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# Ameliorative Efficacy of Taurine and Garlic Extract on Copper Induced Immunotoxic Effect on Total and Differential Leucocyte Counts in African Catfish, *Clarias gariepinus*

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**ABSTRACT:** An experiment was conducted to evaluate the changes in haematological parameters (TLC and DLC) of copper sulphate ($CuSO_4$. $5H_2O$) exposed African catfish, *Clarias gariepinus* with or without simultaneous treatment of water with taurine or garlic extract. Fish of average weight 98.43 ± 24.09 g and length 20.5 ± 2.5 cm were selected as test organism. Fish were divided into seven groups (I to VII) containing 20 fish in each group. The results obtained revealed that chronic exposure of fish to different sublethal concentrations (4 and 8 ppm) of copper sulphate induced marked alterations in total leucocyte count and differential leucocyte count. The results declared significant decrease of total leucocyte count and lymphocytes and non-significant (P>0.05) decrease of eosinophils, while as significant increase of neutrophils and monocytes were observed in copper sulphate treated groups (II and III) in comparison to control. Interestingly on supplementation of 5 ppm each of taurine in groups IV and V and garlic extract in groups VI and VII has appeared to minimize the effects of copper at all-time intervals by significantly increasing the leucocyte count and modulating the deranged differential leucocyte count.

**Key words:** Copper Sulphate, TLC, DLC, Taurine, Garlic Extract, *Clarias gariepinus*

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## INTRODUCTION

Leukocytes are important parameters to evaluate both the fish's state of health and their immune system (Tavares-Dias and Moraes 2006). Immunosuppression can also be observed (Mazon et al., 2002), due to the fact that monocytes and neutrophils are sensitive to heavy metals (Witeska and Wakulska, 2007). The suppression of the immune system increases the susceptibility to disease in fish, a very important aspect considering the presence of heavy metals in natural ecosystem as a result of human activities (Wedemeyer et al., 1990).

Copper is a micronutrient and is an essential part of cytochrome oxidase and many metalloenzymes. However, copper becomes toxic to aquatic biota when its concentration exceeds than the biological requirement. Upon entering the body, copper is carried by ceruloplasmin present in plasma for its transport and delivery to tissue cells (Linder et al., 1998). It is proposed that free reduced Cu (I) in the cell binds to sulfhydryl groups and inactivates enzymes such as glucose-6-phosphate dehydrogenase and glutathione reeducates (Dash, 1989).The toxic effect of copper is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (Lopes et al., 2001). Haemolytic anaemia, a common complication of copper sulphate poisoning, is caused either by direct red blood cell membrane damage (Chuttani et al., 1965) or indirectly as a result of the inactivation of enzymes (including glutathione reductase) which protect against oxidative stress (Mital et al., 1966). Copper can act as Na analogues and competitors in gill transport systems, and out-compete Na, thereby blocking transport systems (Grosell and Wood, 2002).

The currently approved treatment for heavy metal intoxication is to give chelating agents, such as DMSA and MiADMSA, which form an insoluble complex with heavy metals and shield it from biological targets, thereby reducing its toxicity (Flora et al., 2008). However, these chelators are potentially toxic and often fail to remove heavy metals from all body tissues. 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 (2-aminoethane sulphonic acid) is the major free intra cellular non protein sulphur amino acid (Atmaca, 2004) found in milimolar concentrations in many animal tissues (Wright et al., 1986). Taurine is present in high concentration in most tissues particularly in lymphocytes (Fukuda et al., 1982). The potential of taurine as a chelating agent against lead poisoning has been reported by Flora et al. (2004). Taurine is reported 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 (Flora et al., 2008). Direct interaction between taurine and metal ions is mainly attributed to the electric association between metal cations and the sulfonate anion or to the interaction between metal ions is mainly attributed to the electric association

between metal cations and the sulfonate anion or to the interaction between metal ions and the nitrogen's unshared pair of electrons (Wright et al., 1986). 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. The sulphur compounds are responsible both for garlic's pungent odour and many of its medicinal effects. 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 (Block, 1985). Garlic compounds are having tremendous antioxidant property which exerts actions by scavenging ROS (Borek, 2001). Haematological parameters are very sensitive to environmental changes (Hughes and Nemcsok, 1998) and their response to undesirable materials is very fast. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. The objective of present work was to determine the toxic effects of sublethal concentrations of copper sulphate on TLC and DLC and the efficacy of taurine and garlic extract in minimizing the copper toxicity by modulating the deranged haematological parameters in *Clarias gariepinus*.

## MATERIAL 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 ability to acclimatize quickly in the laboratory conditions. *Clarias gariepinus* is an exotic fish and was first time brought to India in 1994 from neighboring country Bangladesh (Thakur, 1998). Healthy and disease free specimens of *Clarias gariepinus* of either sex belonging to a single population were purchased from the local fish market of Sagar (M.P). Before introducing to the aquariums, fish were treated with 0.01% $KMnO_4$ 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 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, taurine and garlic extract during the entire experimental period of 90 days. Dose selection and mode of administration of garlic extract and taurine was based on Kumar et al. (2009). All the fish were fed with commercially available fish pellet feed (Tokyo®, Japan) throughout the experiment.

**Table 1:** Showing the experimental design.

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

**Chemicals:** Taurine was purchased from HiMedia Laboratories, Delhi India. Analytical grade copper sulphate (99%, $CuSO_4.5H_2O$) supplied by WebChem®.

**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.

**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.

**Haematological analysis:** Blood samples were collected from three fish at $15^{th}$, $30^{th}$, $60^{th}$ and $90^{th}$ days from each group. Blood was drawn from the caudal vein

decanted in EDTA vials for the assessment of haematological parameters.

**Enumeration of Total Count of WBC:** Total leucocytes were counted by using improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1968). The final results were expressed as the number of the WBCs/$mm^3$.

**Differential Leucocyte Count (DLC):** A drop of blood was placed on a clean glass slide about 1-2 cm from one end. The blood smear was made with the help of a spreader slide placed at an angle of 45° approximately and then stained by Leishman's stain for a period of 5-7 minutes. The excess stain was drained and washed with water and air dried and observed under microscope. Leucocytes were identified and the movement was repeated till a total 100 cells were counted. The values of different morphological types were expressed as the percentage.

**Statistical Analyses:** The data were analyzed by using GraphPad InState3 for Windows. The Data are expressed as mean ± SD and significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey-Kramer procedure for multiple comparisons. $P<0.05$ was considered statistically significant.

## RESULTS

The chronic 90 days exposure of *Clarias gariepinus* to different sublethal concentrations of copper sulphate induced marked alterations in total leucocyte count and differential leucocyte count, and interestingly the supplementation of sulphur containing antioxidants (taurine and garlic extract) has appeared to minimize the effects of copper at all-time intervals by significantly ($P<0.05$) increasing the leucocyte count. The results also declared significant decrease of lymphocytes and non-significant ($P>0.05$) decrease of eosinophils, while as significant increase of neutrophils and monocytes were observed in copper sulphate treated groups in comparison to negative control and challenged groups.

Increase in the total leucocyte count was reported due to increased lymphocytes in fish treated with supplementation of 5 ppm of garlic extract to groups VI and VII compared to positive control groups II and III however, the values were significantly lower in comparison with the negative control group I (Table 2, 3, 4, 5,6).

**Table 2:** Showing the changes in WBC count ($1x10^3/mm^3$) in blood of *Clarias gariepinus*.

| Days of Exposure | Control Group I | 4ppm $CuSO_4$ Group II | 8ppm $CuSO_4$ Group III | 4ppm $CuSO_4$+T Group IV | 8ppm $CuSO_4$ + T Group V | 4ppm $CuSO_4$ +GE Group VI | 8ppm $CuSO_4$+GE Group VII |
|---|---|---|---|---|---|---|---|
| **15 Days** | 24.99±0.26 | $22.05 \pm .27^{B}$ | $20.64 \pm 0 .48^{D}$ | $23.13 \pm 0.19^{A}$ | $22.36 \pm 0.69^{A}$ | $22.39 \pm 0.27^{B}$ | $21.88 \pm 0.19^{B}$ |
| **30 Days** | $24.32 \pm 0.26^{n}$ | $19.63 \pm 0.47^{Db}$ | $18.93 \pm 0.29^{Dn}$ | $22.11 \pm 0.45^{An}$ | $21.76 \pm 0.24^{Dn}$ | $21.74 \pm 0.31^{Bn}$ | $20.18 \pm 0.17^{Dn}$ |
| **60 Days** | $23.89 \pm .51^{n}$ | $18.6 \pm 0.18^{Dd}$ | $17.86 \pm 0.32^{Da}$ | $20.98 \pm 0.24^{Ba}$ | $20.12 \pm 0.65^{Bn}$ | $20.95 \pm 0.36^{Bn}$ | $19.27 \pm 0.31^{Da}$ |
| **90 Days** | $23.85 \pm .56^{n}$ | $17.9 \pm .24^{Dd}$ | $16.49 \pm 0.67^{Db}$ | $20.29 \pm 0.61^{Bb}$ | $19.26 \pm 0.51^{Ba}$ | $19.09 \pm 0.39^{Dd}$ | $18.91 \pm 0.28^{Db}$ |

**Table 3:** Showing the changes in lymphocyte count (%) in blood of *Clarias gariepinus*.

| Days of Exposure | Control Group I | 4ppm $CuSO_4$ Group II | 8ppm $CuSO_4$ Group III | 4ppm $CuSO_4$ + T Group IV | 8ppm $CuSO_4$ + T Group V | 4ppm $CuSO_4$+EG Group VI | 8ppm $CuSO_4$ +GE Group VII |
|---|---|---|---|---|---|---|---|
| **15 Days** | 66.33 ±0.88 | $62 \pm 0.58^{B}$ | $61.33 \pm 0.88^{A}$ | $63 \pm 0.58^{A}$ | $62 \pm 0.58^{A}$ | $63 \pm 0.58^{A}$ | $61.67 \pm 0.88^{A}$ |
| **30 Days** | $66.67 \pm 0.33^{n}$ | $63 \pm 0.58B^{n}$ | $59.33 \pm 0.33^{Bn}$ | $62.67 \pm 0.88^{Bn}$ | $62.33 \pm 0.88^{An}$ | $63.67 \pm 0.33^{An}$ | $62 \pm 1.52^{An}$ |
| **60 Days** | $67.33 \pm 0.33^{n}$ | $60 \pm 0.58^{Dn}$ | $59 \pm 1^{Dn}$ | $63.33 \pm 0.88^{An}$ | $62.33 \pm 0.67^{Bn}$ | $62.67 \pm 0.67^{Bn}$ | $60.67 \pm 0.67^{Dn}$ |
| **90 Days** | $67.67 \pm 0.88^{n}$ | $58.33 \pm 0.33^{Db}$ | $56.67 \pm 1.20^{Da}$ | $62 \pm 0.58^{Bn}$ | $60.33 \pm 0.88^{Bn}$ | $60.67 \pm 1.33^{Bn}$ | $58.67 \pm 0.88^{Dn}$ |

**Table 4:** Showing the changes in neutrophil count (%) in blood of *Clarias gariepinus*

| Days of Exposure | Control Group I | 4ppm $CuSO_4$ Group II | 8ppm $CuSO_4$ Group III | 4ppm $CuSO_4$ + T Group IV | 8ppm $CuSO_4$ +T Group V | 4ppm $CuSO_4$+EG Group VI | 8ppm $CuSO_4$ +GE Group VII |
|---|---|---|---|---|---|---|---|
| **15 Days** | $19 \pm 0.58$ | $24 \pm 1^{D}$ | $23.33 \pm 0.67^{A}$ | $21.67 \pm 0.33^{N}$ | $21.33 \pm 0.88^{N}$ | $23 \pm 0.58^{A}$ | $22.33 \pm 1.20^{N}$ |
| **30 Days** | $19 \pm 0.58^{n}$ | $23.33 \pm 0.33^{Bn}$ | $25 \pm 0.58B^{n}$ | $21.67 \pm 0.88^{Nn}$ | $22 \pm 0.58^{Nn}$ | $21 \pm 0.58^{Nn}$ | $22.67 \pm 1.45^{Nn}$ |
| **60 Days** | $18.33 \pm 0.33^{n}$ | $24.33 \pm 0.33^{Bn}$ | $25.33 \pm 1.20^{Dn}$ | $21.33 \pm 1.45^{Nn}$ | $22.33 \pm 0.33^{An}$ | $22.33 \pm 0.33^{An}$ | $23 \pm 0.58^{Bn}$ |
| **90 Days** | $18.33 \pm 0.88^{n}$ | $26 \pm 0.58^{Dn}$ | $26.67 \pm 0.88^{Dn}$ | $22.33 \pm 0.67^{An}$ | $22.33 \pm 0.88^{An}$ | $23.33 \pm 0.88^{Bn}$ | $24.33 \pm 0.33^{Bn}$ |

**Table 5:** Showing the changes in monocyte count (%) in blood of *Clarias gariepinus*.

| Days of Exposure | Control Group I | 4ppm $CuSO_4$ Group II | 8ppm $CuSO_4$ Group III | 4ppm $CuSO_4$ + T Group IV | 8ppm $CuSO_4$ + T Group V | 4ppm $CuSO_4$+EG Group VI | 8ppm $CuSO_4$ +GE Group VII |
|---|---|---|---|---|---|---|---|
| **15 Days** | 9.33 ±0.33 | $10.67 \pm 0.33^{N}$ | $12 \pm 0.58^{N}$ | $11 \pm 1^{N}$ | $11.33 \pm 0.67^{N}$ | $10 \pm 0.58^{N}$ | $11 \pm 1^{N}$ |
| **30 Days** | $9.33 \pm 0.3^{n}$ | $11.33 \pm 0.33A^{n}$ | $12.67 \pm 0.33^{Bn}$ | $10.33 \pm 0.33^{Nn}$ | $10.67 \pm 0.67^{Nn}$ | $10.67 \pm 0.67^{Nn}$ | $11.33 \pm 0.33^{An}$ |
| **60 Days** | $10 \pm 0.58^{n}$ | $12.33 \pm 0.33N^{a}$ | $13.33 \pm 0.33^{An}$ | $10 \pm 0.58^{Nn}$ | $11.33 \pm 0.67^{Nn}$ | $11 \pm 0.58^{Nn}$ | $12 \pm 0.58^{Nn}$ |
| **90 Days** | $9.67 \pm 0.8^{n}$ | $13.3 \pm 0.33D^{b}$ | $14.33 \pm .33^{Aa}$ | $11.33 \pm 0.33^{Nn}$ | $13.33 \pm 0.33^{An}$ | $12.33 \pm 0.33^{An}$ | $13 \pm 1.15^{Nn}$ |

**Table 6:** Showing the changes in eosinophil count (%) in blood of *Clarias gariepinus*.

| Days of Exposure | Control Group I | 4ppm $CuSO_4$ Group II | 8ppm $CuSO_4$ Group III | 4ppm $CuSO_4$ + T Group IV | 8ppm $CuSO_4$ + T Group V | 4ppm $CuSO_4$ + GE Group VI | 8ppm $CuSO_4$ + GE Group VII |
|---|---|---|---|---|---|---|---|
| **15 Days** | 5.33 ± 0.33 | $3.33 \pm 0.33^{A}$ | $3.33 \pm 0.33^{N}$ | $4.33 \pm 0.33^{N}$ | $5.33 \pm 0.88^{N}$ | $4 \pm 0.58^{N}$ | $5 \pm 0.58^{N}$ |
| **30 Days** | $5 \pm 0.58^{n}$ | $2.33 \pm 0.33^{Bn}$ | $2.67 \pm 0.33^{Nn}$ | $5.33 \pm 0.33^{Nn}$ | $5 \pm 0.58^{Nn}$ | $4.67 \pm 0.33^{Nn}$ | $4 \pm 0.58^{Nn}$ |
| **60 Days** | $4.33 \pm 0.33^{n}$ | $3.33 \pm 0.33^{Nn}$ | $2.33 \pm 0.33^{An}$ | $5.33 \pm 0.67^{Nn}$ | $4.67 \pm 0.33^{Nn}$ | $4 \pm 1^{Nn}$ | $4.33 \pm 0.33^{Nn}$ |
| **90 Days** | $4.33 \pm 0.88^{n}$ | $2.33 \pm 0.67^{Nn}$ | $2.33 \pm 0.33^{Nn}$ | $4.33 \pm 0.33^{Nn}$ | $4 \pm 0.58^{Nn}$ | $4 \pm 0.58^{Nn}$ | $4 \pm 0.58^{Nn}$ |

**Means with superscripts in small letter in a row and capital letter in a column indicate significances:**
**nN** non-significant (P>0.05); **aA** significant at (P<0.05); **bB** significant at (P<0.01); **dD** significant at (P<0.001).
Capital letters in rows indicate significance from corresponding control and small letters in columns indicate significance from 15 days value.
Data are represented as mean ± SEM, (N=3).

## DISCUSSION

Some of the most common causes of heavy metal toxicity are inflammatory lesions associated with tissue damage, anaemia and neoplasia. The results of study are consistent with the findings by Dethloff et al. (1998) who reported consistent decrease in the percentage of lymphocytes and elevated percentage of monocytes and neutrophils at 26.9 and 6.4 µgCu/l. The decrease in total leucocytes number was observed due to lymphopenia in $HgCl_2$ exposed *O. Niloticus* (Ishikawa et al., 2007), and *Acanthopagrus latus* (Safahieh et al., 2010). In another study (Dethloff et al., 2001) also observed decreased leucocrit and percentage of lymphocytes in rainbow trout at copper contaminated sites. A high copper sulphate content causes immunosuppression, as reported by Mazon et al. (2002) due to secretion of cortisol which shortens the life span of lymphocytes and promotes their apoptosis (Wyets et al., 1998; Verburg van Kemenade et al., 1999) and reduces their proliferation (Espelid et al., 1996), so a decrease in lymphocyte count are often observed. Copper stress may also lead to redistribution of lymphocytes, mainly to lymphoid organs (thymus and anterior kidney) and diminishing their number in blood circulation. Lymphocytes are usually the most common leucocyte type present in the blood of some fish, accounting for as much as 85% of the total leucocyte population, excluding thrombocytes (Groff and Zinkl, 1999).

The present observations get confirmation from the studies of Handy (2003) who reported that Cu exposure lowers the immunity in fish by decreasing lymphocyte level and increasing neutrophils due altered haemopoietic responses in kidney. Mazon et al. (2002) enumerated increased leucocytes with differential leucocyte percentage displaying significant reduction in lymphocytes and an increase in neutrophils in freshwater fish, *Prochilodus scrofa* exposed to 25 and 29 µgCu/l. Likewise, Mishra and Srivastava (1980) also observed significant decrease in the number of lymphocytes in *Colisa fasciatus* exposure to 3 mg/l of copper nitrate. The increase in the TLC was also reported by Saxena and Chauhan (1994) in *Heteropneustes fossilis* due to copper sulphate intoxication and by Singh (1995) in *Channa punctatus* due to copper sulphate and potassium dichromate induced toxicity. Cadmium exposure to *Parachanna africans* is also reported to cause significant decreases in white blood cell (Kori-Siakpere and Ikomi, 2011). Leucopenia may also be the result of bioaccumulation of heavy metal in the kidney and liver (Agarwal and Srivastava, 1980).

Dick and Dixon (1985) reported significant leucopenia with large reduction in lymphocytes on acute copper exposure. Leucocytopenia, an overall reduction in leucocytes, has been also demonstrated in teleosts exposed to other heavy metals (Srivastava and Agrawal, 1979; Gill

and Pant, 1987). Witeska and Wakulska (2007) reported that phagocytes (neutrophils and monocytes) are sensitive to heavy metal intoxication. A mixture of Cu and Zn induced a significant drop in leucocyte count as reported by Bagdonas and Vosyliene (2006). In a situation of monocytopenia and neutropenia in *O. mossambicus*, the migration and phagocytic activity in the gills, liver and kidney were disrupted on exposure to copper (Nussey et al., 1995). However, in contrast to our study Singh et al. (2008) reported leucocytosis with increase in the lymphocytes and eosinophils numbers and was followed by decrease in the numbers of monocytes and basophils in *Channa punctatus*.

Leucocytopenia is a nonspecific response to a variety of stressors mediated by corticosteroid hormones (Ellis, 1981) and cannot be considered a specific cytotoxic action of copper (Dick and Dixon, 1985). However, the leucocytosis reported in *O. mossambicus* after acute exposure to copper (Nussey et al., 1995) may be attributed to increased leucocyte mobilization to protect the body against toxicity in copper damaged tissue (Mazon et al., 2002). Foregoing results revealed increase in neutrophil count in copper exposed fish which could be cortisol-induced since this hormone prevents neutrophil migration into the tissues (inhibiting inflammatory response) and extends their life span by inhibition of apoptosis (Wyets et al., 1998). A four-fold increase in neutrophil percentage in fish subjected to transport was observed by Elsaesser and Clem (1986). Sublethal exposure of copper levels to rainbow trout in water was reported to increase their susceptibility to infectious haematopoietic necrosis virus (Hetrick et al., 1979) which suggests its role in suppression of immune response.

Our study revealed that administration of garlic formulation partly modulated copper induced changes in total leucocyte count and differential leucocyte count. The elevated mean percent of neutrophils due to copper toxicity was also slightly decreased in garlic extract treated groups, but were still significantly higher than the negative control group. The present results are in harmony with the studies of Khan et al. (2008) who revealed protective and curative effect of garlic in lead toxicity by only decreasing the TLC to the extent of 18.92% compared to 31.32% decrease in group of mice treated only with lead acetate in comparison to the control group. Recently, Sharma et al. (2010) reported that oral administration of garlic extract to lead nitrate increased the declined leucocyte and lymphocyte counts to some extent.

Fazlolahzadeh et al. (2011) evaluated immunomodulation due addition of garlic extract in feed of rainbow trout in heat stress and concluded that use of garlic in suitable doses can decrease mortality and increase immunity. Garlic contains a therapeutic factor, germanium, which enhances NK cell and macrophage activity in experimental animals (Aso et al., 1985). Humoral innate factors such as lysozyme were higher in garlic treated fish groups compared with the control fish group (Sahu et al., 2006). Ndong and Fall (2006) reported that 0.5% supplementation of garlic had significantly improved leucocyte count and phagocytic activity, indicating the immunostimulant properties of garlic in juvenile hybrid tilapia. Extracts of fresh garlic contain antioxidant phytochemicals that prevent oxidant damage by enhancing the cellular antioxidant enzymes (Borek, 2001).

Supplementation of *Allium sativum* to diets increased leucocytes, and thrombocytes in fish, *Piaractus mesopotamicus* (Martins et al., 2002) and increased total leucocytes, neutrophils and lymphocyte counts in rats (Oluwole, 2001). Garlic has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Jeorg and Lee, 1998). Garlic components have been reported to decrease the activity of ceruloplasmin which is copper containing oxidase present in plasma for transport of copper to tissue cells (Metwally, 2009). 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 ($NH_2$) groups in their structures. These biologically active compounds might have chelated $Cu^{2+}$ (Dillon et al., 2003) and enhanced its excretion from the body, resulting in least toxic effect of copper. Therefore, utilization of garlic can be beneficial in alleviating the metal induced toxicity in fish when used in balanced doses.

The present investigation revealed elevated total leucocyte count and lymphocyte count in cotreated (taurine and copper) groups of fish in comparison to only copper exposed ones and were decreased significantly when compared with negative control group throughout the experimental duration of 90 days. Therefore, taurine ameliorated the copper induced toxicity in *Clarias gariepinus*. Present findings are comparable to the studies made in male Wister rats by Yeh et al. (2009) in which taurine normalized the white blood cell count altered by toxicity of vitamin A with oxidized fish oil. Kumar et al. (2007) revealed that $CdCl_2$ significantly suppressed lymphocyte proliferation, while as garlic and taurine treated groups showed significant increase in lymphocyte proliferation fish, *Clarias batrachus*. A lack of taurine in the diet is reported to cause a significant leucopenia and abnormalities in the immune system of cats (Schuller-Levis et al., 1990). Cetiner et al. (2005) revealed that leucocyte apoptosis and cell death was significantly ($P<0.05$) decreased on administration of taurine (50mg/kg) in methotrexate treated rats. Anand et al. (2010) reported oral administration of high doses of taurine in Wister rats increased neutrophil count and decreased lymphocyte count without any significant change in TLC.

The results demonstrate that taurine and garlic extract could be beneficial to some extent in fish exposed copper. Moreover, these results can be attributed to great potential of taurine to stabilize the cell membrane and thus could have protected the leucocytes against copper induced toxic damage. However, further studies should be carried out on fishes to determine their leucocyte protective mechanism on copper exposure.

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