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Changes in Liver and Kidney Antioxidant Enzyme Activities in the Rainbow Trout (*Oncorhynchus mykiss*) Exposed Cadmium

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In vivo effects of cadmium on antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GSR) investigated in liver and kidney tissues of rainbow trout (*Oncorhynchus mykiss*). Three test groups of fish were subjected to cadmium in concentrations of 0 (control), 1 and 5 ppm. The duration of exposure was 1, 3, 5 and 7 d. The antioxidant enzyme activities, measured liver and kidney homogenates, were stimulated by both concentration (1 and 5 ppm) of cadmium chloride. Moreover, the dose-response patterns of the antioxidant enzyme activities in the liver and kidney tissue were very similar. All antioxidant enzyme activities were significantly stimulated on the first day of experiment (Day 1) in the tissues at the both dose of Cd ($p < 0.05$) while CAT activity was stimulated after 3 days in the tissues at a dose of 1 ppm Cd ($p < 0.05$). The stimulation effect of Cd on SOD, GPx and GSR activities in the tissues diminished after 7 d cadmium administration. However, this effect of Cd on CAT activity diminished after 5 d. These findings indicate that the tissue antioxidant enzymes function to protect against cadmium toxicity.

Key Words: Rainbow trout, Cadmium, Antioxidant enzymes.

INTRODUCTION

The antioxidant defense (AD) system of organisms provides a means of dealing with oxidative stress refers to the disturbance of the equilibrium between antioxidants and pro-oxidants towards oxidants and includes several enzymes and vitamins[1,2]. Exposure of organisms to oxidants attack can increase antioxidant defenses, for example, by increasing synthesis of antioxidant enzymes[3]. If antioxidant defenses are effective in detoxifying oxidants, then no harmful consequence is resulted in the tissues. However, if the oxidants attack is severe, antioxidant defense systems may be overwhelmed, resulting in inhibition of antioxidant enzymes and oxidative damage to lipids, proteins, DNA and other key molecules. Such processes may in turn provoke alterations in molecular and membrane structures and functions leading to cell and tissue damage[4].

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Most cellular antioxidants can be regarded as part of the innate non-specific immune system[5]. Similarly to other vertebrates, fish possess an AD system both enzymatic and nonenzymatic. The more relevant AD enzymes consist of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and non-enzymatic defenses include the vitamin C, α-tocopherol and vitamin A[6,7].

Cadmium is known to be extremely toxic to mammals, fish and other fauna and flora. It is generally viewed along with mercury as a toxic element and environmental problem[8,9]. Sunda *et al.*[10] and Sprague[11] reported that the free metal ion, Cd^{2+}, is the form of cadmium most available to aquatic organisms, while inorganic cadmium complexes appear not to be taken up by fish[12]. The main uptake route in fresh water fish is from the water *via* the gills[13] while food is the major cadmium source for marine fish[14,15]. May and McKinney[16] reported cadmium concentrations ranging from 0.01 to 1.04 mg/kg (wet weight), for freshwater fish (*Cyprinus carpio* or *Micropterus* spp.) Hardisty *et al.*[17] sampled flounder (*Platichthyes flesus*) with mean cadmium concentrations of 3.4-7.3 mg/kg (dry weight).

Many chemicals at relatively low dosages affect the metabolism of biota by altering normal enzyme activity[18]. Like the other heavy metals such as mercury and lead, cadmium causes significant metabolic alterations such as the enzymatic activities and membrane transport mechanisms[19] and injuries of biological systems at different levels[20].

It is assumed that Cd may cause some of impairments in enzymatic process in fresh water fish and therefore investigation of the *in vivo* effects of Cd on SOD, CAT, GPx and GSR, endogenous antioxidant enzymes from rainbow trout (*Oncorhynchus mykiss*) livers and kidneys is aimed in the present study.

EXPERIMENTAL

Rainbow trout weighing 200 ± 20 g were obtained from the Fisheries Department of Agricultural Faculty at Ataturk University in Erzurum. Fish were placed in tanks (12 fish per tank) where they acclimated for 15 d. Nine cylindrical fiberglass tanks, 1 m in depth (h) and 1 m in diameter (D = 2r) with a total volume of 0.785 m^3 ($V = \pi r^2 h$) per tank were used in the study. Fish in each of three tanks were subjected to single dose of Cd [(0 (control), 1 and 5 ppm)]. Recirculated, aerated and dechlorinated tap water with flow rate of 1.5 L/min was used to maintain a supply of fresh water and the flow rate was equivalent to one full exchange of water every 8.7 h. Other conditions of the water were maintained as follows: the average water temperature was monitored and maintained at 9 ± 1 ºC; the concentration of dissolved oxygen was maintained at 9 ppm; the pH was maintained at 7.8; total water hardness was measured to be 102 mg as $CaCO_3$.

Fish were fed *ad libitum* and divided into 3 groups. Fish in group I served as controls. Fish in groups II and III were exposed to sublethal concentrations of cadmium at dosages of 1 and 5 ppm of Cd, respectively. Six randomly selected fishes from each group (the single control group and the two test groups) were sacrificed and

enzyme levels in the target tissues were tested after 1, 3, 5 and 7 d of exposure to cadmium.

Before taking tissues samples, fish were killed by an overdose of an anesthetic compound (MS-222)[21]. Subsequent to death, their livers and kidneys were removed. Each tissue was homogenized in a Potter-Elvehjem homogenizer and put into homogenization medium[22] and then centrifuged[8] at 4 °C, 10,000×g for 45 min. After centrifugation, the supernatant was removed from the centrifuge tubes and used for these *in vivo* studies.

The following enzyme activities were measured: SOD by the method of Sun *et al.*[23], CAT according to Aebi[24], GPx as descriped by Beutler[25], GSR by the method of Goldberg and Spooner[26].

Protein levels were determined spectrophotometrically (595 nm) according to the Bradford method[27], using bovine serum albumin (BSA) as the standard.

Statistical evaluation of the data was performed using Student's t test. Differences from controls were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

All values of the antioxidant enzymes of the hepatic and renal tissues were summarized in Tables 1 and 2. It was observed that there were no differences ($p > 0.05$) among the enzyme activities determined from control group at 0 (initial), 1, 3, 5 and 7 d for each enzyme. However, the antioxidant enzyme activities, measured liver and kidney homogenates, were stimulated by both concentration (1 and 5 ppm) of cadmium chloride. Moreover, it was determined the dose-response patterns of the antioxidant enzyme activities in the liver and kidney tissue were similar.

TABLE-1
ANTIOXIDANT ENZYME ACTIVITIES IN LIVER OF RAINBOW TROUT EXPOSED 1 AND 5 ppm CONCENTRATION OF CADMIUM

Name of enzyme	Control	1 day	3 days	5 days	7 days
SOD	0.07±0.01	0.10±0.01*	0.11±0.01**	0.10±0.01*	0.08±0.01^{NS}
		0.10±0.01**	**0.12±0.01*****	**0.11±0.01****	**0.09±0.01^{NS}**
CAT	0.25±0.04	0.29±0.36^{OD}	0.34±0.05*	0.27±0.05^{NS}	0.25±0.05^{NS}
		0.42±0.06**	**0.38±0.05***	**0.32±0.06^{NS}**	**0.24±0.04^{NS}**
GPx	0.35±0.04	0.49±0.04*	0.54±0.10**	0.48±0.04*	0.40±0.06^{NS}
		0.59±0.10**	**0.53±0.09***	**0.50±0.08***	**0.38±0.06^{NS}**
GSR	0.11±0.02	0.16±0.02*	0.20±0.02**	0.16±0.03*	0.15±0.02^{NS}
		0.21±0.03**	**0.20±0.03****	**0.19±0.03****	**0.16±0.02^{NS}**

Each value is the mean ± SD of 6 individual observations. Bold values represent the enzyme activities under 5 ppm exposure of cadmium. NS, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Spesifik activity is expressed as SOD, units/mg protein; CAT, μmol H_2O_2 metabolized/mg protein/min; GPx and GSR, μmol NADPH/mg protein/min.

TABLE 2
ANTIOXIDANT ENZYME ACTIVITIES IN KIDNEY OF RAINBOW TROUT EXPOSED 1 AND 5 ppm CONCENTRATION OF CADMIUM

Name of enzyme	Control	1 day	3 days	5 days	7 days
SOD	0.06±0.01	0.08±0.01*	0.08±0.01*	0.08±0.01*	0.06±0.01^{NS}
		0.08±0.01**	**0.09±0.01****	**0.09±0.01****	**0.07±0.01^{NS}**
CAT	0.20±0.03	0.23±0.03^{OD}	0.27±0.04*	0.21±0.04^{NS}	0.20±0.04^{NS}
		0.31±0.05*	**0.30±0.04***	**0.26±0.04^{NS}**	**0.19±0.04^{NS}**
GPx	0.28±0.03	0.40±0.03*	0.42±0.08*	0.38±0.04*	0.32±0.05^{NS}
		0.44±0.08*	**0.42±0.07***	**0.40±0.06***	**0.30±0.05^{NS}**
GSR	0.09±0.02	0.13±0.02*	0.14±0.02*	0.13±0.02*	0.12±0.02^{NS}
		0.16±0.03**	**0.16±0.02****	**0.15±0.02****	**0.13±0.02^{NS}**

Each value is the mean ± SD of 6 individual observations. Bold values represent the enzyme activities under 5 ppm exposure of cadmium. NS, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Spesifik activity is expressed as SOD, units/mg protein; CAT, µmol H_2O_2 metabolized/mg protein/min; GPx and GSR, µmol NADPH/mg protein/min.

The activities of SOD, GPx and GSR in the liver and kidney tissue significantly increased after the first day of experiment at the both dose of Cd ($p < 0.05$). However, the CAT activity increased after 3 d of 1 ppm Cd treatment in the tissues ($p < 0.05$). All enzyme activities except CAT returned to control level after 7 d of Cd treatment ($p > 0.05$). CAT activity returned to control level after 5 d ($p > 0.05$).

The increase in specific activities of liver and kidney antioxidant enzymes shown in Tables 1 and 2 suggested that Cd was strong stimulation of oxidative stress in the exposed fishes and the enzymes had a effective role of liver and kidney that are suffering from oxidative stress[4,28].

Similarly to present experimental findings, cadmium expose of the *Oreochromis mossambicus* resulted in a significant increase in SOD, GPx, CAT activity in the liver and kidney. However, a strong decrease in the GPx, GSR and CAT enzyme activities was reported in the fish *Sparus aurata* following in vivo exposure to Cd[22]. In addition, it was also determined that inhibition effect of Cd on carbonic anhydrase (CA) enzyme activity in rainbow trout erythrocytes, gill, liver and kidney. The inhibition effect started after day 3 in erythrocytes, liver and kidney at dose of 1 ppm Cd, while it was starting immediately after first day in gill and erythrocytes at 3 and 5 ppm dose of Cd was reported[29].

Taken into consideration the results of the present study *i.e.*, the dose-response pattern of the antioxidant enzyme activities in liver and kidney tissue was almost identical in kidney and liver tissue. For these results, it is suggested that cadmium entered in the organs of the fish first through its gills and it then bonded to albumins and erythrocytes in the blood and was transferred into organs kidneys and liver.

The antioxidant enzymes showed significantly different increased activity in the tissues with a decrease after 7 d of Cd treatment. This can be attributed to Cd produced a cumulative dose-dependent the enzyme activation and adapt to oxidative conditions to which fish are exposed. Similarly, Basha and Rani[9] determined that

the antioxidant enzymes showed significantly increased activity in liver and kidney tissues then a slight decrease during a chronic cadmium exposure of 30 days and this probably reflected an adaptation to oxidative conditions.

In conclusion, it was determined that antioxidant enzymes were potential targets for cadmium and they were adaptive and protective responses to exposure to cadmium. Moreover, cadmium produced a cumulative dose-dependent the enzyme activation in liver and kidney and degree of susceptibility to cadmium-induced activation was also similar between the liver and kidney tissue of trout.

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