolation. The epithelium was also found detached from the lamellae at many places (**Fig -1 C**).

A

Figure - 1A. Section passing through gill of fish *Gonoproktopterus kolus* from control (10 X) PGL – Primary gill lamellae, SGL – Secondary gill lamellae.

B

Figure - 1B. Effect of 2.430 ppm of Zinc sulphate on gill of fish *Gonoproktopterus kolus* after 96 hrs. exposure (10 X). DE – Detached epithelium, BC – Clot, SE – Split skeletal epithelium, TPG – Thickened primary gill lamellae.

C

Figure - 1C. Effect of 4.860 ppm of Zinc sulphate on gill of fish *Gonoproktopterus kolus* after 96 hrs. Exposure (10 X). FSL – Fused secondary lamellae, BC – Blood clot, LS – Loss of secondary lamellae.

Histopathology of Liver:-

The normal histological structure and changes induced by the effects of heavy metal at acute concentration are shown in Figure - 2 (Figure A to C).

Control of Acute Test -

The histological structure of liver of fish from control set appeared to be normal. The mass of the liver is comprised of continuous polyhedral hepatocytes. The hepatocytes were organized in continuous cords of cells. Each hepatocyte contained granulated cytoplasm with central nucleus. At few places, blood sinusoids were seen in hepatic mass (**Fig - 2A**).

Zinc Sulphate: Concentration 2.430 ppm:-

Degeneration of cell wall leading to cirrhotic changes were observed. Extrusion of nuclei resulted due to precipitation and loss of cytoplasm. Degeneration of nuclei was also observed. Empty hepatic cells showed the shifting of the nucleus. Hepatocytes showed pyknosis that has given rise to vacuolation in the tissue (**Fig -2B**).

Zinc Sulphate: Concentration 4.860 ppm:-

Hepatic cells showed hypertrophy due to degenerative changes in cellular structure. There was disarray of hepatic cords and large spaces were seen among the hepatic cells. Blood vessels showed dilation with empty blood sinusoids. Hepatic cells showed acute necrosis resulted into cirrhotic changes. Mixing of cytoplasm was seen due to degeneration of cell wall (**Fig – 2C**).

Figure - 2A. Section passing through liver of fish *Gonoproktopterus kolus* from control (400X). N – Nucleus, H – Hepatocytes

Figure - 2B. Effect of 2.430 ppm of Zinc sulphate on liver of fish *Gonoproktopterus kolus* after 96 hrs. exposure (400 X). DC – Degenerating cell wall, DN – Degenerating nucleus, EN – Extruded nucleus, SN – Shifted nucleus, V – Vacuole.

Figure - 2C. Effect of 4.860 ppm of Zinc sulphate on liver of fish *Gonoproktopterus kolus* after 96 hrs. exposure (400 X). EBS – Empty blood sinus, DN – Degenerating nucleus, NE – Necrosis.

Histopathology of Kidney:-

The normal histological structures and changes induced by the effect of heavy metals at acute concentrations are shown in **Figure - 3** (Figures A to C).

Control of Acute Test -

The kidney of the fish from control showed normal histological structure. The functional kidney consists of many renal tubules and hemopoietic tissue. The tubules were lined with epithelial cells, which were nucleated containing dense cytoplasm. The glomeruli of various sizes and shapes were found to be placed in Bowman's capsule. The proximal, distal, and collecting tubules were prominently observed (**Fig – 3A**).

Zinc Sulphate: Concentration 2.430 ppm:-

Degenerative nuclei were seen in the epithelial lining of the tubule. Empty space was observed around the tubule. The lumen of the tubule was occupied by slough from the cells. Disorganization of cells in the epithelium of the tubule was evident. Haemopoietic tissue showed hypertrophy and disarray. Distended glomerulus was observed (**Fig – 3B**).

Zinc Sulphate: Concentration 4.860 ppm:-

Degeneration of nuclei and disorganization of epithelial cell of the tubule was prominent. Renal tubules showed disarrangement. Haemopoietic tissue exhibited hypertrophy and disarray. Many empty spaces were seen among the tissue. Lumen of the tubule was filled with the slough from the cells. At some places blood clots were also seen (**Fig - 3C**).

A

Figure - 3A. Section passing through kidney of fish *Gonoproktopterus kolus* from control (400 X). **T** – Tubule, **HT** – Haemopoietic tissue, **G** – Glomerulus.

B

Figure – 3B. Effect of 2.430 ppm of Zinc sulphate on kidney of fish *Gonoproktopterus kolus* after 96 hrs. exposure (400 X). **DG** – Distended glomerulus, **HHT** – Hypertrophy of haemopoietic tissue, **ES** – Empty space, **DEC** – Disorganized epithelial cells.

C

Figure – 3C. Effect of 4.860 ppm of Zinc sulphate on kidney of fish *Gonoproktopterus kolus* after 96 hrs. exposure (400 X). **DE** – Degenerating epithelium, **DN** – Degenerating nucleus, **HHT** – Hypertrophy of haemopoietic tissue, **ES** – Empty space, **V** – Vacuole.

Entry of heavy metals in the body leads to physiological, biochemical and pathological changes in fish. Histopathological changes in cellular structure of tissue and organs are irreversible resulting in malfunctioning of the organ, which finally results in death of a fish. The gills are main respiratory and osmoregulatory organ, which come in contact with surrounding water. Therefore, gills are the best indicators of any kind of aquatic pollution Pandey [26]. The impact of copper on gill and functional enzymes activity of carp Karan [19], Laurent [20], Au [2] and Camargo [4] showed that histopathological changes in fish gill were among very commonly recognized responses to environmental pollutions.

In present study after acute toxicity of zinc sulphate at LC_{50} concentration (2.430 ppm), the tips and bases of primary gill lamellae showed hyperplasia and hypertrophy. There were degenerative changes in secondary gill lamellae and reduction in their length. At some places, destruction of second lamellae was observed. The fusion of secondary lamellae was also observed (Fig. – 1B). Similar results were observed when freshwater fish *Tilapia mossambica* was treated with cadmium sulphate Jalaludeen [14]. The gill of lead acetate exposed fish showed remarkable histopathological changes like hyperplasia of the epithelial cells that caused the merging of adjacent secondary gill lamellae. Besides this, the secondary lamellae became smaller and lamellar fusion formed swellings. Further, edema between the primary epithelium and secondary lamella, severe degeneration with fibrosis in primary filaments, and disappearance of inter lamellar spaces were also observed Pandi [27].

The liver of fish is a large vital organ. It has much functions comprising detoxifications, protein synthesis, storage of glycogen and production of biochemicals (bile) essential for digestion. Toxicants (Heavy metals) entering the body through the body surface, gills and gastrointestinal tract reach the liver through the blood. The liver is considered as an environmental biomarker, because of its capacity for detoxification and storage of harmful components Stori [31]. The present study after acute exposure, at LC₀ concentration of zinc sulphate the liver showed degeneration of cell wall leading to cirrhotic changes. Extrusion of nuclei resulted due to precipitation and loss of cytoplasm. Degeneration of nuclei was also observed. Empty hepatocytes showed shifting of nucleus. Hepatocytes showed pyknosis that caused vacuolation in the tissue (Fig. 2B). At LC₅₀ concentration of zinc sulphate, the hepatocytes showed hypertrophy due to degenerative changes in cellular structure. There was disarray of hepatic cords and large cavities were seen between the hepatic cells. Blood vessels showed dilation with empty blood sinusoids. Hepatocytes exhibited acute necrosis resulted into cirrhotic changes. Mixing of cytoplasm was seen due to degeneration of cell wall (Fig. 2C). These changes are usually linked with the reaction of hepatocytes to toxicants, Van [33]; Hinton [11]. These pathological changes also may be correlated to the vacuolation of hepatocytes that is interrelated with suppressed protein synthesis, energy depletion, breaking of microtubules, or alterations in substrate utilization, as described by, Ajani [1]. Cadmium treated *Oreochromis niloticus* showed, hepatocyte degeneration, with nuclear pyknosis in majority of the cells and the addition of metal binding proteins in their nuclei was reported by Kaoud [18]. Gaps in cytoplasm, deterioration of nuclei, vacuolation in stroma, hazy swellings, pycnotic nuclei, necrosis, break in blood sinusoids, disorder of hepatic cords, loss of shape of hepatocytes was witnessed in the liver of *Channa gachua* (Ham) exposed to mercury and copper Deore [7]. Increase of hemorrhage, vacuolation in hepatocytes and penetration of sinusoids with WBC's were noticed in liver of Nile Tilapia, *Oreochromis niloticus* after acute exposure to cadmium El-Sayed [8]. *Cirrhinus mrigala* exposed to mercury showed loss of cellular design, necrosis in hepatocytes and accumulation of fat in parenchymal cells of liver, Chavan [5]. Extensive hepatocyte necrosis with long-lasting inflammatory changes was detected in the liver of fishes exposed to Cu solution. Justina [16].

The kidneys are the organs of excretion. They also play a role in osmoregulation and detoxification. The kidney of freshwater fish functions for checking additional solute loss as they contain more salts within their blood than the surrounding water body. The kidney is an important vital organ of the body and proper function is to regulate the homeostasis. The kidney is not only liable for selective reabsorption, which assists in holding volume and pH of blood and body fluids and erythropoiesis Iqbal [13]. The

kidney is one of the first organs to be influenced by contaminants in the water, Thophon [32]. During acute exposure (LC₀ concentration) to zinc sulphate, the epithelial lining of the tubule showed degenerative nuclei. Space was observed around the tubule. The slough from the cells was filled in the lumen of tubule. The epithelium of the tubule showed disorganization of cells. Hypertrophy and disarray was evident in the haemopoietic tissue. Distended glomerulus was observed (Fig. 3B). In LC₅₀, concentration the epithelial cell of the tubule showed disorganization and degeneration of nuclei. Renal tubules showed disarrangement. Hypertrophy and disarray was observed in haemopoietic tissue. Many empty spaces were observed among the tissue. Lumen of the tubule was filled with the slough from the cells. At some places blood clots were also seen (Fig. 3C). Similar findings such as enlargement of renal tubules, desquamation of epithelial lining, edema, severe necrosis, pyknotic nuclei, hypertrophied nuclei, vacuolization, disorganized blood capillaries in glomerulus were observed in the kidney of *Channa punctatus* treated with a sub-lethal concentration of zinc, Gupta [10]. Intracellular spaces between the cells, irregularly shaped cells and necrosis were observed in the kidney of *Hoplias malabaricus* exposed to methyl mercury, Mela [17]. The authors suggested that the cause histopathological abnormalities are due to high accumulation of zinc in the kidney probably dysfunction the detoxification mechanism of renal tissue in it. Aggregation of inflammatory cells, capillary tubes dilation of renal tubule, and hemolysis in the kidney of *Cyprinus carpio*, was observed after exposure to chromium at sub-lethal concentration, Rana [29].

4. Conclusions

Histopathology is a useful method for exploring the effect toxicants in various organs of the treated organisms. In present study, gill, liver, and the kidney of the fishes exposed to acute concentrations of zinc sulphate showed several drastic degenerative histological changes. The result showed that these degenerative changes might lead to malfunctioning of the organ. Any single factor cannot be related to changes in cellular architecture and may be dependent on the cumulative effects of many factors induced by the presence of heavy metals in the body of fishes.

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