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RESEARCH ARTICLE

CATALASE ACTIVITIY IN THE DIGESTIVE GLANDS OF THE FRESHWATER MUSSELS, LAMELLIDENS CONSOBRINUS AFTER HEAVY METAL STRESS

Bhagyashri S. Bhangale[1]* and Mahajan P.R[2]

[1]Department of Zoology, Bhusawal Arts, Science and P.O. Nahata Commerce College, Bhusawal, Dist –Jalgaon (M.S.)
[2]Department of Zoology, Sardar V.P. Arts and Science College, Ainpur, Tal - Raver, Dist – Jalgaon. 425509

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ABSTRACT

Mussels are used as bio-indicators to evaluate the toxic effects of chemical pollutants in freshwater organisms, especially heavy metals, representing an important tool for bio-monitoring environmental pollution in aquatic environment. Antioxidant defence enzymes play an important role in cellular antioxidant defence systems and protect from oxidative damage by reactive oxygen species (ROS). Zinc is an environmental pollutant that causes damage to biological systems. The enzyme activities of catalase was studied in the digestive gland of the freshwater mussels, *Lamellidensconsobrinus* under the two groups. Group A was maintained as control; group B mussels were exposed to chronic $LC_{50/10\ dose}$ of zinc sulphate (0.34 ppm) up to 21 days. After 21 days investigate the potential use of the antioxidant defence enzymes against heavy metal pollution in freshwater mussels, *Lamellidensconsobrinus* as biomarkers of oxidative stress. Catalase activities in digestive gland of control and experimental mussels from A and B groups respectively were estimated after 7, 14 and 21 days. CAT showed a significant increased activity with increasing exposure period of heavy metal salts, $ZnSO_4$ but decrease increase the chronic period of exposure. Means catalase activity was highest in mussels from treatments exposed to the heavy metal concentrations as compared to control group of mussels. However, the lowest concentration of CAT activity after 14 and 21 days exposure to zinc. Catalase activity (CAT) was measured following the decrease of absorbance at 240 nm due to H_2O_2 consumption (Luck H.1974).

INTRODUCTION

The presence of heavy metal salts in the aquatic environment had effects on aquatic organisms; lethal effects resulted in serious physiological disorders or death, while sub lethal effects were manifested by disturbances of metabolism. Make Man caused serious hazards in the quality of water, but also to the aquatic life. Mining and smelting operations and discharge of most of the industrial wastes into the aquatic environment lead to the accumulation of inorganic pollutants like mercury, cadmium, nickel, copper, lead, chromium, iron and zinc in dissolved and suspended forms (Chukwu and Ugbeva, 2003). The environmental risk assessment and ecotoxicological involved the use of biological markers to highlight an early stage of pollution (R. Van der Oost, J. Beyerm, N.P.E. Vermeulen, 2003). Aquatic pollution is of great concern because every organism depends upon water. Zinc which has been recognized as a toxic metal, has become common aquatic pollution in recent year due to it wild spread use in industrial like zinc smelters, electroplating, alloy and pigment factories. Zinc has become hazardous causing metabolic, physiological, structural and functioning disorders of aquatic animals. (Kraak M.H., Scholen M.C., 1991).

Zinc is an element commonly found in the Earth's crust. It is released to the environment from both natural and anthropogenic sources; however, releases from anthropogenic sources are greater than those from natural sources. The primary anthropogenic sources of zinc in the environment (air, water, soil) are related to mining and metallurgic operations involving zinc and use of commercial products containing zinc. Zinc is capable of forming complexes with a variety of organic and inorganic groups (ligands). Biological activity can affect the mobility of zinc in the aquatic environment, although the biota contains relatively little zinc compared to the sediments. Zinc bio concentrates moderately in aquatic organisms; bio concentration is higher in crustaceans and bivalve species than

Corresponding author:* **Bhagyashri S. Bhangale
Department of Zoology, Bhusawal Arts, Science and P.O. Nahata Commerce College, Bhusawal, Dist –Jalgaon (M.S.)

in fish. Hydrogen peroxide is toxic to cells. CAT and GPX are the major primary antioxidant defense component that catalyses the decomposition of H_2O_2 which is produced by the action of superoxide dismutase to H_2O.Many biochemical and cellular biomarkers had beenstudied in aquatic organisms, and particularly in fish and bivalve molluscs. These biomarkers included those that were specific to oxidative stress, recommended for biomonitoring the quality of the aqatic environment, including malondialdehyde (MDA) which is derived from lipidperoxidation of polyunsaturated fatty acids in cell membranes during oxidative stress (Hamza-Chaffai, Pellerin J, Amiard J.C,2003; Dewes I.J, Sandrine J.Z, Monserrat J.M, Yunes J.S,2006; Oruc E.O, Usta D, 2007), reduced glutathione (GSH) involved in the antioxidant defense system (Machreki-Ajmi M, Hamza-Chaffai A, 2006) and catalase (CAT) which is the first line of defense against oxidative stress (Smaoui-Damak W, Hamza Chaffai A, 2003).

Under normal physiological condition, animals maintain a balance between eneration and neutralization of reactive oxygen species (ROS). However when organisms are subjected to xenobiotic compounds, the rate of production of ROS, such as superoxide anion radicals (O2 •−), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH) and peroxyl radicals (ROO−) exceeds their scavenging capacity (Halliwell and Gutteridge, 2007). CAT response to toxic chemicals shows a bell-shaped trend, with an initial increase in activity due to enzyme induction, followed by a decrease in activity due to an enhanced catabolic rate and/or direct inhibition by toxic chemicals (Viarengo *et al*., 2007). Such trends in CAT activity can be found in mussels at polluted sites according to the levels and duration of pollutant exposure (Nasci *et al*., 2002; Regoli *et al*., 2004; Nesto *et al*., 2004; Pampanin *et al*., 2005; Tsangaris *et al*., 2010). The aim of our study was to determine the physiological responses of catalase (CAT) in the digestive gland of freshwatermussels, *Lamellidens consobrinus* after heavy metal stress.

MATERIALS AND METHODS

The mussels, *Lamellidens consobrinus* wereacclimatized to laboratory condition for 2-3 days and healthy active snails of approximately medium size and weight were chosen. These mussels were divided into two groups, such as group A and B. The mussels of group A were maintained as control. The mussels from group B were exposed to chronic concentration ($LC_{50/10}$value of 96 hr.) of heavy metal salt, Zinc sulphate (0.34ppm) up to 21 days. After exposure for 21 days the experimental mussels from A to B groups were dissected after 7,14 and 21 days. The digestive gland from both groups were removed.

Tissue processing

The removed wet tissue was homogenate in blender with M/150 phosphate buffer at 1-4^{0}c and centrifuge.stir sediment with cold phosphate buffer and allowsstanding in the cold with shaking occasional then repeating the extraction once or twice and using the supernatant for assay of catalase.

Biochemical analyses

Catalase activity (CAT) was measured following decrease of absorbance at 240 nm due to H_2O_2 consumption (Luck H, 1974).

OBSERVATION AND RESULTS

CAT showed a significant increased activity with increasing exposure period of heavy metal salts, zinc sulphate but decrease increase the chronic period of exposure. Increase in activity was proportional to days of exposure as well, with highest CAT activity in digestive gland on the 7th day. (Table A) Mean catalase activity was highest in mussels from treatments exposed to the heavy metal concentrations as compared to control group of mussels. However, the lowest concentration of CAT activity after 14 and 21 days exposure to zinc.

DISCUSSION

Antioxidant systems are efficient protective mechanisms against reactive oxygen species (ROS) produced by endogenous metabolismor by the biotransformation of xenobiotic. The activity of these systems may be induced or inhibited after chemical stress. Aninduction can be considered an adaptation, allowing the biological systems to partially or totally overcome stress resulting fromexposure to an unsafe environment.

In contrast, a deficiency of the antioxidant system will induce a precarious state, making biological species more susceptible to toxic agents and preluding toxicity. Indeed, such a deficiency will impair the ability toneutralize ROS and to prevent cell damage. Thus, the parameters of antioxidant systems could be useful biomarkers reflecting not only exposure to contaminants, but also toxicity. Laboratory studies on analysis of stress responses in tissues of organism exposed to metal can help to understand mechanism through which metals exert their toxicity in organisms and hence the results can be used to explain the impact of heavy metal toxicity on organisms in fields.

Table A- Effect of Zinc sulphate on catalase activities in digestive glands of Lamellidens consobrinus, after chronic exposure zinc sulphate.

Treatment	Sr No.	Body Tissue	Catalase activity(U/mg.protein/ min.)		
			7 Days	14Days	21 Days
(A) Control	i	D.G	42.43	41.05	39.44
(B) 0.34 ppm $ZnSO_4$	ii	D.G	46.10, + 0.0367*	35.55,- 0.055*	31.19,- 0.0825*

D.G – Digestive gland,*-Compared with respective A

It is obvious from the present study that exposure of freshwater gastropods snails to ($LC_{50/10}$ concentration of 96 hours) zincs, only influence the oxidative stress on the antioxidant enzymes (CAT) in digestive glands of *Lamellidens consobrinus* Catalase, a well-established biomarker, is an essential enzyme of antioxidant defense system, which is present virtually in all aerobic organisms. This enzyme catalyzes the decomposition of hydrogen peroxide ($H_2O_{2)}$ into water and oxygen. A wide variety of stressors encountered in aquatic environments is able to alter the levels of catalase activity (Chandran *et al*., 2005; Mena *et al*., 2014)

In this study, the tested heavy metal salt, exhibited various levels of catalase activity against fresh water mussels, *Lamellidens consobrinus*, Zn was found to be most effective against this mussels. The CAT activity in digestive glands is increasing significantly increasing exposure period but decrease increase the chronic period of exposure zinc as compared to control groups of mussels. The CAT activity is highest in digestive glands after 7 days as compared to digestive glands in control group of mussels.The digestive glands of mussels is the key organ of metabolism and it is concerned with the production of digestive enzymes, absorption of nutrients, endocytosis of food substances, food storage, and excretion (Dallinger *et al*. 2002). It has been found to be the major site of xenobiotic and oxy-radical-generating bio- transformation enzymes (Livingstone *et al*. 1992).

The antioxidant CAT is an extremely important component of intracellular and antioxidant defenses of organisms (Jamil, 2001). (Siweela A.H and *et.al*,2010) studied, A comparison of metal levels and antioxidant enzymes in freshwater snails, Lymnaeanatalensis, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe and conclude that, Superoxide dismutase (SOD), diphosphotriphosphodiaphorase (DTD) and catalase (CAT) activities were significantly lower whilst malondialdehyde (MDA) levels were significantly higher in tissues of snails exposed to Lower Mguza Dam sediment and water. On the other hand, selenium-dependent glutathione peroxidase (Se-GPX) activity was significantly elevated in tissues of snails exposed to Lower Mguza Dam sediment and water.At high H_2O_2 concentrations, organic peroxides are metabolized by Catalase. According to (Geret, 2002) this inhibition is metal dependent. Mercury is known to be involved in redox reactions (Fenton reactions), which result in the production of oxyradicals.

CONCLUSION

In the present study, significant differences have been recorded in the activities of antioxidant enzyme (CAT) in the freshwater mussels, *Lamellidens consobrinus*after exposed to zinc as compared with the control mussels. This indicates that there is an increased level of oxidative stress due to the presence of heavy metals, and that an imbalance is generated between pro-oxidants and antioxidants. The study made in *Lamellidens consobrinus*can help to understand mechanism through which metals exert their toxicity in organisms and hence the results can be used to explain the impact of heavy metal toxicity on organisms .Zn exposed mussels are likely to adapt themselves even to the highest concentration.

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