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Histopathological alterations induced in gills and liver tissue of *Clarias gariepinus* (Bruchell, 1822) exposed to sublethal doses of copper sulphate

Sabiha Shapoo*, Mohd Asad Khan, Mohd Waseem, Shabnam Banoo
Department of Zoology and Applied Aquaculture, Barkatuallah University, Bhopal, Madhya Pradesh, India

Abstract
The present study was carried out to determine the histopathological alterations induced *in C. gariepinus* when exposed to three different sublethal concentrations of copper sulphate for period of 30 days from October to November 2017 at Department of Zoology and Applied Aquaculture, Barkatuallah University, Bhopal.140 fishes having average weight 60 ± 10 g were stocked in plastic tanks with capacity of 200 L water. Fishes were divided into three groups, Group A and B were treated by 1.5mg/L, 2.0 mg/L, 3.0 mg/L while group C served as control. Gills and liver tissues were fixed by bouin's solution and sectioned in 7 µm and stained with H & E method for microscopic study. Samples of gills and liver tissues were collected at zero day, 15th day, 20th day and 30th and histopahtological changes were observed. Alterations induced were damages like hyperplasia, telangiectasis and edema, necrosis of second filaments, jerky movement, aneurism, hyperaemia and fusion of second filaments in gills; and cell atrophy, necrosis, fatty degeneration, hyperaemia and bile stagnation at different concentrations of copper sulphate as compared to control groups. Liver and Gills tissue damages were increase with the increase of Copper concentration and days. So, copper had adverse toxicity effects on gill and liver tissues in African Catfish at 1.5 mg/L, 2.0mg/L and 3.0mg/L concentrations.

Keywords: gill, liver, histopathology, copper sulphate and *Clarias gariepinus*

Introduction
Metal concentrations in aquatic organisms seems to be of several magnitudes higher than concentrations present in the ecosystem It is due to bioaccumulation where metal ions are taken up from the environment by the organism and accumulated in different organs and tissues. Heavy metals are hazardous for the ecosystem. Heavy metals accumulation in Soil, air and water is a major environmental problem.

Naturally, heavy metals formed lower than 1% of body weight and their concentration fluctuations lead to environmental impermanent and disaster in animals. [1]. Copper is taken from the environment, either in the form of food or directly from water column and gets bind to alpha globulin and is transported to various tissues of body to aquatic fauna especially fishes. Environmental pollutions due to heavy metals were increased in the world and it is bioaccumulated in fish tissues [2]. Heavy metals are the most important pollutant of aquatic ecosystem that is the cause of major problems for human [3]. Copper sulphate is often used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae, and to control certain fish diseases [4]. However, above a specific concentration, copper is toxic to fishes including such cultured species as salmonids, cyprinids and catfishes [5] (Wurtz). The toxic effect of copper is related to its capacity for catalysing oxidative reactions, leading to the production of reactive oxygen species [6, 7, 8, 9]. C. gariepinus is a warm water fish that feed on planktons. Copper can be transferred by plankton to aquatic fauna [10, 11] especially fishes and then to human. *Clarias gariepinus* is used for this research because of its wide Tolerance to adverse conditions [12].

Materials and Methods
The fishes were collected from local fish market of Bhopal and were brought to the Departmental laboratory to carry out the research work. Acclimatisation [14days] was done to get adapted to laboratory conditions. Penta-hydrated copper Sulphate was weighed out and used in stock solution Group – A and Group B were treated with copper sulphate concentrations of 1.5mg/L, 2.0 mg/L and 3.0mg/L [30 days] while as group-C served as the control tank. Chronic toxicity was observed and the set up was monitored daily to observe changes in fish. Gills and liver were removed and washed in saline water to remove blood and they were then dehydrated through graded series of ethanol and embedded in paraffin wax (M.P. 58-68 C). Changes induced by Copper sulphate exposure in the gills and liver (15th 20th 30th day) were analyzed and photographed under photomicroscope (Olympus) along with control group. Samples of gills and liver tissues were collected at zero day, 15th day, 20th day and 30th day and histopathological changes were observed.

Results
Clinical studies results showed some apparent signs such as fast opening and closing of operculum and mouth, fidgety and air swallowing.

Microscopic study of gill
Gill tissues at 15th day (1.5 mg/L concentration); after 15th day of exposure the gill architecture showed acidophilic cells intensification of hyperplasia of lamella. Mucus secretion was Prominent which inhibits oxygen diffusion and reduces oxygen intake capacity. Degeneration of primary and

secondary lamella was seen leading to destruction of protective coverings thereby causing toxicity

Gill tissue at 20th Day (2.0 mg/L Concentration); Necrotic changes were seen in secondary lamellae, intensification of vacuoles [vasodilation] proliferation of filamentary epithelium lamellar aneurysm [telangiectasia] haemorrhage was observed due to rupture of lamellar epithelium

Gills tissues at 30th day (3.0mg concentration); Extensive hyperplasia, fusion of 3-4 secondary gill lamellae, degeneration of cells leading to collapsing and internal bleeding in the gill tissues, intensification of lamellar degeneration which finally allows toxin to enter blood stream and hence leading death of fish by blocking the oxygen intake pathway.

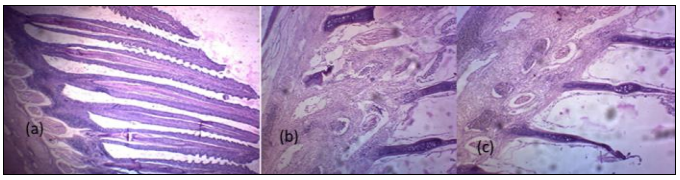

Fig 1: (a) Control gills showing gill lamella and gill rackers. (b)gills at 1.5mg/L and (c) at 2.0mg/L hyperplasia, epithelial lesions, degeneration of secondary lamella (at 100X).

Microscopic Study of Liver

LIVER TISSUES AT 15th day (1.5mg/L concentration) At 15th day, the degeneration of hepatocytes was seen, with intensified necrosis Atrophy, nuclear degeneration, necrosis was seen. Nucleus become pycnotic with degenerated hepatocytes.

Liver tissues at 20th day (2.0 mg/l concentration); At 20th day the hepatocytes showed more degeneration Extensive necrosis with poisonous lesions, nucleus become pycnotic hypertrophy of hepatocytes was seen.

Liver tissue at 30th day (3.0mg/L) Major changes in the hepatocytes such as focal necrosis, severe congestion in sinusoids were also observed. Hypertrophy of hepatocytes with pyknotic nuclei was quite evident in liver tissue exposed to Copper. On treatment with increased concentration, it was seen that the liver cells degenerated and the normal architecture of the liver was markedly disorganized. Dilated sinusoids with congestion signs of pathological processes such as necrosis and lipid infiltration around the blood vessels, regarded as a sign for toxic liver injury.

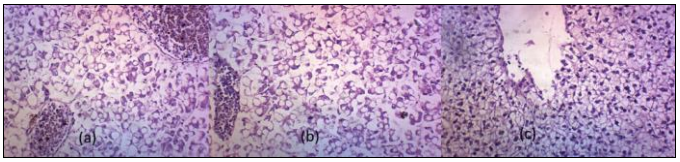

Fig 1; Liver Tissues (a) at 1.5mg/l, (b) at 2.0mg/l and (c) 3.0mg/l copper concentration intoxication. (at 100x)

Conclusion

Copper Sulphate was predominantly toxic to African Catfish, *Clarias gariepinus* and sub-lethal concentrations of Copper Sulphate exhibited wide spectrum of histopathological changes on *Clarias gariepinus* in which significant alterations occurred in the tissue morphology and in the cyto-architecture of the gills and liver tissue. This study reveals that presence of copper sulphate in fresh water reservoirs, even in small concentration, could cause health hazards effects on fish physiology and may potentially disturb their survivability in natural environment. Therefore, controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic chemicals. From our results, it can be concluded that the liver and gills histopathological changes in (*Clarias gariepinus*) have been related to copper sulphate concentrations and duration of its exposure which can serve as biomarkers of environmental pollution. Toxicity rate of Copper in fishes was severe with the increase of concentrations and days. These findings can play an important role in monitoring fish health and risk of assessments during periods of fluctuating levels of pollutants in environment.

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