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International Journal of Current Research
Vol. 7, Issue, 11, pp.22339-22343, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF CADMIUM ON BRAIN ANTIOXIDANT ENZYMES IN FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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ARTICLE INFO

Article History:
Received 19th August, 2015
Received in revised form
06th September, 2015
Accepted 25th October, 2015
Published online 30th November, 2015

Key words:

Cadmium, *Oreochromis mossambicus,*
Antioxidant enzymes, Brain.

ABSTRACT

The objective of this study is to assess the response of *Oreochromis mossambicus* to cadmium by determination of 96 hours LC_{50} value and behavioural response from exposure to different cadmium concentration. In this study conducted for 30 days enzymatic response of antioxidant defense system under heavy metal cadmium exposure was studied in the brain tissue. A decline in activity of superoxide dismutase and catalase was observed while an increase was in the activity of glutathione peroxidase was evaluated. It is proved to have minimal adaptive response in relation to cadmium exposure in polluted aquatic environment.

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***Citation*:** Sasikala, M., Murugan V. and Jothi Narendiran, N. 2015. "Effect of cadmium on brain antioxidant enzymes in freshwater fish *Oreochromis mossambicus*", *International Journal of Current Research*, 7, (11), 22339-22343.

INTRODUCTION

Cadmium (Cd) has become widely employed and is now a major threat to man's environment, due to its many industrial uses such as electroplating, plant dyestuffs, metallurgy and mining industries. Other major sources of contamination include paper, PVC, plastic, pigments and ceramic industries, battery and smoldering units and many modern industries (Farombi *et al.,* 2007; Honggang *et al.,* 2010). Cadmium is released to the biosphere from both natural and anthropogenic sources. 10% of total Cd in the environment is derived from natural sources, whereas remaining 90% is derived form anthropogenic activity (Das and Banerjee, 1980). Anthropogenic activities like smelting operations, use of phosphate fertilizers, pigment, cigarettes smokes, automobiles etc. have contributed to the entry of cadmium into human and animal food chain (WHO, 1992; Okada *et al.,* 1997). Cadmium is a silver-white, blue-tinged, lustrous metal it is insoluble in water, although its chloride and sulphate salts are freely soluble. Cadmium has been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic (Lippmann, 2000). The toxicity of Cd is attributed to its ability to generate reactive oxygen species that may act as signaling molecules in the induction of gene expression and apoptosis, deplete endogenous radical scavengers, and also damage a variety of transport proteins including the Na^+ / K^+ - ATPase (Faverney *et al.,* 2001).

Cadmium not only accumulated in the liver but also increased in the brain, even if levels were far lower than those found in the liver. Accumulation in the brain seems to be dependent on the administration route. Cd has been shown to be taken up by olfactory epithelium and transported to the brain in pikes (Tallkvist *et al.,* 2002), a route that could fit with the waterborne exposure. Cd accumulated in the olfactory rosettes, nerves, and bulbs in rainbow trout (Scott *et al.,* 2003; Vetillard and Bailhache, 2005).

Antioxidant enzymes are crucial in the effort to counteract oxidative stress caused by toxicants once the supply of other antioxidant compounds are depleted (Radi and Matkovics, 1988; Martinez- Alvarez *et al.,* 2005). Cadmium has become the focus of intense research globally because of its toxicity in diversity. Cadmium toxicity to aquatic ectothermal animals depends on complex biochemical interaction and a balance between rates of absorption, detoxification and excretion (Puneet and Ann, 2010). Brain was taken as the tissue for the study, since its controls the behaviour and coordinated movements in fish and is a central organ which regulates

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various body functions and conveys signals to adapt to various conditions. In this pilot study changes of the antioxidant enzymatic defense system under heavy metals cadmium exposure will be evaluated.

MATERIALS AND METHODS

Experimental animals

Freshwater fish *Oreochromis mossambicus* was used as the experimental model to evaluate the toxicity of cadmium. The fish used in this experiment were transferred from natural ponds around Arakonam district and brought to the laboratory and acclimatized for 15 days to laboratory conditions in tub aquaria each measuring (60 cm × 30 cm × 30 cm) filled with 25 litres of dechlorinated tap water with aerator fitted to the aquaria for continuous oxygen supply. The aquaria was disinfected with potassium permanganate solution and washed thoroughly prior to introduction to prevent any fungal infection. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state. Initial mean weight and length of the fish were 20-28 gm and 8-12 cm respectively. The fishes were maintained in normal light dark period and optimal temperature.

Metal toxicity and determination of lethal concentration (LC_{50})

Acute toxicity experiments were conducted for 96 hours using a static bioassay technique. Five groups of 6 fishes each were set for the LC_{50} bioassay method. Five groups of fishes were exposed to a range of five different concentrations of cadmium chloride. The concentration at which 50% mortality occurred after 96 hours was taken as the median lethal concentration. The dead fishes were removed immediately from the aquaria to avoid oxygen depletion. Mortality, behavioral and morphological changes were recorded during the 96 hr LC_{50} observation.

The fishes were maintained in a narrow range concentration. The 96 hour LC_{50} was determined by Probit analysis method (Finney, 1964). LC_{50} was found to be 3.58 mg/L. One fifth (0.71 mg/L) was taken as the sub lethal concentration for the present study. Experiments were conducted with sub-lethal and toxicologically safe concentrations of cadmium chloride for a period of 30 days.

Experimental Design

Group I served as the control while Group II were exposed to heavy metal, cadmium at sub-lethal concentration.

Group I: Control fishes maintained in toxicant free water.
Group II: Fishes maintained at 0.71 mg/L of cadmium.

The control and the experimental animals were fed with normal fish feed. Commercial food pellets with ingredients consisting of fish meal, wheat flour, soybean meal, yeast, vitamins and minerals were fed. Water was changed daily at 8.00 hours which facilitated the removal of un-consumed food. After renewal of water the required quantity of cadmium chloride was added to maintain the concentration of the toxicant in water.

At the end of 30th day five fishes were sacrificed by cervical dislocation brain tissues were dissected out and washed thoroughly with 0.9N saline solutions. Tissues were weighed and homogenized in Tris 0.1M HCL buffer using a homogenizer. The homogenate of the tissue were centrifuged at 2500 rpm for 15 minutes in a high speed centrifuge and clear supernatant was used for biochemical analysis and assay of antioxidant enzyme activity.

Biochemical analysis: Proteins and antioxidant enzyme activity was analysed by standard procedures. Total protein (Lowry *et al.,* 1951), Superoxide dismutase (Misra and Fridorich, 1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme, Catalase (Sinha 1972) and Glutathione peroxidase (Rotruk *et al.,* 1973).

Statistical analysis: The data collected on the different parameters of the control and experimental study were subjected to statistical analysis using statistical software SPSS version 6.0. The statistical significance was tested at 1% and 5% levels using standard 't' test.

RESULTS

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of fishes to cadmium concentration for 96 hours. The fishes were maintained for a period of 30 days in sublethal concentration. The LC_{50} was found to be 3.58 mg/L. From this one fifth (0.71 mg/L) was taken as the sublethal concentration which was considered as safe dose.

The total protein content of the brain in experimental groups was significantly decreased (P<0.001) after 30 days exposure to cadmium, when compared to control fishes (Table 1)

Table 1. Effect of cadmium on protein content in brain of freshwater fish *Oreochromis mossambicus*

Biochemical constituent	Control group	Experimental group	t-value	p-value
Total Protein	15.42 ± 1.39	10.55 ± 2.35	9.1651	<0.001

Value are expressed as Mean ± SD (n=5); Value significant at 1% level.
Protein content expressed as mg/g tissue.

Table 2. Effect of cadmium on antioxidant enzymes in brain of freshwater fish *Oreochromis mossambicus*

Antioxidant Enzyme	Control group	Experimental group	t-value	p-value
Superoxide dismutase	1.30 ± 0.55	0.83 ± 0.98	2.2922	<0.005
Catalase	102.92 ± 8.65	42.86 ± 7.98	11.4112	<0.001
Glutathione Peroxidase	52.49 ± 12.75	109.37 ± 8.65	6.7496	<0.001

Values are expressed as Mean ± SD (n=5); Significant at 5% and 1% level
Activity of enzyme expressed as
SOD = Units / min / mg ptn
CAT - μ moles of H_2O_2 consumed / min / mg ptn.
GPx - μ moles of GSH oxidized / min / mg ptn

Activity of antioxidant enzymes superoxide dismutase (SOD) was significantly decreased ($P<0.05$) in brain tissue in the experimental fishes exposed to cadmium when compared to control, while catalase showed a decline in brain which was significant at ($P<0.001$). Glutathione peroxidase increased in brain tissue also being significant at ($P < 0.001$) (Table 2)

DISCUSSION

Cadmium has an extremely long half life of 20 to 30 years. It is a ubiquitous toxic metal and induce oxidative damage by disturbing the proxidant-antioxidant balance in the tissues (Sobha *et al.,* 2007; Talas *et al.,* 2008). In aquatic systems, as fish occupy the upper trophic level, there are greater chances of transferring cadmium to higher organisms particularly to man due to its bioacuumulation in fish (Liopoulpou and Kotsanis, 2001; Kumar *et al.,* 2005). Bioenhancement of cadmium transfer along food chain was reported by Seebaugh *et al.,* (2005). The major behavioural changes observed during the experiment was erratic swimming, restlessness, upward movement and gulping of air in the initial days of the experiment. Similar behavioural changes were observed in *Channa punctatus* when exposed to acute mercury toxicity (Agarwal, 1991) and seabass exposed to cadmium (Faucher *et al.,* 2008).

Proteins are an important organic constituent of animal cells playing a vital role in the process of interaction between intracellular and extracellular media, being a part of cell membrane (Sharma and Agarwal, 2005). The decrease in protein content in the brain of the experimental fishes could be due to diversification of energy for detoxification. Any change in the environment may alter the synthesis and utilization of protein in brain leading to behavioural changes such as restlessness, tremor, ataxia and depression. Cadmium could have exerted greater stress during the process of detoxification which may have altered the protein status of animal. Changes in brain protein levels may be attributed to the above reasons (Ghosh and Chattajee, 1989).

Similar results is reported in other studies *Gambunia affinis* when exposed to organophosphate pesticide showed considerable change in protein level of the different regions in brain (Joshi and Rage, 1980). Pyrethroid fenvalerate is also reported to have significantly reduced protein content in the brain tissue of *Clarias batrachus* (Tripathi and Verma, 2004b). A decrease in brain protein content levels after 45 days exposure to synthetic pyrethroid lambda cyhalothrin was reported in freshwater catfish *Clarias batrachus* (Suman Gulati, 2007).

The heavy metal toxicity stimulates the oxidative stress and the antioxidant enzymes are induced as a defense mechanism (Hansen *et al.,* 2006). The defensive free radical scavenger superoxide dismutase (SOD), triggers an induction response in heavy metal intoxicated groups (Oruc and Uner, 2000; Orun *et al.,* 2008). This indicates that more protein is required to protect cells against superoxide radicals. The superoxide dismutase level decreased in the brain, indicating stress developed by the heavy metals. The alterations in SOD activity may depend on several factors such as cadmium dose, exposure time to cadmium and the functional state of the fishes.

Catalase (CAT) is a manganese or heme containing enzyme, functions along with dismutase to convert H_2O_2 to water and oxygen (Elia *et al.,* 2003). The decrease in CAT activity in brain tissue of cadmium exposed fishes in the present study could be due to its inactivation by superoxide radical or due to decrease in the rate of reaction as a result of the excess production of H_2O_2. Cadmium by inhibiting the enzyme activity leads to hydroxyl radical formation and subsequently results in cellular damage via the metal catalyzed Haber – Weiss reaction (Pratama Yoga, 2002). The peroxidative damage to the cell membranes may cause injury to cellular components due to interaction of metal ions with cell organelles (Filipovic and Raspor, 2003).

Environmental pollutants may induce glutathione peroxidase activity (Lushchak *et al.,* 2001). The level of glutathione peroxidase in the brain was found to be increased. Significant increase in glutathione peroxidase activity indicates the protective role of the enzyme against lipid peroxidation. This probably reflects an adaptation to the stress conditions to which the fish have been exposed due to cadmium.

Our results are also in agreement with other studies. Lushchak *et al.* (2001) observed oxidative stress and antioxidant defenses in brain tissues of *Carassius auratus* during anoxia and reported a significant elevation in the GPX activity in brain tissue. Bernstsden *et al*. (2003) studied the effects of mercuric chloride on brain lipid peroxidation in *Salmo salar* and reported a significant decrease in SOD and GPX activity. CAT activity was reported to be significantly declined in *Channa punctatus* exposed to deltamethrin (Sayeed *et al.,* 2003). Bagnyukova *et al*. (2005) reported a gradual decrease in the brain catalase activity of *Carassius auratus* in response to aminotriazole induced oxidative stress. Decrease in SOD and CAT activity was found in the brain of *Clarias batrachus* exposed to synthetic pyrethroid lambda cyhalothrin for 45 days (Suman Gulati, 2007).

In order to protect aquatic organisms, it is necessary to determine contamination levels and water quality criteria. The present study suggests that metals and the mode of action cadmium are susceptible to high degree of damage even at very low concentration of toxicant present in aquatic environment, even acute exposure period can bring multiorgan changes.

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