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Acute effect of TBTCL on protein alteration in freshwater bivalve, *Lamellidens marginalis*

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Abstract

The freshwater bivalves *L. marginalis* exposed to 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm concentration of tributyltin chloride on protein content in the tissue of gonad, gill and digestive glands up to 96 hours exposure respectively. Compare to control group there was significant change in protein contents from gonad, gill and digestive glands in tributyltin chloride exposed groups. The result showed that TBTCl induces significant depletion in protein metabolic profiles in gonad, gill and digestive glands might be due to increased proteolysis and possible utilization of the products of their degradation for metabolic purpose.

Keywords: Bivalve, *Lamellidens marginalis*, Tributyltin chloride, Protein alteration

INTRODUCTION

Water pollution is the biggest menace of urbanization, industrialization and modern agricultural practices. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as that of the environment [1]. The biochemical changes in the organs of animal exposed to heavy metals have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract toxic stress.

Tributyltin chloride is one of the organotin compounds which are most toxic groups of xenobiotics ever produced and deliberately introduced into the environment. This compound is known to be harmful to many “non-target” aquatic organisms, particularly molluscs [2]. The biochemical changes occurring in the body gives the important indication of stress [3]. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different.

Proteins are the major biochemical component, which act as source of energy for various physiological functions including reproduction [4]. Higher concentrations of toxicant in aquatic environment cause adverse effect on aquatic organism at cellular or molecular level and ultimately it leads to disorder in biochemical composition. Several workers have reported the impact of various aquatic pollutants on protein metabolism of different species. Jaiswal et al. [5] observed changes in biochemical constituents such as protein, lipid and glycogen when exposed to naphthalene to freshwater prawn, *M. kistnensis*. Machale et al. [6] reported depletion in protein, lipid, and glycogen due to cuprous oxide stress in various tissues of freshwater crab, *B. guerini.* Khan et al. [7] observed changes in levels of protein, lipid and glycogen in the muscle of freshwater crab, *B. guerini* exposed to copper sulphate. Zambare [8] studied the reflections in protein content of freshwater bivalve, *C. striatella* due to heavy metal exposure. Muley [9] observed the alteration in protein content after exposure to monocrotophos, cypermethrin and some heavy metals. Patil and Mane [10] observed the biochemical levels in different body parts of freshwater bivalve, *L. marginalis* exposed to mercury in monsoon season. Bhavani [11] observed the absorption of metals, biochemical components like Proteins, Carbohydrate and Lipids were, the decrease of proteins, carbohydrates and lipids in the body tissue of *Perna viridis,* due to metal toxicity. Satyaparameshwar [12] reported decrease in protein content of different tissues in freshwater mussel, *Lamelidens marginalis* exposed to chromium.

Knowing the importance of organic constituents in the body of organisms, above problem was selected to study the effect of tributyltin chloride on protein alteration of freshwater bivalve, *Lamellidens marginalis*.

MATERIAL AND METHODS

The freshwater bivalves, *L. marginalis* were collected from Godavari River at Paithan, 45 Km. away from Aurangabad city of Maharashtra state. Animals were brought and kept in plastic troughs and acclimatize to them in laboratory conditions for 3 to 4 days. 1ppm stock solution of tributyltin chloride was prepared in acetone [13]. For each experiment 10 animals of approximately similar size were exposed to acute concentrations as3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm for 24, 48, 72 and 96 hours respectively. After the acclimatization, healthy and medium sized bivalves were selected for experiments. The analysis of protein from gonad, gill and digestive glands of *L. marginalis* were made belonging to the control and experimental wet tissues.

Estimation of total protein

100 mg of wet tissue was homogenized in 5 ml of cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected after centrifugation at 3000 rpm for 15 minutes. The protein precipitate at the bottom of centrifuged tubes was dissolved in 10 ml 1.0 N NaOH solution. 0.1 ml of this solution of

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each powder was taken in three test tubes containing 4.0 ml. freshly prepared Lowry's C. After adding 0.5 ml. Folins- phenol reagent, the test tubes were incubated in dark at 37^{0}C for 30 minutes. The O.D. of blue colour developed was read at 530mμ filter. The blank was prepared in same way without dissolved protein precipitate. The protein content of different tissues was calculated referring to standard graph value and it was expressed in terms of mg/100 mg wet weight of the tissue.

RESULTS

The changes in biochemical composition of gonad, gills and digestive gland of freshwater bivalve, *Lamellidens marginalis* exposed to acute concentrations of organotin tri-butyltin chloride were studied along with control animals. The data was supported by various stastical analysis and the standard deviation and standard error of the mean were calculated. Student't' test was used to find out significance. The level of significance was used in the present study (P<0.1, P<0.05, P<0.01, P<0.001) [14].

The gonad of control bivalves, the protein levels were observed (8.3477 ± 0.2337). The bivalves exposed to 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm concentration of tributyltin chloride induced depletion in protein content (7.6730 ± 0.4048, 8.0828%), (7.4031 ± 0.2337, 11.3160%), (6.9982 ± 0.2337, 16.1657%) and (6.5934 ± 0.2337, 21.0154%) % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1 , Fig-1).

In the gill of control bivalves protein content were found (11.4516 ± 0.2337) and the experimental bivalves, protein depletion were recorded (9.9671 ± 0.2337, 12.9626%), (9.0225 ± 0.2337, 21.2116%), (8.0778 ± 0.4048, 29.4605%) and (7.4031 ± 0.2337, 35.3526%), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1 ,Fig-1).

The digestive gland of control bivalves, protein content were (12.7336 ± 0.2024) and in experimental bivalves the protein decreases (10.9117 ± 0.4048, 14.3070%), (9.9671 ± 0.2337, 21.7254%), (8.7526 ± 0.2337, 31.2635%) and (7.1332 ± 0.2337, 43.9808%), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1 ,Fig-1).

Table – 1: Protein content from different body parts of freshwater bivalve, *Lamellidens marginalis,* exposed to acute concentration of TBTCl.

	Tissue	Control	24h. Exp.	48h. Exp	72h. Exp	96h. Exp
1	Gonad	8.3477 ± 0.2337	7.6730 ± 0.4048 8.0828%	7.4031 ± 0.2337 11.3160%	6.9982 ± 0.2337*** 16.1657%	6.5934 ± 0.2337*** 21.0154%
2	Gill	11.4516 ± 0.2337	9.9671 ± 0.2337*** 12.9626%	9.0225 ± 0.2337** 21.2116%	8.0778 ± 0.4048** 29.4605%	7.4031 ± 0.2337* 35.3526%
3	Digestive gland	12.7336 ± 0.2024	10.9117 ± 0.4048*** 14.3070%	9.9671 ± 0.2337** 21.7254%	8.7526 ± 0.2337* 31.2635%	7.1332 ± 0.2337* 43.9808%

1. Values expressed as mg/100mg of wet wt. of tissue.
2. ± indicate S.D. of three observations.
3. P<0.1^{NS}- Non significant, P<0.05***, P<0.01**, P<0.001*.

DISCUSSION

Any stressful condition alters the biochemical composition. The change in metabolic rate leads towards the change in biochemical composition hence, the change in biochemical composition is an indicator of stress of chemical or physical nature in the surrounding which mainly affects protein contents.

There is paucity of information related to effects of heavy metals particularly on organotin compounds of aquatic invertebrate organisms. The present findings indicated decrease of protein in gonad, digestive gland and gills in *L. marginalis* exposed to acute concentration 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm concentration for 24, 48, 72 and 96 hours of tributyltin chloride. In the present study, total protein percentage decreases gradually as a period of tributyltin chloride exposure increases.

The depletion of protein content suggests an increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. They may be mobilized in to TCA cycle through amino acid metabolism system to cope up with the excess demand of energy during toxic stress conditions. The fall in protein level during pollutant exposure may be due to increased catabolism and decreased anabolism of proteins. Depletion in protein content in animal tissue after exposure to organotin pollutant and other heavy metals were reported by few researchers, [15] suggested the effect of tributyltin on pen shell *A. pectinata* and reported that the energy metabolism of pen shell is disrupted by exposure to TBT and simultaneously decrease in the level of important organic constituent and elevated lactate, pyruvate and fumarate concentrations. [16] reported the reduction in total protein content in the ovary, hepatopancreas, gill and muscles of the *Macrobrachium kistnensis* exposed to different concentrations of tributyltin chloride, and it was possibly due to stress condition caused by toxicity of tributyltin chloride on protein metabolism similar results were reported by [17,18,19].

The results of total protein contents in all tissues clearly indicated that digestive gland was the most affected organ followed by gonad and gill. The higher depletion of protein in the digestive gland might be due to high metabolic potency and efficiency of the gland under pollutant stress .The digestive gland may be the site of action of pollutant in the body of bivalve or digestive gland seems to be the main site of degradation and detoxification of toxicants and hence has the largest demand of energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein in digestive gland provided better indication of the extent of toxicity. [20, 21] and [22] supported the most alteration of protein in digestive gland under stress. Many researchers documented similar results. [23] reported depletion of protein content in various body tissues of snails, *B. bengalensis* after malathion treatment. [24] recorded significant decline in protein content in fresh water bivalve, *L. marginalis* exposed to flodit and metacid. Similar observations were made by number of workers in mollusc [25,26, 21] on exposure to acute concentrations of copper observed a decline in protein level in the bivalve, *S. scripta* after prolonged exposure. Thus the results obtained in the present study indicate severe disturbances in the protein metabolism of the bivalve,

L. marginalis exposed to tributyltin chloride. [27] showed the depletion of the protein contents in different tissues such as gonad, gill and hepatopancreas of bivalve, after exposure to $HgCl_2$ and $CuSo_4$.

There was scanty information available on the toxicity of organotin compounds on the biochemical constituents in freshwater mollusk, particularly bivalve. and hence the above discussion and all the available literature, we can conclude that the TBTCl is very toxic to the freshwater bivalve, *Lamellidens marginalis.* The release of organotin compounds in aquatic environment especially in freshwater ecosystem might be controlled.

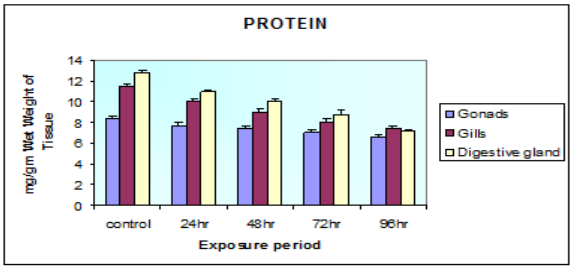

Fig. 1: Effect of 24h, 48h, 72h and 96h LC_{50} of TBTCl on protein constituents in Gonads, Gills and Digestive gland of freshwater bivalve, *Lamellidens marginalis*.

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