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Original Article

The acute effect of copper exposure on serum biochemical characteristics of Common carp (*Cyprinus carpio* L.)

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Abstract: Effects of ambient copper was investigated on serum stress markers, sodium and enzyme levels in Common carp (*Cyprinus carpio* L.) over a 14-d exposure period. Fish were exposed to 0, 25 and 100 μg L^{-1} copper (as copper sulfate) and blood was sampled at 0, 3, 7 and 14 d after exposure. Serum profile was significantly affected by copper concentration, sampling time and their interaction. Increase in serum levels of cortisol, glucose, alanine aminotransferase and aspartate aminotransferase and decrease in serum sodium levels were observed in both copper-exposed groups, 3 d after copper exposure, which lasted until the end of the experiment. It is concluded that copper exposure causes stress response and sodium loss in common carp. Likewise alanine aminotransferase and aspartate aminotransferase increase after exposure which might be as results of either tissue damage or stress.

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Introduction

Aquatic organisms are threatened by heavy metal pollution, since aquatic habitats are the final destination of effluents and wastes. Fish may be exposed to waterborne metals including copper. Copper resources in the water bodies are industrial waste or copper sulfate as a therapeutic or algicide agent. Thus, fish may experience copper toxicity both in their natural habitat and rearing systems. Copper sulfate is highly soluble in water producing Cu^{2+} which is an important xenobiotic in aquatic ecosystems and a non-degradable and cumulative pollutant. The toxicity of Cu^{2+} to teleosts has been extensively studied, as summarized by Sorensen (1991). Generally, copper attacks mainly the gill, an important organ in gas exchange and hydromineral balance in fish. Therefore, waterborne copper exposure causes stress response in fishes (Flik et al., 2002; Fırat et al., 2011).

Common carp (*Cyprinus carpio* L.) is an important pond-aquaculture species reared in Iran. It is an important species of the Caspian Sea and plays an important role in producing food supply for people. This species may expose to ambient copper as result of copper sulfate, used as algicide or therapeutic. Therefore, the water source of the Common carp ponds might be contaminated by copper due to waste water introduction. Also, this species might face copper toxicity in the Caspian Sea, as de Mora et al. (2004) reported the occurrence of copper in the southern Caspian coasts.

The present study aimed to investigate the acute effect of copper (as copper sulfate) on serum characteristics of Common carp (*C. carpio* L.) over a 14-day period.

Materials and methods

Fish maintenance and condition: Two hundred and forty Common carp (~ 100 g in weight) were randomly distributed in 12 fiberglass tanks, filled with 350 L decholorinated tap water. The fish were fed (2% of biomass) with an artificial diet (35.5%

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Table 1. Significance of copper concentration (Dose), period of exposure (Time) and their interaction on serum biochemical characteristics of common carp *C. carpio*

P_{value}	Cortisol	Glucose	Sodium	ALT	AST
Dose	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Dose × Time	0.014	< 0.0001	0.0014	< 0.0001	< 0.0001

Two-way ANOVA, n = 4.

protein, 10.1% lipid) twice a day. Water of the tanks was exchanged 80%, daily. Water quality was as following: temperature = 25-27˚C, dissolved oxygen > 5.5 mg L^{-1}, pH = 7.03-7.1, total hardness = 210 mg L^{-1} ($CaCO_3$), alkalinity = 200 mg L^{-1} ($CaCO_3$), magnesium = 1 mg L^{-1}, calcium = 84 mg L^{-1}, iron = 0.01 mg L^{-1}, potassium = 8.5 mg L^{-1}, sulfate = 7.2 mg L^{-1} and free copper = undetectable. Continuous aeration was supplied for all tanks. The fish remained under these conditions over a 2-week period.

Toxicity trail: After 2 weeks of acclimation period, the tanks were assigned as 3 treatments (4 tanks per treatment). One of the treatments served as control, which received freshwater (as acclimation period), while the two others received same water with 25 and 100 μg L^{-1} copper, as copper sulfate. Feeding and the other conditions were similar to those of the acclimation period. The specimens remained under these conditions for 2 weeks.

Sampling and analyses: Specimens from all treatments were blood-sampled before exposing to copper as well as at 3, 7 and 14 day after exposure. At each sampling point, two specimens were caught using a dip net from each tank (4 fish per treatment) and anesthetized with clove solution bath (3000 ppm) in less than 1 min. Then blood samples were collected using caudal severance. All samples were kept at room temperature for 2 h prior to centrifugation (3000 rpm, 5 min). Serum samples were stored at -80°C until further analyses.

Serum levels of cortisol were determined by ELISA method using commercial kit (IBL, Gesellschaft für Immunchemieund Immunbiologie, Germany). Serum glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured spectrophotometerically using commercial kits (Pars Azmun Co. Ltd, Tehran, Iran). Sodium levels were determined using a flame photometer (SEAC, Florence, Italy).

Dissolved oxygen and pH were determined by portable multiparameter meter (sensION 156, USA). Water total hardness, alkalinity, magnesium, calcium, iron, potassium, sulfate and free copper were determined using a portable photometer with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK).

Statistical analyses: All data were tested for normality and homogeneity of variances using the Shapiro-Wilk's and the Levene test, respectively. All data were examined using a two-way ANOVA with copper concentration and sampling time as factors, and significant difference among the means was delineated by LSMeans' test. All analyses performed using the statistical software of SAS version 9. Data are presented as mean ± SD. The values of $P<0.05$ were considered significantly different.

Results

The results showed that all tested parameters were significantly affected by copper concentration, sampling point and their interactions (Table 1).

There were no significant differences in cortisol, glucose, sodium, ALT and AST levels over the time, in the control group (Table 2). In addition, there were no significant differences among the treatments before exposing to copper (Table 2).

The levels of cortisol increased significantly at the days 3 and 7 and showed further increase at the day 14 compared to that of 0 d, in 25 μg L^{-1} treatment. In Also, its levels in 100 μg L^{-1} treatment showed a significant increase at the day 3 and remained elevated until the day 14.

Table 2. Effect of copper concentration and period of exposure on Common carp *C. carpio* serum levels (mean ± SD) of cortisol (ng ml^{-1}), glucose (mg dl^{-1}), sodium (mEq L^{-1}), ALT (IU L^{-1}) and AST (IU L^{-1}). Different lowercase letters show significant difference over the time in each copper concentration. Different symbols show significant difference among the treatments at each sampling point

	Cu ($\mu g\ L^{-1}$)	Exposure period (day)			
		0	3	7	14
Cortisol	0	38.7 ± 2.5 a †	41.3 ± 4.3 a †	40.3 ± 4.3 a †	43.3 ± 4.4 a †
	25	37.5 ± 2.4 a †	47.8 ± 2.5 b † ‡	50.5 ± 4.5 bc ‡	55.8 ± 7.3 c ‡
	100	36.7 ± 4.3 a †	52.3 ± 2.5 b ‡	52.5 ± 6 b ‡	58.2 ± 6.2 b ‡
Glucose	0	42.1 ± 2.2 a †	43.8 ± 3.9 a †	43.1 ± 4.1 a †	43.3 ± 3.9 a †
	25	44.5 ± 3.2 a †	88.3 ± 6.2 b ‡	85.8 ± 6.5 b ‡	104 ± 4.4 c ‡
	100	43.2 ± 4.8 a †	92.2 ± 6.7 b ‡	100 ± 3.6 c §	115 ± 4.5 d §
Sodium	0	140 ± 1.3 a †	140 ± 1.8 a †	140 ± 1.7 a †	139 ± 1.7 a †
	25	140 ± 2.8 a †	132 ± 2.2 b ‡	133 ± 2.2 b ‡	132 ± 1.9 b ‡
	100	139 ± 1.8 a †	132 ± 1.3 b ‡	131 ± 1.8 b ‡	131 ± 2.5 b ‡
ALT	0	13.8 ± 1.7 a †	15.5 ± 2.1 a †	13.5 ± 3.4 a †	14.3 ± 1.7 a †
	25	14.8 ± 2.5 a †	28.8 ± 2.9 b ‡	33.2 ± 2.2 c ‡	40.5 ± 3.1 d ‡
	100	15.8 ± 2.2 a †	35.3 ± 2.6 b §	44.5 ± 2.1 c §	45.3 ± 2.9 c §
AST	0	118 ± 6.2 a †	121 ± 2.8 a †	121 ± 2.6 a †	120 ± 4.3 a †
	25	118 ± 1.7 a †	146 ± 5.1 b ‡	175 ± 5.3 c ‡	203 ± 5.4 d ‡
	100	120 ± 4.6 a †	150 ± 3.6 b ‡	184 ± 4.1 c §	208 ± 5.4 d ‡

The glucose increased over the time in both 25 and 100 $\mu g\ L^{-1}$ treatments, however, the fish exposed to 100 $\mu g\ L^{-1}$ copper showed higher glucose compared to that of 25 $\mu g\ L^{-1}$ at the days 7 and 14.

The levels of sodium decreased at the day 3 d in both copper-exposed groups and remained low until the day 14. ALT and AST increased over the time in both copper-exposed treatments.

Discussion

The present study showed that copper exposure alters serum characteristics of Common carp. Since, the experimental condition and sampling procedure was constant over the experiment, no significant change was observed in the measured parameters over the trial time, in the control group. In addition, there were no significant differences among the treatments at 0 d suggesting no tank effects before the experiment initiation. Thus, any change in serum profile of the copper-exposed groups, assumed to be related to ambient copper.

The levels of cortisol increased significantly in both copper-exposed treatments. Cortisol is an important indicator of stress in fish (Wendelaar Bonga, 1997; Barton, 2002). Its elevation after stress and how long it remains elevated are the means for evaluating degree of stress. Accordingly, both copper-exposed groups experienced stress after exposure and did not recover until the end of the experiment. Fırat et al. (2011) showed increase in cortisol levels after copper exposure in Nile tilapia, (*Oreochromis niloticus* L.).

The glucose also increased over the time in both groups with higher rate in 100 $\mu g\ L^{-1}$ treatment. Glucose is a secondary stress response which increased parallel to increase in cortisol with a certain delay (Barton, 2002). Glucose elevation supplies demanded energy to cope stress condition (Wendelaar Bonga, 1997; Barton, 2002). Stress caused by copper exposure found to be as a result of respiration stress (Grosell et al., 2007) and oxidative stress (Roméo et al., 2000). The levels of glucose found to remain elevated during the stress and even during recovery in Common carp (Ruane et al., 2002; Hoseini, 2010). It is suggested that 100 $\mu g\ L^{-1}$ treatmrnt experienced more severe stress than that of 25 $\mu g\ L^{-1}$ which led to further glucose elevation at 7 and 14 d. Previous studies (Abdel-Tawwab et al.,

2007; Fırat et al., 2011) showed hyperglycemia after copper exposure.

The levels of sodium decreased in both copper-exposed groups and remained low until 14 d. Grosell et al. (2007) found the main toxic effect of copper was due to loss of body sodium as a result of impact on gill function. Hence, the constant sodium levels of both copper-exposed groups suggests that fish were partially capable to counteract the adverse effects of copper on sodium balance. Mc Donald and Milligan (1997) suggested ion loss might be stressful for fish and caused increase in cortisol and glucose levels. This is, at least in part, the reason of higher levels of cortisol and glucose in the copper-exposed fish. Similarly, previous studies (Grosell et al., 2007; Öner et al., 2008; Fırat et al., 2011) showed loss of sodium after copper exposure.

ALT and AST increased over the time in both copper-exposed groups. ALT and AST are cytosolic enzyme found in cell cytoplasm in high concentration. Higher levels of these enzymes in circulation might be as a result of both leakage from cells (due to high concentrations) as well as cell damage which is caused enzyme release to peripheral fluids. Copper is caused oxidative stress which in turn leads to lipid proxidation, mainly cell membrane lipids. Damage to cell membrane fails its normal function and causes the cytosolic enzyme to escape from the cell into peripheral fluids and causes increase in enzyme activity levels in blood. On the other hand, these enzymes are involved in gluconeogenesis and increase during stress to supply amino acids for gluconeogenesis (Tejpal et al., 2009). In the present study, the cortisol and glucose levels suggest that fish experienced stress until the end of the experiment, in both copper-exposed groups. Thus, increase in enzymes' levels might be as a result of activation of gluconeogenesis. Increase in ALT and AST after copper exposure in this study is in agreement with previous studies (Abdel-Tawwab et al., 2007; Fırat et al., 2011).

It is concluded that sublethal copper exposure caused stress response and sodium loss in Common carp which lasted until 14 d. Moreover, copper causes ALT and AST elevation which might be as a result of tissue damage or stress.

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