Hepatoprotective role of taurine and garlic extract in minimizing the histopathological changes in liver induced by long-term exposure to copper sulphate in *Clarias gariepinus*

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Abstract

The present study was undertaken to elucidate whether sulphur containing antioxidants such as taurine and garlic extract could ameliorate copper sulphate induced histopathological alterations in liver of African catfish, *Clarias gariepinus*. To carry out this investigation, experiment was setup in seven groups (I to VII) containing 20 fishes in each group. The histopathological investigation of liver of fishes subjected to copper sulphate at 4 ppm and 8 ppm levels and sampled at 15th, 30th, 60th and 90th day revealed, severe alterations in dose and time dependent manner. Moreover, addition of 5 ppm each of taurine and garlic extract was found partly to minimize the histopathological changes in liver throughout the course of experimental duration. In conclusion, it appears that taurine and garlic extracts have beneficial effect in protecting against copper sulphate induced hepatotoxicity to some extent by minimized histopathological changes. So, taurine and garlic extracts have a promising role and these are worth to be considered as natural antidotes for copper intoxication.

Key words: Taurine, Garlic extract, Copper sulphate, Liver, Histopathology, *Clarias gariepinus*

Introduction

Copper is non-biodegradable but easily assimilated and bioaccumulated (1). Copper is an essential element for the entire biota. The lack of this nutrient can trigger enzymatic dysfunctions (2), but it can also be toxic to fish when present at high concentrations in the water (2). Copper concentration rarely exceeds 50 nmol$^{-1}$ in natural water, but in polluted water, the level surpass 1 µmol$^{-1}$ and makes it toxic (4). Copper is quite toxic to *Clarias gariepinus* (5) and is also reported to reduce the reproductive potential of many species of fish (6).

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 (7). When fish gets exposed to any heavy metals there is every possibility that they may accumulate in various organs, resulting in the generation of free radicals thereby causing oxidative stress. 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 in urine or faeces.

Taurine is sulphur containing conditionally essential 6-amino acid found especially in tissues that are excitable, rich in membranes and generates oxidants (8). The sulfonate group in taurine is a strong acid that makes it completely zwitterionic over the physiological pH range (9). Taurine is known to maintain calcium homeostasis, osmoregulation, removal of hypochlorous acid, and stabilizing the membranes (9). Taurine is reported to prevent the bioaccumulation of cadmium in the tissues of goldfish, *Carassius auratus* (10). Taurine was also shown to form less stable metal complexes with various transition metals such as Cu$^{+2}$, Ni$^{+2}$, Zn$^{+2}$, Fe$^{+2}$, Mg$^{+2}$, Mn$^{+2}$, compared to other amino acids (11).

Garlic and garlic extracts, used for millennia in folk medicine is still a mainstay for various torments. It contains at least 33 sulphur compounds, several enzymes, 17 amino acids, and minerals such as selenium (12). The consequence of synergism between various compounds is responsible for the antioxidant activity of garlic. One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut. The injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin (S-allylcysteine sulfoxide) to allicin (13).

Taurine is having tremendous antioxidant property which exerts actions by scavenging ROS (14). Its potential as a chelating agent against lead poisoning has been reported by Flora et al (15). 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* (16). The aim of the present study is to evaluate the protective role of supplementation of taurine and garlic extract in minimizing the histopathological changes 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 from neighbouring country Bangladesh (17). Alive, healthy and disease free specimens of catfish, *Clarias gariepinus* of either sex belonging to a single population were purchased on order from the local fish market of Sagar (M.P). The specimens were transported in plastic containers filled with oxygenated and cool water to reduce their activity and stress before reaching the fish laboratory. Before introducing those in the aquariums, fishes were treated with 0.01% KMnO$_4$ solution for 15 minutes to obviate any dermal infection. Fishes were then kept for a period of fifteen days for acclimatization in laboratory conditions. Faecal remains and food residues were removed by net every other day.

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 (WebChem®), taurine (Hi-Media Laboratories, Delhi India) and garlic extract during the entire experiment period of 90 days (Table 1). Dose selection and mode of administration of garlic extract and taurine was based on published studies (18). All the fish were fed with commercially available fish pellet feed (Tokyo®, Japan) throughout the experiment.

**Table 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.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Copper Sulphate</th>
<th>Garlic Extract</th>
<th>Taurine</th>
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<tbody>
<tr>
<td>I</td>
<td>Control</td>
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<tr>
<td>II</td>
<td>4 ppm</td>
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<td>III</td>
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<td>IV</td>
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<td>V</td>
<td>8 ppm</td>
<td>-</td>
<td>5 ppm</td>
</tr>
<tr>
<td>VI</td>
<td>4 ppm</td>
<td>5 ppm</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>8 ppm</td>
<td>5 ppm</td>
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**Preparation of copper stock solution**

Dilution of copper sulphate (CuSO$_4$) 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.
Results and Discussion

Histopathological observations of liver

Group I (Control): The microscopic examination of liver of Clarias gariepinus revealed the normal histological details (Fig. 1 and 2) and did not show any pathological lesions in any fish of control group throughout the experiment. The liver consisted of typical parenchymatous polyhedral hepatic cells, known as hepatocytes. Each hepatocyte contains homogeneous cytoplasm around the distinct centrally or subcentrally located spherical nucleus with deeply stained chromatin. The nucleolus in nucleus was densely stained. The hepatocytes were radially arranged in cords around a central sinusoid. The sinusoids were observed in continuous communication and they were reported to converge into the central vein. The wall of the sinuses was seen to contain Kupffer cells.

Group II (4ppm CuSO₄): The liver sections of fishes after 15 days revealed the presence of dilated sinusoidal space with mild infiltration of macrophages. There were vacuoles within the cytoplasm resulting in cell membrane degeneration of few hepatocytes (Fig. 3). After 30 days, hepatocyte degeneration and necrosis were observed at few places with severe infiltration of macrophages in the hepatic tissue. Hypertrophy of few hepatocytes around the engorged and ruptured blood vessel was observed. Cell membrane of the hepatocytes was ruptured and showing syntial appearance at few places (Fig. 4). After prolonged exposures (60 and 90 days) hepatic tissue revealed more frequent alteration including extensive sinusoids dilation. Some Kupffer cells were pushed into the sinusoidal lumens. Cells were swollen, the cell outline become indistinguishable. The cytoplasm of some hepatocytes showed a higher density of granules (Fig. 5). After 90 days, hypertrophy of hepatocytes and slight infiltration of leucocytes were observed. Vacular degeneration of cytoplasm was more prominent, vacuoles being either large or small. The hepatic cells exhibited nuclear degeneration (karyolysis) and pyknosis (Fig. 6).

Group III (8ppm CuSO₄): The liver parenchyma cells after 15 days revealed severe nuclear hypertrophy with irregular in shape. Accumulation of golden-brown granules (bile stagnation) and formation of large necrotic spots was seen in certain areas of hepatic tissue. Dilation of sinusoids was reported with infiltration of macrophages (Fig. 7). Severe dilation of sinusoid was observed after 30 days with infiltration of mononuclear leucocytes. The hepatocytes showed extensive cytoplasmic vacuolation and few exhibited atrophy of nuclei (karyolysis) (Fig. 8). There were more severe changes in the hepatic parenchyma after 60 days and exhibited engorged and congested vasculature with degenerative changes in the hepatocytes around blood vessels (Fig. 9). More severe histoarchitectural deformities were seen after 90 days including extensively dilated central veins with thickened walls and sinusoidal. Degeneration of hepatocytes around the ruptured blood vessel was reported. Haemorrhage and haemolysis was also reported due to rupture of blood vessels (Fig. 10).

Group IV (4 ppm CuSO₄ and 5 ppm taurine): The hepatic...
tissue after 15 days exhibited normal histoarchitecture with polygonal hepatocytes. However, mild cytoplasmic vacuolation and infiltration of few mononuclear leucocytes was reported occasionally (Fig. 11). After 30 days, mild cytoplasmic vacuolation and increase in number of Kupffer cells within the sinusoids was reported. In hepatocytes, the granularity of cytoplasm was seen increased with bile stagnation (Fig. 12). The histopathology of liver after 60 days showed dilated and congested central blood vessel with thickened walls. Occasionally, there were few hepatocytes with pyknotic nuclei, bile stagnation and mild cytoplasmic vacuolation (Fig. 13). After 90 days, degeneration of few hepatocytes was observed due to hypertrophy and cytoplasmic vacuolation (Fig. 14).

**Group V (8 ppm CuSO₄ and 5 ppm taurine):** The histopathology after 15 days revealed less severe congested and dilated blood vessels. Occasionally, there were some hepatocytes with irregular, eccentric and pyknotic nuclei (Fig. 15). Necrosis and cytoplasmic vacuolation was reported in hepatocytes around the congested blood vessel after 30 days (Fig. 16). The hepatic tissue after 60 days revealed mild necrosis of hepatic cells due to hypertrophy of hepatocytes. There was severe infiltration of mononuclear leucocytes in the slightly dilated sinusoids (Fig. 17). The liver parenchyma revealed severe dilation of blood vessels with haemolysis after 90 days. Aggregation of macrophages was also observed near the ruptured blood vessel. Necrosis and cytoplasmic vacuolation of hepatocytes was clearly seen (Fig. 18).

**Group VI (4 ppm CuSO₄ and 5 ppm garlic extract):** The liver parenchyma exhibited normal appearance with deeply stained hepatocytic cytoplasm and nucleus after 15 days. The histopathological changes were less severe with few irregular shaped hepatocytic nuclei, mild cytoplasmic vacuolation and infiltration of mononuclear cells (Fig. 19). After 30 days, liver sections revealed centrally located nuclei with the presence of minor cytoplasmic vacuoles. The nuclei were normal in shape, however, few pyknotic nuclei were also observed. Increased infiltration of leucocytes was reported in dilated sinusoidal lumen. Occasionally, necrosis and accumulation of yellow-brownish pigment was also observed in hepatocytes (Fig. 20). The histopathology of liver after 60 revealed presence of mild cytoplasmic vacuolation and slight widening of sinusoids. Atrophy of nucleus was reported in few hepatocytes while majority of hepatic cells contain condensed and slightly eccentrically located nucleus (Fig. 21). After 90 days hepatic parenchyma revealed slight disorganization of cells with certain areas exhibit degeneration and mild cytoplasmic vacuolation. Diffused infiltration of leucocytes was observed within the sinusoids and throughout the hepatic tissue (Fig. 22).

**Group VII (8 ppm CuSO₄ and 5 ppm garlic extract):** The histopathology of liver after 15 days revealed mild cytoplasmic vacuolation in combination with nuclear pycnosis. Slight rupture of blood vessel with mild intravascular haemolysis and damage of few hepatic cells around blood vessel was observed (Fig. 23). After 30 days, cytoplasm of hepatocytes was mostly occupied by large vacuoles (Fig. 24). The hepatic tissue revealed mild cytoplasmic vacuolar degeneration of hepatocytes with extensive pyknotic nuclei after 60 days. Mild intravascular haemolysis was reported within slightly engorged blood vessels (Fig. 25). Moderate damage in hepatic tissue was seen with syncytial appearance due to ruptured cell membranes of hepatocytes after 90 days. The semiquantitative scoring of liver lesion is shown in Table 2. Aggregation of macrophages and bile stagnation was also observed in hepatocytes (Fig. 26).

The liver of teleosts is the major site of the cytochrome P450-mediated, mixed function oxidase system (21). This system inactivates some xenobiotics, while activating others to their toxic forms. Liver histology has been proven to be indicative of exposure to pollution (22). The results of histopathological investigation of liver of fish, *Clarias gariepinus* subjected to copper sulphate exposure at 4 ppm and 8 ppm levels and sampled at 15th, 30th, 60th and 90th day revealed severe histopathological alterations. Moreover, the ameliorative potential of taurine and garlic extract found partly to minimize the histopathological changes in liver throughout the course of experimental duration. Occurrence of normal histological structure in the liver of fish of control group I in comparison to copper sulphate exposed group II and group III, envisages that histopathological changes in liver were dose and time dependent. In present study, the histopathological changes observed in the liver were mainly represented by sinusoidal dilation with blood congestion, swelling of hepatocytes, cytoplasmic vacuolization and granulation of the hepatocytes, congestion of blood vessels, haemolysis and haemorrhage, nuclear atrophy and pycnosis, bile stagnation, leucocytic infiltration and focal necrosis. The results of this study were similar to that of Figueiredo-Fernandes et
Plate I: Histology of *C. gariepinus* Liver in control. **Fig. 1 and 2:** copper sulphate exposed groups II and III (3-10). **Fig. 1 and 2:** Normal hepatocytes (HP), sinusoids (SN), blood vessel (BV), nucleus (N) Kupffer cells (KF), X100, X400, respectively. **Fig. 3:** exposed to 4 ppm CuSO$_4$ after 15 days showing necrosis of hepatocytes (NC), cell membrane degeneration of hepatocytes (CMD), infiltration of macrophages (MCP), dilation of sinusoids (DSN), cytoplasmic vacuolation, [X400]. **Fig. 4:** exposed to 4 ppm CuSO$_4$ after 30 days showing haemolysis (HL), haemorrhage (HG), ruptured blood vessel (RBV), hypertrophy of hepatocytes (HHP), [X400]. **Fig. 5:** exposed to 4 ppm CuSO$_4$ after 60 days showing dense cytoplasmic granules (CG), dilation of sinusoids (DS), pycnotic nuclei (PN), [X400]. **Fig. 6:** exposed to CuSO$_4$ after 90 days showing ruptured blood vessel (RBV), atrophy of nucleus (AN), hypertrophy of hepatocytes (HHC) [X400]. **Fig. 7:** exposed to 8 ppm CuSO$_4$ after 15 days infiltration of macrophages (MCP), hypertrophy of nucleus (HN), necrosis (NC), bile stagnation (BS), increased in number of Kupffer cells (KF) [X400]. **Fig. 8:** exposed to 8 ppm CuSO$_4$ after 30 days showing extensive cytoplasmic vacuolation (CV), extensive dilation of sinusoid (DSN), atrophy of nuclei (AN), infiltration of leucocytes (LC) focal necrosis (NC), [X400]. **Fig. 9:** exposed to 8 ppm CuSO$_4$ after 60 days showing hypertrophy of hepatocytes (HHC), cytoplasmic vacuolation (CV), dilated blood vessel (DBV), haemolysis (HL), eccentric nuclei (EN), [X400]. **Fig. 10:** exposed to 8 ppm CuSO$_4$ after 90 days showing extensively dilated blood vessel (DBV), necrosis (NC), haemolysis (HL), dilation of sinusoid (DSN) and haemorrhage (HG) [X400].
Plate II: Histology of *C. gariepinus* liver in groups IV and V exposed copper sulphate and 5ppm taurine (12-19). **Fig. 11:** exposed to 4 ppm CuSO₄ and 5 ppm taurine after 15 days showing mild cytoplasmic vacuolation (CV), infiltration of leucocytes (LC), pycnotic nuclei (PN), [X400]. **Fig. 12:** exposed to 4 ppm CuSO₄ and 5 ppm taurine after 30 days showing necrosis of few hepatocytes (NC), bile stagnation (BS), infiltration of leucocytes (LC), increase in number of Kupfer cells (KF), [X280]. **Fig. 13:** exposed to 4 ppm CuSO₄ and 5 ppm taurine after 60 days showing mild cytoplasmic vacuolation (CV), bile stagnation (BS), dilated blood vessel (DBV), [X400]. **Fig. 14:** exposed to 4 ppm CuSO₄ 5 ppm and taurine after 90 days showing mild infiltration of leucocytes (LC), dilated sinusoids (DSN), disorganization of hepatocytes (DOH), hypertrophy of hepatocytes (HHP), [X400]. **Fig. 15:** exposed to 8 ppm CuSO₄ 5 ppm & taurine after 15 days showing pycnotic nuclei (PN), slight hypertrophy of hepatocytes (HHP), [X400]. **Fig. 16:** exposed to 8 ppm CuSO₄ and 5 ppm taurine after 30 days showing mild cytoplasmic vacuolation (CV), slight necrosis of hepatocytes (NC), few pycnotic nuclei (PN), haemorrhage (HG), [X400]. **Fig. 17:** exposed to 8 ppm CuSO₄ and 5 ppm taurine after 60 days showing hypertrophy of hepatocytes (HHP), cytoplasmic vacuolation (CV), infiltration of leucocytes (LC), [X400]. **Fig. 18:** exposed to 8 ppm CuSO₄ and 5 ppm taurine after 90 days showing dilated blood vessel (DBV), haemolysis (HL), pycnotic nuclei (PN), aggregation of macrophages (AM), [X400]
Plate III: Histology of *C. gariepinus* liver in groups VI and VII exposed to copper sulphate and 5 ppm garlic extract (19-26). **Fig. 19:** exposed to 4 ppm CuSO$_4$ & 5ppm garlic extract after 15 days showing mild cytoplasmic vacuolation (CV), irregular nuclei (IN) infiltration of few leucocytes (LC), [X400]. **Fig. 20:** exposed to 4 ppm CuSO$_4$ and 5 ppm garlic extract after 30 days showing mild bile stagnation (BS), few pyknotic nuclei (PN), infiltration of leucocytes (LC), increased number of Kupfer cells (KF), [X400]. **Fig. 21:** exposed to 4 ppm CuSO$_4$ and 5 ppm garlic extract after 60 days showing dilated sinusoids (DSN), atrophy of nucleus (AN), cytoplasmic vacuolation (CV), [X400]. **Fig. 22:** exposed to 4 ppm CuSO$_4$ and 5 ppm garlic extract after 90 days showing infiltration of leucocytes (LC), pyknotic nuclei (PN), deposition of dense cytoplasmic granules (CG) cytoplasmic vacuolation (CV), [X400]. **Fig. 23:** exposed to 8 ppm CuSO$_4$ and 5 ppm garlic extract after 15 days showing few damaged hepatocytes (DHC), mild cytoplasmic vacuolation (CV), pyknotic nuclei (PN), infiltration of few leucocytes (LC). [X400]. **Fig. 24:** exposed to 8 ppm CuSO$_4$ and 5 ppm garlic extract after 30 days showing hypertrophy of hepatocytes (HHP), mild focal necrosis (NC), disorganization of hepatocytes (DOH), eccentric nuclei (EN), irregular nuclei (IR), [X400]. **Fig. 25:** exposed to 8ppm CuSO$_4$ & 5ppm garlic extract after 60 days showing pycnotic nuclei (PN), slightly dilated sinusoids (DSN), mild haemolysis (HL) pyknotic nuclei (PN), [H. and E. X400]. **Fig. 26:** exposed to 8 ppm CuSO$_4$ and 5 ppm garlic extract after 90 days showing slight disorganization of hepatocytes (DOH), bile stagnation (BS), cytoplasmic vacuolation (CV) aggregation of macrophages (AM), [X400].

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The present work showed that the atrophy of hepatocytes nuclei in hepatic tissue increases with the copper concentration. Alterations in the size of nucleus have been previously reported by Paris-Palacios et al. (30) in Brachydanio rerio exposed to sublethal concentrations of copper sulphate. Alterations in size and shape of nucleus have often been regarded as signs of increased metabolic activity but may be of pathological origin (31). Foregoing studies revealed bile stagnation in liver of copper exposed fish which might be due to the metabolic problems. This lesion is characterized by the remains of the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes (32), indicates that the bile is not being released from the liver. This accumulation of bile indicates possible damage to the hepatic metabolism (33).

Our results indicate that copper promotes the invasion of leucocytes in the liver of fish followed by the development of necrosis and cellular vacuolization, as a response of the body to presence of damaged tissue. Leucocyte infiltration with increased number of melano-macrophage centers and necrotic areas were seen by Mela and co-workers (34) in liver of Hoplias malabaricus on exposure to methylmercury. In general, the presence of necrotic areas in liver is related to an increase in leucocyte infiltration and formation of MMCs (35), which was also observed in this study. The function of the melanomacrophages in the liver of fishes remains uncertain, but some studies have suggested that it is related to destruction, detoxification or recycling of endogenous and exogenous compounds (36). According to Rabitto et al. (37) the occurrence of melano-macrophage centers increase with the levels of lesions in liver of R. quelen, so the presence of MMCs can be considered as a representative change after exposure to copper, signalizing to cell and tissue degeneration and tissue failure.

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

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Group I Control</th>
<th>Group II 4 ppm CuSO&lt;sub&gt;4&lt;/sub&gt;</th>
<th>Group III 8 ppm CuSO&lt;sub&gt;4&lt;/sub&gt;</th>
<th>Group IV 4 ppm CuSO&lt;sub&gt;4&lt;/sub&gt; + T</th>
<th>Group V 8 ppm CuSO&lt;sub&gt;4&lt;/sub&gt; + T</th>
<th>Group VI 4 ppm CuSO&lt;sub&gt;4&lt;/sub&gt; + GE</th>
<th>Group VII 8 ppm CuSO&lt;sub&gt;4&lt;/sub&gt; + GE</th>
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<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Vacuolation</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>***</td>
<td>***</td>
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<td>***</td>
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<tr>
<td>Pyknotic nuclei</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
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<tr>
<td>Necrosis</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sinusoid dilation</td>
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</tr>
<tr>
<td>Haemorrhage</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>BV rupture</td>
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<td>Bile stagnation</td>
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Score value (-) None, (+) Mild, (++) Moderate, (+++) severe occurrence
Hepatocytes with irregular shaped and eccentrically situated nuclei and necrosis of hepatic tissue was reported by Arellano et al (38) in the liver of Solea senegalensis exposed to 0.5 mg/l copper sulphate for 45 days. Moreover, it was also reported by several studies that chronic copper accumulation in the liver of fish causes necrosis of hepatic tissue, cirrhosis and ultimately death (39, 40). Hypertrophy of hepatocytes is a closely related condition that occurs under chronic toxicity (41). According to Gingerich (42) the vacuolization of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in hepatocytes. In addition, (34) suggested that the increase of cytoplasmic vacuolization in liver of fish may result from disturbances in lipid metabolism or in cytoskeleton structure. According to Roncero et al (43) the accumulation of haemoglobinemic pigment produced by intense haemolysis in the cytoplasm of hepatocytes and culminated in the massive necrosis of liver parenchyma on exposure of copper sulphate to Tinca tinca.

The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or dilation of blood vessels (44). The liver of the copper exposed fish in present study revealed severely cytoplasmic vacuolated cells which is an indication of fatty degeneration of hepatocytes. Cellular necrosis as observed in this work probably resulted from excessive work required by the fish to get rid of the copper from its body during the process of detoxification. Ultrastructural changes in hepatocytes on exposure to copper in butterfish, Poronotus triacanthus was studied by Jiraungkoorskul et al (45) and reported extensive proliferation of the smooth endoplasmic reticulum and dilation of the rough endoplasmic reticulum, suggesting an active detoxification attempt by the liver.

Alterations of liver such as those described for C. gariepinus in this work might not be related only to copper exposition and should not be considered a specific biomarker of copper exposition. It is reported that absorbed Cu especially accumulates in lysosomes of cells. Excessive metal accumulation in lysosomes disrupts the normal process of lysosomal biogenesis causing impairment of this essential cellular system (46). Again, degenerative changes occur when Cu accumulates in nucleus (47). Vascular disorders were revealed in histopathology of liver in current study due to toxic effect of copper sulphate. Increased arterial flow also leads to sinusoidal dilatation (48). The toxic impact of Cu on fish induced histo-cytological perturbations and led to physiometabolic dysfunction in the fish liver hepatocytes with histopathological alterations (30).

Histopathological examinations indicated that administration of taurine (5 ppm) exhibited an obvious preventive effect against the hepatic tissue damages induced by copper in groups IV and V throughout the course of experiment. Therefore, the hepatoprotective role of taurine against copper toxicity could be attributed to its potential of scavenging the oxygen free radicals and elevating the level of antioxidant enzymes (49) or by stabilizing the biomembranes (50).

The results of present investigation support the views, in this regard, forwarded by Hassan and co-workers (51) who reported histopathological changes in the rats treated with CCl₄ alone, which were severe than those seen in the animals treated with CCl₄ plus taurine. In addition, (52) stated that 200 mg/kg of taurine significantly attenuated acetaminophen induced liver injury and prevented hepatic DNA fragmentation and hepatocyte necrosis. Supplementation of taurine is known to protect rats from acute ozone induced inflammation and hyperplasia (53). Ameliorative potential of taurine was also studied by several authors (54, 55) in alleviating the severe degenerative changes in liver of rats induced by chemotherapeutics.

Administration of taurine before monosodium glutamate (therapeutically) is reported to restore the hepatic injury, evidenced from reduction of hepatocyte necrosis with slight hydropic degeneration of few hepatocytes (56). In the case of hepatic fibrosis, taurine mitigates the liver injury, decreases the expression of transforming growth factor beta-1 and relieves hepatic fibrosis (57). In addition, it is effective in the prevention of lipopolysaccharide induced hepatotoxicity and pro-oxidant status (58), reduces oxidative stress and prevents progression of hepatic fibrosis in CCl₄ induced hepatic damage in rats as well as inhibits transformation of the hepatic stellate cells (59), and plays a beneficial role for the prevention of cisplatin hepatotoxicity (60).

Addition of garlic extract at 5 ppm to group VI and VII produced protective effects on histological structure of the liver against copper toxicity. Garlic extract was able to diminish
necrosis, congestion, and hepatocyte vacuolation with less congested and dilated vasculature in hepatic tissue. The efficiency of garlic was perhaps due to the presence of the sulfur-containing amino acids and compounds having free carboxyl (C=O) and amino (NH₂) groups in their structures. These biologically active compounds might have chelated Cu²⁺ (61) and enhanced its excretion from the body, resulting in least toxic effect of copper on hepatic tissue.

The present observations get confirmation from the studies of Sharma et al (62) who revealed improvement in liver histopathology on treatment with high dose of garlic (250 mg/l) in lead nitrate exposed mice. Similarly, Khan et al (63) found slight haemorrhage in mice on co-administration of garlic and vitamin B-complex along with lead acetate. Co-administration of garlic with cadmium remarkably alleviated the lesions, induced by Cd (64). Bioaccumulation of copper was significantly (P<0.01) decreased in liver tissues of Oreochromis niloticus after treatment with Allium sativum (65). 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. Lead acetate was found to induce cellular damage by (66) and disruption of cytoarchitectural features were at their minimum on pretreatment with 50 mg/kg/day of aqueous garlic extract in rats. Similarly, Abdel-Naim and co-workers (67) observed remarkable hepatoprotective effect of garlic oil against ethanol and carbon tetrachloride induced histopathological changes in rats.

However, high doses of garlic have been reported by several authors to cause mild histopathological changes in liver of rats (68) and fish, Chrysichthys auratus (69). Also, (70) found that garlic in low doses has the potential to enhance the endogenous antioxidant status, while as at higher doses (1000 mg/kg/day) the reverse of this effect is observed with marked histopathological and ultrastructural changes in liver. However, Sumiyoshi and co-workers (71) found no toxicity symptoms in rats due to much higher dietary garlic extract concentration at 2 g/kg 5 times/week for 5 months.

Our study showed that taurine and garlic extract were effective in protecting copper induced hepatic damage, however further investigations are required to elaborate and understand the way taurine and garlic extract operates to prevent hepatotoxic effect of copper sulphate especially in fishes.

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