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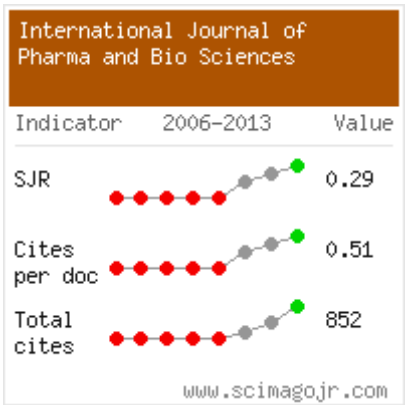

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Int J Pharm Bio Sci 2015 April; 6(2): (B) 237 - 243

Research Article **Allied Science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF COPPER SULPHATE ($CUSO_4$) ON BEHAVIOUR AND ON LIVER OF CLARIAS BATRACHUS (LINN. 1758)

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ABSTRACT

The heavy metal toxicants accumulate in the fish through the general body surface which affects severely their life support system. Once these toxic substance enter into the fish body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders. The present study proved that the heavy metal salts, i.e. copper sulphate impacts on behavioral responses in fresh water breathing fish *Clarias batrachus* (Linn.). Histological studies have revealed that the liver sections from control fishes showed normal histoarchitecture, The liver is characterized by polygonal shaped hepatocytes with granular cytoplasm and centrally placed round nuclei. Hepatocytes were arranged in well organized hepatic cords and separated by narrow blood sinusoids. Liver of fishes exposed to 1.5 ppm, 2.0 ppm and 3.0 ppm copper sulphate for 30 days resulted in the loosening of hepatic tissue, vacuolization, enucleated and distended hepatocytes and the centrally situated nuclei have shifted to the periphery of the cells.

KEY WORDS: ***Clarias batrachus*, Heavy metals, Hepatocytes, Liver, Vacuolization.**

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INTRODUCTION

Aquatic pollution from sources like effluents from industries, power plants, untreated domestic and sewage waste etc. have adverse effects on aquatic ecosystems. Due to which the animals thriving in these water bodies are exposed to unnaturally high levels of contaminants. Evidence of toxic effect of heavy metals has been reported on fishes and population eating contaminated food[1]. Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metal contamination may have lethal effects on the ecological balance of recipient environment and diversity of aquatic organism[2]. These heavy metal pollution posses a great threat to fishes. When fishes are exposed to elevated level of metal in polluted aquatic ecosystem, they tend to take these metals up from their direct environment[3]. The fish constitutes a valuable commodity from the stand point of human consumption. So heavy metal contamination of fissh bodies and aquatic biota becomes a serious concern from human health point of view. Copper, a heavy metal is widely used in various industrial plants. Effluents from such plants are sources of copper into aquatic environments. Most aquatic organisms have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. When their concentration exceeds, they become harmful[4]. Histopathological study thus gives us useful data concerning tissue change prior to external manifestation. The fish liver plays an important and vital function in basic metabolism. It is the major organ of accumulation, biotransformation and excretion of contaminants in fish. The measurement of suitable biomarkers in liver becomes useful and gives an idea about health status of fish. Toxicological studies have shown that the impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of the fish that respond specially to the degree and type of contamination, also the liver histology is used as biomarker for the environmental pollution and there have been numerous reports of histopathological changes in the liver of fish exposed to a wide range of organic compounds and heavy metals.

MATERIALS AND METHODS

The fresh water Indian cat fish *Clarias batrachus* was procured from the local market machi bazaar, Paltan market Dehradun, and was transferred to large plastic troughs. They were treated with 0.5% $KMnO_4$ *solution in minutes for dermal disinfection. Then they were acclimatized for period of fortnight to* laboratory condition and were fed on artificial food.

(i) Behavioural Changes

In order to investigate the behavioral changes in the experimental fishes. three different concentrations of heavy metal was selected and divided into four groups (three experimental and one control) comprising 10 fishes each, placed in individual treated aquaria of 30litres capacity and used for experiment. The experiment lasted for thirty days. Control fishes were mentioned along with the toxicant concentrations to provide a reference for assessing any behavioral and morphological changes. Responses were recorded if they differ from the control and occurred, in 10% of the fish in each test tank.

(ii) Histopathological Changes

Clarias batrachus was used for this study. The fish was killed by decapitation and the entire digestive tract was exposed through a mid-ventral incision and removed from the body. Small pieces of liver after washing in phosphate buffer we*re fixed by immersion in Bouin's solution. After fixation, the specimens were rinsed in* water, dehydrated in graded ethanol solutions (50% to absolute), cleared in xylene and embedded in paraffin. Transverse paraffin sections (5-7 µm) of each portion of th*e liver were prepared and processed for staining through haematoxylins and eosin (H&E). Photomicrographs were taken with a video camera connected to microscope (Olympus, Tokyo, Japan).*

RESULTS

Behavioural change

The experimental fish exposed to sub lethal concentration of copper sulphate exhibited abnormal behavioral response. During exposure time, fish initially showed rapid movement, faster opercular activity, surfacing and gulping air. They showed erratic swimming with jerky movements, hyper excitability, convulsions and tendency of escaping from aquaria. These activities were increased initially and subsequently reduced. Beside an interesting observation was noted that there was remarkable reduced body pigmentation along with profuse mucus secretion and its coagulation all over the body. This was followed by loss of equilibrium and fish slowly moved upward in a vertical direction. There after fish became progressively lethargic and lost their sense of equilibrium completely. Ultimately the fish lay down on the bottom of the aquaria with their belly upward before death. In conclusion, the present study proved that the heavy metal salts i.e. copper sulphate impacts on behavioral responses in fresh water breathing fish *Clarias batrachus* (Linn.).

Table 1
Diagnostic behavioural effects of copper sulphate on fresh water catfish Clarias batrachus (Linn.)

Behavioural and Morphological Symptoms	Diagnosis
Loss of equilibrium	Yes (Fish roll over on side or back)
General activity	Hyperactive (Fish swim faster than the control fish: that's around tank without being provoked at initial exposure time) *Hypoactive (Fish swim slower than control fish with ncreased exposure period of more than 48 hours exposure when severely intoxicate) Vertical position (Fish occasionally assumed a vertical position before death) Lateral flexure (Lateral flexure was in the fish in the caudal region)*
Startle response	*Under reactive (Fish that away from stimuli slower than the control fish with increased exposure time)*
Hemorrhage	*None*
Deformities	*Posturing of pectoral fins (Posturing of pectoral fins Distinguishable behavioral /morphological change or signs of stress)*

Histopathological study of liver

Histological studies revealed that the liver sections from control fishes showed normal histoarchitecture, liver is characterized by polygonal shaped hepatocytes with granular cytoplasm and centrally placed round nuclei. Hepatocytes were arranged in well organized hepatic cords and separated by narrow blood sinusoids (Figure 1). Liver of fishes exposed to 1.5 ppm, 2.0 ppm and 3.0 ppm copper sulphate for 30 days resulted in the loosening of hepatic tissue, vacuolization, enucleated and distended hepatocytes and the centrally situated nuclei have shifted to the periphery of the cells (Figure 2 & 3). Liver of fishes exposed to 2 ppm and 3 ppm copper sulphate for 30 days resulted in severe loosening and necrosis of hepatic tissue, hepatic cells lost their original shape, got excessively distended and vacuolated. Extensive vacuolization and necrosis was seen in 3.0 ppm concentration (Figure 4& 5).

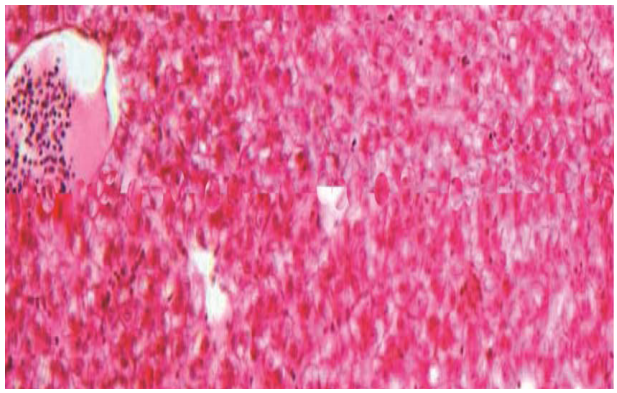

Figure 1
Tranverse Section of Liver of Clarias batrachus in control showing normal hepatocytes and blood vessels

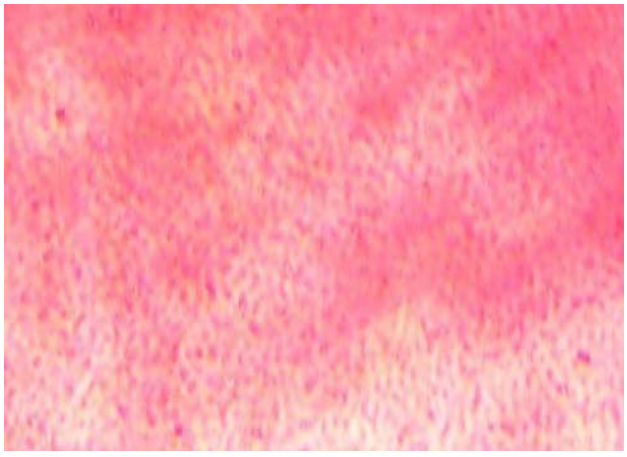

Figure 2
Transverse Section of Liver of Clarias batrachus exposed to 1.5ppm Concentration of $CuSO_4$ showing loosening of hepatic tissue

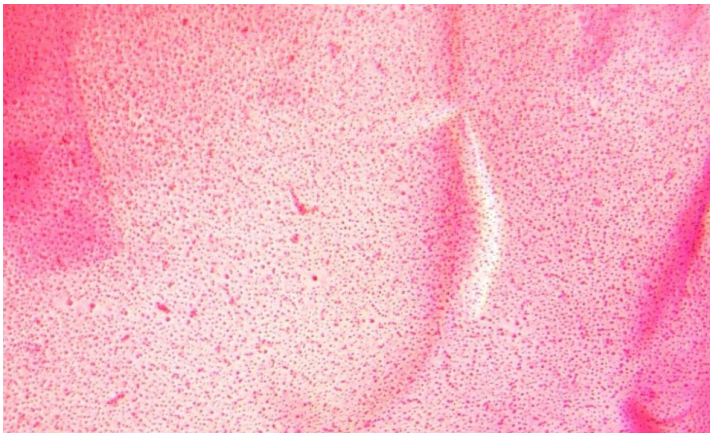

Figure 3
Transverse Section of Liver of Clarias batrachus exposed to 2.0 ppm concentration of $CuSO_4$ showing vacuolization (double headed arrow) and necrosis (single headed arrow)

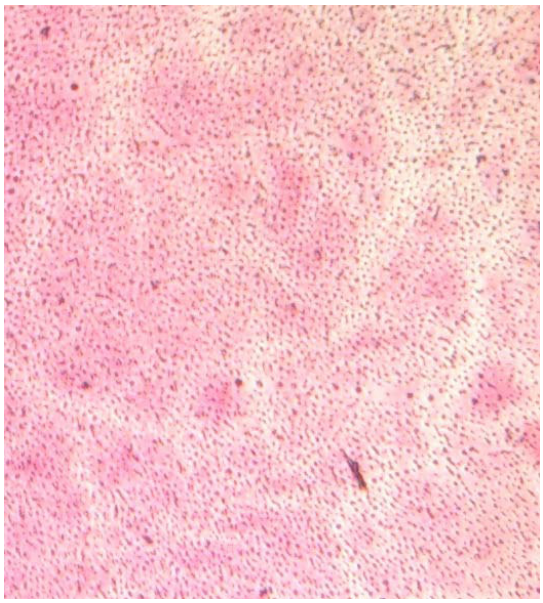

Figure 4
Transverse Section of Liver of Clarias batrachus exposed to 3.0 ppm concentration of $CuSO_4$ showing necrosis (double headed arrow)

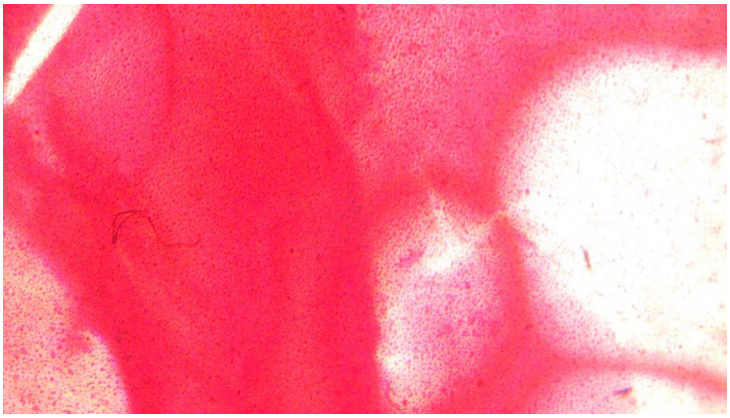

Figure 5
Transverse Section of Liver of Clarias batrachus exposed to 3.0 ppm concentration of $CuSO_4$ showing extensive vacuolization (double headed arrow)

DISCUSSION

Behavioral abnormalities in various fish species on exposure to heavy metal have been reported by several researchers[5]. According to some worker *Notiemigonus crysoleucus,* when exposed to 5 ppm copper piped at the surface, became restless and finally lost equilibrium[6]. Similar results obtained when *Lepomis macrochiurs* treated with different concentration of copper[7]. Lethargic response and frequent surfacing along with gulping of air in exposure to 0.25 ppm copper were observed in *Heteropneusta fossilis*[8]. *Eutroplus maculatus* on exposure to copper, mercury and selenium showed irregular erratic swimming, frequent surfacing, gulping of air, revolving, convulsions and accelerated ventilation with rapid arrhythmic opercular and mouthmovements[9]. Behavioural abnormalities have been attributed to nervous impairment due to blockage of nervous transmission between the nervous system and various effectors sites[10]. The present study revealed that copper sulphate

exposure induced histopathological alterations in liver of a freshwater catfish, *Clarias batrachus,* severity of the lesions was dose and duration dependent. Under present investigation, it has been observed that the liver of fishes exposed to 1.5 ppm, 2.0 and 3.0 ppm copper sulphate for 30 days exhibited several histological alterations like deshaping of hepatocytes, eccentric position of nuclei, enucleation, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue. The magnitude of changes increased in proportion to increased dose and time period. Similar results have been reported by another worker in *Tilapia mossambica (Oreochromis mossambicus)* exposed to cadmium chloride at 5 and 50 ppm for 1, 7, 15 and 30 days[11], in *Oreochromismossambicus* exposed to cadmium and zinc[12], *Clarias batrachus* exposed to 4 ppm and 8 ppm cadmium chloride for 90 days[13]and *Clarias gariepinus* exposed to cadmium[14]. White Sea bass, *Lates calcarifer* exposed to 5mg/l cadmium chloride for 3 weeks[15]. The present findings are in accordance with above studies.

CONCLUSION

Thus the present study revealed that copper sulphate exposure induced altered behavioral response as well as histopathological changes viz., necrosis, vacuolization and loosening of hepatic cells in *Clarias batrachus* (Linn.1758).

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