



Effect of Superoxide Dismutase and Catalase in Erythrocytes of Catfish (*Heteropneustes fossilis* Bloch.) Exposed to Cadmium

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ABSTRACT

Effect of sublethal concentration of cadmium chloride (12 ppm; 10% of 96h LC₅₀) in erythrocytes of catfish was studied for a period of 5, 10, 20 and 45 days. It was found that the activity of superoxide dismutase (SOD) in red blood cells (RBC) significantly decreased after 5 and 10 days of cadmium exposure. However, the SOD activity increased after 20 and 45 days of cadmium treatment. Elevated activity of catalase (CAT) was found in erythrocytes of cadmium treated fish after 45 days. These results indicate oxidative stress and cell damage in the exposed fish.

INTRODUCTION

In freshwater fishes cadmium enter through gills and binds to albumins and erythrocytes in the blood, and then transferred into tissues and organs where it is bound to proteins of low molecular mass producing metallothioneins by the induction of metallothionein mRNA synthesis (Gould & Karolus 1974, George et al. 1996). It causes significant metabolic alterations and injuries at different levels (Pratap & Bonga 1990, Brown et al. 1984). The synthesis of metallothioneins in certain organs decreases to some extent the toxicity of non-bound cadmium (Thornalley & Vasak 1985, Wormser et al. 1990, Olsson & Kille 1997). In fish, cadmium causes destruction of erythrocytes and leads to anaemia (Pushpa 2008). Cadmium increases the production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defense system (Jackim et al. 1970, Pruell & Engelhardt 1980, Zikic et al. 1996, 2001). There are only scarce data available on influence of cadmium on the activity of enzymes of the antioxidative defense system in erythrocytes of fishes (Thomas & Wofford 1993, Palace et al. 1993, Zikic et al. 1997). Hence, the present investigation has been designed to understand the influence of cadmium on the activity of superoxide dismutase (SOD, EC 1, 15. 1.1) and catalase (CAT, EC 1.11.1.6) in erythrocytes of catfish (*Heteropneustes fossilis*) after exposure lasting 5, 10, 20 and 45 days.

MATERIALS AND METHODS

The experimental catfish (*Heteropneustes fossilis* Bloch.) weighing 82-85 g were adapted for 30 days to the laboratory conditions with water temperature of $28 \pm 2^\circ\text{C}$, pH 7.4 and concentration of dissolved oxygen 5.6 ppm (APHA 1998) dechlorinated and aerated water. The fish were fed with minced goat liver. After the period of acclimation, four experimental groups of fish were exposed to cadmium in a concentration of 12 ppm in water (10% of 96 h LC₅₀ = 120 ppm) (Hamilton et al. 1977). Control fish were resided in non-polluted water. The fish were sacrificed in groups after exposure to cadmium for 5, 10, 20 and 45 days, each group consisting of 10 fish.

Table 1: The activity of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes of control catfish (C) and catfish exposed to 12 ppm cadmium for 5, 10, 20 and 45 days.

Parameters	Control	Experimental			
		5 d	10 d	20 d	45 d
SOD	430 ± 1.12	352 ± 0.75*	410 ± 1.15	598 ± 2.10*	657 ± 1.05*
CAT	1.75 ± 0.75	1.60 ± 0.25	1.42 ± 0.25	1.60 ± 0.75	1.68 ± 0.00

Note: The values are expressed in units of enzyme activities per mL of red blood cells (U/mL RBC). Mean ± S.E.M from seven fish in each group. Significantly different from controls; * $p < 0.05$.

After sacrificing the fish, freshly heparinised blood samples were collected immediately and prepared for further processing as recommended by Mazeud et al. (1979) and Wdzieczak et al. (1982). The activity of superoxide dismutase (SOD) was determined spectrophotometrically at 480 nm by the epinephrine method, and it was expressed in units of enzyme activity per mL of red blood cells (U/mL RBC). The activity of catalase (CAT) was determined spectrophotometrically at 570 nm (Sinha 1972), and it was expressed in mmoles of decomposed hydrogen peroxide per second per mL RBC ($\text{mmol H}_2\text{O}_2/\text{sec/mL/RBC}$). Data were analysed using the non-parametric Mann-Whitney two-tailed test and differences at $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

The results are represented in Table 1. Cadmium causes a significant decrease of SOD activity ($p < 0.05$) after 5 days of exposure. No significant differences were found after 10 days of exposure compared to the control values, whereas after 20 and 45 days of exposure, values were significantly increased ($p < 0.05$) when exposed in U/mL RBC. The obtained data show that cadmium did not change the activity of CAT expressed in $\text{mmol H}_2\text{O}_2/\text{sec/mL RBC}$.

In the present study, the activity of SOD was significantly decreased after 5 days of exposure to sublethal cadmium. Similar results were obtained in previous investigations (Zikic et al. 1997) on carp during acute cadmium exposure. However, after 10 days, the activity of this enzyme was non-significant with control values. During prolonged exposure, the activity of SOD was significantly increased after 20 and 45 days. These results indicate the activation of protective mechanisms necessary for scavenging of produced free radicals of oxygen in erythrocytes. Kostic et al. (1993), while studying the effect of cadmium on antioxidant and metabolic status in RBC of rats, showed the enhanced activity of this enzyme. In the present study cadmium did not exhibit any effects on the activity of CAT when expressed per mL RBC. Petronijevic et al. (1995) observed significant alterations of energy metabolism in RBC when rats exposed to cadmium. It also increased concentration of non-enzymatic components of the antioxidant defense system in plasma (ascorbic acid-AsA, Vitamin E) (Zikic et al. 1995) of rats and enhanced activity of enzymes of antioxidant defense system SOD, CAT, glutathione peroxidase and glutathione reductase in RBC of rats (Kostic et al. 1993).

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