

International Journal of Pharmacy and Biological Sciences ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online) IJPBS | Volume 8 | Issue 1 | JAN-MAR | 2018 | 01-12



Research Article | Biological Sciences | Open Access | MCI Approved | ज्ञान-विज्ञान विमुक्तये

UGC Approved Journal

# **NEPHROPROTECTIVE ACTION OF TAURINE AND** GARLIC EXTRACT AGAINST COPPER SULPHATE **INDUCED RENAL TOXICITY IN CLARIAS GARIEPINUS**

Adil A. Wani, Malvika Sikdar, S.M. Zuber\*

Department of Zoology, Dr. Harisingh Gour University, Sagar (M.P)-470003, India \* Govt. Degree College, Bijbehara (J&K)

\*Corresponding Author Email: malvikabar@gmail.com

# ABSTRACT

The present study was performed to investigate whether sulphur containing antioxidants such as taurine and garlic extract could ameliorate copper sulphate induced histopathological alterations in kidney of Clarias gariepinus. Fishes were divided into seven groups (I to VII) containing 20 in each group. The histopathological investigation of kidney of fishes exposed to copper sulphate at 4 ppm and 8 ppm concentrations and sampled at 15th, 30th, 60th and 90th day revealed severe alterations in dose and time dependent manner. The major histopathological changes include fragmentation of glomerulus, desquamation of luminal epithelium, degeneration and disorganization of haematopoietic tissue, haemorrhage and cytoplasmic vacuolation with necrosis of renal tubules. Moreover, addition of 5 ppm each of taurine or garlic extract was found partly to minimize the histopathological changes in kidney. In conclusion, it appears that taurine and garlic extract have beneficial effect in protecting against copper sulphate induced nephrotoxicity to some extent by minimizing histopathological changes. The amelioration of copper induced toxicity on histopathological parameter was highest in taurine treated groups followed by garlic extract treatment. The results reflected that 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, Kidney, Histopathology, Clarias gariepinus

# 1. INTRODUCTION

Copper is a trace element essential for all living organisms and important life functions cannot function properly in its absence (Steenkamp et al., 1994). However, copper becomes toxic to icththyofauna when its concentration exceeds than the biological requirement (Grosell et al., 2003; Wani et al., 2013). Copper salts are intentionally introduced into water bodies as aquatic plant herbicides, algaecides and molluscicides (Dueck et al., 1987). Upon entering the body, copper is being carried by ceruloplasmin present in plasma for its transport (Linder et al., 1998). The toxic effect of copper is related to its capacity for catalyzing

oxidative reactions, leading to the production of ROS (Lopes et al., 2001; Abdel-Khalek et al., 2015).

The kidney in teleosts is important organ comprising excretory and endocrine elements with haematopoietic tissue responsible for blood formation (Takashima and Hibiya, 1995), therefore are at major toxicological risk (Romeo et al., 2000). In recent years, more studies have incorporated the kidney in biomarker research and histological changes have been identified in the renal tissue after exposure to environmental pollutants (Afzali et al., 2013).

Dimercaptosuccinic acid (DMSA) and Monoisoamyl DMSA (MiADMSA) are used in treatment for heavy

1



metal intoxication, which form an insoluble complex with heavy metals and thereby reduce its toxicity (Flora et al., 2008). However, these chelators are potentially toxic and often fail to remove heavy metals from all body tissues. Thus it seems to be logical to look into natural antidotal agents, which could chelate heavy metals into less toxic complex to be excreted in urine or faeces (Hwang et al., 1998; Maha, 2015).

Taurine (2-aminoethanesufonic acid) is the major free intra cellular non-protein sulphur amino acid (Atmaca, 2004). 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-Levis and Park, 2009). The zwitterionic nature of the taurine gives it high water solubility and low lipophilicity (Huxtable, 1992). Taurine is found in greater concentration in aquatic foods than in land animal foods and consequently higher fish meal than in meat meal (Divakaran, 2006).

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 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 (diallyl disulfide) does not exist in garlic until it is crushed. The injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin (S-allylcysteine sulfoxide) to allicin (Block, 1985). Garlic compounds are having tremendous antioxidant property which exerts action by scavenging ROS (Borek, 2001). Ansari, 2015 reported the nephroprotective role of garlic against the mercuric chloride induced renal damage.

The aim of the present study was to evaluate the potential nephroprotective role of taurine and garlic extract in minimizing the histopathological changes in kidney induced by sublethal long-term exposure to copper sulphate in Clarias gariepinus.

# 2. MATERIALS AND METHODS

#### 2.1. Test animal 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 animal because of its ability to acclimatize quickly in the laboratory conditions. Clarias gariepinus is an exotic fish and was first time brought to India in 1994 from neighbouring country Bangladesh (Thakur, 1998). Healthy and disease-free specimens of Clarias gariepinus belonging to a single population were procured on order from the fish market of District, Sagar (M.P., India). Before introducing in the aquariums, fish were treated with 0.01 % KMnO4 solution for 15 minutes to obviate any dermal infection. Fish were then kept for a period of fifteen days for acclimatization in laboratory conditions. Faecal remains and food residues were removed by net every second 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). All the fish were fed with commercially available fish pellet feed (Tokyo®, Japan) throughout the experiment.

Group	Copper Sulphate Conc. (ppm)	Garlic Extract Conc. (ppm)	Taurine Conc. (ppm)						
I (Control)	00	00	00						
II	4.0	00	00						
III	8.0	00	00						
IV	4.0	00	5.0						
V	8.0	00	5.0						
VI	4.0	5.0	00						
VII	8.0	5.0	00						

# Table 1: Showing experimental design of 90 days exposure to Clarias gariepinus

#### 2.2. Preparation of copper stock solution

Dilution of copper sulphate 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

2



provide the 4 and 8 ppm copper sulphate concentrations in water.

# 2.3. 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.

#### 2.4. Light microscopic study

Kidneys of both control and treated fishes were removed aseptically and were fixed in aqueous Bouin's fluid. Preserved tissues were washed under tap water and dehydrated, clarified with xylene and embedded in paraffin blocks. They were cut at 4-5  $\mu$  thickness by using microtome and stained routinely with haematoxylin and eosin (H & E) for histological examination (Luna, 1968). Stained histopathological sections were examined under binocular microscope (Zeiss, PrimoStar®) on different magnifications and photographed.

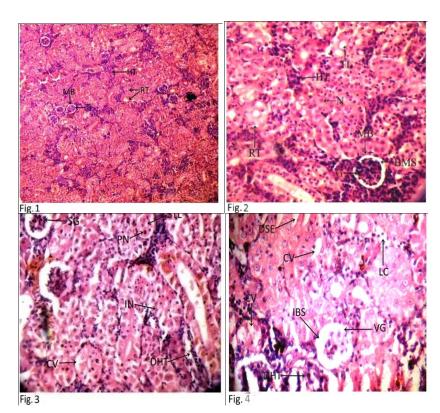
#### 2.5. Semiquantitative Scoring

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.

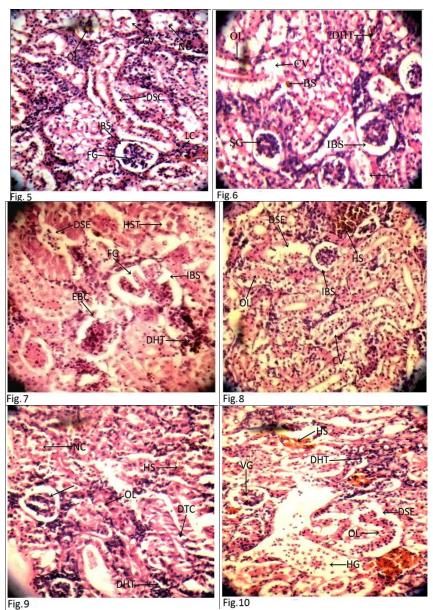
# 3. RESULTS

# 3.1. Histopathological observation

**Group I (Control):** The microscopic examination of kidney of Clarias gariepinus revealed the normal histoarchitecture (Figure 1 & 2) and did not show any recognizable alterations in any fish of control group throughout the experiment. The renal tissue possessed a number of nephrons within a surrounding matrix of haematopoietic tissue.







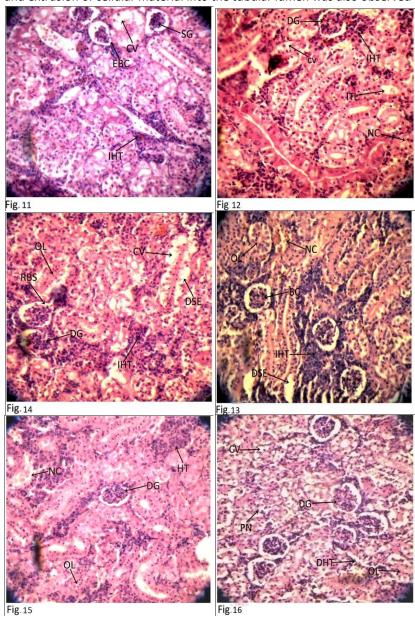
Histology of *C. gariepinus* kidney in control group I (Figure 1- H. & E. X100 and 2- H. & E. X400) and copper sulphate exposed groups II and III (3-10- H. & E. X400).

**Fig 1** and **2** showing normal Malpighian body (MB), Bowman's capsule (B), Bowman's space (BS), renal tubules (RT), haematopoietic tissue (HT). **Figure 3:** exposed to 4ppm CuSO<sub>4</sub> after 15 days showing shrunken glomerulus (SG), cytoplasmic vacuolation (CV), damaged hematopoietic (DHT), shrunken tubular lumen (STL). **Figure 4:** showing vacuolated glomerulus (VG), severely damaged haematopoietic tissue (DHT), extensive cytoplasmic vacuolation (CV) desquamation of epithelial layer (DSE), increased Bowman's space (IBS). **Figure 5:** exposed to 4ppm CuSO<sub>4</sub> after 60 days showing fragmentation of glomerulus (FG), extensive cytoplasmic vacuolation (CV). **Figure 6:** exposed to CuSO<sub>4</sub> after 90 days showing cytoplasmic vacuolation (CV), shrunken glomerulus (SG), damaged haematopoietic tissue (DHT). **Figure 7:** exposed to 8ppm CuSO<sub>4</sub> after 15 days showing extensive fragmentation of glomerulus (FG), damaged haematopoietic tissue (DHT). **Figure 8:** exposed to 8ppm CuSO<sub>4</sub> after 30 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 60 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 60 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL). **Figure 9:** exposed to 8ppm CuSO<sub>4</sub> after 90 days showing occlusion of tubular lumen (OL), haemorrhage (HG), desquamation of epithelial layer (DSE).



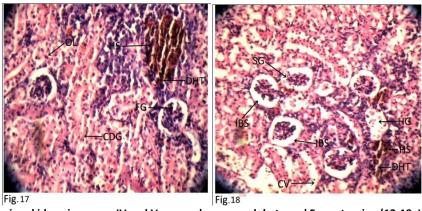
**Group II (4 ppm CuSO**<sub>4</sub>): The histological study of renal tissue after 15 days revealed shrunken lumen and mild cytoplasmic vacuolation of tubules with irregular. Increase in Bowman's space was observed due to shrinkage of glomerulus tuft (Figure 3). Slight haemorrhage and damage of interstitial haematopoietic tissue was reported after 30 days (Figure 4). There were severe changes in the renal tissue after prolonged exposures (30 days) with extensive shrinkage and fragmentation of glomeruli with increased Bowman's space (Figure 5). More severe histoarchitectural deformities were seen after 90 days including hydropic swelling of renal tubules with cytoplasmic vacuolation, extrusion of cellular material into the lumen due to complete degeneration of the tubular epithelium with moderate loss of haematopoietic tissue (Figure 6).

**Group III (8 ppm CuSO<sub>4</sub>):** After 15 days the histoarchitecture of kidney revealed ectasy of Bowman's capsules with extensive fragmentation and slightly degenerated haematopoietic tissue (Figure 7). After 60 days renal tissue exhibited hemosiderin deposition and haemorrhage with moderate infiltration of leucocytes within slightly deteriorated haematopoietic tissue in some areas (Figure 8). After prolonged exposures (60 and 90 days), the kidney tissue exhibited necrosis and complete destruction of tubule architect (Figure 9). Severe haemorrhage and haemolysis with aggregation of inflammatory cells and hemosiderin was reported after 90 days. Desquamation of epithelial cell layer and extrusion of cellular material into the tubular lumen was also observed (Figure 10)



www.ijpbs.com or www.ijpbsonline.com





Histology of *C. gariepinus* kidney in groups IV and V exposed copper sulphate and 5ppm taurine (12-19, H. & E. X400). Figure11: exposed to 4ppm CuSO<sub>4</sub> & 5ppm taurine after 15 days showing increased haematopoietic tissue (IHT), ectasy of Bowman's capsule (EBC), mild cytoplasmic vacuolation (CV) Figure12: exposed to 4ppm CuSO<sub>4</sub> & 5ppm taurine after 30 days showing slightly damaged haematopoietic tissue (DHT), increased tubular lumen (ITL), mild cytoplasmic vacuolation (CV). Figure13: exposed to 4ppm CuSO<sub>4</sub> & 5ppm taurine after 60 days showing increased haematopoietic tissue (IHT), necrosis of renal tubules (NC), occlusion of tubular lumen (OL), cytoplasmic vacuolation (CV). Figure 14: exposed to 4ppm CuSO<sub>4</sub> 5ppm & taurine after 90 days showing cytoplasmic vacuolation (CV), occlusion of tubular lumen (OL), desquamation of epithelial layer (DSE). Figure 15: exposed to 8ppm CuSO<sub>4</sub> 5ppm & taurine after 15 days showing occlusion of tubular lumen (OL), mild necrosis of few tubules (NC). Figure 16: exposed to 8ppm CuSO<sub>4</sub> & 5ppm taurine after 30 showing dilated glomerulus (DG), cytoplasmic vacuolation (CV), damaged haematopoietic tissue (DHT), haemorrhage (HG). Figure 17: exposed to 8ppm CuSO<sub>4</sub> & 5ppm taurine after 60 days showing fragmented glomerulus (FG), damaged haematopoietic tissue (DHT), occlusion of tubular lumen (OL). Figure18: exposed 8ppm CuSO<sub>4</sub> & 5ppm taurine after 90 days showing increased Bowman's space, shrunken glomerulus (SG), cytoplasmic vacuolation (CV), haemorrhage (HG).

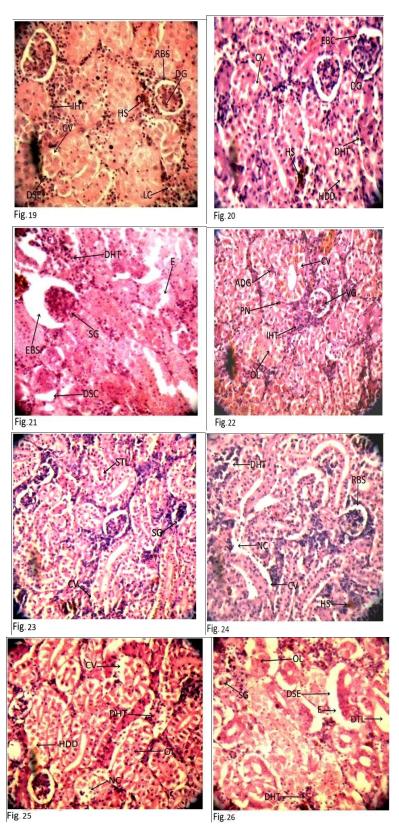
Group IV (4 ppm CuSO<sub>4</sub> and 5 ppm taurine): After 15 days the renal tissue showed no recognizable histopathological changes in majority of uriniferous tubules with uniformly stained cytoplasm. However, mild shrunken glomeruli and ectasy of Bowman's capsule was reported (Figure 11). After 30 days, the histoarchitecture of kidney exhibited minor alterations including cytoplasmic vacuolation with occlusion of few renal tubules (Fig 12). Minor necrotic spots with cytoplasmic granulation and vacuolar degeneration with occlusion of lumen was noted in several tubules after 60 days (Figure 13). Moderate damage in was seen in renal tissue with necrosis and cytoplasmic vacuolation in few renal tubules after 90 days. However, the haematopoietic tissue was seen uniformly distributed in renal tissue (Figure 14).

**Group V (8 ppm CuSO**<sup>4</sup> **and 5 ppm taurine):** The histoarchitecture of kidney after 15 days exhibited minor and less frequent histopathological changes including mild expansion of glomeruli. Occasionally, necrosis was reported in few renal tubules with occluded lumen, while majority of uriniferous tubules were having uniformly stained cytoplasm with centrally located nuclei and normal tubular lumen (Figure 15). After 30 days the renal tissue revealed mild expansion

of glomeruli, ectasy of few Bowman's capsules and mild haemorrhage within interstitial haematopoietic tissue (Figure 16). Accumulation of hemosiderin and disorganized haematopoietic tissue with slight fragmentation of glomeruli was also observed after 60 days (Figure 17). Cytoplasmic vacuolation and occluded lumen was seen after 90 days in tubules with nuclear pyknosis and haemorrhage (Figure 18).

Group VI (4 ppm CuSO4 and 5 ppm garlic extract): The histological analysis of kidney after 15 days showed renal tubules with mild cytoplasmic vacuolation, slight desquamation of epithelial layer and occlusion of tubular lumen with round normal euchromatic nuclei (Figure 19). After 30 days, the renal tissue exhibited dilation of glomeruli and mild cytoplasmic vacuolation. Occasionally, hyaline droplet degeneration in some cells of the tubular epithelium also was reported (Figure 20). Glomeruli were severely shrunken resulted in enlargement of Bowman's space with mild desquamation and formation of edematous spaces in renal tubules was observed after 60 days (Figure 21). The renal histoarchitecture after 90 days exhibited tubular cells with mild vacuolated cytoplasm and pycnotic nuclei (Figure 22).





Histology of *C. gariepinus* kidney in groups VI and VII exposed to copper sulphate and 5ppm garlic extract (19-26, H. & E. X400).

Figure 19: exposed to 4ppm CuSO<sub>4</sub> & 5ppm garlic extract after 15 days showing dilated glomerulus (DG), mild cytoplasmic vacuolation (CV), slight desquamation of epithelial layer (DSE), increased haematopoietic tissue (IHT). Figure 20: exposed to 4ppm CuSO<sub>4</sub> & 5ppm garlic extract after 30 days showing slight damage in haematopoietic tissue (DHT), dilated glomerulus (DG), hyaline droplet degeneration (HDD). Figure 21: exposed to 4ppm CuSO<sub>4</sub> & 5ppm garlic extract after 60 days showing

7



severely shrunken glomerulus (SG), increased Bowman's space (IBS),. Figure 22: exposed to 4ppm CuSO<sub>4</sub> & 5ppm garlic extract after 90 days showing cytoplasmic vacuolation (CV), pycnotic nuclei (PN), occlusion of tubular lumen (OL). Figure 23: exposed to 8ppm CuSO<sub>4</sub> & 5ppm garlic extract after 15 days showing ectasy of Bowman's capsule (EBC), shrunken tubular lumen (STL). Figure 24: exposed to 8ppm CuSO<sub>4</sub> & 5ppm garlic extract after 30 days showing damage in haematopoietic tissue (DHT), desquamation of epithelial layer (DSE). Figure 25: exposed to 8ppm CuSO<sub>4</sub> & 5ppm garlic extract after 60 days showing hyaline droplet degeneration (HDD), occlusion of tubular lumen (OL), cytoplasmic vacuolation (CV). Figure 26: exposed to 8ppm CuSO<sub>4</sub> & 5ppm garlic extract after 90 days showing severe desquamation of epithelial layer (DSE), shrunken glomerulus (SG).

Group VII (8 ppm CuSO4 and 5 ppm garlic extract): The histopathology of kidney after 15 days showed ectasy of Bowman's capsules and slightly increased haematopoietic tissue. Mostly the uriniferous tubules were in normal shape while as few tubules possessed shrunken lumen with mild cytoplasmic vacuolation (Figure 23). After 30 days, desquamation and cytoplasmic vacuolation along with occluded lumen and some necrotic spots in uriniferous tubules were reported (Figure 24). Moderate vacuolar degeneration and necrosis of uriniferous tubules due to ruptured epithelial lining in addition to hyaline droplet degeneration was observed after 60 days (Figure 25). After 90 days renal tissue exhibited severe desquamation of epithelial lining in tubules with formation large edematous spaces (Figure 26).

Semiquantitative scoring of the lesion of kidneys after long-term exposure to with or without simultaneous treatment of water with copper sulphate, taurine and garlic extract during the entire 90 days of experiment shown in Table 2.

# 4. DISCUSSION

Kidney serves as a major route of excretion for excretion for metabolites of various xenobiotics to which fish have been exposed (Naeemi, 2013). Histopathological findings in present study revealed severe histopathological changes in the kidney of fish, Clarias gariepinus in groups II and III exposed to 4 and 8 ppm of copper sulphate, respectively while as control group I revealed normal histoarchitecture throughout the course of experimental duration of 90 days. Moreover, the ameliorative efficacy of taurine and garlic extract found to be good mitigator of the histopathological changes in kidney of fish groups IV, V, VI and VII.

The copper induced histopathological changes in kidney were more severe in fishes of group III, exposed to 8 ppm of copper sulphate. These findings were in accord findings of Jiraungkoorskul et al. (2007) who found vacuolar degeneration and nuclear deformities in tubules after the sub-acute exposure of butterfish, P. triacanthus to 25µg/l copper. Similarly, Mourad and Wahby (1999) reported tubular destruction, epithelial cell edema and pycknosis of nuclei in kidney of Tilapia zillii exposed to effluent from Egyptian copper works. The toxic effect of Cu is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species and DNA damage (Lopes et al. 2001). These highly reactive compounds may also induce tissue alterations in fish (Varanka et al., 2001). The degeneration of haematopoietic tissue in kidney due to copper exposure could be correlated to the decreased haematological parameters (Wani and Sikdar, 2013), which is also supported by views of Handy (2003), in this regard. Copper has a great capacity to alter membrane structural lipids and could provoke membranous disruption (Roncero et al., 1992).



Lesion	Cont				4 ppm CuSO₄ Group II				8 ppm CuSO₄ Group III				4 ppm CuSO <sub>4</sub> + 5 ppm Taurine Group IV			8 ppm CuSO <sub>4</sub> + 5 ppm Taurine Group V			4 ppm CuSO <sub>4</sub> + 5 ppm Garlic Extract Group VI				8 ppm CuSO <sub>4</sub> + 5 ppm Garlic Extract Group VII					
	15	30	60	90	15	30	60	90	15	30	60	90	15	3 0	6	90	1 5	30	60	90	15	30	60	90	15	30		90
Shrinkage of glomerulus	-	-	-	-	++	+++	++	++	++	+	++	++	+	-	-	-	-	-	++	+	-	-	++	+	+	-	-	+
Haematopoietic tissue damage	-	-	-	-	+	++	++	++	++	++	++	++	-	-	-	-	-	+	+	+	-	+	+	+	+	+		+ ++
Epithelial layer desquamation	-	-	-	-	+	++	+++	+++	++	+++	++	+++	-	-	+	++	-	+	+	+	+	+	++	+	+ +	++	++	+ + +
Necrosis of renal tubules	-	-	-	-	+	+	++	++	++	++	++	+++	-	+	+	+	+	+	++	+	-	+	+	++	+	+	+	+
Cytoplasmic vacuolation	-	-	-	-	+	++	+++	+++	+	+++	++	+++	+	+	+	++	-	+	+	++	+	+	++	++	+	+	++	+
Occlusion of tubular lumen	-	-	-	-	+	++	++	++	+	++	++	+++	-	-	+	++	+	+ +	+	++	-	+	+	++	+	+	++	+

# Table 2: Semiquantitative scoring of kidney lesion in Clarias gariepinus

(-) none, (+) mild occurrence, (++) moderate occurrence, (+++) severe occurrence



The degenerative changes in renal tubules were also reported by Osman et al. (2009) on exposure to copper sulphate O. niloticus. Hypertrophied tubular epithelium and severe epithelial degeneration were seen in Puntius conchonius (Gill et al., 1989) and Lates calcairifer (Thophon et al., 2003) exposed to CdCl2. The accumulation of hyaline droplets in tubular cells seen in this study might be the result of a glomerular alteration (Bucher and Hofer, 1993). Occlusion of the renal tubule can occur by the accumulation of certain materials in the lumen and also as consequence of the swelling of the epithelial cells (Takashima and Hibiya, 1995).

The present findings also revealed that the histopathological changes in kidney were efficiently minimized in cotreated (taurine and copper sulphate) groups IV and V of fish in comparison to groups exposed only to copper sulphate. In agreement with the present observations, other studies have also shown that taurine exert morphological and functional protection against acute renal damage induced by gentamicin (Erdem et al., 2000) and acetaminophen (Das et al., 2010). Al-Kahtani (2010) reported that treatment of taurine shortly after aluminium exposure resulted in a significant improvement of the histology and ultrastructural pattern in the glomeruli as well as PCT. The nephroprotective effects of taurine against the degenerative effects of copper could have been achieved via mechanisms, including calcium modulation, osmoregulation and preservation of membrane integrity (Erdem et al., 2000). The treatment of taurine is reported to significantly reduce the pathological alterations in the kidney caused by mercuric chloride intoxication (Agha et al., 2014) and aluminium chloride (Abdel-Moneim, 2013) in rats.

The possible nephroprotective potential of garlic against the heavy metals has been reported by several authors (Kumar et al., 2009, Akande et al. (2014). Supplementations of garlic extract at 5 ppm in groups VI and VII was found to be effective to reduce the copper sulphate induced histopathological changes in kidney, which could be attributed to its potential of preventing organs against oxidative and inflammatory injuries (Chowdhury et al., 2008). Our results agree with those reported earlier by Metwally and Hashem (2009) who observed few swollen glomeruli on administration of garlic in cadmium intoxicated rats. Similarly, Gulnaz et al. (2010) reported that pretreatment of garlic oil counteracted the nephrotoxicity of acetaminophen by efficiently restoring the normal histoarchitecture of kidney in rats. However, the present study is in contrast with the findings of Banerjee et al. (2001) who revealed that garlic caused some degrees of alterations in the kidney parenchyma, especially at very high doses. Garlic extract is also reported to significantly decrease the bioaccumulation of cadmium in Clarias batrachus (Kumar et al., 2009) and copper in Oreochromis niloticus by decreasing the activity of ceruloplasmin (Metwally, 2009).

Our study showed that garlic extract and taurine in particular were effective in ameliorating copper sulphate induced renal injury. Further investigations are required to elaborate and understanding the way garlic and taurine operates to prevent nephrotoxicity of copper in fishes.

#### Acknowledgement

Authors are thankful to Prof. J.D. Ahi, HOD of Zoology, and Prof. U.S. Gupta (DSW), Dr. Harisingh Gour University, Sagar (M.P) for their valuable guidance for carrying out this work.

# REFERENCES

- Abdel-Khalek A, Kadry M, Badran S, Marie M (2015). Comparative toxicity of copper oxide bulk and nano particles in Nile Tilapia, Oreochromis niloticus: Biochemical and oxidative stress. J Basic Appl Zool 72: 43– 57.
- Abdel-Moneim A (2013). Effects of taurine against histomorphological and ultrastructural changes in the testes of mice exposed to aluminium chloride. Arh Hig Rada Toksikol 64: 405-414.
- Afzali F, Sharifpour I, Soltani M (2013). Study of pathological effects of an organic germicide bathing on rainbow trout. Iran J Fish Sci 12: 500-510.
- Agha E, Youness R, Selim M, Ahmed H (2014). Nephroprotective potential of selenium and taurine against mercuric chloride induced nephropathy in rats. Renal Failure 36: 704-716.
- Akande M, Aliu O, Ambali S, Ayo J (2014) taurine alleviated biochemical alterations in male Wistar rats co-exposed to chlorpyrifos and lead. J Toxicol Environ Health Sci 6:13-25.
- Al-Kahtani AM (2010). Renal damage mediated by oxidative stress in mice treated with aluminium chloride: Protective effects of taurine. J Biol Sci 10: 584-595.
- 7) Ansari S (2015). Effect of metal exposure in rats: amelioration by diallylsulphide. Toxin Reviews, 1-4.
- 8) Atmaca G (2004). Antioxidant effects of sulphur containing amino acids. Yonsei Med J 45: 776-788.



- Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK (2001). Garlic induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem Toxicol 39: 793-797.
- 10) Block E (1985). The chemistry of garlic and onions. Sci Am 252: 114-119.
- 11) Borek C (2001). Antioxidant health effects of aged garlic extract. J Nutr 131: 1010-1015.
- 12) Bucher F, Hofer R (1993). The effects of domestic sewage on three organs (gills, kidney, liver) of brown trout, Salmo trutta. Water Res 27: 255-261.
- Chowdhury R, Dutta A, Chaudhuri SR, Sharma N, Giri AK, Chaudhuri, K (2008). In vitro and in vivo reduction of sodium arsenite induced toxicity by aqueous garlic extract. Food Chem Toxicol 46: 740-751.
- 14) Das J, Ghosh J, Manna P, Sil PC (2010). Taurine protects acetaminophen induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation. Toxicol 269: 24-34
- Divakaran S (2006). Taurine: an aminoacid rich in fish meal. Monterrey, Nuevo Leon, Mexico. PP: 310-317, SSBN970-694-333-5.
- 16) Dueck H, Tensen D, Duijh J, Pasman M (1987). Nutrient fertilization, copper toxicity and growth in three grassland species in the Netherlands. J Appl Ecol 24: 1001–1010.
- 17) Erdem A, Gondogan NU, Usubatan A, Kilinic K, Erdem SR, Kara A, Bozkurt A (2000). The protective effect of taurine against gentamicin induced acute tubular necrosis in rats. Nephrol Dial Transpl 15: 1175-1182.
- 18) Flora S, Saxena G, Mehta A (2008). Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res 128: 501-523.
- 19) Gill TS, Pant JC, Tewari H (1989). Cadmium nephropathy in a freshwater fish, Puntius conchonius. Ecotox Environ Safe 18: 165-172.
- 20) Grosell M, Wood CM, Walsh PJ (2003). Copper homeostasis and toxicity in the elasmobranch Raja erinacea and the teleost Myoxocephalus octodecemspinosus during exposure to elevated waterborne copper. Comp. Biochem. Physiol. Toxicol. Pharmacol 135: 179–190.
- 21) Gulnaz H, Tahir M, Munir B, Sami W (2010). Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. Biomedica 26: 9-15.
- 22) Handy (2003). Chronic effects of copper exposure versus endocrine toxicity: two sides of the same toxicological process. Comp Biochem Phys A 135: 25-38.
- 23) Hinton DE, Lauren DJ (1990). Integrative histopathological approaches to detecting effects of environmental stressors on fishes. Am Fish Soc Symp 8: 51-66.
- 24) Huxtable RJ (1992). Physiological actions of taurine. Physiological Reviews 72: 101-163.

- 25) Hwang DF, Wang LC, Cheng HM (1998). Effect of taurine on toxicity of copper in rats. Food Chem Toxicol 36: 239-244.
- 26) Jiraungkoorskul W, Sahaphong S, Kangwanrangsan N (2007). Toxicity of copper in butterfish, Poronotus triacanthus: tissues accumulation and ultrastructural changes. Environ Toxicol 22: 92-100.
- 27) Kumar P, Prasad Y, Patra AK, Ranjan R, Swarup D, Patra RC, Pal S (2009). Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish, Clarias batrachus. Sci Total Environ 407: 5024-5030.
- 28) Linder MC, Wooten L, Cerveza P, Cotton S, Shulze R, Lomeli N (1998). Copper transport. Am J Clin Nutr 67: 965-971.
- 29) Lopes PA, Pinheiro T, Santos MC, Mathias M, Collares-Pereira MJ, Viegas-Crespo AM., (2001). Response of antioxidant enzymes in freshwater fish populations (Leuciscus alburnoides complex) to inorganic pollutants exposure. Sci Total Environ 280: 153-163.
- 30) Luna LC (1968). Manual of histologic staining methods. Armed forces institute of Pathology, 3rd Ed. McGraw Hill Book Company, New York.
- 31) Maha A (2015). Amelioration of nandrolone decanoateinduced testicular and sperm toxicity in rats by taurine: Effects on steroidogenesis, redox and inflammatory cascades, and intrinsic apoptotic pathway. Toxicol Appl Pharmacol 282: 285–296.
- 32) Metwally M, Hashem MA (2009). Protective role of garlic against cadmium toxicity in rats: clinicopathological and histopathological studies. Egypt J Comp Pathol Clin Pathol 22: 114-140.
- 33) Metwally, M., 2009. Effect of garlic (Allium sativum) on some heavy metal (copper and zinc) induced alteration in serum lipid profile of Oreochromis niloticus. World J Fish Marine Sci 1: 01-06
- 34) Mourad M, Wahby O (1999). Physiological and histological changes in Tilapia zillii exposed to sublethal concentrations of the effluent of the Egyptian Copper Works. Acta Ichthyol Piscat 29: 73-80.
- 35) Naeemi A, Jamili S, Shabanipour N, Mashinchian A, Shariati Feizabadi S (2013). Histopathological changes of gill, liver and kidney in Caspian kutum exposed to Linear Alkylbenzene Sulfonate. Iran J Fish Sci 12: 887-897.
- 36) Newall CA, Anderson LA, Phillipson JD (1996). Herbal medicines a guide for health-care professionals. London: Pharmaceutical Press. 9, 296.
- 37) Osman M, El-Fiky S, Soheir Y, Abeer A, (2009). Impact of water pollution on histopathological and electrophoretic characters of Oreochromis niloticus. Res J Environ Toxicol 3: 9-23.
- 38) Romeo M, Bennani N, Gnassia-Barelli M, Lafaurie M, Girard JP (2000). Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass Dicentrarchus labrax. Aquat Toxicol 48: 185-194.



- 39) Roncero V, Duran E, Soler F, Masot J, Gomez L (1992). Morphometric, structural and ultrastructural studies of tench, Tinca tinca hepatocytes after copper sulphate administration. Environ Res 57: 45-58.
- 40) Schuller-Lewis, G. B, Park, E., 2003. Taurine: new implications for an old amino acid. Fems Microbiol Lett 226: 195-202.
- 41) Steenkamp VE, Du Preez HH, Schoonbee HJ (1994). Bioaccumulation of copper in the tissues of Potamonautes warren (Decapoda) from industrial, mine and sewage polluted freshwater ecosystems. S Afr J Zool 29: 152-161.
- 42) Takashima F, Hibiya T (1995). An atlas of fish histology: normal and pathological features. 2. ed. Kodansha, Tokyo.
- 43) Thakur NK (1998). A biological profile of the African catfish, Clarias gariepinus and impacts of its introduction in Asia. Natcon Publications, Muzzafarnagar (UP) India.
- 44) Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S (2003). Histopathological

alterations of white seabass, Lates calcacifer, in acute and subacute cadmium exposure. Environ Pollut 121: 307-320.

- 45) Van der Oost R, Beyar J, Vermeulen NP (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Phar 13, 57-149.
- 46) Varanka Z, Rojik I, Varanka I, Nemcsok J, Abraham M (2001). Biochemical and morphological changes in carp, Cyprinus carpio liver following exposure to copper sulfate and tannic acid. Comp Biochem Physiol 128: 467-478.
- 47) Wani AA, Sikdar-Bar M, Khan HA (2013). Acute toxicity of copper sulphate to African catfish, Clarias gariepinus. GERF Bull Biosci 4: 14-18.
- 48) Wani AA, Sikdar-Bar M (2013). Ameliorative efficacy of taurine and garlic extract on copper induced immunotoxic effect on total and differential leucocyte counts in African Catfish, Clarias gariepinus. Asian J Med Pharm Res 4: 122-129.

\*Corresponding Author: S.M. Zuber\*

Email: malvikabar@gmail.com