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Impact of copper on haematological profile of freshwater fish, *Channa punctatus*

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Abstract: *The present study was to evaluate heavy metal toxicity stress symptoms in fish blood during long-term exposure of sublethal concentration of copper sulphate ($CuSO_4$). The effects of copper on various heamatological parameters were evaluated exposing Indian fresh water fish, Channa punctatus to a sub-lethal concentration of copper sulphate (0.36 mg l^{-1}) for different periods. Exposure of fish to copper showed a significant decrease in the haemoglobin (Hb) content from 10.73 to 6.60%, red blood cells (RBC) from 2.86 to 1.84 x10^6/ mm^3 and packed cell volume (PCV) from 31.00 to 23.33% at the end of 45th day as compared to control. Whereas the white blood cells (WBC) increased from 60.00 to 92.48 x10^3/ mm^3, clotting time (CT) from 27.66 to 43.00 second, erythrocyte sedimentation rate from 5.0 to 13.66 mm / hr and mean corpuscular volume from 108.11 to 126.85 cμ, significantly, with increase in exposure periods. Although mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) values showed significant increase during 15 and 30 days exposures,, both the values were found significantly increased at the end of the experimental period. The differential leucocytes count (DLC) showed a significant increase in the populations of lymphocytes and eosinophils, whereas neutrophils, monocytes and basophiles were found decreased after exposure to copper.*

Key words: *Channa punctatus, Blood, Copper sulphate*
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Introduction

Intensive industrial developments in last few decades have increased the concentration of copper in river and lake, affected fishes and deplete natural resources. Heavy metals have become major environmental hazards, although they have great biological significance as micronutrients. Major sources of Cu in aquatic environment are sewage and industrial effluents. Cu toxicity to fishes had already been reported by many workers (Eriksen *et al.,* 2001; Wepener *et al*., 2001; Paquin *et al.,* 2002; Dhanapakiam *et al.*, 2006; Lodhi *et al*., 2006; Ketpadung and Tangkrock-olan, 2006). Copper sulphate is widely used as an algaecide for controlling phytoplankton in fish ponds and lakes as well as a herbicide, used in aquatic weed control since 1882 (Effler *et al.*, 1980; Carbonell and Tarazona, 1993). Copper sulphate has many industrial applications including the preparation of Bordeaux mixture (a fungicide) and the manufacturing of other copper compounds.

Fishes are the simple and reliable biomarker of copper pollution of aquatic bodies (Taylor *et al.*, 2000; Lodhi *et al*., 2006). The metallic ion present in water enters the fish body and gets accumulated in various organs like liver and kidney (Al-Mohanna, 1994; Shukla *et al.*, 2007). The blood parameters have been used as sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, *etc*. These metallic ions are the probable major cause of the physiological abnormalities in fish. A fall in RBC count, Hb% and PCV%, in the fish, *Channa punctatus* upon treatment with both copper and chromium was noticed along with acute anaemia (Singh, 1995). The metal entering into fish system are slowly eliminated (Newman and Mitz, 1988; James and Sampath, 1996; James *et al.*, 1996), hence the blood parameters get affected on account of metal toxicity. The oxygen carrying capacity of blood at 95% saturation, the iron content of the blood and number of red blood cells in fishes often vary with life history, stage, habits and environmental conditions. Transport of oxygen in the blood depends on the amount of haemoglobin, a respiratory pigment found in the blood of all vertebrates. There is a large number of inherent conditions in which the red blood cell membrane shows abnormal fragility which may be manifested in abnormal shape (spherocytoma), shortened red blood cell life time (often accompanied by reticulocytosis) or in impaired response to a challenge by a drug or other chemical). Acute exposure of *Colisa fasciatus*, *Oreochromis mossambicus* to sub-lethal concentrations of lead, copper and zinc has been shown to produce haemolytic anaemia due to lysis of erythrocytes with concomitant decrease in Hb%, PCV% value and the number of erythrocytes (Soiveo and Nikinmaa, 1981; Sampath *et al.*, 1998). In metals affected water, fishes are killed after association with runoff storm events. The metals responsible for toxicity are those whose toxicity and or mobility are enhanced by the depression of pH, hardness, alkalinity and decomposable organic-carbon that typically accompany these events. Activated oxygen species produced via oxygen gas are able to increase the hemolytic effects of cupric salts evoking risks of side effects. A variety of heavy metals including copper are in use from the ages for industrial progress. Both flora and fauna including human beings have suffered a loss on account of copper pollution of water resources through out the globes. The present investigation is carried out to evaluate the changes in various haematological parameters of the freshwater fish, *Channa punctatus* after exposing to sublethal concentration of copper sulphate.

Materials and Methods

The haematological effect of copper was studied by static bioassay using tap water (University water supply) as dilution medium was estimated by the method of APHA (2005). The changes in physico-chemical characteristics, such as temperature, pH, TDS (total dissolved solids), DO (dissolved oxygen), hardness, alkalinity, chloride and iron of experimental water were recorded throughout the experimental period. The small size freshwater fish, *Channa punctatus,* weighing 15±2 g and measuring11±2 cm, were collected with the help of local fisherman from water bodies located in the sub-region of Lucknow. The fish was properly washed in tap water and treated with 0.02% $KMNO_4$ and 0.004% formalin solution to remove external infection of fungi, algae, etc. Prior to the experimentation the normal uninfected healthy fish were selected for experiment. The fish were acclimatized to laboratory conditions for 15 days before taken for experimentation. The animals were fed TOKYO made in Japan on a day at 8 pm everyday. The fish were divided into 4 equal groups consisting of 10 each and each group was transferred separately to glass aquaria of 100 l volume. While the group I fish were maintained as control without any treatment, the group II, III and IV fish were exposed to sublethal concentration (0.36 mg l^{-1}) of copper sulphate for 15, 30 and 45 days. The waste products were removed from aquaria water by using good quality of aquaria water filter. The LC_{50} was estimated employing Trimmed Spearman Karber Method (Hamilton *et al.*, 1977). The sublethal toxicity test was conducted at 1/10 of median lethal concentration (3.60 mg l^{-1} LC_{50} value) of fish. Sub-lethal concentration of $CuSO_4.5H_2O$ (MERCK) test chemical was freshly prepared in distilled water before mixing in aquaria water. The blood from the caudal vein of control and treated fish was collected for heamatological investigation. Hb, RBC, WBC, DLC, ESR and PCV were examined following the procedures of Wintrobe (1957) and Sood (1996). The anticoagulant heparin liquid power (5,000I.U) was used for estimation of ESR and PCV.

The data observed in the experiment were statistically analyzed for the calculation of standard error of mean (SEM). One way ANOVA and Duncan Multiple range test for individual group wise comparison was administrated for testing the hypothesis. The data shown are the average of three replicates ± SE and statistical significance was tested at $p<0.05$ level.

Results and Discussion

In the present study, exposure of fish to sub-lethal concentration of copper for 15, 30 and 45 days caused significant alterations in haematological parameters of Indian freshwater fish, *Channa punctatus*. The alterations observed in various physico-chemical parameters, such as temperature, pH, TDS, DO, hardness, alkalinity, chloride and iron of control and experimental systems are indicated in Table 1. Hb%, RBC and PCV% decreased significantly after 15, 30 and 45 days of exposure periods, respectively, in comparison with control (Table 2). On the contrary, WBC counts, ESR, CT and MCV values were found significantly increased after 15, 30 and 45 days of exposure, as compared to control. After an initial increase over and above control in MCH for 15 and 30 days, it was found significantly decreased after 45 days of exposure. Although the MCHC and MCH values were found significantly elevated during the exposure of 15 and 30 days. The increase in MCH was also significant after 15 and 30 days exposures. Both values decreased significantly after 45 days of exposure in comparison with control. The result of Duncan Multiple range for individual groups' wise comparison are shown in Table 3. The lymphocytes and eosinophils registered an increasing trend over and above the control (Fig. 1). The percentage of neutrophils, monocytes and basophils were found decreased as exposure periods increased. Maximum decrease in neutrophils, monocytes and basophils was recorded after 45 days of exposure.

The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as heavy metals, biocides, pesticides, industrial effluents, *etc*. The exposure of *Channa punctatus* to sub-lethal concentration of copper significantly decreased Hb%, RBC count and PCV% values leading to anaemia. The anaemia might have led to a fall in the red blood cell count, haemoglobin concentration, and haematocrit volume. Anaemia, under copper induced stress, may also be due to blood cell injury and disrupted heamoglobin synthesis (Mckim *et al.*, 1970; Gross *et al.*, 1975). The anaemic condition in fish results from an unusually low number of red blood cells or too little haemoglobin in the red blood cells. Similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals have been reported previously by Goel *et al.* (1985) and Goel and Sharma (1987). According to Pamila *et al.* (1991), the reduction in haemoglobin content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for synthesis of haemoglobin. Joshi *et al.* (2002) suggested that heavy metal exposure also decreased the RBC, Hb% and PCV% due to impaired intestinal absorption of iron. Anaemia is an early manifestation of acute and chronic intoxication of heavy metals. Significance of these changes may be understood in terms of reduced oxygen consumption in fish resulting in death due to heavy metal pollution (Christensen *et al.*, 1972).

High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. In most cases, abnormal red cell morphology is noted. White blood cell counts were found increased following copper exposure as shown in Table 2. Similar findings were also documented significantly higher in fish exposed to increased copper concentration (Nath and Banerjee, 1995; Mazon *et al.*, 2002). Mishra and Srivastava(1980) also reported an increase in leucocytes count when they exposed fishes to heavy metals. Some of the most common causes of heavy metal toxicity are inflammatory lesions associated with tissue damage, anaemia and neoplasia. Further, an increase in fibrinogen or serum globulins or a decrease in serum albumin, may also cause an increase in the ESR. Increase in ESR and MCV values and total leucocytes count suggested that the anaemia was of macrocytic type (Sampath *et al.,* 1998; Sinha *et al.*, 2000). A clot is formed as the end product of blood coagulation. The clot under normal conditions undergoes contraction, when serum is expressed from the clot, and

Table - 1: Effect of copper sulphate on physico-chemical profile of water

Parameters	Days of exposure period						
	Zero hr control + treatment	15 day control	15 day treatment	30 day control	30 day treatment	45 day control	45 day treatment
Temperature ^{0}C	22	24	24	25	25	26	26
pH	7.5	7.5	7.2	7.4	6.5	7.3	5.8
TDS (mg l^{-1})	100	105	200	110	230	130	280
DO (mg l^{-1})	7.8	7.5	6.7	7.6	6.8	7.0	6.8
Hardness(mg l^{-1})	110	100	96	95	80	85	50
Alkalinity mg l^{-1})	350	350	300	340	250	300	200
Chloride (mg l^{-1})	43.50	42.50	36.75	43.00	33.50	40.00	29.70
Iron (mg l^{-1})	0.41	0.35	0.31	0.34	0.29	0.34	0.26

TDS =Total dissolved solids, DO = Dissolved oxygen

Table- 2: Effect of copper sulphate on haematological parameters of *Channa punctatus*

Parameters	Days of exposure period				
	Control	15 day treatment	30 day treatment	45 day treatment	F value
Hb gm (%)	10.73 ± 0.17	10.06 ± 0.13*	8.80 ± 0.11*	6.60 ± 0.11*	175.27
RBC (x10^6/ mm^3)	2.86 ± 0.06	2.46 ± 0.06*	2.08 ± 0.91*	1.84 ± 0.02*	47.55
WBC(x10^3/ mm^3)	60.00 ± 1.04	81.30 ± 1.95*	91.88 ± 1.35*	92.48 ± 0.91*	121.38
ESR (mm / hr)	5.00 ± 0.57	7.00 ± 0.57*	9.66 ± 0.33*	13.66 ± 0.66*	45.93
PCV (%)	31.00 ± 1.00	27.7 ± 0.33*	24.66 ± 1.20*	23.33 ± 0.33*	17.44
CT (Second)	27.66 ± 1.45	33.7 ± 1.85*	39.00 ± 2.08*	43.00 ± 1.15*	15.77
MCV (cμ)	108.11 ± 2.00	112.31 ± 3.10^{NS}	118.16 ± 0.70*	126.85 ± 2.38*	12.97
MCHC (%)	34.87 ± 0.61	35.95 ± 1.65^{NS}	37.08 ± 1.03*	28.29 ± 0.64*	13.63
MCH (μμg %)	37.67 ± 0.05	41.10 ± 0.65*	43.28 ± 1.23*	35.86 ± 0.17^{NS}	24.83

Values are mean ± SEM, n = 3, * = significant at p<0.05 level, Hb% = Haemoglobin, RBC = Red blood cells, WBC = White blood cells, ESR = Erythrocyte sedimentation rate, PCV% = Packed cell volume, CT = Clotting time, MCV = Mean Corpuscular Volume, MCHC = Mean corpuscular Haemoglobin concentration, MCH = Mean corpuscular Haemoglobin

Table - 3: Duncan Multiple Range test for individual groups wise comparison (values are given in difference of mean) for haematological aspects of *Channa punctatus*

Comparison	Hb	RBC	WBC	ESR	PVC	CT	MCV	MCHC	MCH
Control vs. 45 day treatment	4.13	1.02	32.48	8.66	7.66	15.33	18.73	6.57	1.80^{NS}
Control vs. 30 day treatment	1.93	0.78	31.88	4.66	6.33	11.33	10.05	2.21	6.14
Control vs. 15 day treatment	0.66	0.40	21.30	2.00	3.33	6.00	4.19^{NS}	1.08^{NS}	3.16
15 day T vs. 45 day treatment	3.46	0.62	11.18	6.66	4.33	9.33	14.54	7.65	4.97
15 day T vs. 30 day treatment	1.26	0.38	10.58	2.66	3.00	5.33^{NS}	5.85^{NS}	1.13^{NS}	2.98
30 day T vs. 45 day treatment	2.20	0.24	0.60^{NS}	4.00	1.33^{NS}	4.00^{NS}	8.68	8.79	7.95

Values are significant at p<0.05, NS = Not significant

finally the clot becomes denser. The blood clotting substance in fish blood is prothrombin which is present in high percentage. However, it is less than that of mammalian blood level (Table 2). A substance released by the platelet (thrombosthenin) is responsible for clot retraction (Cassilas and Smith, 1977; Pandey and Shukla, 2005). Comparable results have also been reported in *Labeo rohita* exposed to copper sulphate (Sinha *et al.,* 2000), and *Catla catla* exposed to cadmium (Vincent *et al.*,1996).

The lymphocytes are reported to be responsible for immune response. The nucleus occupies virtually the whole of the cell, leaving only a narrow rim of basophilic cytoplasm in which there are a few mitochondria and isolated ribosomes. The number of lymphocytes in the blood is noticeably greater in fishes than in mammals. Monocytes are partially differentiated end cells, which under appropriate circumstances develop into mature cells of mononuclear phagocyte system but are not capable of further division. In teleosts, this system is organized as in other vertebrates, with circulating monocytes arising from renal haemopoietic tissue and being readily able to take up a functional tissue role. Monocytes in fishes have been observed to take up foreign particulate material such as carbon. Neutrophils and monocytes are important white blood cells to protect the body, through their elevated phagocytic activity, against bacterial infection in damaged tissue. Morphologically fish neutrophils closely resemble their mammalian counter parts though the degree of nuclear polymorphism in teleosts varies considerably. Release of neutrophils

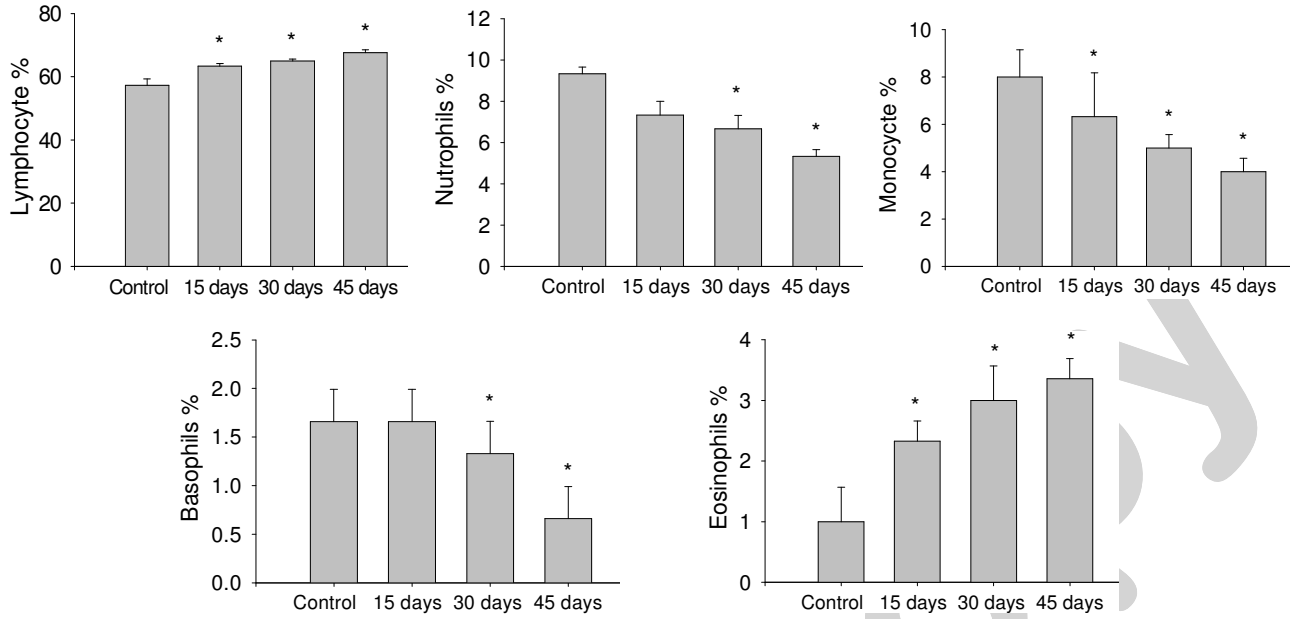

Fig. 1: Effect of copper sulphate on differential leucocytes count (DLC) of *Channa punctatus*. Values are mean ± SEM, n = 3, * = significant of p< 0.05 level

in to the blood, causing a disease neutrophilia, is known to occur as a non-specific response to a variety of stress stimuli in mammals and fishes. The percentage of these cell types generally decreases during acute exposure to copper (Nussey *et al*., 1995; Svobodova *et al*., 1994), and in situations of chronic copper exposure, the neutrophils percentage has been reported to increase (Dick and Dixon, 1985). Eosinophils are putatively considered to play a role in defence mechanisms in mammals by phagocytosing antibody / antigen complexes. The fish eosinophils have been implicated in inflammation and some reports of their phagocytic activity do also exist (Kelenyi and Nemeth, 1969). Thus, it is concluded that the haematological parameters are the most sensitive parameters in monitoring the toxicity of copper especially at sublethal concentrations.

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