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ACUTE AND CHRONIC TRIBUTYLTIN INDUCED ALTERATIONS IN DIGESTIVE ENZYMES OF THE PRAWN, *CARIDINA RAJADHARI*

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Effect of both lethal and sublethal concentrations of TBTO on digestive amylase, protease and lipase in hepatopancreas, stomach and midgut was studied in the prawn, *Caridina rajadhari*. Both the acute and chronic exposures exhibit inhibition of all the enzyme activity in all the tissues. However maximum depletion of enzyme activity was observed in prolonged exposure period and also tissue specific impact was noticed. The observed effects were due to the severe damage to the secretory cells of digestive organs or by neurotoxic effect caused by the TBTO poisoning.

INTRODUCTION

The mechanism of enzymes is itself one of the most fascinating fields of scientific investigation being pursued at the present time with relation to the enviornmental changes. The poisoning of any single enzyme involved in the main metabolic chain will render the whole chain inoperative and will have a profound or even fatal effects upon the organism. The increasing realization of these facts have changed the environmentalists towards enzymatic studies from the usual toxicity tests, which is now being recognized by many pharmacologists and toxicologists as of fundamental importance to their subjects.

The usefulness of organisms in environmental pollution monitoring or surveillance programmed has been well established (Topping, 1983). A particular significant attribution of lethal and sublethal physiological response is that it is amenable to both laboratory and field measurements unlike traditional toxicant testing.

The enzyme profiles may be altered by changes in metabolism, cellular integrity, membrance permeability, exogenous stressers or may be combination of these. The alterations in the enzyme pattern and distribution of individual enzymes are to be observed well before morphological changes became evidenced microscopically. In the present study, tributyltin oxide, an organotin antifouling compound was used, which is a major pollutant and is used as a biocide in paper, paint and agriculture industry extensively. The digestive enzymes are the key enzymes for the survillance or organism and are mainly useful in the digestive physiology. Hence the present study is carried out to work out the impact of acute and chronic Tributyltin oxide impact on the digestive enzymes of the prawn, *Caridina rajadhari*.

MATERIAL AND METHODS

The prawns, *C. rajadhari* were procured form Kham river near Aurangabad and were acclimated to the laboratory conditions (12L : 12D, 27 ± 2^0C, Do_2, 5.95 ml O_2/1, pH 7.2 ± 0.3). To study the impact of tributyltin oxide on digestive enzymes, adult, healthy, intermolt (stage C) female of same size (26 ± 2mm) were selected from the

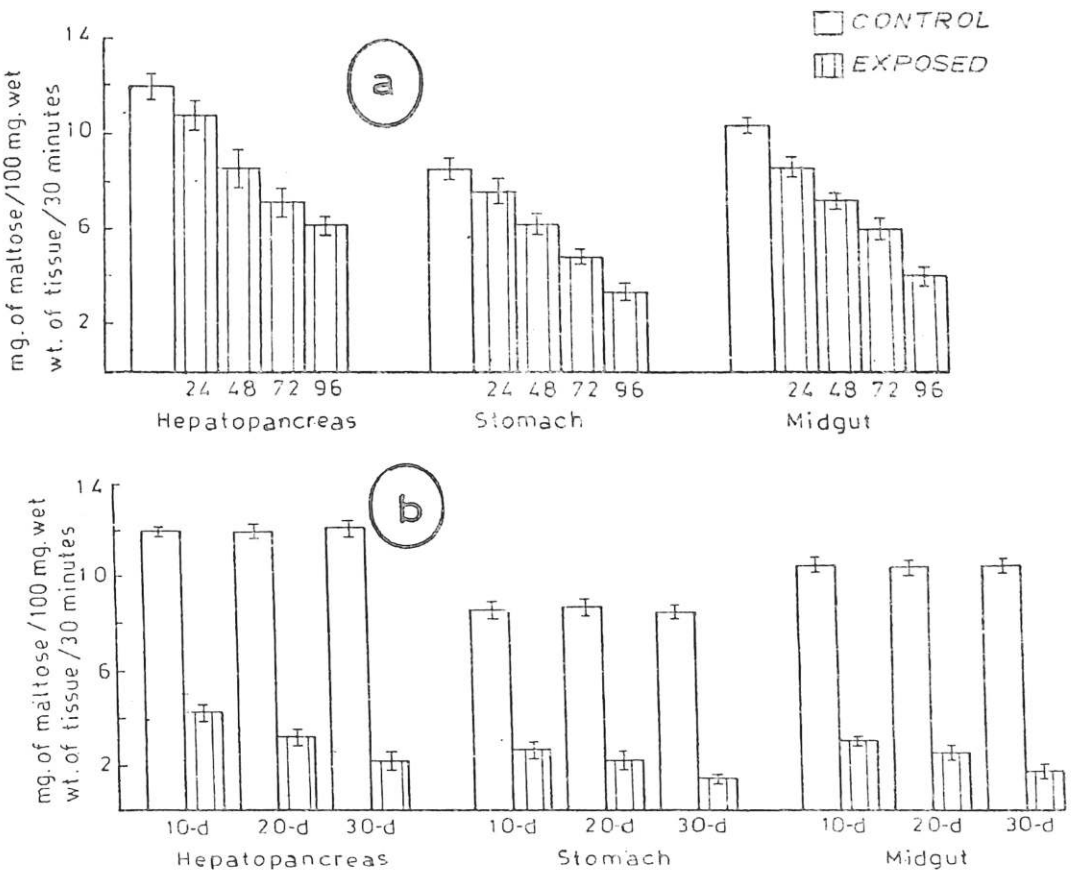

Fig. 1 : Profiles of amylase activity in hepatopancreas, stomach and midgut of the prawn, *C. rajadhari* exposed to (a) lethal and (b) sublethal concentrations of tributyltin oxide (Mean ± SD; n=3).

laboratory acclimatized prawns. Sufficient number of animals were exposed to 0.102, 0.097, 0.091, and 0.084 ppm TBTO (LC_{50} values of 24, 48, 72, and 96 hrs) for acute and for chornic studies 1/10 of 48 hrs. LC_{50} (0.0097ppm) were used. Simultaneous control prawns also maintained upto the completion of experiment and both control and experimental animals were fed with green algae and wheat flour twice in a week. At the end of exposure period all the animals were sacrificed and tissues like hepatopancreas, stomach, and midgut were isolated and kept in ice bath separately. The tissues were thoroughly cleared with ice cold distilled water and were homogenised in a glass homogeniser and 1% aqueous extract was prepared by centrifuging at 3000 rpm for 15 minutes and the supernatants were used for the quantitative estimations. Amylase activity was determined by the method described by Neolting & Bernfeld (1948) as modified by Dhage & Mohamad (1977). Values are expressed in mg of maltose/100 mg of wet weight of tissue/30 minutes. Lipase activity was determined by the method of Sinha (1975) and is expressed as ml of 0.1N sodium hydroxide over control. Proteolytic activity was determined by Sorenson's method as adopted by Prosser & Van Weel (1968). The amount of amino acids liberated in terms of ml. of 0.1N potassium hydroxide was taken as an index of the enzyme activity.

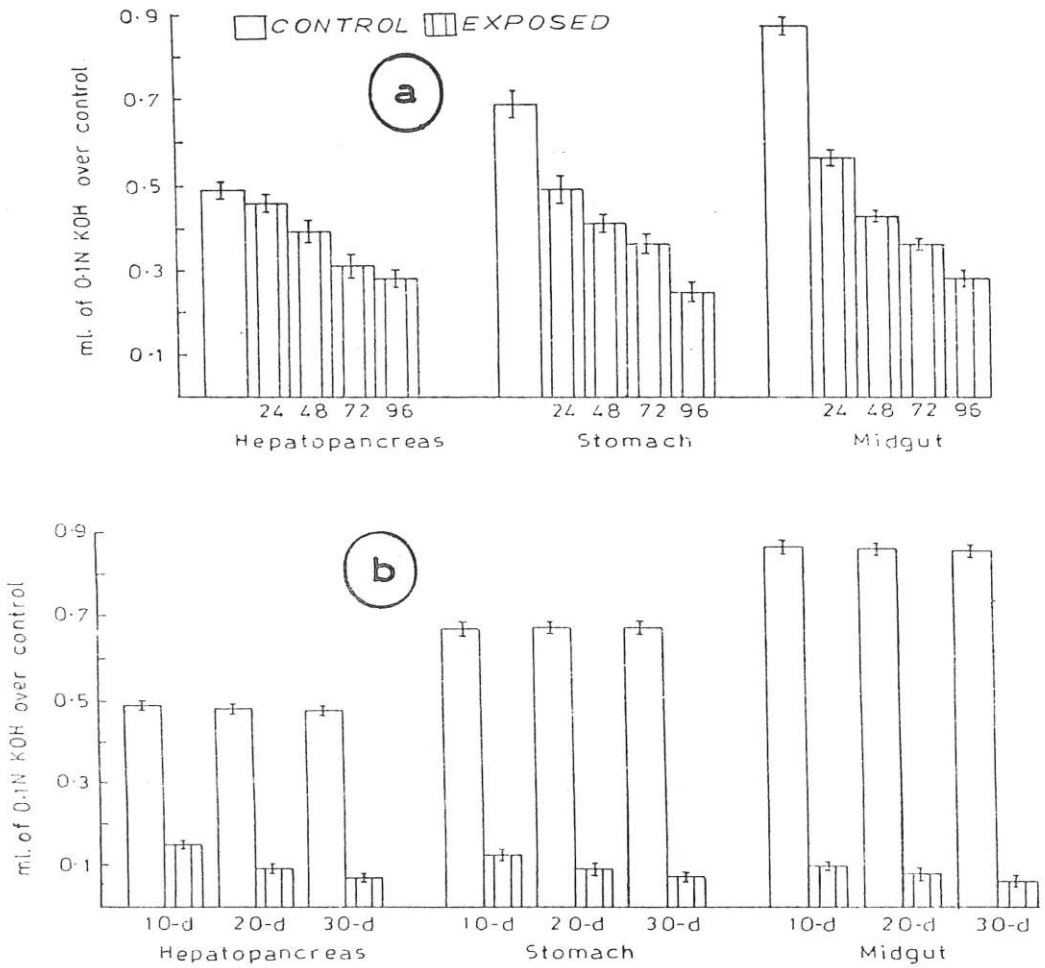

Fig. 2 : Profiles of protease activity in hepatopancreas, stomach and midgut of the prawn, *C. rajadhari* exposed to (a) lethal and (b) sublethal concentrations of tributyltin oxide (Mean ± SD; n=3).

RESULTS AND DISCUSSION

Enzymatic profiles of some digestive enzymes like amylase, lipase and protease in the tissue of hepatopancreas, stomach and midgut are shown in Figs. 1 to 3. Impact of tributyltin oxide showed an overall retardation of enzyme activity of hepatopancreas, stomach and midgut to both lethal and sublethal exposures in the prawn, *C. rajadhari*. Highly significant ($P < 0.001$) inhibition of enzyme activity was observed in all the above said tissues during chronic exposure. However, tissue specificity to TBTO was noticed.

In the current study tissue specific alterations of digestive enzymes like amylase, lipase, and protease activity were noticed in hepatopancreas, midgut and stomach, when exposed to acute and chronic periods. Prolonged exposure to sublethal exposure showed

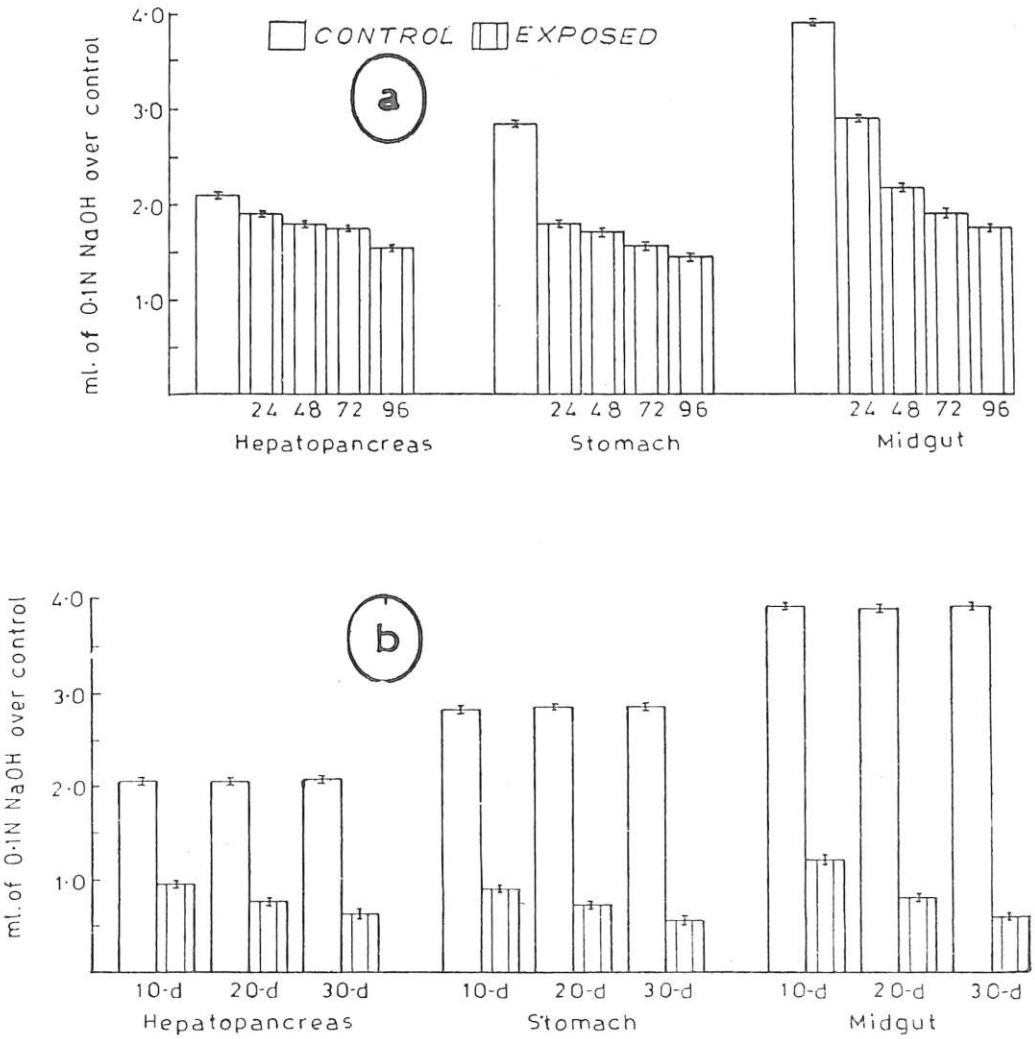

Fig. 3 : Profiles of lipase activity in hepatopancreas, stomach and midgut of the prawn, *C. rajadhari* exposed to (a) lethal and (b) sublethal concentrations of tributyltin oxide. (Mean ± SD; n=3).

maximum inhibition of digestive enzyme activity in all the tissues. These results supported by findings of Maqdom (1986) who reported greater inhibition of amylase, protease and lipase activity in hepatopancreas, stomach and intestine of the crab, *Barytelphusa guerini* exposed to DDT and sevin. Maximum depletion of amylase, protease and lipase activity by xylene stress in *M. lamerrii* was reported by Jaiswal (1989).

Much information is accumulated indicating that increase or decrease in serum and tissue enzyme activity are directly related to the cellular damage. The fibrillar cells manufactures the digestive enzymes in Crustacea (Well, 1970). Yamamoto (1960) demonstrated that the decreased enzyme activity in *Procambarus clarkii* might be due to the degeneration of hepatopancreatic cells. Indira (1988) reported that prolonged exposure of *C. weberi* to tributyltin oxide causes severe damage to hepatopancreas

showing vacuolization and reduction of globular mass in the secretory cells of hepatopancreas. By considering the above reported evidence it was suggested that decrease in the enzymatic activities of hepatopancreas, stomach and midgut might be due to the irreversible damage to the secretory cells of the tissues caused by TBTO impact in the prawn, *C. rajadhari.*

The control of digestive secretion by neurohormones have examined very extensively (Fingerman *et al.*, 1967; Nagabhushanam & Diwan, 1974; Kleinholz, 1976; Fleischer, 1981) in Crustacea. Recently, Nilawar (1989) reported that eyestalks of *Macrobrachium kistnensis* contain the inhibitory factors whereas brain and thoracic ganglion contain acceleratory factors of digestion. It is well known that TBTO is neurotoxic (Watanabe, 1980) to mammals.Cytological evidences shown by Sarojini *et al.* (1990) states that TBTO poisoning cause alterations of neurosecretory materials in eyestalk of the prawn, *C. rajadhari.* Hence it was thought that TBTO by its neurotoxic action stimulates the inhibitory factors release leading to the retardation of digestive enzymes in the tissues of the prawn *C. rajadhari.*

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