Effect of cadmium on catalase activity in four tissues of freshwater fish Heteropneustes fossilis (Bloch.)

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Citation

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Abstract

Effect of sublethal concentration cadmium chloride (12ppm; 10% 0f 96h LC_{50}) on Catalase (CAT, EC 1.11.1.6) activity in brain, gill, kidney and liver of freshwater fish Heteropneustes fossilis was studied for 5,10,20 and 45 days exposure periods. In brain, gill, and kidney the catalase activity was significantly inhibited. But in liver non significant increase was noted upto 20 days, but significant increase was noted after 45 days exposure period. These results showed the role of catalase in antioxidant defense system to protect the animals from oxidative stress, hence it can be considered as a sensitive bioindicator of the antioxidant defense system.

INTRODUCTION

Extensive industrialization and urbanization has increased the concentration of heavy metals into the aquatic environment [1] . The stress of heavy metals increase in highly reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical leading to oxidative stress in fish [2,3,4,5] . Fish tissues, specifically the liver and kidney are endowed with antioxidant defense systems consisting of CAT, superoxide dismutase (SOD) etc. to protect them from oxidative stress caused by metals [6] . Antioxidant enzymes contribute to the maintenance of a relatively low level of the reactive and harmful hydroxyl radical, the superoxide radical and hydrogen peroxide in the presence of Cu^{2+} and/or Fe^{3+} [7,8] . CAT, primary antioxidant defense component, eliminates hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O+O_2$) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes. Various responses of CAT activity have been observed in animals exposed to organic or metallic contaminants in both field and laboratory experiments and CAT has been shown to be either induced or inhibited by metals depending on the dose, the species or the route of exposure [8,9] . The oxidative stress has gained considerable interest in the field of ecotoxicology. Therefore, CAT activity is considered as a sensitive biomarker of oxidative stress before hazardous effects occur in fish [8,10] . This research aims to investigate the response of CAT activity to different heavy metals in the tissues of H. fossilis. The data may provide a useful database for future investigations of pollutant effects on antioxidant system in aquatic environment.

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 28 ± 2° C , pH 7.4 and concentration of dissolved oxygen 5.6 ppm [11] 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 96h LC_{50} = 120) [12] . 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. At the end of each exposure periods, all fish were taken out and dissected with clean equipment. The brain, gill, kidney and liver tissues sampled immediately and stored at −80 °C until the enzyme analysis. The tissues were homogenized (1 : 10, w/v) in 20 mM Tris buffer (pH 7.8) containing 0.25 M sucrose and 1 mM EDTA at 9500 rpm for 3 min. Homogenates were centrifuged at 13000 g (Hettich Universal 30 RF) for 20 min at +4 °C and supernatant was used as enzyme source. CAT activity was determined [13,14] . CAT activity was measured spectrophotometrically at 240 nm using a specific absorption coefficient at 0.0392 cm^2 μmol^{-1} H_2O_2. 2.5 mL of substrate solution made up of 25 mM H_2O_2 in a 75 mM phosphate buffer at pH 7.0 and 20 µL of supernatant which contains 0.2 mg protein/mL were

mixed at 25 °C for 2 min and reaction was stopped by adding 0.5 mL of 1 M HCl. CAT activity was calculated as mol H_2O_2 decomposed/mg protein/min. The protein contents of the homogenates were determined [15] using bovine serum albumin as a standard. The 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

Figure 1

Table 1: The activity of catalase (CAT) (mol HO/mg protein/min) in brain,gill,kidney and liver of control catfish and catfish exposed to 12ppm cadmium for 5,10,20 and 45 days.

Tissues	Control	Experimental			
		5d	10d	20d	45d
Brain	121.21±12.10	NS 118.22 ± 12.10	* 102.25 ± 11.72	** 98.12 ± 10.15	** 92.75 ± 8.16
Gill	157.04±12.25	NS 153.18 ±11.05	NS 146.28 ±11.75	NS 144.22±7.85	* 138.10±10.20
Kidney	312.16 ±10.25	NS 310.14 ± 7.15	NS 307.10 ±10.25	NS 301.11 ± 6.10	NS 299.08 ± 9.28
Liver	522.07± 21.02	NS 526.18 ± 20.21	NS 534.25 ± 18.58	NS 538.17± 16.25	** 541.15± 10.42

Note : Mean ± S.E.M from seven fish in each group. Significantly different from controls; *p<0.05; NS = non-significant.

Catalase (CAT, EC 1.11.1.6) is an important enzyme in antioxidant defense system protecting animals from oxidative stress. The effect of 12ppm of cadmium chloride solution on the four tissues (brain,gill,kidney and liver) are given in table1. The brain is very susceptible to oxidative damage through free radicals as it contains high amounts of unsaturated lipids and utilizes about 20% of total oxygen demand of the body. While CAT is highly active in the liver and kidney, the brain is not particularly enriched in antioxidant enzymes. It was determined that CAT activity was found to be maximal in liver and minimal in the brain of seven animal species [16] . In the present study, the specific activity of brain CAT was found to be lower, which may be related to the direct binding of metal ions to –SH groups on the enzyme molecule, increased hydrogen peroxide and superoxide radical due to oxidative stress. It was indicated that rapid inactivation of CAT at high hydrogen peroxide concentration was due to the converting of active enzyme compound to inactive compounds [17] . In addition, stimulation of CAT activity can be associated with effective antioxidant defense system acting against oxidative stress and/or compensating for the decrease in other antioxidant enzymes such as Mn-SOD and GPX. On the other hand, no significant change in CAT activity may be attributed to the increase in other antioxidant enzymes such as GPX and/or non-enzymatic mechanisms such as GSH and metallothioneins. In general inhibition of CAT activity in all tissues of H. fossilis may be resulted due to the direct effect of metals. The modification of CAT activity in the sea bass erythrocytes in response to metallic ions in vitro that Zn and Cr activated while Cu inhibited the activity is highly dependent on the nature of the metal [3] and also suggested that deterioration of the protective defence system can possibly occur due to the formation of oxyradicals caused by metals.

Gill is also the first affected organ when fish are exposed to metals. It was determined that there was no significant change in CAT activity in the liver and gill and this was associated with the high activity of GPX, which acts as a defense against the formation of H_2O_2 or effective antioxidant responses due to a higher renovation of gill epithelium. The lowest activity of CAT was measured in the gill tissue; this was explained by the increased generation of H_2O_2, which led to a decreased CAT activity [2,15] . The highest inhibition was observed in the kidney, This can be associated with the effective antioxidant system in this tissue where the higher metal bioaccumulation and related to metal binding protein synthesis and non-enzymatic antioxidant mechanisms were observed [6, 19] . Moreover, this can be attributed to the possible induction of stress proteins and/or non-enzymatic antioxidant formation [6] . The results showed that the Cd^{2+} can activate redox cycling and demonstrate both in vivo and in vitro responses which are typical of the damage to the membranes and oxidative stress [9] .

The highest CAT activity was determined in liver tissue compared to other tissues, which are in agreement with other literatures [7, 10] . These data are in accordance with those reported in other fish species where CAT activity is distributed in a decreasing order, as follows: liver, kidney, heart, brain and muscle [7] . In the present study, cadmium decreased the CAT activity in the liver. The reduction may be associated with the direct binding of metal to –SH groups on the enzyme molecule. Liver CAT activity was found to be inhibited following both in vivo and in vitro exposure to dissolved Cd^{2+} at a concentration greater than 1 mg/L in the killifish, Fundulus heteroclitus and the authors suggested a direct effect of Cd^{2+} on high molecular weight compounds like catalase [20] . In previous studies, the liver was found to be stronger into the face of oxidative stress than the other tissues and a uniform organ with the highest antioxidant enzyme activities (SOD, CAT). This could be related to the fact that the liver is the site of multiple oxidative reactions and maximal free radical generation [10, 21] .

CONCLUSION

The response of CAT activity in different tissues of H .fossilis exposed to sublethal concentration of cadmium chloride solution (12ppm) was found to be variable depending on tissues and duration of exposure periods. Hence the CAT activity can be considered as a sensitive biomarker for biomonitoring the aquatic environment contaminated with chemicals and this may provide a useful data for future investigations.

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