ASIAN J. EXP. BIOL. SCI. VOL 2(2) 2011: 278-282

ISSN 0975-5845
AJEBS
http://www.ajebs.com

ORIGINAL ARTICLE

Histopathological Alterations Induced in Gill Epithelium of African Catfish, *Clarias gariepinus*, Exposed to Copper Sulphate

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ABSTRACT

African catfish, Clarias gariepinus were exposed over 60 days period to different sublethal concentrations(2.0 and 5.0 mg/l) of copper sulphate to observe histopathological alterations in gills. Control group was maintained simultaneously. The gills showed marked alterations in the epithelia in response to copper sulphate exposure. There were fusion of three to four secondary gill lamellae, edema, lifting of lamellar epithelia and hyperplasia of gill epithelium.Long-term exposure to high concentration caused hypertrophy of lamellar epithelial cells, aneurysms (telangiectasia) and haemorrhage due to rupture of lamellar epithelium of gill filaments. The toxic responses evaluated from histology of gills of C. gariepinus exposed to copper sulphate can serve as potential biomarkers for assessment of copper toxicity in environmental biomonitoring.

KEY WORDS*: Copper sulphate, Clarias gariepinus, Histopathology, Gills*

INTRODUCTION

Human destructive influence on the aquatic environment is in the form of sublethal pollution which results in chronic stress conditions that have negative effect on aquatic life. The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent and run-off from agricultural fields. Stress response is characterized by physiological changes and effect of pollutants on fish is assessed by acute and chronic toxicity tests. Copper is an essential trace metal in small concentrations for several fish metabolic functions. Copper forms an essential part of variety of enzymes (free radical defence) and liver proteins homocuprien and heptacuprien [1]. It is also used as fungicide, algaecide and herbicide and in municipal water treatment systems [2].

Despite the essential role of copper in a number of vital biochemical processes, the metal is known to induce several histopathological changes in gills when present in higher concentration in water [3, 4]. Copper accumulation in organs of animals of polluted water bodies [5, 6] leads to generation of free radicals which causes the biochemical and morphological alterations in them [7, 8]. The effect of copper sulphate on fish has been studied comprehensively and some species have been found to be more susceptible to copper than others [9, 10, 11]. *Clarias gariepinus* was used for this experiment because of its hardy nature and economic importance. Hence, this study was undertaken to examine the effect of different sublethal copper sulphate concentrations on histological aspects of gills of African catfish, *Clarias gariepinus* (Burchell, 1822).

MATERIALS AND METHODS

Adult specimens of fish, *Clarias gariepinus* of weight and length measuring 100-110 gm and 18-20 cm respectively were obtained from the local fish market of Bhopal and brought to the laboratory. The fish were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing in the aquarium fish were treated with

0.1% KMnO4 solution to obviate any dermal infection. Fish were acclimatized to laboratory conditions for a period of 15 days. Both control and treated fish were fed with chopped meat once daily.

Pentahydrate copper sulphate (Ranbaxy, India) was used for the preparation of various concentrations (stock solution) by adopting the dilution techniques. The fish were divided into three groups kept in three transparent glass aquariums (200 L), group A and B were treated with copper sulphate concentrations of 2 and 5 mg/l respectively, while as group C served as the control. In order to maintain the constant concentration of copper sulphate water was changed after 5 days during the experimental period.

Histological Procedure

On the 30 and 60 days of the exposure of two different sublethal concentrations of copper sulphate, one fish from each exposed group and control group were sacrificed by giving a sharp blow on head and dissected out. Gills were removed and washed in saline water to remove blood and fixed in aqueous Bouin's fixative for 24 hrs. They were then dehydrated through graded series of ethanol and embedded in paraffin wax (M.P. 58-68^{0}C). Blocks were prepared and sectioned at a thickness of 6-7 microns. The sections were deparaffinized in xylene and stained with haematoxylin-eosin (HE). Changes induced by $CuSO_4$ exposure in the gills were analyzed and photographed under photomicroscope (Olympus) along with control group.

RESULTS

Control group

The gills after 30 and 60 days possess double rows of filaments or primary lamellae from which arise perpendicularly the secondary lamellae. The epithelium is composed of pavement cells. Chloride and mucus cells are present between secondary lamellae (Fig. 1).

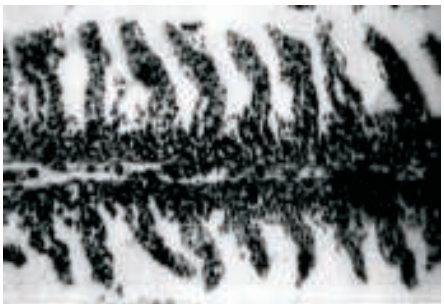
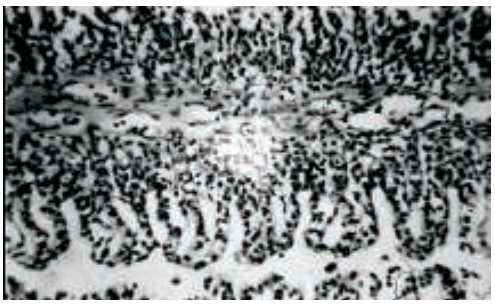


Figure 1. Photomicrograph of T.S of gills of *C. gariepinus* of control fish. (Haematoxylin Eosin) 100 X, showing normal aspect of the gill with filament and distinct separate secondary gill lamellae.

Treated groups

2mg/l of $CuSO_4$ after 30 and 60 days of exposure

The histopathological alterations observed after 30 days were lifting of lamellar epithelium and edema in the filamentary epithelium (Fig.2) while as lamellar disorganization, swollen and fusion of secondary gill lamellae tips were noticed after 60 days of exposure (Fig. 3).



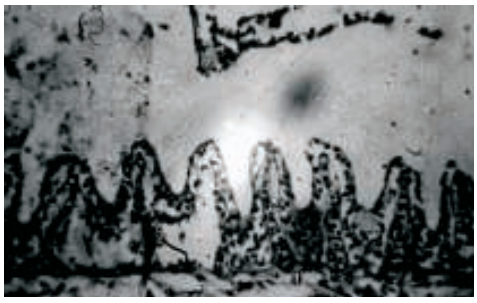


Fig 2. Photomicrograph of T.S of gills of 2 mg /l of copper sulphate intoxication after 30 days. (HaematoxylinEosin)280 X, showing lifting of lamellar epithelium and edema.

Fig 3. Photomicrograph of T.S of gills of 2 mg/l of copper sulphate intoxication after 60 days. (Haematoxylin Eosin) 280 X,

5 mg/l of $CuSO_4$ after 30 and 60 days of exposure

Proliferation of filamentary epithelium was observed which resulted in fusion of 3 to 4 secondary gill lamellae, hypertrophy and hyperplasia of lamellar epithelium were also noticed after 30 days (Fig.4). After 60 days severe histopathological alterations were found including complete fusion of secondary gill lamellae (Fig 5), lamellar aneurysm (telangiectasia) and haemorrhage due to rupture of lamellar epithelium (Fig. 6).

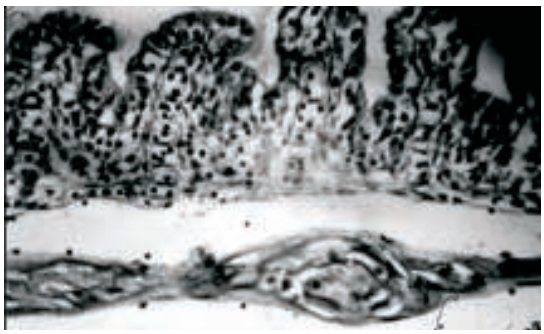


Fig 4. Photomicrograph of T.S of gills of 5 mg/l of copper sulphate intoxication after 30 days. (Haematoxylin Eosin) 280 X, showing hyperplasia and hypertrophy of lamellar epithelium.

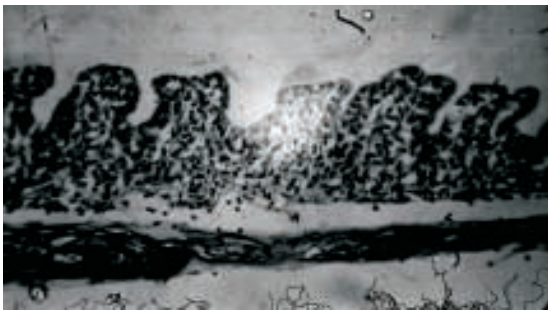


Fig 5. Photomicrograph of T.S of gills of 5 mg/l of copper sulphate intoxication after 60 days. (Haematoxylin Eosin) 280 X, showing fusion of secondary gill lamellae due to proliferation of epithelium.

Fig 6. Photomicrograph of T.S of gills of 5 mg/l of copper sulphate intoxication after 60 days. (Haematoxylin Eosin) 280 X, showing lamellar aneurysm and epithelial rupture with haemorrhage.

DISCUSSION

There are many routes for the entry of heavy metals into the body of fish, namely oral ingestion, absorption through gills [12], general body surface and gastrointestinal tract. After absorption the metal makes its way into the target organ, where it produces various types of disturbances. The gills carry out the functions of respiration, osmoregulation and excretion, remain in contact with external environment and is particularly sensitive to changes in the quality of

water are considered to be the primary target of the contamination[13, 14, 15, 16] . In the present study *Clarias gariepinus* from control group shows normal structural organization of gills while as treated groups shows histopathological alterations namely epithelial lifting, edema in the filament, fusion of lamellae, epithelium proliferation, haemorrhage and lamellar aneurisms.

The lifting of lamellar epithelium could be serve as a defence mechanism, because separation of epithelium of the lamellae increases the distance across which heavy metals must diffuse to reach the blood stream [17,18]. The lifting of lamellar epithelium is probably due to severe edema [19, 20].Cell proliferation which results in hyperplasia is one of the major histological change observed in fishes exposed to copper sulphate is reported by several authors [21, 22, 23] and which leads to lamellar fusion observed in the present study. Hyperplasia of gill epithelium would not only decrease the surface area available for oxygen diffusion [24] but would increase the oxygen distance between water and blood [25] which in turn could cause tissue hypoxia [26].

The present findings suggested that gill histopathological alterations are due to toxicity of copper sulphate. Thus, it can be concluded that alterations in gill histology can serve as a potential biomarker of heavy metals and will be helpful in the understanding the toxic mechanism of copper in aquatic system.

ACKNOWLEDGEMENT

Authors are thankful to Prof. Mrs. Mangla Bhide, HOD of Zoology, Dr. H.S. Gour University, Sagar (M.P) for their valuable guidance for carrying out this work.

REFERENCES

[1]. Seymore, T. (1994): Bioaccumulation of metals in *Barbus marequensis* from the Olifants River, Kruger National Park and the lethal levels of manganese to juvenile *Oreochromis mossambicus*. M.Sc. Thesis, Rand Afrikaans University, South Africa.

[2]. Stoskospf, M.K. (1993): Fish medicine. W.S. Saundes Company, London.

[3]. Vutukuru, S.S., Suma, Ch., Radha K., Juveria, Smitha, J., Venkateswara J. and Anjaneyula Y. (2005): Studies on the development of potential biomarker for rapid assessment of copper toxicity to freshwater, fish using *Esomus danricus* as model. *Int. J. Environ. Res. Public Hlth.,* 2(2), 253-257.

[4]. Peyghan, R., Oskoiizadeh, K., Esmailzadeh, S. and Rasekh. A. (2007): Pathological study of short and long-term copper sulphate bath on gill in grass carp, *Ctenopharyngodon idella. Asian Fisheries Sci*., 20, 109-116.

[5]. Hota, A.K., Nanda, B.R., and Mishera, D, K. (1993): Toxicity and accumulation of heavy metals in a freshwater fish, *Heteropneustes fossilis* (Bloch.). *Bull*. *Environ*. *Sci*., 11, 36-39.

[6]. Al-Kahtani, M.A., (2009): Accumulation of heavy metals in tilapia fish (*Oreochromis niloticus*) from Al-Khadoud spring, Al-Hassa, Saudi Arabia. *American J. Applied Sci*., *6*(12), 2024-2029.

[7]. Varanka, Z., Rojik, I., Varanka, I., Nemcsok, J. and Abraham, M. (2001): Biochemical and morphological changes in carp (*Cyprinus carpio*) liver following exposure to copper sulphate and tannic. *Comp*. *Biochem*. *Physiol*., C 128, 467-478.

[8]. Monteiro, S..M., Mancera, J.M., Fontainhas-Fernandes, A. and Sousa, M. (2005): Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. *Comp*. *Biochem*. *Physio*. C. 141, 375-383.

[9]. Boyd, C.E. (1982): Water quality management for Pond Fish Culture. *Elsevier,* Amsterdam. 317.

[10]. Wedemeyer, G.A. (1996): Physiology of fish intensive culture systems. *Chapman and Hall*, New York, 231.

[11]. Darwish, A.M., Straus, D.L., Griffin, B.R. (2004): Coppersulphate Target Animal Safety in Channel Catfish. *Annl. Eastn. Fish Hlth. Workshop*. 71.

[12]. Lloyd, R. (1992): Pollution and freshwater fish. Fishing new books, *Blackwell scientific Pub*. *Ltd*., London.

[13]. Olurin, K., Olojo, E.A.A., Mbaka, G.O., and Akindele, A.T. (2006): Histopathological response of the gills and liver tissues of *Clarias gariepinus* to herbicidie glyphosat. *African J*. *Biotechnol*., 5(24), 2480-2487.

[14]. Camargo, M..M and Martinez, C.B. (2007): Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. Ichthyol*., 5(3) 327-336.

[15]. Fernandes, M.N. and Mazon, A.F. (2003): Environmental pollution and fish gill morphology. In Val, A. L. and B.G. Kapoor (Eds). Fish adaptations. *Enfield, Sci. Pub.*, 203-231.

[16]. Mohamed, F.A. (2009): Histopathological studies *on Tilapia zillii* and *Solea vulgaris* from Lake Qaqrun, Egypt. *World J. Fish and Marine Sci*., *1*(1), 29-39.

[17]. Laurene, D.J. McDonald, D.G. (1987): Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. *Can*. *J. Fish. Aquat. Sci*., 44, 00-104.

[18]. Arellano, J.M., Storch, V. and Sarasquete C. (1999): Histological changes and copper accumulation in liver and gills of the Senegales sole, *Solea senegalensis*. *Ecotoxicol. Environ. Saf*. 44, 62-72.

[19]. Pane, E.F., Haque, A. and Wood, C.M. (2004): Mechanistic analysis of acute, nickel induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon. *Aquat. Toxicol*., 69, 11-24.

[20]. Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. and Triebskorn, R. (1997): The use of histopathological indicators to evaluate contamination-related stress in fish. *J. Aqua. Ecosyst., Stress and Recov.,* 6, 75-86.

[21]. De Boeck, G., Vlaeminck, A., Balm, P.H., Lock, R.A., De Wachter, B. and Blust, R. (2001): Morphological and metabolic changes in common carp, *Cyprinus carpio*, during short-term copper exposure: interactions between copper ions and plasma cortisol elevation. *Environ. Toxicol. Chem*., 20, 374-381.

[22]. Van Heerden, D., Tiedt, L.R., and Vosloo, A. (2004): Gill damage in *Oreochromis mossambicus* and *Tilapia sparrmanii* after short-term copper exposure. Elsevier *Int. Cong. Ser*., 1275, 195-200.
[23]. Figueiredo, A., Ferreira, J.V., Garcia, S., Monnteiro, S.M., Carrola, J., Matos, P., and Fontainhas, A. (2007): Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesq. Vet. Bras.,* 27 (3), 103 109.
[24]. Lappivaara, J., Nikinmaa, M., and Tuurala, H. (1995): Arterial oxygen tension and the structure of the secondary lamellae of the gills in rainbow trout (*Oncorhynchus mykiss*) after acute exposure to zinc and during recovery, *Aquat*. *Toxicol*., 32, 321331.
[25]. Skidmore, J.F. and Towell, P.W.A. (1972): Toxic effects of zinc sulphate on the gills of rainbow trout. *Water Res*., 6, 271-230.
[26]. Cerqureira, C.C.C. and Fernandes, M.N. (2002): Gill tissue recovery after copper exposure and blood parameter response in the tropical fish *Prochilodus scofa*, *Ecotox. Environ. Saf*. 52, 83-91.

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