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CATALASE ACTIVITIES IN THE DIGESTIVE GLAND AND GILLS OF THE FRESHWATER MUSSELS, *LAMELLIDENS MARGINALIS* AFTER HEAVY METAL INTOXICATION

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ABSTRACT
Present study investigate the potential use of the antioxidant defence enzymes in freshwater mussels, *Lamellidens marginalis* as biomarkers of oxidative stress. The enzyme activities of catalase was examined in the digestive gland and gill of the freshwater mussels,*Lamellidens marginalis* after chronic exposure of heavy metal salts, mercury chloride (0.169ppm) up to 9 days. Catalase activities in digestive gland and gills of control and experimental mussels from A and B groups respectively were estimated after 3, 6 and 9 days. Catalase activities were decrease significantly after 3, 6 and 9 days after exposure of mussels in heavy metal salt. Catalase activity (CAT) was measured following the decrease of absorbance at 240 nm due to H_2O_2 consumption.

Keywords: *Mercury, Catalase Activity, Lamellidens marginalis*

INTRODUCTION

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). Mercury is considered global pollutants of high concern regarding their adverse effects on environmental and human health (Barboza *et al.,* 2018; Guilhermino *et al.,* 2018; Wright and Kelly, 2017).There are some heavy metals and mercury (Hg) is one of the most toxic heavy metals in our environment including the lithosphere, hydrosphere, atmosphere and biosphere **(**Barbosa *et al.,* 2001). Mercury is used in a variety of industrial, consumer and medical products. It is also released into the environment through natural phenomena (volcanoes, degradation of minerals or evaporation from soils) and manmade processes.The analyses of biomarkers in these bivalves have been also incorporated into biomonitoring studies to evaluate the effects of pollutants (Viarengo *et al.,* 2000).

Antioxidant defense enzymes are important scavengers of these radicals. In aquatic organisms, two important antioxidant defense enzymes are superoxide dismutase (SOD) and catalase (CAT). 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.Catalase (CAT)which is the first line of defense against oxidative stress (Smaoui-Damak and Hamza , 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 (Regoli *et al.,* 2004; Nesto *et al.,* 2004; Pampanin *et al.,* 2005 and Tsangaris *et al.,* 2010). The aim of our study was

to determine the physiological responses of catalase (CAT) in the digestive gland and gills of freshwater mussels, *Lamellidens marginalis* after heavy metal (Hg) stress.

MATERIALS AND METHODS

The mussels, *Lamellidens marginalis* were acclimatized to laboratory condition for 2-3 days and healthy active bivalves 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, mercury chloride (0.169ppm) up to 9 days.The control group A and experimental group of mussels B were dissected after 3,6 and 9 days. The digestive glands and gills from both groups were removed.

Tissue processing

The removed wet tissue of digestive glnds and gills were homogenate in blender with M/150 phosphate buffer at 1- 4^0c and centrifuge.stir sediment with cold phosphate buffer and allows standing 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).

RESULTS

CAT activity showed a significant increased activity with increasing exposure period of heavy metal salts, $HgCl_2$. The catalase activity in digestive system after 3,6 and 9 days is 39.12 ± 0.030 , 40.15 ± 0.030 and 39.21 ± 0.020 U/mg.protein/ min. in control while in experimental mussels is 42.73 ± 0.028, 49.05 ± 0.039 and 54.28 ± 0.031 respectively.It is in gills in control mussels is 18.53 ± 0.022, 18.79 ± 0.048 and18.63 ± 0.027 while in experimental group is 23.42 ± 0.031 , 25.02 ± 0.042 and 26.13 ± 0.018 U/mg.protein/ min. The figure 1 and 2 indicates, catalase activity was highest indigestive glands as compared to gills.

Table A: Catalase activites in digestive glands and gills of fresh water mussels, *Lamellidens marginalis*, after chronic exposure mercuric chloride

Treatment	Sr No.	Body Tissue	Catalase activity (U/mg.protein/ min.)		
			3 Days	6 Days	9 Days
(A) Control	i	D.G	39.12 ± 0.030	40.15 ± 0.030	39.21 ± 0.020
	ii	G	18.53 ± 0.022	18.79 ± 0.048	18.63 ± 0.027
(B) $HgCl_2$ (0.169 ppm)	i	D.G	42.73 ± 0.028 (9.228 %)*	49.05 ± 0.039 (22.166 %)*	54.28 ± 0.031 (38.434)*
	ii	G	23.42 ± 0.031 (26.389 %)*	25.02 ± 0.042 (33.155%)*	26.13 ± 0.018 (40.257 %)*

D.G - Digestive gland, G – Gills **- % Variation compared with respective*

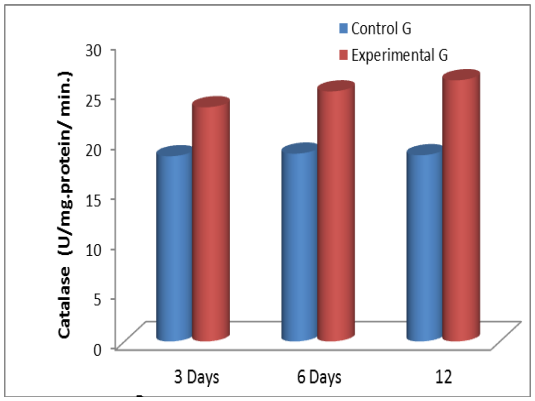

Figure -1

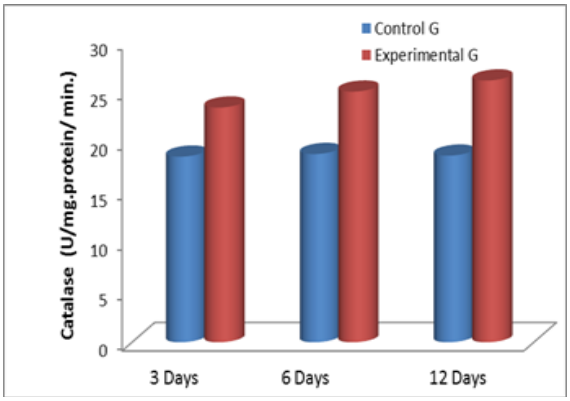

Figure -2

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, makingbiological 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. Livingstone *et al.,* (2000) found increased CAT activity in the digestive gland of *M. edulis* after menadion treatment.

CAT is the primary scavengers of H_2O_2 in the cell. Increased CAT activity presently seen exposed to trace metals in *P. viridis*, further indicate that pollution stress has elevated the formation rate of H_2O_2. These results are comparable to those found in other studies, where CAT activity increases at sites contaminated with metals (Lima *et al.,* 2007). Increased activities of CAT have been reported in several fish and invertebrate species (Stephensen *et al.,* 2000). Heavy metals could induce increased CAT activity in bivalves (Verlecar *et al.,* 2008). Elevated activity of CAT was reported in *Mytilus galloprovincialis* in the Adriatic Sea (Borkovic *et al.,* 2005).

In present study that exposure of freshwater bivalves to ($LC_{50/10}$ concentration of 96 hours) mercury; only influence the oxidative stress on the antioxidant enzymes (CAT) in digestive glands and gills of *Lamellidens marginals.* 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, Hg exhibited various levels of catalase activity against fresh water mussels, *Lamellidens arginalis*, Hg was found to be most effective against these mussels. The CAT activity in digestive glands is increasing significantly increasing exposure period (42.73 ± 0.028 to 54.28 ± 0.031),same result obtained in CAT activity in gills (23.42 ± 0.031 to 26.13 ± 0.018). The antioxidant CAT is an extremely important component of intracellular and antioxidant defenses of organisms (Jamil, 2001). 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 marginalis* after exposed to Hg 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 marginalis* 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. Hg exposed mussels are likely to adapt themselves even to the highest concentration.

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