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Original Research

Effect of copper sulphate on the antioxidant parameters in the rainbow trout fry, *Oncorhynchus mykiss*

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Abstract: The aim of this study was to evaluate antioxidant responses of rainbow trout (*Oncorhynchus mykiss*) exposed to different concentrations of copper sulphate ($CuSO_4$). Fish were exposed to 0 (Group I-control), 5 (Group II), 15 (Group III) and 30 µg/L (Group IV) concentrations of $CuSO_4$ for 14 days. Liver and gills samples were collected at the end of the experiment, and analysed for their oxidant–antioxidant status, including the malondialdehyde (MDA) level, the catalase (CAT) and glutathione peroxidase (GSH-Px) activities as well as the reduced glutathione (GSH) concentration. Results obtained showed that the levels of MDA increased in tissues of fish. Compared to control, GSH level and GSH-Px and CAT activities were significantly reduced in the fish that were exposed to different concentrations of $CuSO_4$. The result demonstrated that $CuSO_4$ has an oxidative-stress-inducing potential in fish.

Key words: Copper sulphate, rainbow trout, oxidative stress, antioxidant system.

Introduction

In aquatic animals, many natural and anthropogenic factors can induce an imbalance between the production of reactive oxygen species (ROS) and their removal, and as a result, oxidative stress occurs (1). Oxidative stress is one of the most important factors that exacerbate damage by certain drugs, pesticide and other environmental chemicals (2,3). Toxicity biomarkers, such as malondialdehyde (MDA), have been proposed to reflect the oxidative status of species. The MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation (4).

Antioxidant defence system consist of antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), glutathione reductase (GR)), and low -molecular weight antioxidants (e.g., such as reduced glutathione (GSH), vitamin A, vitamin C, and vitamin E) (5). Levels of antioxidants can be used as an in dicator of the antioxidant status of the organism and can serve as biomarkers of oxidative stress (6). When antioxidant defences are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation (4).

Copper sulphate (CuSO4) is an economical treatment with relatively unrealized potentials for controlling fish pathogens (7). $CuSO_4$ is used extensively as a prophylactic or disinfective treatments in a differents doses, but used in treatment doses among 0.2-1.7 mg/L (8,9). It is active against saprolegniosis (10), *Ichthyophthirius multifiliis* (11), *Yersinia ruckeri* (12), *Vibrio anguillarum* (13), *Edwardsiella ictaluri* (14) and *Flavobacterium columnare* (15) infections. On the other hand, numerous studies indicated an increased oxidative stress in the several organs of fish and other animals exposed to $CuSO_4$ (16,17,18,19,20,21,22).

The aim of the present study was to examine the effects of $CuSO_4$ exposure on rainbow trout, using oxidative stres biomarkers to observe toxic effects. The results obtained from this study will be useful in monitoring the effects of bathing rainbow trout with $CuSO_4$.

Materials and Methods

Experimental fish

Rainbow trout, *Oncorhynchus mykiss* (3.47 ± 0.24 g, mean weight), were kindly supplied by a commercial fish farm. Two weeks prior to the experiments, the fish were acclimatized in 50-L glass aquaria. The aquaria were aerated with air stones. The fish were fed with a commercial diet daily to satiation. Exposure chambers were cleaned as needed. For the duration of the study, the dissolved oxygen, temperature, pH, salinity, hardness, ammonia and nitrite levels were monitored and maintained within acceptable ranges. The water quality characteristics were determined according to the American Public Health Association (23) guidelines. The mean quality parameters of water were as follows: dissolved oxygen of 7.58± 0.5 mg/L, pH 7.3±0.2, temperature of 14.0 ± 2 °C. The fish were exposed to light and dark intervals of 12:12 h.

The fish were divided equally into four groups. The first group was maintained in tap water as a control group. The fish in group 2 were exposed to 5 ppm of $CuSO_4$ for 14 days. The fish in group 3 were exposed to 15 ppm of $CuSO_4$ for 14 days. The fish in group 4 were exposed to 30 ppm of $CuSO_4$ for 14 days. The entire

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experiment was repeated two independent times; each replicate for each group contained ten fish, for a total of 80 fish. No mortality was observed during the experiment.

At the end of the test, the fish were anaesthetized in a anaesthetic matter (50 ppm, benzocaine) and liver and gills tissues were isolated, washed with physiological saline (0.9 % NaCl) and stored at -40 °C until the antioxidant assays, which were performed within 1 month after extraction. Feeding was discontinued 1 day preceding collection of gillss and liver. Homogenates was extracted from a pool of liver and gills of 3 fish, which were used for biochemical tests. The homogenization was carried out in a Teflon-glass homogenizer, with a buffer containing 1.15% KCl, to obtain 1:10 (w/v) the whole homogenate. The homogenates were centrifuged at 18.000×*g* for 30 min at 4°C to determine MDA and reduced glutathione (GSH) levels, CAT and GSH-Px activities.

Determination of oxidative stress and antioxidant status

The levels of MDA as indices of oxidative stress were determined according to a modified method of Placer et al. (24) based on the reaction with thiobarbituric acid, and were expressed as nmol/mg protein. The CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (25), and was expressed as k/mg protein, where k is the first-order rate constant. The GSH-Px activity was determined by the procedure described by Beutler (26). The procedure of analysis performed was based on the oxidation of GSH by GSH-Px coupled to the disappearance of NADPH by glutathione reductase measured at 37 °C and 340 nm and were expressed as U/mg protein. The GSH levels were measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman (27) and was expressed as µmol/mg protein. Protein concentrations were measured according to Lowry et al. (28).

Statistical analysis

The data were analyzed statistically by using one-way analysis of variance (ANOVA) and Duncan's new multiple range test (SPSS 15.0). Significant differences were based on the $p < 0.05$ level. Each value was expressed as mean ± standard error (SE).

Results

The effects of the $CuSO_4$ exposure on the tissue MDA levels in the control (group I) and experimental fish (group II, III and IV) are presented in Table 1, where it can be seen that the liver and gills MDA levels were significantly increased ($p < 0.05$). When compared to the group I (control group), the increases in the liver MDA levels in the group II, III and IV (experimental groups) were 13.48 %, 39.71%, and 80.14%, respectively. There were 35.58 %, 41.72 % and 63.19 % decreases in the gills MDA levels the of fish in the group II, III and IV, respectively.

Changes in the liver and gills CAT activities of the control and experimental fish are shown in Table 1. The liver and gills CAT activities at all the concentrations (group II, III and IV) were lower than in the group I (control group). There were statistically significant decrease in the liver and gills CAT activities of the experimental groups (group II, III and IV) compared to the group I (control group) ($p < 0.05$).

Treatment of rainbow trout with $CuSO_4$ caused a dose-dependent change in the liver and gillss GSH-Px activity of the experimental groups (group II, III and IV). The GSH-Px activity was significantly decreased in the liver and gillss of the groups that exposed to $CuSO_4$ in different concentrations ($p < 0.05$). These data are shown in Table 1.

Changes in the liver and gills GSH levels are shown Table 1. In all experimental groups (group II, III and IV), the liver GSH level was lower than in the control group (group I) ($p < 0.05$). The gills GSH level was decreased significantly ($p < 0.05$) in gillss of fish induced by 30 ppm $CuSO_4$.

Discussion

We observed that lipid peroxidation levels in the gills and liver were increased by $CuSO_4$, while the CAT and GSH-Px activities and GSH level decreased. Therefore, $CuSO_4$-induced stress in the fishes was characterized by decreased antioxidant enzymes and increased lipid peroxidation.

The response of the antioxidant system to oxida-

Table 1. Effect of $CuSO_4$ on some antioxidant parameters in liver and gills tissues of the control and experimental groups.

		Parameters**			
	Groups*	**MDA**	**CAT**	**GSH-Px**	**GSH**
Liver	**I**	1.41 ± 12.85^{a}	7.20 ± 0.74^{a}	6.15 ± 0.74^{a}	26.29 ± 2.74^{a}
	II	1.60 ± 14.76^{b}	5.17 ± 0.62^{b}	5.46 ± 0.40^{b}	22.24 ± 3.10^{b}
	III	1.97 ± 20.41^{c}	4.97 ± 0.45^{b}	5.33 ± 0.62^{b}	21.04 ± 3.41^{b}
	IV	2.54 ± 19.88^{d}	3.83 ± 0.68^{c}	5.20 ± 0.39^{b}	20.84 ± 2.93^{b}
Gills	**I**	1.63 ± 15.20^{a}	5.13 ± 0.42^{a}	4.26 ± 0.32^{a}	14.41 ± 2.03^{a}
	II	2.21 ± 22.47^{b}	3.49 ± 0.61^{b}	3.70 ± 0.29^{b}	13.36 ± 2.40^{a}
	III	2.31 ± 20.14^{b}	2.55 ± 0.22^{c}	3.20 ± 0.71^{c}	13.21 ± 1.98^{a}
	IV	2.66 ± 25.03^{c}	1.62 ± 0.19^{d}	3.06 ± 0.59^{c}	11.62 ± 1.36^{b}

* I: Control, II: 5 µg/L $CuSO_4$, II: 15 µg/L $CuSO_4$, II: 30 µg/L $CuSO_4$

** MDA: malondialdehyde level (nmol/mg protein), CAT: catalase activity (k/mg protein, where k is the first-order rate constant), GSH-Px: glutathione peroxidase activity (U/mg protein), GSH: reduced glutathione level (µmol/mg protein). Different superscripts in the same column indicate significant differences between groups ($p < 0.05$). Values are means ± SE

tive stress in various tissues shows differences that are due to tissue-specific antioxidant potentials (29). In our study, significant differences were similarly found in the antioxidant responses and capacities of liver and gills. The tissue differences could be due to different rates of free radical generation, differences in susceptibility to oxidative damage or different antioxidant capacities of the liver and gills.

Lipid peroxidation has been extensively used as a biomarkers of oxidative stress (30,31,32). MDA are produced by lipid peroxidation and considered as indicators of oxidative stres, which results from the free radicals damage to membrane complements of cells (33). Several researchers have reported a relation between MDA and $CuSO_4$-induced stress in the fish (18,19,20). The findings of this study demonstrated that the MDA levels in the liver and gills were higher than in the control group (Table 1 and 2). This status can be most likely explained with an excessive production of reactive oxygen species (ROS), which could be related to antioxidant enzyme leakeage, thus leading to lipid peroxidation.

CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2, which is metabolized to molecular oxygen and water (34,35). $CuSO_4$-induced inhibition and induction in CAT activity have been reported in various studies in different fish species. For example, Vutukuru et al. (22) reported that copper caused a decrease in CAT activity. Another study revealed that Cu exposure caused significant decreases in CAT activities in tissues of *Dicentrarchus labrax* (18). On the other hand, Paris-Palacios et al. (17) assessed CAT and GST activities of zebrafish (*Danio rerio*) following exposure to various concentrations of copper. The results showed that copper at different concentrations stimulated CAT and GST activities. In our case, the CAT activity was significantly decreased in the liver and gills of fish exposed to different $CuSO_4$ concentrations. This decrease in CAT activity could be due to the flux of superoxide radical, which have been reported to inhibit CAT activity (36).

GSH-Px catalyses the reduction of hydrogen peroxide and lipid peroxides and is considered an efficient protective enzyme against lipid peroxidation at the expense of GSH (37,38,39). The present results showed the GSH-Px activity in liver and gills was reduced in the $CuSO_4$-induced groups. This finding agrees with previous observations by Sanchez et al. (40) and Liu et al. (41) that $CuSO_4$ significantly decreased the GSH-Px activity in fish. Similarly, Trivedi et al. (19) reported that copper caused a decrease in the GSH-Px activity. The reduction could be due to its exhaustion or restriction as a result of the increased production of free radicals. This result signify that the increase in the levels of MDA in the liver and gillss of fish exposure to different concentrations of $CuSO_4$ may be related to the diminish in the CAT and GSH-Px activities.

Glutathione redox cycle is very important in intracellular antioxidant system and is essential for the tissues to protect themselves against the ROS damage. Glutathione is one of the necessary compounds for providing cell stability because of its reducing properties (32,42,43,44). Copper binds thiol-containing molecules such as glutathione (45). At the present study, the GSH levels in the liver and gills of rainbow trout treated with $CuSO_4$ were significantly differed compared to the control group. The inhibition of total GSH has been observed in the liver of three-spined sticklebacks *Gasterosteus aculeatus* exposed to $CuSO_4$ (40). The depletion of GSH in fish muscle after copper sulphate exposure has been reported (46). Similar findings have also been reported by Lauren and McDonald (47), Berntssen et al. (48), Parvez and Raisuddin (49) and Vutukuru et al. (22).

$CuSO_4$ have proved attractive to fish farmers for use in bacterial and parasites control. This compound are, however, highly toxic to fish. In this study, different doses of $CuSO_4$ caused a dose-dependent increased in the level of MDA as well as decreased in the CAT and GSH-Px activities and GSH level in the liver and gills. These results indicate the effect of $CuSO_4$ in the depletion of antioxidant mechanisms. Future studies should be carried out to elucidate the underlying mechanisms involved in the long-term toxicity profile of $CuSO_4$ in rainbow trout.

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