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Histopathological Alterations in the Gills of Freshwater Fish *Garra mullya* (Sykes) Exposed to Lethal and Sublethal Concentrations of Copper Sulphate

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Abstract
The study of pathological alterations brought about in bodily tissue's microscopic structure is useful for diagnosis the change in cell architecture. Any change occurs in peculiar alteration of cells; it may indicate disease either the effect of toxic substance. In aquatic animals such as fishes, it was observed that due to toxic chemicals affect the external organs and causes loss of equilibrium, verticular movements, increase in opercular movements and finally death of fishes, as a result damage to internal organs. Studies on histopathology provide important information on tissue alterations before them show symptoms externally. The extensive use of heavy metals has been made to improve farming on land and in water. Due to their great toxic effects on fishes, these metals are potential pollutants and are affecting the fishes either directly or indirectly on oral membrane of gills. General body surface and gastrointestinal mucosa are the main routes for the entry of toxicant into the body and through circulation of blood, these toxicants reached to different parts of the body of aquatic organisms. The present study was the effects on of both lethal and sublethal concentrations of copper sulfate on the gills of freshwater fish Garra mullya.
Keywords: *- Histopathology , Gills, copper sulphate, Garra mullya*

Introduction

The industrial development and green revolution is intimately associated with the toxic substances, which reach the aquatic ecosystems and exert serious effects on physiology of fishes. Water contaminated by heavy metals is one of the biggest environmental issues the modern world is experiencing. The environment is becoming more polluted with heavy metals worldwide as a result of increased human activity. Moreover, it is becoming more and more significant every day because of its clear effects on human health via the food chain. A number of environmental issues, most notably the poisoning of water with heavy metals, have become increasingly concerning due to the industrial sectors' explosive development.

An indiscriminate disposal of chemical and heavy metal wastes from agriculture, industries and mining activity has disastrous effects on aquatic biota and the water quality. As such, increasing attention is being focused on the stress caused on aquatic environments. Several ingredients of these wastes disrupt the integrity of the cellular structures and affect the vital physiological processes Bryan, (1971). The toxic effects of copper sulphate are seen on aquatic organisms first of all and due to its tendency of contamination, it is also hazardous to human beings through food chain. Fishes are sensitive so the effect of pollutants can be observed first in them among the aquatic organisms. The toxic levels of most heavy metals run closely to their level in environment.

The physiological studies project a partial picture of deleterious effects of the heavy metals and histopathological observations were seen in gills. Some toxicants exert their primary effect locally, at the port of entry. Thus different toxicants affect different tissues and bring about structural modifications resulting in the death of organism.

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Materials and methods:

Without suffering any chemical injuries, the freshwater fish *Garra mullya* were taken from a Asna river , about 4 km from Nanded city and transported to a lab. Fish with an average weight of 11±2 gram and a length of 9±2 cm are chosen for the experiment. 1% $KmN0_4$ was used to treat fish in order to prevent skin infection. The fish were then acclimatized 15 days becoming used to the lab environment before the experiment. The last day of feeding was reached before the experiment.

The aging of water is must be remove residual chlorine, which is extremely harmful to fish, and to stabilize its composition. During the experimental period, the physicochemical parameters are analyzed using standard methods recommended by IAAB (1998) and APHA (1998).

Three batches of 10 healthy fish each, A, B, and C, were sorted. A batch that did not expose the fish to the heavy metals was considered as the control. Fish have been exposed to different

concentrations of copper sulfate in two further batches. Fish in batch B were exposed to 96 hours of a lethal dosage of copper sulphate i.e. 96 hrs. LC_{50} value 3.0 ppm. The C batch was exposed to sublethal concentration of copper salphate is $1/10^{th}$ of 96 hrs. LC_{50} value 0.3 ppm.
For 96 hours, lethal and sublethal dosages of copper sulphate were given to the test fish, *Garra mullya.* After every 24 hours up to 96 hours of exposure, the rate of oxygen consumption of fish exposed to lethal and sublethal concentrations of copper sulphate was determined.
The acclimatised fish, *Garra mullya*, was exposed to copper sulphate concentrations that were both lethal and sub lethal. At the end of exposure period i.e.96 hrs. The survived fish were randomly selected for histopathological examination. After they were executed, tissues like gills, were removed from the control and treated animals. The tissues were then cleaned from unwanted tissues and fixed in aqueous Bouin's fluid for 24 hours. After that, the tissues were cleaned in xylene, processed in a graded series of alcohol, and embedded in paraffin wax. The sections were taken at 5µ thickness and it stained with Ehrich hematoxylene and Eosin in accordance with the standard histology protocol. The sections were later screened and photographed by microphotography.

Result (Observation)

Aquatic dissociation of copper sulfate releases bioavailable cupric ions. These free metal ions come into direct contact with the external epithelial surface of the freshwater fish *Garra mullya* gills leading to initial structural and membrane damage throughout the branchial epithelium. This results in physical and functional damage causing a serious disorder of ion regulation, disturbing the active transport of vital electrolytes such as sodium and chloride. Thus, copper accumulation in the cell and loss of osmotic balance lead to oxidative stress and damage to the cell through generation of reactive oxygen species.
In response to the continuing cellular injury and osmotic imbalance, protective but pathological structural alterations occur, i.e. epithelial lifting, hyperplasia (excessive proliferation of cells) and tissue edema. The adjacent secondary lamellae fuse and show marked degeneration as the lesion progresses. In the end, extensive cellular necrosis destroys the architecture of the gill, resulting in a significant reduction of the functional respiratory surface area and respiratory distress or asphyxiation.
The impact of mercuric chloride and copper sulphate concentrations that are lethal and sublethal on gills of *Garra mullya* were studied.

Histology of Gill

Fish gills perform a multitude of important functions for these aquatic animals, including gas exchange, ionic regulation, and the excretion of nitrogenous waste. The structure in the control fish revealed that axis or bases is roughly triangular in outline and consist of central core of connective tissue enclosed by a layer of epidermis, which is protected by a thin cuticle. Each branchial arch of epithelium is subdivided into two dorsoventral columns of filaments, each containing a row of secondary lamellae on the dorsal and ventral surfaces. Gas exchange occurs in the secondary lamellae, where water is diffused into blood. Adjacent gill filaments come into contact with one another. All secondary gill lamellae are delicate, flattened structures made up of two layers of epithelial sheets held in place by different basement membranes. The epithelial layers of two sides are supported by pillar cells or pilaster cells. Each surface of the secondary gill lamellae is occupied by rows of pillar cells. The epithelium covering adjacent pillar cells fuses on the basement membrane to form the entire lamellar vessel lining, including the blood channels that connect the afferent and efferent lamellar vessels. The gill capillaries in the gill filaments are clearly seen. The gill rakers from which the gill lamellae are also normally seen in the control group. Histopathological alterations in fish tissue, particularly in the gills, have been extensively employed in both laboratory and field studies for toxicological testing(Mela *et. al*,2007)
The effects of a sublethal dose of fenvalerate in Cirrhinus mrigala gills were assessed by Anita Susan and Tilak (2003). They saw significant pathological alterations in the gill tissues, such as necrosis and progressive degeneration. Following a prolonged exposure to the azo dye methyl red, Shweta Sharma et al. (2006) conducted histological analyses of a few key organs of the freshwater fish Poecilia reticulata. They observed that the primary and secondary gill lamellae

had deformed appearances, with the former showing degenerative changes such as loss of blood, mucus, and respiratory epithelial cells along with thickening of the basement membrane and enlargement of cartilaginous axis cells, and the second one showing nearly total disintegration. They stated that such adverse affect in the gills of fish might have caused respiratory stress in them. Similar such observations were also noted by Sakthivel (1994) in Cyprinus carpio exposed to dye wastewater.

Histopathology

After exposure of copper sulphate, the gills of fish showed some histopathological changes. The partial degradation of secondary gill lamellae's epithelium was the primary characteristic seen in gills subjected to sublethal concentrations of copper sulphate.; secondary gill lamellae vacuolization; sometimes adjacent secondary gill lamellae seemed connected to one another. The primary gill lamellae did not alter. Further gills of the fish exposed to lethal concentration of copper sulphate, showed disruption of tissue structure in gill epithelium, pillar cells and blood capillaries. Cartilage cells were accumulated in the tip and in the middle of gill filaments in association with blood cells thus showing hemorrhage. The epithelium of secondary gill was found completely destructed. It is well known that one of the most well recognized effects to environmental contaminants in fish is a change in their gills.(Mallatt.1985,Laurent and Perry 1991). Oladimeji *et. al*, (1984). reported that the fish gill is able to excrete the adminis-tered arsenic.

C.K.C. Wong and Wong reported a similar finding and provided an indirect evidence of role of CC in excretion of Cd. In the present study, an increase in apical membrane area and CC density could be considered as an adaptive response against metal exposure, especially in the fish exposed for a longer duration. Al-Balawi, *et. al*, (2013), which were in agreement with the current study. These changes in the gills epithelia were observed by Thophon *et. al*, (2003) in Lates calcarifer exposed to heavy metal cadmium. These results are also reported similar changes in gills of fresh water fish, Tilapia zillii, exposed to aluminum. Similar results are also observed by Figueiredo-Fernandes *et. al*, (2007) in Nile tilapia, Oreochromis niloticus, exposed to copper. These findings also, were found in fish exposed to other pollutants in the freshwater fish (*Puntius gonionotus, Oreochromis niloticus*), exposed to pesticides paraquat, and dimethoate (Cengiz and Unlu, 2006).

I. Ahmad & *et. al*,(2006) found that the metal exposure caused a time-dependent increase in LPO indicating per oxidative damage to gill membrane. These findings were confirmed by increasing severity of morphological and histological changes with increasing exposure duration. Heavy metals can interact with cell membrane structures and alter the normal physiology by stimulating LPO. Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats reported by S. Athikesavan & *et. al*, (2006). Metals in particular are one of the most deleterious environmental toxicants affecting gills by changing general morphology and ultrastructure of fish gills. Heavy metal ions interfere with respiration and osmoregulation by causing cellular damage to gill cells

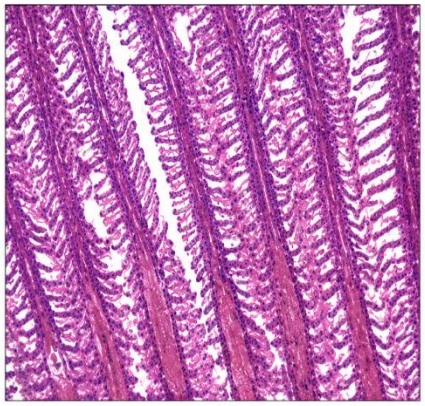


Fig.1: Microphotograph of T. S. of Gill Control fish. HE X 100

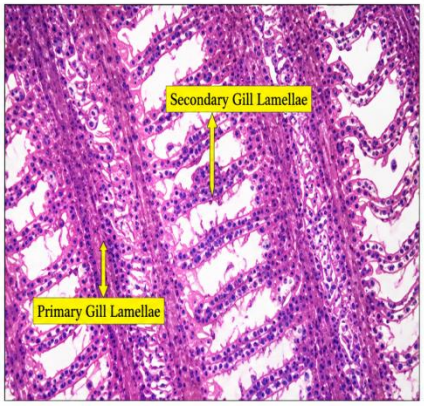


Fig.2: Microphotograph of T. S. of Gill Control fish. HE X 400

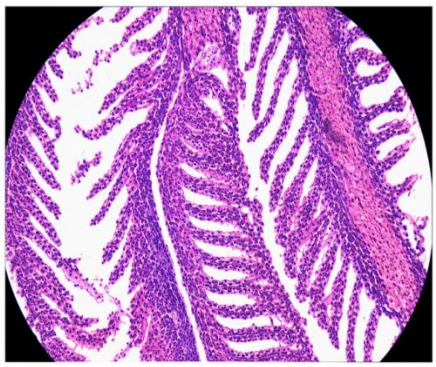


Fig 3: Microphotograph of T. S. of Gill after acute exposure to 0.35 ppm copper sulphate. HE X 400



Fig. 4: Microphotograph of T. S. of Gill after acute exposure to 3.51 ppm copper sulphate. HE X 400

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