Evaluation of the Effects of Exposure to Copper Sulfate on some Eco-physiological Parameters in Silver Carp (*Hypophthalmichthys molitrix*)

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ABSTRACT

Background: Copper is a trace element vital to life, but the toxic effect of water pollution with it on fish is now exhibited. Fish blood is susceptible to contamination-induced stress and its alternation due to the hematological and immunological parameters can be utilized as toxicity indices of xenobiotics.

Methods: In this study, juvenile species of silver carp with average weight of 200gr were kept in laboratory conditions for seven days in a 400l tank. One control group consisted of 21 fish stored in a fiberglass tank. The fish were rendered unconscious immediately with 200ppm clove powder. Blood samples were drawn from tail blood vessels by heparinized syringes and saved without delay in ice. Data analysis was done by t-test and ANOVA using SPSS version 19.

Results: The immunological (lymphocyte, neutrophil), hematological (Hb, Ht, RBC, MCH, MCHC, WBC) and biochemical (Glucose, Cortisol) indices of silver carp (*Hypophthalmichthys molitrix*) were investigated at low (10%LC50) and high (50%LC50) concentrations of CuSO4 for 96h. At both concentrations, Ht, Hb, MCV, RBC, and lymphocyte were significantly ($P<0.05$, $P<0.01$) lower than control group whereas MCH, MCHC, WBC, glucose, cortisol, neutrophil were significantly ($P<0.05$, $P<0.01$) larger in comparison with the corresponding control group.

Conclusion: The main finding of this study is that CuSO4 concentrations (low and high) may cause some alternations in the hematological, biochemical and immunological parameters of the studied fish. Assessment of these indices can supply a useful indicator of CuSO4 of water bodies. It appears that MCHC is an appropriate biomarker of CuSO4 in silver carp (*H. molitrix*).

Keywords: Aquatic Ecosystems, Heavy Metals, Pollutions, Toxicology.

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INTRODUCTION

Copper contamination from normal and anthropogenic sources, like mine washing, agricultural leaching and proximate implementation appears as algaecide and molluscicide in the aquatic environment [1]

High densities of copper are observed in some ecological aquatic systems collecting vineyard runoff water and groundwater [2]. Copper is a trace element vital to life, but the toxic influence of water pollution with this metal on fish is now manifestly exhibited [3].

As shown in the past, in multiple vertebrates, copper chiefly accumulates in fish liver wherein its biological effects are significant [3]. Low copper concentrations quickly cause hepatic disturbances in fish [4].

The toxic influence of copper is connected to its valence for catalyzing oxidative responses, leading to the yield of reactive oxygen species [5]. These extremely reactive compounds may also cause tissue changes and alteration of some physiological reactions of fish, leading to oxidative stress [6].Furthermore, copper can influence the immunological status of fish [7].

The main body part organ for copper storage in fish is liver [4]. Fish blood is susceptible to contamination-induced stress and its interchanging caused by the

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hematological and immunological parameters can be utilized as toxicity indices of xenobiotics [8]. In action, fish blood is greatly utilized in toxicological investigation and environmental monitoring as a favorable indicator of physiological and pathological alterations. Hematological indices and blood parameters could be practical to the assessment of the effects of contaminants on fish [9]. Hematological indices, such as MCV[1], MCH[2] and MCHC[3], hematological parameters, like Hb[1] Hct[2], RBC[3], and WBC[4] amount, and biochemical parameters, like glucose, are greatly used to assess the toxic effect of environmental pollutants [10].

Silver carp is probably the most plentiful freshwater fish because of its fast growth [11]. Although the produce of silver carp is expanding annually, the processing of this fish is restricted. In this study, tested variations of biochemical parameters, including glucose, cortisol, and hematological and immunological changes in silver carp (*Hypophthalmichthys molitrix*) were exposed to $CuSO_4$.

MATERIALS AND METHODS

Preparation of Fish in Experimental Conditions

Juvenile species of silver carp with average weight of 200gr were provided by Fisheries Research Laboratory. The fish were adapted to laboratory conditions for seven days in a 400-liter tank. All fish were nourished with trading pellet twice a day. They were exposed to a high concentration of 50% LC50 (0.49 mg/l) $CuSO_4$ and a low concentration of 10% LC50 (0.09 mg/l) for periods of 24h, 48h, and 96h for static toxicity examination, presented in a tank of 400L, groups of 21 each. A control group consisting of 21 fish was preserved in a fiberglass tank without exposure to the toxicant. Three repeats were executed for each experimental stage. Temperature, dissolved oxygen, pH, and conductivity were purged during the experiment [12].

Process of Collecting Fish Specimens and Blood Appraisal

Fish were rendered unconscious immediately with 200ppm clove powder. Blood samples were quickly obtained from tail blood vessel by heparinized syringes and saved without delay on ice [13]. Calculation of the blood indices were carried out on fresh blood. Numbers of blood leukocytes and erythrocytes were executed by diluting heparinized blood with Giemsa stain at 1:30 dilution and the cells were counted using a Neubauer hemocytometer through light microscopy [14]. The leukocyte differential computation was done in peripheral blood spots stained by Merck Giemsa [15], giving the neutrophils quantity of differential neutrophils and the mononuclear quantity of differential lymphocytes, eosinophils, and monocytes. Hematocrit values (Ht%) were shortly determined after gathering samples by locating fresh blood in glass capillary tubes and centrifuging them via a micro-hematocrit centrifuge for 5 minutes at 10000 rpm (Hettich, Germany). Then dimensions of the packed cell capacity were determined [16]. Hematocrite interpretation was carried out with the help of a microhematocrit reader. Hemoglobin levels (Hb mg/l) were colorimetrically measured in cyanomethemoglobin [17]. Erythrocytes indices, MCH (mean corpuscular hemoglobin), MCHC (mean cell hemoglobin concentration), and MCV (mean corpuscular volume) were evaluated in RBC, Ht, and Hb [17].

Statistical Analysis

To find out meaningful differences for examining the effect of copper on blood parameters an analysis of variance (ANOVA) with Duncan Post Hoc was utilized.

Pearson coefficients of correlation(r) were computed between copper concentrations and blood parameters to examine the relationship between bioaccumulation and its impacts. To ascertain the kinship between copper concentration and blood parameters multiple regressions were used. Data were analyzed statistically at $P<0.05$ and expressed as mean±SD ($\overline{X} \pm SD$).

Ethical Issues

The experimental protocols were approved by the Medical Ethics Committee of the organizations that supported this study.

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RESULTS

Lc50 Value (96h)

The 96h Lc50 value of copper sulfate to silver carp in the present study was evaluated and located to be 0.98±1.98 mg/l. Blood factors at 10% LC50 (low) and 50%LC50(high) concentrations were measured.

Hematological Indices

Some of the hematological indices (Ht, Hb, MCV, and RBC) significantly ($P<0.05$) declined in copper sulfate treated fish from that of the control group in both of the low (10% LC50) and high (50% LC50) concentration (Figures 1-3), whereas other hematological indices (MCH, MCHC, and WBC) in fish exposed to the high concentration of 50% LC50 $CuSO_4$ and low concentration of 10% LC50 for 96h significantly increased compared to the control group (Figures 2 and 4).

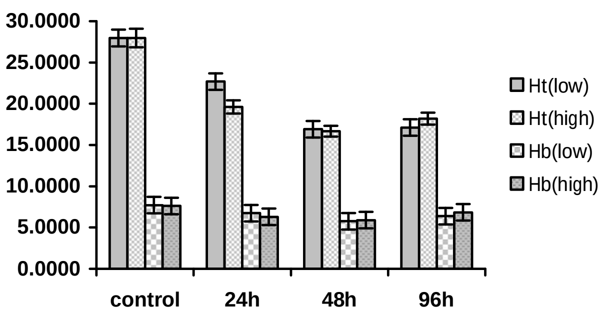

Figure1. Hematocrite and hemoglobin changes during exposure to low and high concentrations of copper sulfate in silver carp.

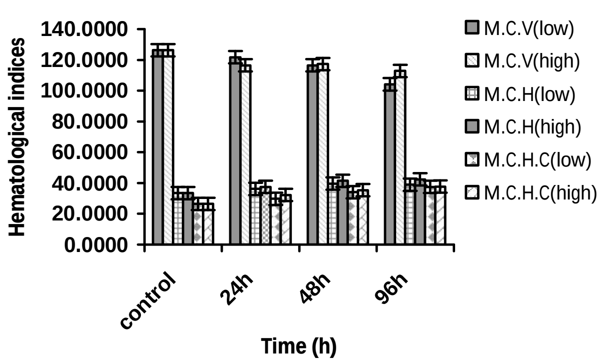

Figure 2. MCV, MCH, and MCHC changes during exposure to low and high concentrations of copper sulfate in silver carp.

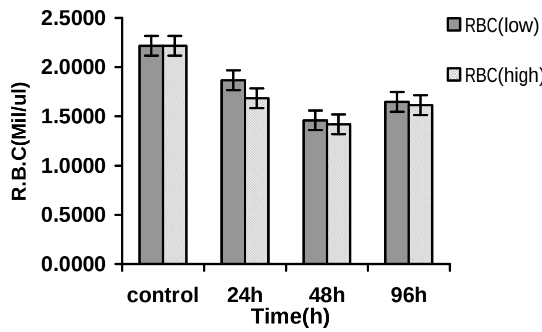

Figure 3. Red blood cell changes during exposure to low and high concentrations of copper sulfate in silver carp.

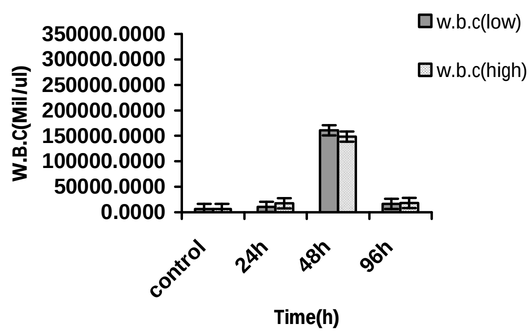

Figure 4. White blood cell changes during exposure to low and high concentrations of copper sulfate in silver carp.

The correlations between $CuSO_4$ and all parameters were statistically examined by analyzing the data procured during exposure to $CuSO_4$ for 96h.

At high concentration (50%LC50), MCH and MCHC levels indicated significant positive correlations ($P<0.01$), whereas RBC, Ht, and MCV levels showed significant negative correlations ($P<0.05$) with exposure to $CuSO_4$. However, at low concentration (10%LC50), RBC and Hb ($P<0.05$) and Ht and MCV ($P<0.01$) levels presented significant negative correlations while MCH ($P<0.05$) and MCHC ($P<0.01$) levels showed significant positive correlations with exposure to $CuSO_4$ for 96h.

Biochemical Analysis

Glucose and cortisol levels significantly ($P<0.05$) increased in the fish treated with both 10% and 50% concentrations of LC50

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copper sulfate when contrasted with the fish in the control group (Figure 5). Glucose level at 50% LC50 concentration indicated a significant positive correlation (P<0.01) with exposure to CuSO4. At 10% LC50 concentration, glucose and cortisol showed significant positive correlations (P<0.01) with exposure to CuSO4.

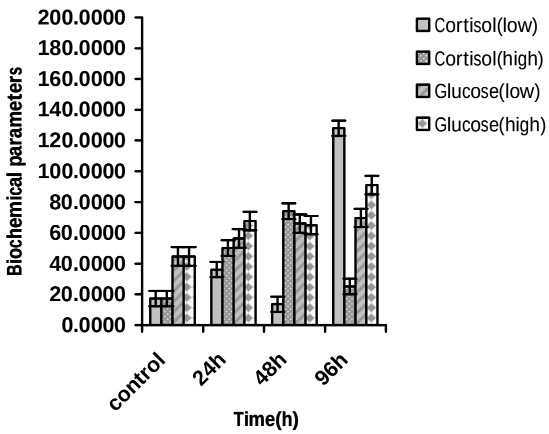

Figure 5. Cortisol and glucose changes during exposure to low and high concentrations of copper sulfate in silver carp.

Immunological Indices

Lymphocyte level significantly (P<0.05) decreased when exposed to 10% and 50% LC50 copper sulfate in comparison with the control group but neutrophil was increased in copper sulfate for 96h (Figure 6).

The neutrophil level at low concentration showed a significant positive correlation (P<0.05) and lymphocyte indicated a significant negative correlation (P<0.05) with exposure to CuSO4. Eosinophils did not show significant correlations at both concentrations.

To determine the relationship between CuSO4 concentration and hematological, biochemical, and immunological activities, curve estimation regressions were utilized. At high concentrations, MCHC, MCH, and eosinophils levels indicated a significant linear regression (P<0.05) Y = a ± bX with CuSO4.

MCHC, glucose, and MCV levels at low concentration showed a significant linear regression (P<0.05) Y = a ± bX with CuSO4.

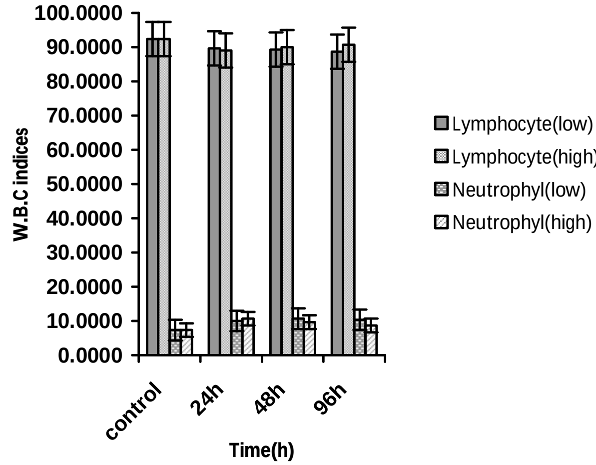

Figure 6. Lymphocyte and neutrophil changes during exposure to low and high concentrations of copper sulfate in silver carp.

DISCUSSION

Metal compounds extant in wastes may accumulate in various organs of animals or cause significant alterations in plant and fish biomass production [18]. Statistical findings in this study, indicate that copper is toxic to silver carp (*H. molitrix*). In this study, a number of hematological, biochemical, and immunological parameters were examined in silver carp after exposure to 10% and 50% LC50 doses. The results showed increase in M.C.H, M.C.H.C, netrophil, cortisol, glucose, eosinophil and decrease in Ht, Hb, lymphocyte, M.C.V, and R.B.C at both concentrations. Blood is a very good indicator of toxic stress and analysis of hematological parameters in fish is greatly used to estimate toxic stress and practical status of the animals' health. During the study period, hemoglobin content and hematocrit values decreased in the fish in the experimental group with the exposure for 96h compared to the controls group.

Exposure to poisons leads to diminished hemoglobin content and hematocrit due to disturbances in haemopoietic processes and acceleration of disintegration of erythrocyte cells [19, 20].

Further lysing of erythrocytes due to toxicant stress may too result in a reduction in hemoglobin and hematocrit amounts in the fish [21].

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Erythropaenia and decreases in haemoglobin and haematocrit levels were the manifestations in *Barbus conchonius* in Gill and Pant's study [22] as well as in *Cyprinus carpio* with sublethal density of carbofuran in the study done by Chandra *et al*. [23].

The erythrocyte level was observed to decline in fishes exposed to demanding qualifications. An anemic condition is usually shown by lower RBC count and hemoglobin amount as seen in fishes exposed to environmental contamination [24]. A significant decline in RBC amount was noticed in this study. The reduction in erythrocyte amount and hematocrit count and, as a result, anemia could be attributed to the following elements: 1) haemodilution of blood caused by weakened osmoregulation and gill harm [25,26] and 2) compensatory reaction enhancing O2 carrying volume to support the gas exchange, which also shows an alteration in the water blood barrier because of gas exchange in gill lamellae [27].

The high proportion of unripe red blood cells in blood circulation might be the cause for MCV reduction in the present examination.

The related decrease in hematological indices proved the toxic effect of copper that affects both metabolic and hemopoietic activities of silver carp.

In the present study, the significant expansion of MCH shows the bulge of red blood cells or the high concentration of smaller premature erythrocytes containing less hemoglobin in circulation due to hyperplasia in the erythropoietic sites, aroused by high claim for oxygen and carbon dioxide delivery [28].

The notable addition of MCHC value might be followed from sphaerocytosis as implied by Sobecka [29]. The results of the present study indicated an increase in differential neutrophils and a decline in lymphocytes.

The significant increase in WBC count reflects a widespread state of toxaemia to an adjustment manifested in leucocytosis for coping with such a stressful situation by the fish [30]. Lymphocytes are the most common current leukocytes established in fish. The heterophils have been changeably nominated heterophil or neutrophil conditional upon the size of cytoplasmic granules in fishes. Unlike fowl and reptile heterophils, fish heterophils include a big quantity of myeloperoxidase and their macrophages manufacture nitric oxide and reactive oxygen [31].

There is an absence of knowledge respecting the full work of fish eosinophils, but they peer to work in a mode similar to mammalian mucosal mast cells. The eosinophils have been associated with antigenic arousal and parasitic invasions [32].

The monocyte and neutrophil expansion and lymphocyte reduction during exposure to different stressors have been confirmed in cultured fish *Oreochromis aureus* [33].

It is assumed that neutrophils and monocytes have phagocytic stir which might describe their increased percentage during the exposure period.

Brandão *et al*. [34] observed a reduction in some immunological parameters (platelet, leukocyte, and lymphocyte counts) and an enlargement in neutrophil and monocyte percentages in exposure to $HgCl_2$.

The consequential decline of mononuclear (differential lymphocytes plus monocytes) and multiplied of differential neutrophils to methyl mercury and inorganic lead were noted by Oliveira Ribeiro *et al* [9].

Glucose is liberated by corticosteroids that their elevation has been delineated as a primary answer to most stressors [35].

Hypothalamus-pituitary-interrenal axis, started by multiple stressors, elevated blood levels of cortisol. This endocrine reaction continued by a spurt of catecholamine-stimulated steps (lipolysis, glycogenolysis, and gluconeogenesis), which are prominent energy mobilizing procedures [36]. Increased blood glucose concentrations result from an instability between the hepatic yield of glucose and the peripheral uptake of the sugar [37]. Mostly, the existence of contaminants in aquatic environment exercises its effect at cellular or molecular level which results in an important alterations in biochemical response. For monitoring aquatic environments, analysis of biochemical ways, it offers as consequential biomarkers. However, to date, few perusals have used dietary exposure to examine the

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effects of pollutants in aquatic organisms [38], and the present findings indicated that under experimental conditions, blood parameters were susceptible to different effects of copper.

CONCLUSION

The main findings of this study is that copper concentrations (low and high) may cause some alternations in the hematological, biochemical and immunological parameters of the studied fish. Therefore, assessment of these indices could supply a useful indicator of copper of aquatic environments. It appears that MCHC is an appropriate biomarker of copper in silver carp *(H. molitrix)*.

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