
Role of taurine and garlic extract in alleviating the histopathological changes in gills induced by long-term exposure to copper sulphate in *Clarias gariepinus*

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Abstract: This investigation was intended to test the possible protective role of taurine and garlic extract against the toxicity of copper sulphate to *Clarias gariepinus*. For this purpose experiment was setup in seven groups (I-VII) containing 20 fish in each group. The fish of group I were kept as control. The fish of groups of II, IV and VI were challenged with 4 ppm solution of copper sulphate, where as groups III, V and VII were exposed to 8 ppm copper sulphate. Simultaneously, groups II and III were maintained as copper sulphate exposed non antioxidant treated control whereas, groups IV and V were treated with taurine (5 ppm) and groups VI and VII were treated with garlic extract (5 ppm) during the entire experiment period of 90 days. Histopathological observation of the gills after 15, 30, 60 and 90 days on exposure of sublethal copper sulphate concentrations revealed severe histopathological changes including, lamellar epithelium lifting, disintegration in pillar cell system with formation of aneurysms, increased infiltration of erythrocytes and leucocytes, haemolysis and haemorrhage, hyperplasia, complete fusion of secondary lamellae. While as addition of garlic extract and taurine has comparatively minimized histopathological alterations in groups VI and VII, respectively. It seems that simultaneous exposure of fish to taurine or garlic extract with copper sulphate were found to partly mitigate its toxicity indicating their potential therapeutic activity against copper toxicity in fish.

Key words: Copper sulphate, *Clarias gariepinus*, gills, taurine, garlic extract

Introduction

Copper is a micronutrient and is an essential part of cytochrome oxidase, is a component of many metalloenzymes and is involved in essential redox reactions within the cell (Halfdanarson *et al.*, 2008). However, copper

becomes toxic to aquatic biota when its concentration exceeds than the biological requirement. The toxic effect of copper is related to its capacity for catalyzing oxidative reactions, leading to the production of ROS

(Lopes *et al.*, 2001). In freshwater environments, copper can act as Na analogue and competes in gill transport systems, and out-compete Na, thereby blocking transport systems (Grosell and Wood, 2002). Copper is highly toxic and lethal to *Clarias gariepinus* at lower concentrations (Wani *et al.*, 2013).

Recent trends in controlling and treating diseases favour natural antioxidant, which could chelate heavy metals into non-ionized and less toxic complex to be excreted through urine or faeces. Taurine (2-aminoethane sulphonic acid, $^+\text{NH}_3\text{CH}_2\text{CH}_2\text{SO}_3^-$) is the major free intra cellular non protein sulphur amino acid (Atmaca, 2004) found in millimolar concentrations in many animal tissues (Wright *et al.*, 1986). It is a conditionally essential amino acid and is either derived from food/feed or biosynthesized in the liver (Divakaran, 2006). Taurine is unique in that it is not linked to any protein by a peptide bond and it is not part of any protein (Schuller-Lewis and Park, 2003). Taurine is present in high concentration in most tissues particularly in lymphocytes (Fukuda *et al.*, 1982). The zwitterionic nature of the taurine gives it high water solubility and low lipophilicity (Huxtable, 1992). Taurine is involved in a number of physiological processes including bile acid conjugation, osmoregulation, detoxification of xenobiotics, cell membrane stabilization, modulation of cellular calcium flux, and modulation of neuronal excitability (Huxtable, 1992).

Garlic extract contains at least 33 sulphur compounds, several enzymes, 17 amino acids, and minerals such as selenium (Newall *et al.*, 1996). The consequence of synergism between various compounds is responsible for the antioxidant activity of garlic. One of the most biologically active compounds, allicin does not exist in garlic until it is crushed or cut. Garlic compounds are having tremendous antioxidant property which exerts actions by scavenging ROS (Borek, 2001). Garlic extract has been found to suppress the activity of ceruloplasmin and accumulation of heavy metals (copper and zinc) in the tissues of fish, *Oreochromis niloticus* (Metwally, 2009).

Fishes are ideal organisms to monitor aquatic systems because they occupy positions towards the apex of food pyramids and may, therefore, reflect effects of heavy metals on other organisms including human beings as well as direct stresses on themselves (Van der Oost *et al.*, 2003). Histopathological indicators are beneficial in that they show the net effect of biochemical and molecular changes in the organism resulting from exposure to a contaminant. This work was designed to elucidate the efficacy of taurine and garlic extract in alleviating the histopathological alterations induced by long-term exposure to copper sulphate in *Clarias gariepinus*.

Materials and Methods

Test organism and experimental design

The African catfish, *Clarias gariepinus* of average weight 98.43 ± 24.09 g and length 20.5 ± 2.5 cm was selected as test organism in this study because of its hardy nature and ability to acclimatise quickly in the laboratory conditions. *Clarias gariepinus* is an exotic fish and was first time brought to India in 1994 (Thakur, 1998). Healthy specimens of catfish of either sex belonging to a single population were purchased on order from the local fish market of Sagar (M.P). Fish were then kept for a period of fifteen days for acclimatization in laboratory conditions.

Experiment was setup in seven groups containing 20 fish in each group and kept in fiberglass aquariums (120L) with or without simultaneous treatment of water with copper sulphate, taurine and garlic extract during the entire experiment period of 90 days (Tab. 1). Dose selection and mode of administration of garlic extract and taurine (HiMedia Laboratories) was based on Kumar *et al.*, 2009. All the fish were fed with commercially available fish pellet feed (Tokyo®, Japan) throughout the experiment.

Preparation of copper stock solution

Dilution of pentahydrate copper sulphate (WebChem®) for bioassay test was carried out by preparing a stock solution by dissolving the 50 g of copper sulphate in 1 litre of distilled water. This solution was diluted directly into 40 liters of tap water in 120 liters capacity

aquariums in sufficient amounts to provide the 4 and 8 ppm copper sulphate concentrations in water.

Tab. 1: Showing experimental design of 90 days exposure to *Clarias gariepinus* with or without simultaneous treatment of water with copper sulphate, taurine and garlic extract

Group	Copper Sulphate	Garlic Extract	Taurine
I	Control	-	-
II	4 ppm	-	-
III	8 ppm	-	-
IV	4 ppm	-	5 ppm
V	8 ppm	-	5 ppm
VI	4 ppm	5 ppm	-
VII	8 ppm	5 ppm	-

Preparation of ethanolic garlic extract

The ethanolic garlic extract was prepared with slight modification of Kumar *et al.*, 2009. Dried garlic powder (100 g) was dissolved in absolute 100 ml ethanol and 50 ml distilled water and left for 24h at room temperature. The mixture was filtered through filter paper and the filtrate was then subjected to evaporation in laminar air flow for the separation of ethanol from garlic extract. Thereafter, 5 ppm of the extract was prepared as and when required for experimentation.

Histological study

Gills of (control and treated) fish were

removed aseptically and were fixed in aqueous Bouin's fluid, dehydrated in graded series of alcohol, clarified with xylene and embedded in paraffin blocks. They were cut at 4-5 μ thickness by using microtome and stained routinely with haematoxylin/eosin (Luna, 1968). Stained histopathological sections were examined under binocular compound microscope (Zeiss, PrimoStar) on different magnifications and photographed.

Semiquantitative scoring

Histopathological alterations was assessed according to (Thophon *et al.*, 2003) by using a scoring ranging from - to +++ depending on the degree and extend of the alterations: (-) none, (+) mild occurrence, (++) moderate occurrence, (+++) severe occurrence. Five slides were observed from each organ and treatment.

Results

Histopathological Study of Gills

Group I

The microscopic examination of gills of *Clarias gariepinus* revealed the normal histoarchitecture (Fig. 1) and did not show recognizable alterations in any fish of control group throughout the experiment. Gill arch was composed of primary lamellae with two rows of numerous semicircular secondary lamellae that ran perpendicularly to each filament. These primary lamellae consisted of cartilaginous

supporting rod, a vascular system with traces of sinusoidal blood spaces and multilayered epithelium. Secondary gill lamellae were lined by a wavy single layer of respiratory simple squamous epithelium composed of pavement cells which are separated from the lamellar blood sinuses by a basement membrane. The secondary lamellar epithelium was supported by pillar cells, which are contractile and separate the capillary channels. Each lamella was composed of a network of interconnected spaces called lacuna. Chloride cells or ionocytes were present at the trailing edges of the filament and lamellae. Mucous goblet cells were abundant on the surface of lamellae, appearing as granular domes or vacuolated cells.

Group II

The histopathological examination of gills after 15 and 30 days exposure to copper sulphate exhibited distinct histopathologies including proliferation and hypertrophy of chloride cells and mucous cells at the base of gill filament. Abnormal increase in size of few secondary lamellae was observed (Fig. 2). The tips of few secondary lamellae were swollen due to blood cell congestion and breakdown of pillar cell system and disappearance of lacunae (Fig. 3). After 60 and 90 days severe histopathological alterations were detected in gill filaments and characterized primarily by dilation of the secondary lamellar blood sinuses and vascular congestions (Fig. 4). Lifting of

respiratory epithelia with excessive infiltration of leucocytes and erythrocytes was observed in secondary lamellae. The tips of secondary lamellae were swollen indicating circulatory anomaly. The filamentary epithelium of gills after 90 days revealed decrease in interlamellar space due to extensive hypertrophy and hyperplasia of the chloride and pavement cells (Fig. 5).

Group III

After 15 days exposure the most common histopathological gill changes were lifting of the lamellar epithelium and increase in intracellular vacuolation in pavement cells which resulted in edematous changes due to collapse of pillar cells in secondary lamellae (Fig. 6). After 60 days gills exhibited severe distinct histopathologies including hyperplasia of interlamellar epithelial cells resulted in complete fusion of secondary lamellae and disappearance of the space between contiguous lamellae. Haemolysis and haemorrhage were also reported due to severe congestion of blood (Fig. 7). After prolonged exposures (60 and 90 days) the gill tissue revealed most frequent alteration in the secondary lamellae with severe edema and rupture of pavement cell layer (Fig. 8). The fusion of secondary lamellae along the entire length was observed due to severe hyperplasia and hypertrophy of interlamellar cells (Fig. 9).

Group IV

The histoarchitecture of filamentary epithelium of gill exhibited mild histopathological alterations including hypertrophy of chloride cells at base of secondary lamellae with very slight hyperplasia of mucous cells after 15 days (Fig. 10). Occasionally, slight lifting of epithelium was also seen at the distal portion of secondary lamellae after 30 days (Fig. 11). The histopathological changes observed after 60 days were mild lifting of lamellar epithelium and mild hyperplasia of chloride and mucous cells. At many places rupture of lamellar epithelium was also reported. Occasionally, blood channel of secondary lamellae were dilated and disorganized (Fig. 12). Severe infiltration of leucocytes due to hyperemia was observed after 90 days in secondary lamellae with formation of lamellar aneurysms. However, less frequent rupture of lamellar epithelium with mild haemorrhage was also seen (Fig. 13).

Group V

The histoarchitecture of gill exhibited mild histopathological alterations including slight hyperplasia of interlamellar cells with interstitial edema. Occasionally, the blood sinuses at the proximal portion were dilated with accumulation of few erythrocytes after 15 days (Fig. 14). After 30 days gill revealed slight hyperplasia and hypertrophy of epithelial cells with infiltration of leucocytes was also reported in secondary lamellae (Fig. 15). The blood channels of secondary lamellae were dilated

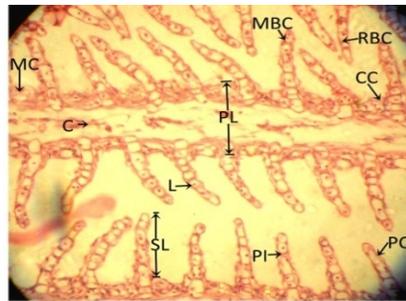


Fig. 1 200 x

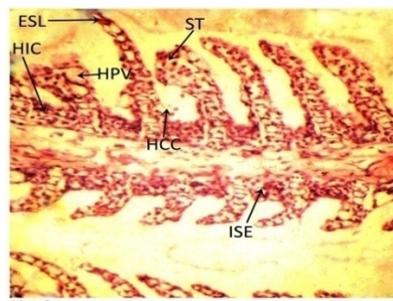


Fig. 2

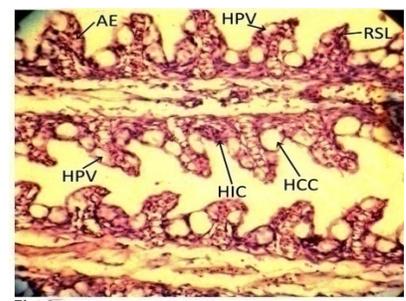


Fig. 3

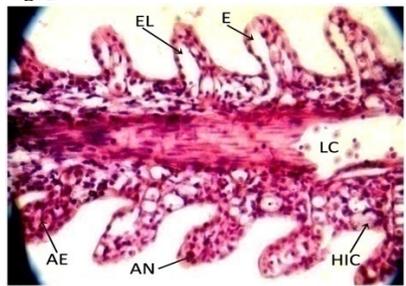


Fig. 4

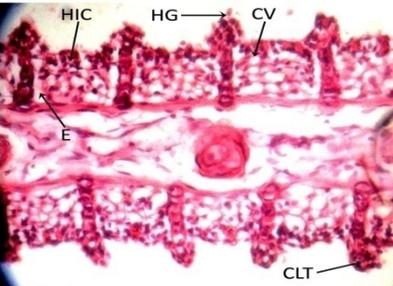


Fig. 5

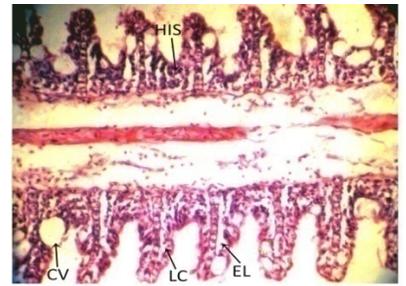


Fig. 6

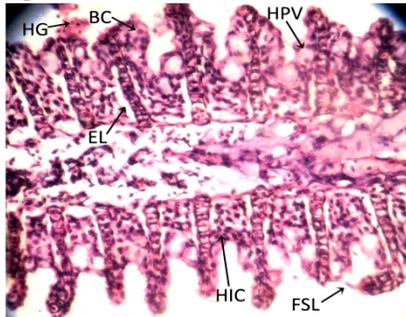


Fig. 7

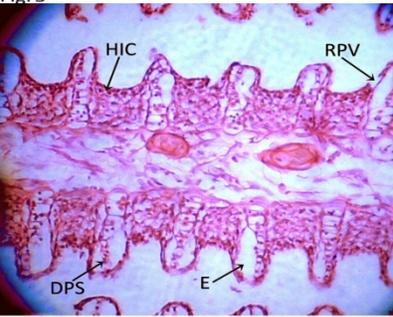


Fig. 8

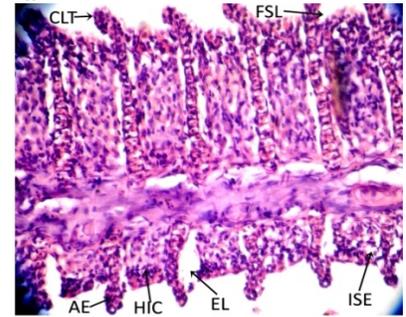


Fig. 9

Plate I- Histology of *Clarias gariepinus* gills in control and copper sulphate exposed groups II and III (Fig. 1 and 9). (Fig 1) Normal gill showing primary (PL), secondary lamellae (SL), [200X]. (Fig. 2) exposed to 4ppm CuSO₄ after 15 days showing swollen tips (ST), unusual elongation of secondary lamella (ESL), hypertrophy of chloride cells (HCC). [X280]. (Fig. 3) exposed to 4ppm CuSO₄ after 30 days showing hypertrophy of pavement cells (HPV), hyperplasia of interlamellar cells (HIC) [X280]. (Fig. 4) exposed to 4ppm CuSO₄ after 60 days showing epithelial lifting (EL), lamellar aneurysms (AY) [X280]. (Fig. 5) exposed to 4ppm CuSO₄ after 90 days showing hyperplasia of interlamellar cells (HIC), club-like lamellae tips (CLT), haemorrhage (RG), [X280]. (Fig. 6) exposed to 8ppm CuSO₄ after 15 days showing cytoplasmic vacuolation (CV), hyperplasia of interlamellar cells (HIC) [X280]. (Fig. 7) exposed to 8ppm CuSO₄ after 30 days showing haemorrhage (RG), hypertrophy of pavement cells (HPV), fusion of secondary lamellae (FSL) [X280]. (Fig. 8) exposed to 8ppm CuSO₄ after 60 days showing epithelial lifting (EL), hyperplasia of interlamellar cells (HIC), disintegration of pillar cell system (DPS), [X280]. (Fig. 9) exposed to 8ppm CuSO₄ after 90 days showing extensive hyperplasia of interlamellar cells (HIC), club-like lamellae tips (CLT), fusion of secondary lamellae (FSL), epithelial lifting, (EL), [X280].

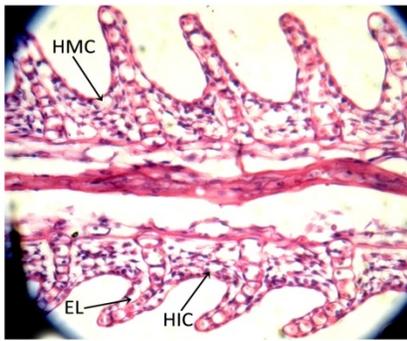


Fig.10

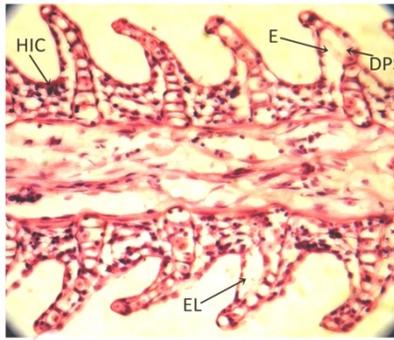


Fig. 11

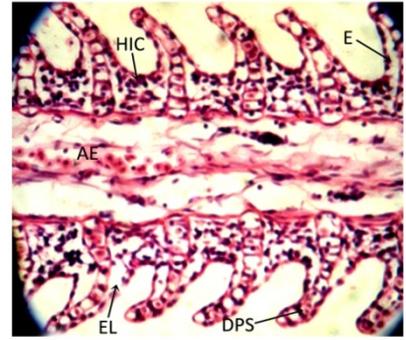


Fig.12

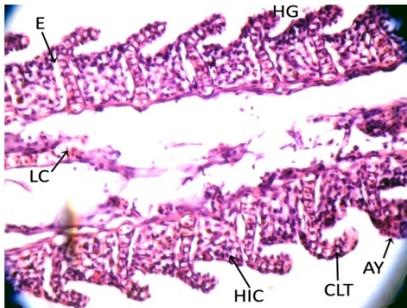


Fig.13

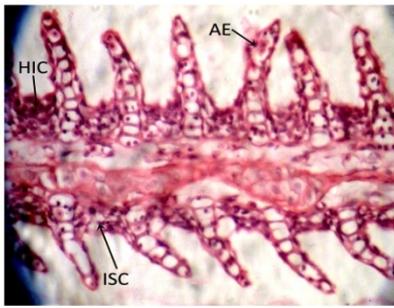


Fig. 14

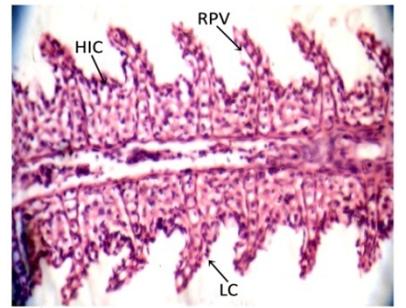


Fig.15

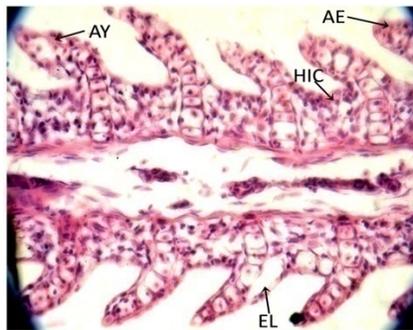


Fig.16

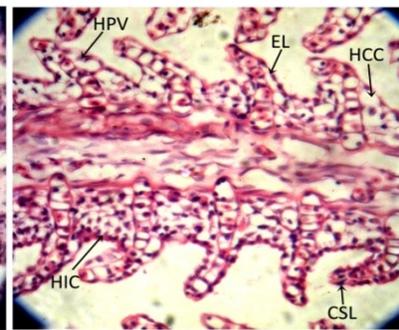


Fig.17

Plate II- Histology of *Clarias gariepinus* gill in groups IV and V exposed copper sulphate and 5ppm taurine (Fig. 10-17). (Fig 10) exposed to 4ppm CuSO₄ and 5ppm taurine after 15 days showing hypertrophy of mucous cells (HMC), mild lamellar epithelial lifting (EL), [X280]. (Fig. 11) exposed to 4ppm CuSO₄ and 5ppm taurine after 30 days showing lamellar epithelial lifting (EL), edema (E), hyperplasia of interlamellar cells (HIC), disintegration of pillar cell system (DPS), [X280]. (Fig. 12) exposed to 4ppm CuSO₄ and 5ppm taurine after 60 days showing epithelial lifting (EL), edema (E), disintegration of pillar cell system (DPS) [X280]. (Fig. 13) exposed to 4ppm CuSO₄ and 5ppm taurine after 90 days showing mild hyperplasia of interlamellar cells (HIC), mild haemorrhage (HG), slight haemolysis (HL), club-like lamellae tips (CLT), lamellar aneurysms (AY), [X280]. (Fig. 14) exposed to 8ppm CuSO₄ and 5ppm taurine after 15 days showing accumulation of erythrocytes (AE), mild hyperplasia of interlamellar cells (HIC), [X280]. (Fig. 15) exposed to 8ppm CuSO₄ and 5ppm taurine after 30 days showing infiltration of leucocytes (LC), hyperplasia of interlamellar cells (HIC), rupture of pavement cell layer (RPV), [X280]. (Fig. 16) exposed to 8ppm CuSO₄ and 5ppm taurine after 60 days showing mild lamellar aneurysms (AN), hyperplasia of interlamellar cells (HIC), epithelial lifting (EL), [X280]. (Fig. 17) exposed to 8ppm CuSO₄ and 5ppm

taurine after 90 days showing curling of secondary lamellae (CSL), hypertrophy of chloride cells (HCC), hypertrophy of pavement cells (HPV) [X280].

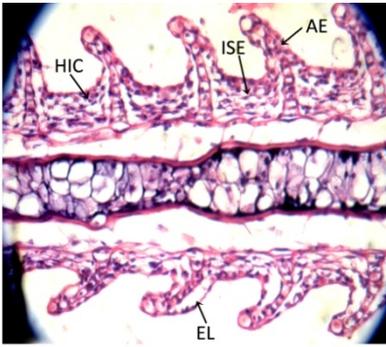


Fig. 18

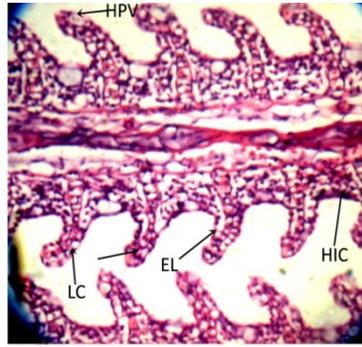


Fig.19

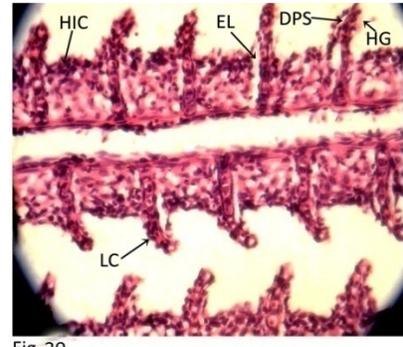


Fig. 20

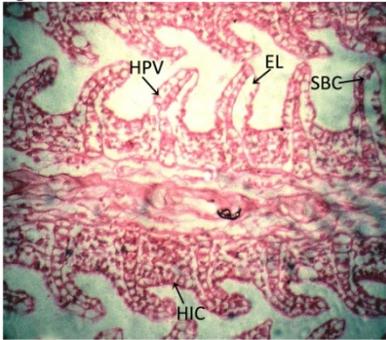


Fig. 21

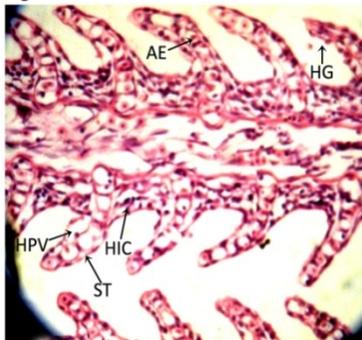


Fig. 22

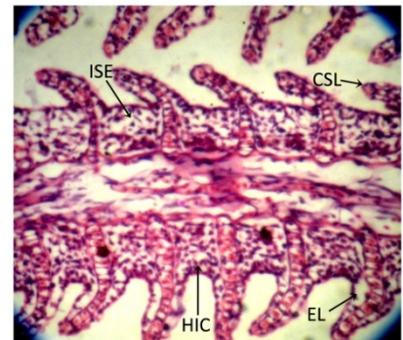


Fig. 23

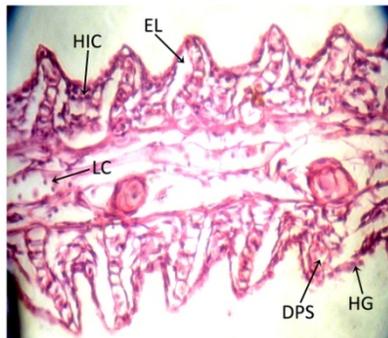


Fig. 24

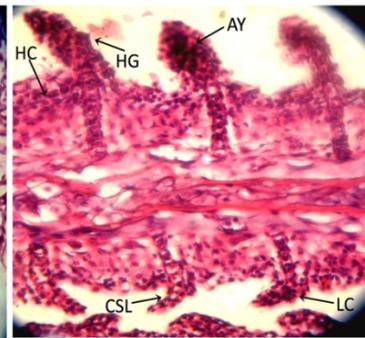


Fig. 25

Plate III- Histology of *Clarias gariepinus* gill in groups VI and VII exposed copper sulphate and 5ppm garlic extract (Fig. 18-25). (Fig. 18) exposed to 4ppm CuSO₄ and 5ppm garlic extract after 15 days showing mild epithelial lifting (EL) and mild hyperplasia of interlamellar cells (HIC), [X280]. (Fig. 19) exposed to 4ppm CuSO₄ and 5ppm garlic extract after 30 days showing mild hypertrophy of pavement cells (HPV), slight hyperplasia of interlamellar cells (HIC), [X280]. (Fig. 20) exposed to 4ppm CuSO₄ and 5ppm garlic extract after 60 days showing hyperplasia of interlamellar cells (HIC), mild haemorrhage (RG), infiltration of leucocytes (LC) [X280]. (Fig. 21) exposed to 4ppm CuSO₄ and 5ppm garlic extract after 90 days showing severe epithelial lifting (EL), hypertrophy of pavement cells (HPV), shrinkage of blood channel (SBC) [X280]. (Fig. 22) exposed to 8ppm CuSO₄ and 5ppm garlic extract after 15 days

showing slight hypertrophy of pavement cells (HPV), mild haemorrhage (RG) with few swollen secondary lamellae tips (ST), [X280]. (Fig. 23) exposed to 8ppm CuSO₄ and 5ppm garlic extract after 30 days showing interstitial edema (ISE), epithelial lifting (EL) curling of secondary lamellae (CSL), [X280]. (Fig. 24) exposed to 8ppm CuSO₄ and 5ppm garlic extract after 60 days showing severe epithelial lifting (EL), disintegration of pillar cell system (DPS), edema (E), [X280]. (Fig. 25) exposed to 8ppm CuSO₄ and 5ppm garlic extract after 90 days showing aneurysms (AY), hyperplasia of interlamellar cells (HIC), haemorrhage (HG), [X280].

with formation of aneurysm after 60 days (Fig. 16). Hyperplasia of interlamellar cells was seen after 90 days with partial fusion of adjacent lamellae. Occasionally, edema and epithelial lifting were reported at bases of lamellae (Fig. 17).

Group VI

The histopathological examination of gills after 15 days revealed slight lifting of secondary lamellae with accumulation of few blood cells in the edematous spaces in filamentary epithelium (Fig. 18). After 30 days the gill sections revealed mild histopathological changes including hypertrophy and hyperplasia of the mucous and chloride cells at the base of the secondary lamellae (Fig. 19). Degeneration of blood channel at the proximal portion and epithelial lifting in secondary lamellae were observed after 60 days. However, less frequent hyperplasia and hypertrophy of chloride and mucous cells were also seen (Fig. 20). After 90 days hypertrophy of pavement cell layer and epithelial lifting in secondary lamellae with formation of edematous spaces was reported.

Shrinkage of blood channel was observed in proximal portion of few secondary lamellae due to degeneration of pillar cells (Fig. 21).

Group VII

The gill revealed minor histopathological alterations including hypertrophy of lamellar epithelium at the base of secondary lamellae. Increased infiltration of blood cells was reported in the dilated blood sinuses of secondary lamellae and in central venous sinuses after 15 days (Fig. 22). After 30 days curling and mild epithelial lifting of secondary lamellae was observed (Fig. 23). Severe lifting of lamellar epithelium with formation edematous spaces was seen after 60 days (Fig. 24). Disorganization of blood sinuses and mild haemorrhage from secondary lamellae were found together with necrosis. After 90 days severe hyperemia, lamellar aneurysms (teleangiectasia) and haemorrhages with rupture of lamellar epithelium was seen (Fig. 25). The Semiquantitative scoring of liver lesion is shown in Table 2.

Tab. 2: Semiquantitative scoring of gill lesion in *Clarias gariepinus* treated with 4 and 8 ppm copper sulphate plus garlic extract (5 ppm) and taurine (5 ppm) for the time period of 90 days

Lesion	Control (Group I)				4 ppm CuSO ₄ (Group II)				8 ppm CuSO ₄ (Group III)				4 ppm CuSO ₄ + T (Group IV)			
	15	30	60	90	15	30	60	90	15	30	60	90	15	30	60	90
Hyperplasia	-	-	-	-	+	++	++	+++	+	++	+++	+++	-	+	+	++
Hypertrophy	-	-	-	-	++	+++	+	++	+++	+++	+	+	+	-	-	-
Epithelial lifting	-	-	-	-	+	+	+++	++	+	+	+++	+	+	+	++	+
Aneurysms	-	-	-	-	+	+	+++	++	-	+	-	++	--	-	-	+
Edema	-	-	-	-	+	+	+++	++	+	-	+++	++	-	+	+	+
Lamellae fusion	-	-	-	-	-	-	-	++	-	+++	++	+++	-	-	-	+
Lesion	8 ppm CuSO ₄ + T (Group V)				4 ppm CuSO ₄ + GE (Group VI)				8 ppm CuSO ₄ + GE (Group VII)							
	15	30	60	90	15	30	60	90	15	30	60	90				
Hyperplasia	+	++	+	+	+	+	++	+	+	++	+	+				
Hypertrophy	-	+	+	++	-	+	-	+	-	+	+	++				
Epithelial lifting	-	+	+	+	+	+	++	+	-	+	+	+				
Aneurysms	-	+	+	-	-	-	-	-	-	+	+	-				
Edema	+	-	-	++	+	-	+	++	+	-	-	++				
Lamellae fusion	-	-	-	+	-	-	-	-	-	-	-	+				

Score value: (-) None, (+) Mild, (++) Moderate, (+++) severe occurrence

Discussion

The toxicological effects of copper on fish are well documented, the variability of the reported results are large (Hodson *et al.*, 1979, Saxena *et al.*, 1982). The gills have an extensive surface area with minimum diffusion distance, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Poleksic and Mitrovic-Tutundzic, 1994, Fernandes and Mazon, 2003).

The fish exposed to 4 ppm and 8 ppm concentrations of copper sulphate in group II and III respectively after 15, 30, 60 and 90 days revealed several histopathological alterations. Moreover, the ameliorative efficacy of antioxidants was found to minimize the histopathological changes in gills on respective intervals. These gill histopathological alterations has been previously observed by several authors in fish submitted to copper (Karan, *et al.*, 1998, Vutukuru *et al.*, 2005, De Boeck *et al.*, 2001, Darwish *et al.*, 2005, Campagna *et al.*, 2008). In *Prochilodus scrofa* as a result of copper exposure hyperplasia and thickening in

the gill as well as lamellar telangiectasis was reported by (Cerqueira and Fernandes, 2002). Some pathological changes like thickening of the epithelium as well as telangiectasis was reported in the of gills of fish exposed copper sulphate (Heerden *et al.*, 2004), similarly same lesions in the gill of rainbow trout were also reported after acute exposure to 0.135 mg/l copper sulphate at 48hours (Daoust *et al.*, 1984). According to Mallatt (1985) such alterations are non-specific and may be induced by different types of contaminants.

The foregoing results of histopathological investigation of gills are in good agreement with Figueiredo-Fernandes *et al.*, (2007) who reported several histological alterations on exposure of 0.5, 1.0 and 2.5 mg/l to copper for a period of 21 days in Nile tilapia, *Oreochromis niloticus*. Similarly, histopathological changes in gills of *O. niloticus* which were positively correlated in its effects with the increase of copper concentration and time of exposure (Osman *et al.*, 2009). Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals (Mallatt, 1985). The results of this study confirm the occurrence of edema independently of copper levels, as in other fish species (Sola *et al.*, 1995, Bury *et al.*, 1998). The lifting of lamellar epithelium is other histological change observed, probably induced by the incidence of severe edema (Arellano *et al.*, 1999, Pane *et al.*, 2004, Schwaiger *et al.*,

1996). According to (Garcia-Santos *et al.*, 2006) this lesion can induce changes in pillar cell normal structure, with consequent loss of their support function and probably, and was responsible for the emergence of lamellar aneurysms in fish exposed to cadmium.

Edema with lifting of lamellar epithelium could serve as a mechanism of defense, because separation of epithelia of the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream (Arellano *et al.*, 1999). As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003). The compensatory changes may become maladaptive if the duration of the stress factor(s) exceeds the biological tolerance limits (Wedemeyer *et al.*, 1990). According to Pneuranen *et al.*, (1996) any discontinuity of epithelial lining of the gill due to massive wear and tear may lead to a negative ion balance and to changes in haematocrit and mean cellular haemoglobin values of blood. The edematous spaces, along with hypertrophied epithelial lining, results in inadequate gas exchange and consequently a reduced diffusion capacity, although they have created an additional barrier for prevention of penetration of waterborne xenobiotics.

Most part of the gill lesions caused by

sublethal exposures affects lamellar epithelium however, some alterations in blood vessels may also occur, when fishes suffer a more severe type of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Rosety-Rodriguez *et al.*, 2002). The formation of an aneurysm is related to the loss of adhesion between the epithelial cells and underlying pillar cell system accompanied by a collapse of structural entity of secondary lamella (Martinez *et al.*, 2004) due to a larger quantities of blood flow that push the lamellar epithelium outward (Alazemi *et al.*, 1996) or even because of the direct effects of copper (Fernandes and Mazon, 2003, Wani *et al.*, 2011) on these cells. The teleangiectasia may affect blood circulation leading to respiratory impairment. Evaluating the effects of the copper ion on *P. scrofa* juveniles during 96h of exposure (Mazon *et al.*, 2002) found aneurysms in the secondary lamellae of specimens exposed to 20.0, 25.0 and 29.0 $\mu\text{gCu/l}$, and in some cases could observe rupture of the secondary lamellae and bleeding. These results corroborate those of the present study, where copper caused haemolysis and haemorrhage at high concentrations. It is the condition in which blood congests in the gill, due to the presence of metabolites and an overall change. The blood vessels near the injury site dilate, the permeability of the capillary walls increases, which produces an

exudation of the fluid which leads to a congestion of the blood cells in these vessels. The blood-derived exudate can enter nearby epithelia (Roberts, 1978).

Due to continuation of sublethal copper sulphate exposure in present study, uncontrolled degeneration of gills takes place and pavement cells become haphazardly arranged. Consequently, the space between the neighbouring secondary lamellae was almost entirely filled with polygonal epithelium and gill filaments appeared a solid mass of cells. Accumulation of cellular debris on the gill lamella was one of the histopathologic findings. Contact of fish gill with copper sulphate can cause excess mucus secretion and because of substantial net negative charge of gill surface, gill have a high affinity for cationic metals. Therefore, the accumulation of superficial debris may be a result of precipitation of copper ions in mucus secretions (Peyghan *et al.*, 2007). Hypertrophy and proliferation of mucous cells was also reported in present investigation in the fused surface of the secondary lamellae. This may be considered as a protective response to carry out the transport of toxins. Cell proliferation with thickening of gill filament epithelium is one histological change found in fish exposed to copper by several authors (Mourad and Wahby, 1999, Van Heerden *et al.*, 2004).

During the present investigation, the number of the chloride cells in the epithelial

linings of *C. gariepinus* increased significantly following exposure to copper sulphate solution. Proliferation of chloride cells are thought to be compensatory response to ion loss and was observed following exposure to water-borne copper in by Peyghan *et al.*, (2007) in *Ctenopharyngodon idella*, and therefore chloride cell hyperplasia may therefore be good biomarker of adaptation (Hinton *et al.*, 1992). The chloride cell hyperplasia in the lamellae following exposure to heavy metal salts have also been observed by Thophon *et al.*, (2003), Parashar and Banerjee, (2002). The pathological changes in the chloride cells on exposure to heavy metals may indicate osmoregulatory dysfunction, which is the main function of the chloride cells (Virtanen, 1986). Chloride cells proliferation may be due to an added function of oxygen transport due to injury to gill tissue proper. Leukocytes infiltration caused their accumulation in the subepithelial spaces of secondary lamellae and necrotic gill tissues. This may be an inflammatory reaction response to copper (El-Feki, 1998) or to phagocyte the copper particles and tissue debris (Muhvich *et al.*, 1995).

Taurine supplementation through water at 5 ppm in groups IV and V seems to be beneficial upto some extent to alleviate copper sulphate induced toxicity in tissue damage in gill as observed in microscopic changes in the present study. The lesions were less severe in gills when compared to those observed in the fish from

group II and V. The beneficial effects could be attributed its zwitterionic nature and concur to those of Huxtable (1992). However, fish do contain high concentration of taurine (Divakaran, 2006) and the extra-supplementation might have protected the gills to alleviate Cu toxicity. The pretreatment with taurine after cyclophosphamide injection produced a significant decrease in urinary bladder weight (edema) and a marked decrease in vascular congestion and haemorrhage, as well as a profound improvement in histological structure (Abd-Allah *et al.*, 2004).

Mild degeneration of chloride cells found in taurine treated fishes might be due an antioxidant property of taurine to maintain membrane organization and thus prevents ion leakage and water influx, and subsequently, avoid cell swelling or hypertrophy (Trachtman, 1992; Chen, 1993). The stabilizing effect of taurine on cellular membrane has been suggested to be associated with the interaction between taurine and polyunsaturated fatty acids in the membrane (Birdsall, 1998). It has been suggested that it may bind copper (Hwang *et al.*, 1998) and cadmium (Hwang and Wang, 2001), forming a complex that is readily excreted in faeces. In other words, it may act by reducing the overall bioavailability of absorbed copper. This property of taurine may also partly account for its protection against copper induced gill necrosis.

Addition of garlic extract at 5 ppm to group

VI and VII found to be effective to reduce copper sulphate induced histopathological changes in gills. Occurrence of mild hyperplasia of interlamellar cells and less frequent hypertrophy of chloride and mucous cells explicates that garlic extract might be involved in the alleviation of copper toxicity due to its antidotal and immunomodulatory activities Agarwal *et al.*, 1996; Banerjee *et al.*, 2003) and presence of selenium (Ingrid and Jacques, 2006). This finding concur to those of Metwally and Hashem, (2009) who noted that cadmium induced histopathological alterations in rats were significantly alleviated by garlic administration and attributed this protective role of garlic to its potential to chelate metal and enhance the antioxidant defense system. Similarly, Harisa *et al.*, (2009) used aqueous garlic extract to ameliorate the histopathological changes induced by acetic acid in colon of rats. Bioaccumulation of copper was significantly ($P < 0.01$) decreased in tissues of *Oreochromis niloticus* after treatment with *Allium sativum* (Metwally, 2009). Thus, it can be suggested that garlic diminished copper induced histopathological changes observed in present study by decreasing its availability and bioaccumulation in the liver of *Clarias gariepinus*.

It could be concluded that the intoxication by copper resulted severe histopathological changes in the gills of *Clarias gariepinus*. Garlic and taurine supplementation counteracted

these toxic effects partly and bringing structural improvement in gills. This could be due to their cytoprotective properties and antioxidant nature, which combines free radical scavenging with metal chelating properties.

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