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Research Article

EFFECT OF TBTCL ON PHOSPHATASES ACTIVITY IN ESTUARINE EDIBLE CLAM, *ANADARA RHOMBEA* BORN (BIVALVIA: MOLLUSCA)

P. Ranilalitha[1]*, M. Sukumaran[1] and S. Raveendran[2]

[1]Post Graduate and Research Department of Zoology, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, India

[2]P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamil Nadu, India

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ABSTRACT

Estuaries are being polluted by a variety of persistent toxic substances (PTS[s]) such as pesticides, heavy metals and organotins. Bivalve molluscs are known as biomarkers of estuarine pollution, since they store and concentrate such toxic substances in their vital body tissues. More recently, changes in metabolites and enzymes are being employed as biomarkers in the evaluation of toxicological studies. Variations in acid and alkaline phosphatases activity in the digestive gland of the edible clam, *Anadara rhombea* under sublethal concentrations (10%, 20% and 30% of Lc_{50} value) of Tributylin chloride (TBTCL) over a period of 15 days were studied in the laboratory conditions. There were increases in the levels of both phosphatases activity in the digestive gland of *A.rhombea* with increase in the concentration of TBTCL and time of exposures. Significant differences ($p<0.05$) were noticed in the treated clams. The increase was associated with the decrease in stability of digestive gland lysosomal membrane or with digestive gland damage which reflects proliferation of lysosome in attempt to sequester the toxic organotin, TBTCL.

Keywords: *Anadara rhombea;* Phosphatases activity; Digestive gland; *Tributyltin chloride*.

INTRODUCTION

Estuaries play a unique role in the functioning of life on this planet. They are also nurseries to many species of finfishes, shellfishes, crustaceans, birds and mammals (Pritchard, 1967). Estuaries are important for the production of protein rich fish and bivalve species. In many parts of the world, communities living near estuaries depend upon them for food and their livelihoods. Valuable nutrient bearing sediments in estuaries are capable and dispersing a variety of pollutants including pesticides, heavy metals and organotins which some estuarine organisms are known to store and concentrate in their body tissues (Galdhar *et al.*, 1978, Langstone and Burt 1979; Langston 1986; Bourgoin and Risk 1987; Wade *et al.*, 1988; Wadlock *et al.*, 1990; Deshpande *et al.*, 1999: Sole *et al.*, 2000; Saha *et al.*, 2009; Revathi *et al.*, 2013).

Organotin compounds (OT[s]) such as Tributyltin (TBT) have been employed as antifouling agents in paints applied on the bottom of boats and mechanical vessels used for fishing activity in estuaries. TBT is poisonous to a range of organisms from plankton to high level organisms. TBT compounds have been detected in water, sediments and biota of estuaries where fishing activity is at large. Once in estuaries, TBT can be taken up by estuarine organisms through exposure to TBT contaminated water or sediments or ingestion of TBT contaminated food sources namely plankton and/or detritus (Alzieu, 2000: Bhosle *et al.*, 2004).

Several laboratory studies showed biological and biochemical changes by organotin residues found in fishes and bivalve molluscs. Moreover, the toxic effects of pesticides and heavy metals

*Corresponding author e-mail: lalithara_06@rediffmail.com

on phosphatases activity in crabs, fishes and gastropods have been documented by some workers (Hirth, 1964; Joshi and Desai 1981; Surendran 1994; Sontakke and Sunita Jadhav, 1997; Ahamed *et al.*, 2010*;* Magar and Shaikh 2013). Very few literatures are available on TBT toxicity on metabolites and enzyme activity in estuarine clams and mussels. Therefore, the present paper is an attempt to investigate the effect of sublethal doses of Tributylin chloride (TBTCL) on acid phosphatase (AcPase) and alkaline phosphatase (AlkPase) in the chief metabolic organ, digestive gland of the estuarine edible clam, *Anadara rhombea.*

MATERIALS AND METHODS

Test Animals

Edible blood clams, *Anadara rhombea* were collected from the mudflats of Agniyar esturary near Mallipattinam, Pudukkottai District, Tamil Nadu, India and kept in glass circular pneumatic troughs (40 x 30 x 30 cm), each containing 10L of freshly collected estuarine water, for three days of acclimitization to the laboratory conditions (Temp: 26±2°C; pH: 7.6±0.3). The medium water was renewed to avoid accumulation of faeces.

Preparation of TBTCL concentrations

Tributylin chloride (TBTCL) concentrations for bioassay experiments were prepared in 60-65% acetone from 1% stock solution. Varied test concentrations ranging from 0.15 to 0.75 ppm were prepared according to method described by Laughlin *et al.* (1983).

Lc_{50} determination

For the determination of Lc_{50} concentration, five groups, each consisting of acclimatized clams (n=20) were formed. Each group was exposed to 0.15, 0.30, 0.45, 0.60 and 0.75 ppm TBTCL concentrations in a glass pneumatic trough containing 10L of estuarine water individually. Mortality of clams was recorded in each group for 96hr. Lc_{50} value was determined by the methodology of Litchifield and Wilcoxon (1949). The results were confirmed repeating the experiment three times.

Bioassay studies

Acclimatized *A. rhombea* of uniform body (shell) length (70-75 mm) were selected for bioassay studies. Four groups were formed. One group was treated without TBTCL and was considered as control. The other three groups were considered as experimental. Each group (n=10) was taken in glass pneumatic trough containing 10L of estuarine water and exposed to each of three sublethal doses Viz. 10%, 20% and 30% of Lc_{50}/96 h for 15 days. Both medium water and sublethal dose were renewed daily.

Enzyme assays

For enzyme assay studies, laboratory acclimatized clams were exposed to each of sublethal doses (1/10, 1/20 and 1/30 of Lc_{50} / 96 h) in TBTCL solution. Experiments were carried out upto 15 days. The clams were taken out, valves of shell opened immediately at the end of 5, 10 and 15 days of exposure both in control and treated groups. Digestive gland was dissected out and the tissue was homogenised in ICE-cold glass distilled water using a hand driven all glass homogenizer and 10% (W/V) homogenate was prepared. The homogenate was centriguged at 1000 rpm for 10 min and the clear supernatant was used as enzyme source. The activities of enzymes AcPase and AlkPase were assessed by using methods as given by Jafee and Badansky (1943) and Rec (1972) respectively. The enzyme activity was expressed as micromole p-nitrophenol/mg protein /hr.

Data analysis

All experiments were replicated three times, Data were subjected to various statistical analysis and the variance, standard deviation and standard error of the mean were calculated (Sokal and Rohlf, 1994). Students ‘t’ test was use to find out the significance. The levels of significance used in the present study was from $p<0.001$ to $p<0.05$.

RESULTS

Mortality studies showed that the Lc_{50} of *Anadara rhombea* for 96 h exposure was 0.37ppm for TBTCL. The minimum effective doses 10% Lc_{50} (0.037 ppm) and 20% of Lc_{50}

(0.076 ppm) and 30% of Lc_{50} (0.114 ppm) were chosen for experimental studies.

The quantitative changes of enzymes, acid phosphatase and alkaline phosphatase in the digestive gland of estuarine clam, *Anadara rhombea* both in control and sublethal concentrations of TBTCL exposed after 5, 10 and 15 days are given in Tables: 1, 2 and 3.

I. Effect of 1/10 of Lc_{50}/96 hr TBTCL toxicity on phosphatases

The activity of Acid phosphatase was 6.04 mg pi/g/h in the control for 5th day of exposure while 6.26 and 6.48 mg/pi/g/h were recorded at 10th day and 15th day of exposure in controls (Table 1).

With the exposure of 0.035 ppm, the increased enzyme activity measured were 7.13, 8.04 and 8.49 mg/pi/g/h at 5,10 and 15 days of TBTCL exposure respectively.

Alkaline phosphatase activity measured in controls were 10.87, 11.02 and 11.58 mg/pi/g/h during 5, 10 and 15 days of observation. Increased levels of Alkaline phosphatise activity were calculated as 11.84, 12.03 and 13.36 mg pi/g/h at 5, 10 and 15 days of 10% of Lc_{50}/96 h (0.038 ppm) TBTCL respectively.

II. Effect of 1/20 of Lc_{50} /96hr TBTCL toxicity on phosphatases

The acid phosphatase enzyme levels were 6.54, 6.62 and 6.71 mg/pi/g/h in controls at 5, 10 and 15 days of observation (Table 2). Acid phosphatase activity measured were 9.68, 10.18 and 11.38 mg/pi/g/h at the exposure of 0.076 ppm sublethal concentration of TBTCL during 5, 10 and 15 days of observation.

Alkaline phosphatase activities measured in controls were 12.03, 12.41 and 12.56 mg/pi/g/h at 5, 10 and 15 days of observation. Alkaline phosphatise enzyme levels were increased as 13.12, 13.88 and 15.28 mg/pi/g/hr at 5, 10 and 15 days of 0.076 sublethal exposures respectively.

III. Effect of 1/30 of Lc_{50} / 96 hr of TBTCL toxicity on phosphatises

With the exposure of 0.411 ppm, the increased acid phosphatase enzyme activity measured were 12.08, 14.22 and 15.14 mg/pi/g/h as against 7.04, 7.14 and 7.36 mg/pi/g/h in controls at 5, 10 and 15 days respectively (Table 3).

Likewise, increased Alkaline phosphatase enzyme activity levels were 15.24, 17.62 and 20.34 mg/pi/g/h when compared to 13.14, 14.32 and 15.36 as observed in controls during 5, 10 and 15 days of sublethal exposure of 0.114 ppm TBTCL.

Table 1. Alterations in phosphatase activity in the digestive gland of estuarine edible clam, *Anadara rhombea* exposed to sublethal (0.038 ppm) of TBTCL at different days.

Exposure period (days)	Treatments	Phosphatases (mg/pi/g/h)	
		Ac Pase	Alk Pase
5	Control	6.04 ± 0.16	10.87 ± 0.19
	Experimental	7.13 ± 0.24	11.84 ± 0.16
		(18.05)	(08.93)
10	Control	6.26 ± 0.18	11.02 ± 0.33
	Experimental	8.04 ± 0.41	12.03 ± 0.24
		(28.44)	(9.16)
15	Control	6.48 ± 0.24	11.58 ± 0.41
	Experimental	8.49 ± 0.19	13.36 ± 0.33
		(31.02)	(15.37)

Lc_{50} /96h = 0.38 ppm. Values are means ± S.E. of three observations. Values in parentheses denote % change over control.

Table 2. Alterations in phosphatase activity in the digestive gland of estuarine edible clam, *Anadara rhombea* exposed to sublethal (0.076 ppm) of TBTCL at different days.

Exposure period (days)	Treatments	Phosphatases (mg/pi/g/h)	
		Ac Pase	Alk Pase
5	Control	6.54 + 0.14	12.03 + 0.24
	Experimental	9.68 + 0.36	13.12 + 0.18
		(48.02)	(09.06)
10	Control	6.62 + 0.16	12.41 + 0.36
	Experimental	10.18 + 0.11	13.88 + 0.19
		(53.78)	(11.85)
15	Control	6.71 + 0.21	12.56 + 0.44
	Experimental	11.38 + 0.19	15.28 + 0.21
		(69.6)	(21.66)

Lc_{50} /96h = 0.38 ppm. Values are means ± S.E. of three observations. Values in parentheses denote % change over control.

Table 3. Alterations in phosphatase activity in the digestive gland of estuarine edible clam, *Anadara rhombea* exposed to sublethal (0.114 ppm) of TBTCL at different days.

Exposure period (days)	Treatments	Phosphatases (mg/pi/g/h)	
		Ac Pase	Alk Pase
5	Control	7.04 + 0.34	13.14 + 0.11
	Experimental	12.08 + 0.24	15.24 + 0.49
		(71.59)	(15.98)
10	Control	7.14 + 0.19	14.32 + 0.41
	Experimental	14.22 + 0.33	17.62 + 0.21
		(99.16)	(23.04)
15	Control	7.36 + 0.17	15.36 + 0.09
	Experimental	15.14 + 0.24	20.34 + 0.07
		(105.7)	(32.43)

Lc_{50} /96h = 0.38ppm. Values are means ± S.E. of three observations. Values in parentheses denote % change over control.

DISCUSSION

In general, the edible estuarine clams constitute the major sources of nutritious food for humans. In the present study, the edible clams, *A. rhombea* were subjected to different sublethal doses of an organotin compound, Tributyltin chloride (TBTCL) and alterations in Acid phosphatise enzyme activity were studied.

Acid phosphatise is a lysosomal enzyme that hydrolyses the phosphorous esters in acidic medium. It is known as inducible enzyme whose activity in animal tissue goes up when there is a toxic impact and the enzyme being to counteract. The increase in digestive gland acid phosphatase activity in intoxicated clams as observed in the present investigation may be due to the destruction of lysosomal membrane which resulted in the release of enzyme. Our results are in corroboration with the results who have reported increased acid phosphatise activities in animal tissues which has been reported to be induced by the action of varied chemicals (Joshi and Desai, 1981; Kumar and Kumar 1997; Jaroli and Sharma 2005; Abdul Naveed *et al.*, 2011). In the present study, the acid phosphatise activity of digestive gland of *A. rhombea* increased in all three sublethal doses. Such increase is also found as a function of exposure period.

Alkaline phosphatase splits various phosphorous esters at alkaline pH and its activity is related to cellular damage. As in Acid

phosphate levels increased, alkaline phosphatise enzyme activity was also increased due to three sublethal doses of TBTCL, in the digestive gland of *A.rhombea*. The increase in tissues alkaline phosphatise has been reported in the fish, *Channa punctatus* (Sherekar and Kullkarni, 1987) and in the fish, *Sarotherodon mossambica* (Kumble and Muley, 2000) and in freshwater catfish, *Clarias batrachus* (Sudish Chandra, 2013). The significant difference in phosphatases activities between the control and experimental groups of clam following the exposure of TBTCL may be considered due to the damage of digestive gland tissue with disturbed normal digestive function.

CONCLUSIONS

It may be concluded that decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hansen, *et al.*, 1992) Analysis of variance results showed that there was some significant difference ($p<0.05$) observed in the means of enzymes, ACP and ALP levels in the treated clams, with increase in the concentration and exposure duration, the entire response was mainly days dependent.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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