ACTIVITY OF PHOSPHATASES IN THE HEMOCYTES OF A COMMERCIALLY IMPORTANT VIVIPAROUS MOLLUSC Bellamya bengalensis EXPOSED TO A SYNTHETIC FENVALERATE

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Abstract: ***Bellamya bengalensis is an aquatic mollusc distributed in the freshwater reservoirs of different states of India including West Bengal. B. bengalensis is a benthic mollusc and is capable of filterfeeding. Aquatic molluscs are involved in bioaccumulation of toxic materials. This edible species is a cheap source of proteinaceous diet of human being, poultry and component of the artificial fish feed. Fenvalerate is an agricultural pesticide which is used to control insect pest and domestic uses. Agricultural runoff contaminates freshwater reservoirs of India. In this present investigation, phosphatase activity both acid and alkaline of hemocytes of B. bengalensis were estimated under the sublethal exposure of fenvalerate in controlled laboratory condition for 24, 48, 72, 96 hours and 15 days time span. In the large sized male B. bengalensis, the highest inhibition of acid phosphatase was recorded as 0.12 ± 0.03 µM/mg protein/ min against the concentration of 3ppm of fenvalerate for 96 hours of exposure. In the large sized female B. bengalensis the highest inhibition of acid phosphatase activity recorded as 0.14 ± 0.03 µM/mg protein/min against the concentration of 3 ppm of fenvalerate for 96 hrs exposure. In the large sized male B. bengalensis the highest activity of ALP was recorded as 2.78 ± 0.42 µM/mg protein/min against the concentration of 1 ppm of fenvalerate for 96 hours of exposure. In the large sized female B. bengalensis the highest activity of ALP was recorded as 2.62 ± 0.82 µM/mg protein/min against the concentration of 2 ppm of fenvalerate for 96 hours of exposure. The lowest activity of ALP was recorded as 0.48 ± 0.1 µM/mg protein/min against the concentration of 2 ppm of fenvalerate for 48 hour exposure. Fenvalerate exposure resulted in a dose independent decrease in the activities of two specific phosphatases and may be determined as a suitable biomarker of freshwater ecosystem of India.***

Key words: Fenvalerate, Bellamya bengalensis, Hemocytes, Phosphatases

INTRODUCTION

Molluscs in ecotoxicology, occupy a distinct place and are often considered as agents for passive and active biomonitoring for ecologigal risk assessment (Salanki, J.,1986). Krieger *et al*. (2010) estimated that the collective filtration ability of molluscs in the Delaware Estuary watershed of the USA which exceeds 100 billion litres per hour. Through the process of filtering suspended matter, molluscs link benthic and pelagic compartments by transferring energy and nutrients from the water column to the sediment, biodepositing organic substances and excreting nutrients (Elderkin *et al*.,2008). *Bellamya bengalensis is* (Mollusca : Gastropoda) a freshwater mollusc and an important resident of the freshwater ecosystem of India (Ray *et al*.,2013b). This edible mollusc is cheap source of proteinaceous food item of human being, poultry and component of artificial fish feed (Baby *et al*., 2010). It bears an important role in ecology, economy, ethnomedicine and nutrition. Prabhakar *et al*. (2009) reported that body parts of *B. bengalensis* is exclusively used as medicinal agent for the cure of a number of ailments such as rheumatism, asthma, arthritis, joint pain, conjunctivitis among the tribal people of the Northern Bihar.

Due to increasing trend of human population and rapid urbanization, natural habitats of the freshwater molluscs have been restricted to a limited geographical area. In agricultural field pesticides are primarily used for controlling insect pest in an unrestricted way. Synthetic pyrethroid such as fenvalerate is exclusively used as pesticide in agriculture. Fenvalerate, a type II synthetic pyrethroid with an α- cyano group, has emerged as a major agricultural pesticide in developing countries due to its wide insecticidal range and moderate toxicity in mammalian groups (Gu *et al*., 2010). This pesticide enters the freshwater ecosystem through different routes and poses a serious threat to the non target aquatic organism including *B. bengalensis.*

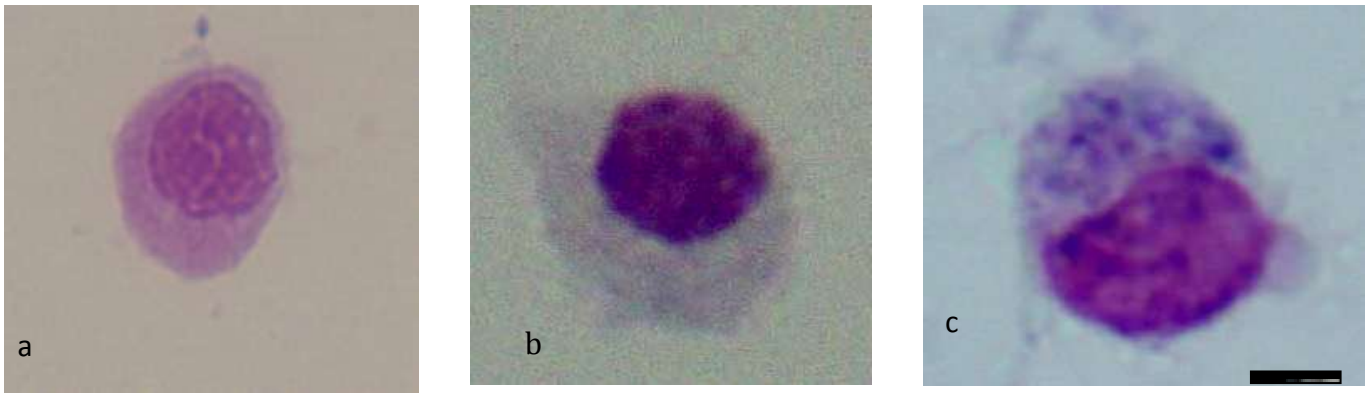

Figure:1. Bright field microscopic image of *B. bengalensis* hemocyte subpopulations (a-c) (magnification X1000).

Hemocytes (Figure:1) are reported as the chief immunoeffector cells of molluscan hemolymph and responsive to the toxic challenge of diverse xenobiotics. Hemocytes play an important role in the non specific defence mechanism and various hemocytic parameters are considered as immuno-toxicological diagnostic tools (Auffret, 2005). Acid and alkaline phosphatases are hydrolytic enzymes. These enzymes are involved with a variety of metabolic processes such as protein synthesis, growth and differentiation, metabolism of carbohydrate, absorption and transportation of molecules etc. (Vijayavel and Balasubramanian, 2006). Phosphatases can be considered as the bioindicator of metal contamination with respect to type and concentration of metal (Atli and Canli, 2007). In *B. bengalensis* concentration of copper causes a severe damage to the tissues which could have been resulted due to diffusion of lysosomal acid phosphatase into cell cytoplasm (Rao *et al*., 2013). In mollusc, cytoplasmic lysosomal granules of phagocytic blood cells contain various enzymes such as acid phosphatase, alkaline phosphatase (Cima *et al*., 2000) which are functionally involved in phagocytosis. Present investigation is aimed to estimate the toxic effects of fenvalerate on acid and alkaline phosphatase activity level in hemocyte of *B. bengalensis.*

MATERIALS AND METHODS

Collection of *B. bengalensis* from natural habitat and transportation

The *B. bengalensis* of various shell lengths were collected from selected freshwater ponds of South 24 Parganas district of West Bengal. Live and healthy specimens were transported to the laboratory in plastic containers with a dimension of 12 inch × 18 inch × 6 inch at a density 10-15 individuals per box in humid condition.

Laboratory acclimation and maintenance of *B. bengalensis*

Live healthy specimens were acclimated in toxin free freshwater in borosilicate glass jars for 6-8 days. The maintenance of animals was carried out in airy and well illuminated laboratory condition. Replenishment of water was done in every 24 hours to avoid residual toxicity. During the experiment animals were properly maintained according to the protocol and methods of Raut (1991).

Treatment with fenvalerate

For the study of phosphatase activity, both sexes of *B. bengalensis* of large sized i.e. 35-44 mm in shell length were considered for exposure 0.5, 1, 2 and 3 ppm fenvalerate (Chemical name- alpha-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methyl butyrate, CAS registry number:51630-58-1, Molecular formula:$C_{25}H_{22}ClNO_3$, marketed by Rallis Tata enterprise) for different spans of time period i.e. 24, 48, 72, 96 hours and 15 days along with control set. Formulations of fenvalerate and water were replenished at every 24 hours.

Collection of hemolymph

Hemolymph was asceptically collected under laminar flow hood by foot prodding method (Sminia, 1972). The collected hemolymph was stored in prechilled microfuge tube to prevent hemocyte aggregation at 4º c.

Preparation of cell lysate

For cell lysate preparation, aseptically collected hemocytes were sedimented by centrifugation of hemolymph at 3000 rpm for 5-10 minutes and the cell density was adjusted with unit volume of sterile snail saline. The pellet of hemocytes with unit density was mixed with 0.1% TritonX-100 (1:1) (SRL India) for 15 minutes for cell lysis. The hemocytes were centrifuged at 10,000 rpm for 20 minutes at 4º c and the cell lysate supernatant was collected.

Acid phosphatase activity(ACP, EC 3.1.3.2)

The cell lysate, 5mM p-nitrophenol phosphate (PNPP) in 50mM sodium acetate buffer (pH 5.0) were mixed and incubated for 30 minutes at 37ºc in a humid chamber. After the incubation, the reaction was halted by addition of 0.1(N) sodium hydroxide (NaOH) and incubated again for 30 minutes in a humid chamber. The absorbance was recorded spectrophotometrically (CECIL-CE 4002,Germany) at 420nm against a standard blank. The enzyme activity was expressed dephosphorylation of p-nitrophenol phosphate to P-nitrophenol in µM/mg protein/min.

Alkaline phosphatase activity(ALP, EC 3.1.3.1)

The hemocyte lysate with 5mM PNPP in 50mM glycine buffer (pH 10.0) and 10mM $Mgcl_2$ was added to incubate for 30 minutes at 37º c in a humid chamber. After proper incubation, the reaction was stopped by addition of 0.02 (N) NaOH and incubated for 30 minutes. The absorbance was determined in a spectrophotometer (CECIL-CE 4002, Germany) at 420nm against a standard blank. The enzyme activity was estimated using a standard curve of P-nitrophenol (PNP). The ALP was expressed dephosphorylation of p-nitrophenol phosphate to P- nitrophenol in µM/mg protein/min.

Statistical analysis

All experiments were repeated for five times and values are expressed as mean ± S.D. The data are compared in Fisher's t-test and symbols denote values that are significantly different from the control set. *p<0.05, **p<0.01, ***p<0.001

RESULTS

The activity of acid phosphatase in the respective sets of control of large sized male *B. bengalensis* ranged from 1.83 ± 0.19 µM/mg protein/min. under different spans of exposure (Figure-2). A significant decrease in the activity of acid phosphatase was recorded against all experimental concentrations of fenvalerate for 24, 48, 72, 96 hours and 15 days of exposure. The highest inhibition of acid phosphatase activity was recorded as 0.12 ± 0.03 µM/mg protein/min. against the concentration of 3 ppm of fenvalerate for 96 hours of exposure. The activity of acid phosphatase in the respective sets of control of large sized female *B. bengalensis* ranged from 1.75 ± 0.43 µM/mg protein/min. to2.75 ± 0.70µM/mg protein/ min.under different spans of exposure (Figure-3). A significant decrease in the activity of acid phosphatase was recorded against all experimental concentrations of fenvalerate for 24, 48, 72, 96 hours and 15 days exposure. The highest inhibition of acid phosphatase activity was recorded as 0.14 ± 0.03µM/mg protein/min. against the concentration of 3 ppm of fenvalerate for 96 hours of exposure (Figure-3).

The activity of alkaline phosphatase of the hemocytes of untreated large sized male *B.bengalensis* ranged between 1.64 ± 0.41 µM/mg protein/min. and 1.92 ± 0.62 µM/mg protein/min. under different spans of exposure. A non linear and dose independent alteration in the activity of the alkaline phosphatase was recorded under all experimental concentrations (0.5, 1, 2 and 3 ppm) of fenvalerate for multiple spans of exposure. The highest activity of ALP was recorded as 2.78 ± 0.42 µM/mg protein/min. against the concentrations of 1 ppm of fenvalerate for 96 hours of exposure (Figure -4). The activity of alkaline phosphatase exhibited by the hemocytes of untreated large sized female *B. bengalensis* ranged between 1.64 ± 0.20 µM/mg protein/ min. and 1.93 ± 0.48µM/mg protein/min. under different spans of experiment (Figure-4). The highest activity of alkaline phosphatase was recorded as 2.62 ± 0.82 µM/mg protein/min. against the concentration of 2 ppm of fenvalerate for 96 hours of exposure (Figure-5). The lowest activity of alkaline phosphatase was recorded as 0.48 ± 0.1 µM/ mg protein/ min. against the concentration of 2 ppm of fenvalerate for 48 hours of exposure (Figure-4). However the pattern of fenvalerate induced alteration in activity of alkaline phosphatase in hemocytes of male and female *B. bengalensis* appeared to be similar.

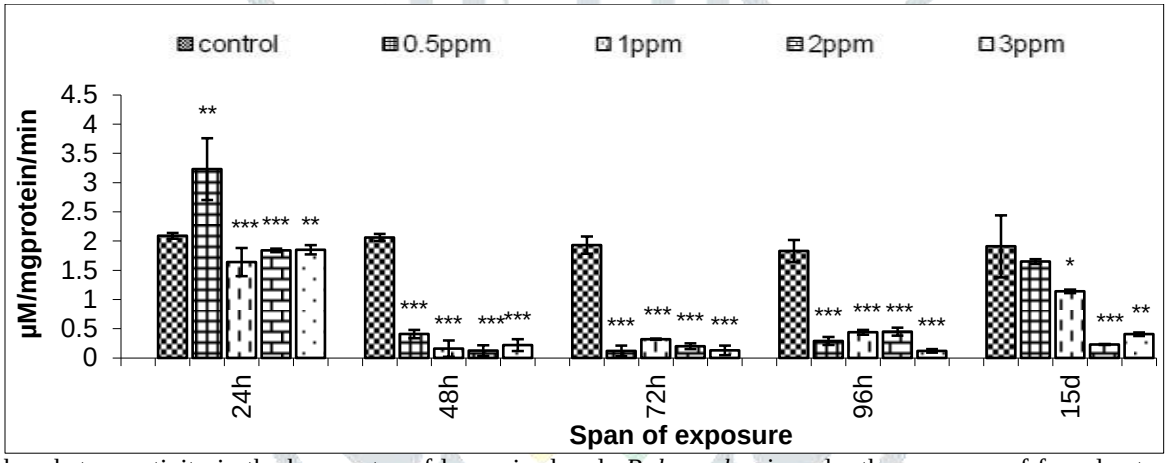

Figure: 2. Acid phosphatase activity in the hemocytes of large sized male *B. bengalensis* under the exposure of fenvalerate. Data presented as mean ± S.D. (n = 5) *p<0.05, **p<0.01, ***p<0.001.

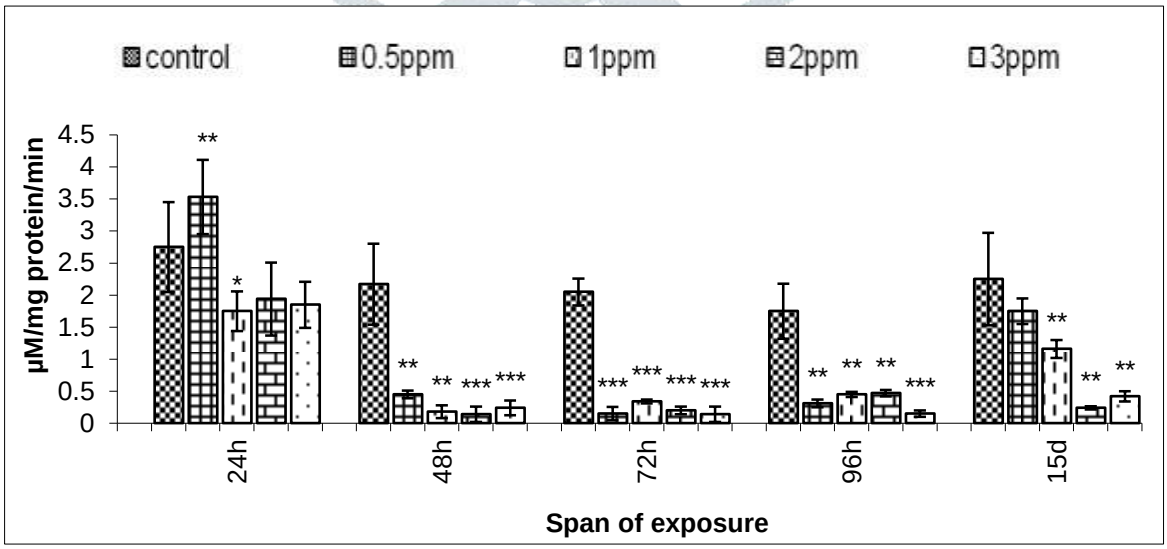

Figure: 3. Acid phosphatase activity in the hemocytes of large sized female *B. bengalensis* under the exposure of fenvalerate. Data presented as mean ± S.D. (n = 5) *p<0.05, **p<0.01, ***p<0.001.

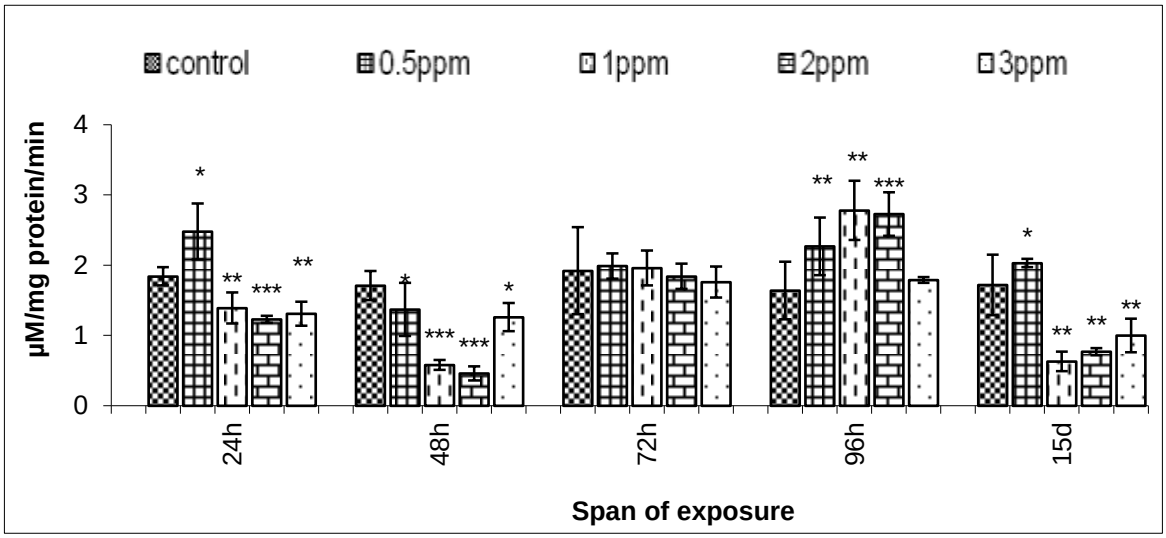

Figure: 4. Alkaline phosphatase activity in the hemocytes of large sized male *B. bengalensis* under the exposure of fenvalerate. Data presented as mean ± S.D. (n = 5) *p<0.05, **p<0.01, ***p<0.001.

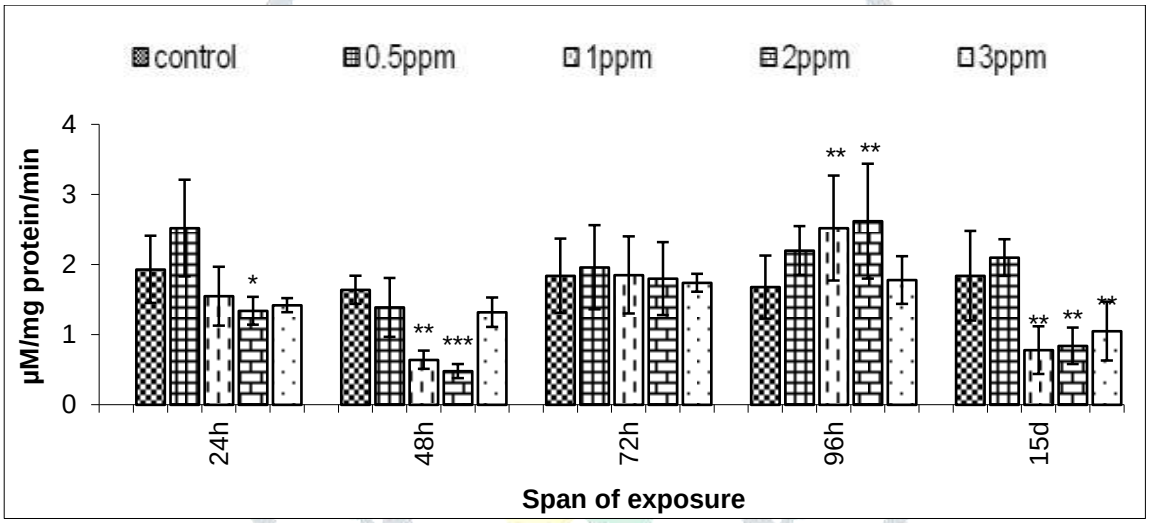

Figure: 5. Alkaline phosphatase activity in the hemocytes of large sized female *B. bengalensis* under the exposure of fenvalerate. Data presented as mean ± S.D. (n = 5) *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

Acid and alkaline phosphatases are hydrolytic marker enzymes reported both in invertebrate and vertebrate (Rahman and Siddiqui, 2004). Acid phosphatase is an intralysosomal enzyme of metabolic importance. In both large sized male and female *B. bengalensis* a significant decrease in the activity of acid phosphatase were recorded against all experimental concentrations of fenvalerate for 24, 48, 72, 96 hours and 15 days of exposure (Figure2 and 3). Acid phosphatase and alkaline phosphatase are sensitive to heavy metal exposure. Variations in the enzyme activity based on the concentration and time span of exposure of metal is of great diagnostic value (Rajalakshmi and Mohandas, 2005). It was reported that the effect of heavy metal in lysosomal acid phosphatase depends on three factors viz. toxicological nature of the toxicant, duration of exposure and morphophysiological status of the concerned organ or tissue. ACP plays a vital role in the process of detoxification of toxic materials entering the animal's body (Zhang *et al*.,2007). A significant decrease of ACP activity was suggestive to impairment of hydrolytic functions of phosphatases and disturbance in nutrient dynamics system in *B. bengalensis* distributed in contaminated habitat.

Alkaline phosphatase is associated with the transport and transphosphorylation mechanisms of cells. This enzyme is considered as a biomarker for a variety of different organisms due to its high sensitivity and less variability and for the non complexity to estimate its activity as stress index (Vijayavel and Balasubramanian, 2006). In both large sized male and female *B. bengalensis*, a nonlinear alterations in the activity of alkaline phosphatase were recorded against all experimental concentrations of fenvalerate for 24, 48, 72, 96 hours and 15 days of exposure (Figure 4 and 5). ALP activity was increased during heavy metal toxicity in *Perna viridis* (Anand *et al*., 2010). Chakraborty *et al*. (2010), reported that the suppression of the activity of acid phosphatase and alkaline phosphatase in the tissue by arsenite might cripple the immune system and nutrient mobility in the gills of freshwater mollusc *Lamellidens marginalis*. Alteration of ALP activity in *B. bengalensis* under the exposure of fenvalerate was indicative to a state of immuological stress in the organism. Both of these enzymes may thus be considered as an effective monitoring tool to estimate the toxicity in *B. bengalensis* a neglected bioresource of India.

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