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ACID PHOSPHATASE AS A BIOMARKER OF HEAVY METAL TOXICITY IN FRESHWATER GASTROPOD, *BELLAMYA BENGALENSIS.*

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Abstract:- :In the present investigation, alterations in acid phosphatase activity of *Bellamya bengalensis* subjected to acute toxicity of copper (96 hrs LC_{50} during summer season= 0.60 ppm) over a period of 30 days (exposure period) was used to assess toxicity. Samples of digestive glands were collected from both exposure and control groups and subjected for biochemical analysis of enzyme activity. There was generally a gradual increase in the levels of acid phosphatase activity in digestive gland when compared to respective control groups. Significant differences were observed in acid phosphatase levels in the copper treated gastropods with an increase in the exposure of time to the copper. Our results demonstrated that, acid phosphatase enzyme may be sensitive to heavy metal and can be affected by heavy metal ions hence it can be used as a biomarker to evaluate toxicity in aquatic organisms.

Keywords: Heavy metal pollution, acute toxicity, copper, acid phosphatase, biomarker, *Bellamya bengalensis*

INTRODUCTION:

Ecotoxicology deals with the harmful effects of chemicals usually of anthropogenic origin on ecosystem. At present, ecotoxicolgy or ecotoxicity is the one branch of toxicology which concerns with the unfavourable effects of toxicants on ecosystems such as aquatic ecosystems (freshwater, marine, etc), terrestrial ecosystems, etc. Aquatic toxicology is the study of the effects and fate of foreign substances or chemicals on aquatic organisms. Heavy metal pollution in aquatic ecosystem has been recognized as a serious environmental problem due to the non-biodegradability and the tendency of the heavy metals to accumulate in animal tissues (Soegianto *et al*., 2008)

Copper and their compounds are used for various applications such as in wire and cable, electronics and related devices, in electric motors, in architectural material, as an antibiofouling agent, as an antimicrobial agent in fabrics (textile industry), as a wood preservative, in electroplating, and as pesticides, fungicides, algaecides and molluscicide. Copper particulates are released into the atmosphere by windblown dust, volcanic eruption and anthropogenic sources. Anthropogenic sources of copper in water are extensive in addition to natural such as weathering of rocks, atmospheric deposition, volcanic eruption, etc. Molluscs are considered as good indicators of heavy metal pollution. Authors have reported their importance as the indicators for monitoring heavy metals (Nurnberg et al., 1984).

Acid and alkaline phosphatases (hydrolytic enzymes) are the marker enzymes both differ in the distribution (Rahman and Siddiqui, 2004). Acid phosphatase is a lysosomal marker enzyme whereas alkaline phosphatase is a plasma membrane enzyme. Both are hydrolytic enzymes. The activities of these enzymes are involved in a variety of metabolic processes such as protein synthesis, growth and differentiation of cell, absorption and transport of molecules, steroidogenesis, etc. (Ram and Sathyanesan, 1985). Copper and mercury at varying degree of concentration have shown to influence the activity of acid phosphatase in freshwater mussels (Rajalakshmi and Mohandas, 2005). Therefore, enzymes can be used as the bioindicators of metal contamination with respect to type and concentration of metal (Atli and Canli, 2007).

Specimens of *Bellamya bengalensis* employed in this study, were collected from Hotgi Tank, Solapur, Maharashtra. Hotgi tank is situated in south direction and 10 km away from Solapur city. This tank is also facing gross pollution problems following the release of untreated effluents from industries and domestic sectors. Anthropogenic impacts have resulted in the accumulation of heavy metals in the tank. As this tank is located in agricultural area many pesticides, fungicides, fertilizers are used for agricultural purpose are responsible for the pollution of this tank. By considering the adverse impact of environmental

pollution due to heavy metals, effects of copper on freshwater gastropod *Bellamya bengalensis* was studied with respect to hydrolytic enzyme.

MATERIALS AND METHOD:

Test organism and their maintenance:

The freshwater gastropods, *Bellamya bengalensis* were collected from Hotgi Tank, Solapur. They were brought to the laboratory and acclimated to the laboratory conditions in a well aerated aquarium. The water was changed for every 24 hrs.

Experimental design:

Before actual experiment, 96 hrs LC50 value of copper was determined with the method described by Finney (1971). The 96 hrs LC50 was found to be 0.60 ppm during summer season. Gastropods were exposed to 96 hrs of LC_{50} (0.60 ppm) of copper sulphate. A control group without toxicant was also run simultaneously. The experiment was conducted for 96 hrs with an interval of 24 hrs. Digestive glands were collected for further estimation from both control and experimental groups.

Preparation of sample for Enzyme assay:

At each interval animals were sacrificed separately to collect various digestive glands for the enzyme assay. They were homogenized separately in glass homogenizers by using buffer. Tissue homogenate were centrifuged and the supernatants were collected which were used for the enzyme assay.

Enzyme analysis:

Acid phosphatase was determined spectrophotometrically with the *p*-nitrophenol method with slight modifications. The specific activity of enzymes is expressed as µg PNP released/ml.

Statistical Analysis:

The data obtained was analyzed with the level of significance by using Student's't' test (Bailey, 1965). Values were taken in triplicate and expressed as Mean±SE. Graphs were prepared by using Graph Pad prism (version 5.00).

Results:

Alterations in acid phosphatase activity from digestive gland of freshwater gastropod, *Bellamya bengalensis* after exposure to copper have been summarized in Table no. 1.

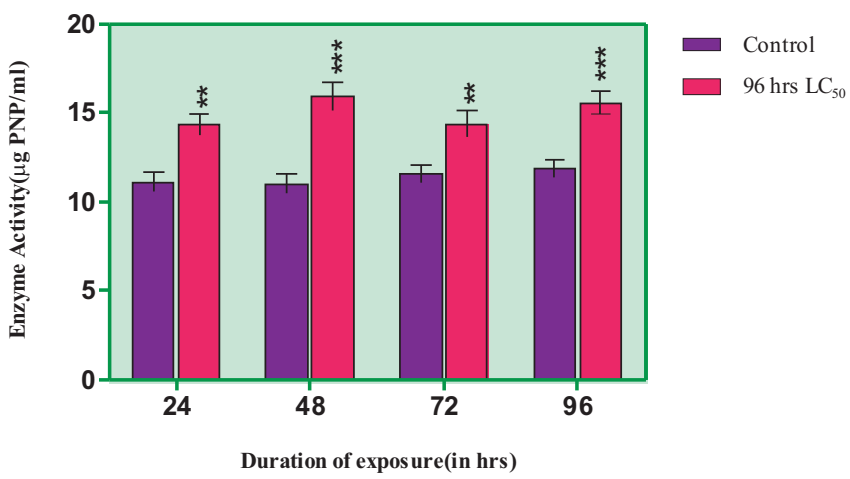
Activity of acid phosphatase in digestive gland of gastropods from control group was found to be in the range of 11.03±0.56 to 11.85±0.49 ug PNP/ml. Highest activity (44.42%) was observed after 48 hrs of exposure. Activity was enhanced in all exposure period as compared to control. Similar increment in acid phosphatase level was reported after 96 hrs (31.48%), 24 hrs (29.10%) and 72 hrs (24.16%) of exposure to acute toxicity of copper when compared to respective control groups. Overall acid phosphatase activity in copper exposed gastropods revealed significant increase ($p<0.01$, $p<0.001$) in activity as the duration of exposure to copper enhanced (Fig.1). Maximum increase was found after 48 hrs of exposure as compared to control.

Table No.1: Variations in the acid phosphatase activity from digestive gland of *Bellamya bengalensis* after exposed to 96 hrs LC_{50} of copper

Duration in hours	Groups	
	Control	0.60 ppm
24	11.10±0.53	14.33±0.58 ** (29.10%)
48	11.03±0.56	15.93±0.78 *** (44.42%)
72	11.55±0.51	14.34±0.74 ** (24.16%)
96	11.85±0.49	15.58±0.68 *** (31.48%)

(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's 't' test, values are significant at * = $p < 0.05$,
** = $p < 0.01$, *** = $p < 0.001$.
ns - Non significant, (Mean±SD, n=3)
Values in ( ) indicate percent change over control.

Fig.no.1 Activity of acid phosphatase from digestive gland of *B. bengalensis* exposed to copper



DISCUSSION:

Phosphatase enzymes play an important role in a variety of metabolic processes such as detoxifation, biosynthesis and metabolism (Rahman and Siddiqui, 2004). Acid phosphatase mainly resides within lysosomes which are primary responding organelles in response to foreign particles (http://en.wikipedia.org/wiki/Lysosome).

In our findings, an increase in the activity of acid phosphatase might have resulted due to the leakage of enzyme from lysosomes into cell cytoplasm, tissues and further they might have entered into blood stream. For the reason that increased activity of acid phosphatase was noticed in digestive glands compared to control. Meanwhile, another reason for the same effect is that copper ions might have responsible for the destabilisation of lysosomal membranes (Moore, 1976). Further, enhancement in acid phosphatase content may also be due to the hypersynthesis of enzyme as it plays a protective role in removal of inflammation (Cheng, 1983) with the enhancement in immune defence system against toxic stress (Giannapas *et*

al., 2012). Sharma and Thomas (2007) have speculated that enhanced acid phosphatase activity in green mussel (*Perna viridis*) at sublethal level of copper may be due to hypersynthesis of enzyme during stress. Variations in the enzyme activity based on the concentration and length of exposure of metal is of great diagnostic value (Rajalakshmi and Mohandas, 2005). It was earlier reported that the effect of heavy metal in lysosomal enzyme acid phosphatase depends on three factors viz, toxicological nature of toxicant, duration of exposure and morphophysiological status of concerned organ or tissue (Jayakumar et al., 2008)

Karan et al. (1998) observed similar enhancement in acid phosphatase activity in gill and serum of carp (*Cyprinus carpio* L) exposed to copper with decrease in enzyme activity after a recovery period. Sharma *et al*. (2006) demonstrated the hypersynthesis of lysozyme by mercury and acid phosphatase by copper.

Pronounced enhancement in enzyme activity during exposure period to copper could have made burden on organ to cope with metal stress for increasing metabolic energy demand which might have increased immune response of exposed gastropods to deal effectively with the metal bioaccumulation which led to enhanced activity level of acid phosphatase. It might be due to that digestive gland is a vital digestive and detoxication organ as well as critical site for heavy metal accumulation (Fallah et al., 2011; Li et al., 2008). Digestive gland is the major detoxifying organ and performs the function of neutralisation of toxic effects of heavy metals with the help of lysosomes containing acid hydrolase enzyme.

CONCLUSION:

From our study it is revealed that activity of hydrolytic enzyme (acid phosphatase) depends on duration of exposure to metal. Acid phosphatase is a sensitive enzyme to copper toxicity. So, it can be effectively used as a lysosomal biomarker enzyme to assess copper contamination in aquatic environment.

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