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Effect of Endosulfan 35 EC on ATPases in the Tissues of a Freshwater Field Crab *Barytelphusa guerini*

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Pesticides are synthetic organic chemicals introduced intentionally in order to augment the agricultural productivity. But the injudicious and inadvertent use of these chemicals during the last three decades resulted in unprecedented chemical pollution, posing a threat to many nontarget organisms in aquatic ecosystems. Organochlorine insecticides are quite persistent and consequently undergo biomagnification and accumulate in the tissues (Wilber 1971; Grav and Peterle 1979). Endosulfan (1,4, 5, 6,7,7-hexachloro 8, 9, 10- Try-norbonn 5,2,3-enylene dimethyl sulphite) is a broad - spectrum organochlorine insecticide, widely used and is known for its potential for insect knock-down capacity. The deleterious effects of endosulfan on fish models have been well documented (Davis and Wedmeyer 1971; Yap et al. 1981). However, information on freshwater crustaceans, particularly on freshwater field crabs, that inhabit paddy fields, is very scanty. Barytelphusa guerini, a freshwater field crab of high edible importance to rural populations, has received little attention with regard to endosulfan toxicity.

ATPases are the enzymes concerned with the immediate release of energy are useful for all types of physiological activities. ATPase activity can be taken as meaningful index of cellular activity and forms a useful toxicological tool. Hence the present investigation is aimed to understand the in vivo effect of an emulsifiable concentrate (35 EC) of endosulfan on ATPase system in the tissues of the field crab, Barytelphusa guerini.

MATERIALS AND METHODS

Healthy, uniform-sized (25.00±0.5) male crabs Barytelphusa guerini collected from and around

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Hyderabad were acclimated to laboratory conditions for a period of 15d. The animals were fed fish meat ad libitum. A density of 10 crabs per 8L of water was used with 10 individuals in each test container. The physico-chemical characteristics of the test water were as follows: pH 7.2-7.4; dissolved oxygen 7.8-8.0 mg/L; salinity 0.190 g/L; chlorinity 0.110 g/L; alkalinity 102 mg/L hardness of water 112 mg/L and carbon dioxide 2.08 mg/L. Endosulfan (Hoechest India) is a brownish crystalline solid, and stock solution was prepared in acetone and mixed in water in required dilutions. To determine the LC_{50} value, the crabs were exposed to six serial concentrations of endosulfan. The bioassay experiment of each concentration was repeated six times with parallel controls, and mortality was noted in each concentration at the end of 96 hr. The LC_{50} value (17.78 mg/L for 96hr was determined according to Finney (1964).

The crabs were divided into two groups of 24 each. Group I served as control and group II were subsequently exposed to a sublethal concentration of endosulfan (1/3 of LC_{50} i.e. 6 mg/L for a period of 4d. The toxicant water and control water were renewed every 24 hours after feeding. The crabs were starved a day prior to experimentation to avoid metabolic differences, if any, due to differential feeding and food reserves. Six crabs each from experimental and control groups were sacrificed every 24 hr over a period of 4 days. The tissues, chelate leg muscle, hepatopancreas, heart, gills and thoracic ganglion were isolated from both control and toxicant-treated animals and were immediately transferred to a deep-freezer for enzyme assays, i.e., ATPases.

ATPase (Adenosine triphosphatase: ATPase phosphorylase E.C. 3.6.1.3) activity was assayed by the method of Kaplay (1978). The tissues were homogenized in 0.25M ice-cold sucrose solution containing 1mM EDTA and 30mM histidine (pH 7.4). The reaction mixture of the enzyme assay in a volume of 1 mL contained: 3mM ATP, 3mM $MgCl_2$, 140 mM NaCl, 14mM KCl, 20mM Tris-HCl buffer (pH 7.4) and 0.2ml of ouabain (for Mg^{++} ATPases). The reaction was initiated by adding ATP and allowed for 10 min at 37°C. The reaction was stopped by adding 1mL of 10% TCA. The inorganic phosphate liberated was measured by the method of Taussaky and Shorr (1953). The difference in the activity of enzyme in the absence and presence of ouabain was taken as Na^+ -K^+ ATPase activity. Total activity in the presence of ouabain was Mg^{++} ATPase activity. The protein content in the enzyme source was estimated according to Lowry et al.(1951), using bovine serum albumin (Sigma) as a

standard. Enzyme activity was expressed as μmoles of inorganic phosphate formed/mg protein/hr. Students 't' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

ATPase activities measured in the present investigation to assess the toxicological effect of a sublethal concentration of endosulfan on the energy metabolism of tissues of crab showed significant alterations in enzyme activities, reflecting sensitivity of crabs to endosulfan toxicity. ATPases, both Na^+ -K^+ ATPase and Mg^{++} - ATPase, exhibited a progressive inhibition. Na^+ - K^+ ATPase is a key enzyme in cellular water balance and in osmoregulation in whole animal. It plays an important role in aquatic organisms. There was almost an inhibition in the activity of Na^+ -K^+ ATPase. The inhibition in the activity was, however, tissue-specific (Table 1). However, a negligible enhancement in the enzyme activity in gills and muscle on 1st day of exposure suggests the stimulation of anaerobic metabolism at the expense of aerobic processes. There was a subsequent decrease in the Na^+ -K^+ ATPase activity in all tissues during rest of the exposure. Several classes of pesticides were known to inhibit ATPases activities (Koch 1969; 1970; Desaiah et al. 1972; Cutkomp et al 1976). Na^+ -K^+ ATPases are highly susceptible to organochlorine pesticides (Davis and Wedmeyer 1971). Several opinions were expressed for such an inhibition in the ATPase activity in the cellular system. Inhibition indicates an overall disruption in the energy metabolism. Na^+ -K^+ ATPase enzyme is associated with lipoprotein in the form of a complex (Nakao et al. 1974). Kinter et al. (1972) speculated that lipophilic pesticides exert biologic effect on ATPase system which would induce partitioning in the enzyme complex. The lipophilic partitioning of ATPase enzyme by endosulfan may produce allosteric change resulting in decreased ATPase activity. Similar conclusion was drawn by La Rocca and Carlson (1979) for the inhibition of ATPase by PCB. Endosulfan toxicosis in fish models exhibited symptoms of hyperexcitabiliity, tremors, and convulsions (Singh and Srivastava 1981). These symptoms can be caused by the inhibition of Na^+ - K^+ ATPases.

Oligomycin sensitive (mitochondrial) Mg^{++} -ATPase in the present study exhibited a progressive inhibition when exposed to endosulfan. Similar observations were made in a teleost fish Channa gachua when exposed to endosulfan (Dalela et al. 1979). Mg^{++} ATPases are associated with synthesis of ATP in

Table 1. Na^+ -K^+ ATPase activity in the tissues of Barytelphusa guerini during endosulfan toxicity

Tissues		24hr	48hr	72hr	96hr
Gills	Control	1.566 ± 0.032	1.588 ± 0.140	1.564 ± 0.026	1.592 ± 0.092
	Experimental	1.648 ± 0.086	1.556 ± 0.034	1.334 ± 0.112***	1.246 ± 0.098***
	% Difference	+ 5.23	- 2.01	-14.70	-21.73
Muscle	Control	2.274 ± 0.052	2.264 ± 0.068	2.288 ± 0.024	2.256 ± 0.126
	Experimental	2.368 ± 0.120	2.122 ± 0.082*	1.992 ± 0.018***	1.818 ± 0.078***
	% Difference	+ 4.13	- 6.27	-12.93	-19.41
Hapato-pancreas	Control	1.658 ± 0.076	1.642 ± 0.048	1.644 ± 0.086	1.658 ± 0.084
	Experimental	1.634 ± 0.022	1.572 ± 0.032	1.452 ± 0.064**	1.392 ± 0.068***
	% Difference	- 1.44	- 4.27	-11.676	-16.04
Heart	Control	2.394 ± 0.116	2.326 ± 0.112	2.432 ± 0.048	2.424 ± 0.142
	Experimental	2.280 ± 0.042	2.144 ± 0.078**	2.122 ± 0.062***	2.152 ± 0.038***
	% Difference	- 4.76	- 7.82	-12.74	-11.22
Thoracic-ganglion	Control	2.214 ± 0.038	2.242 ± 0.022	2.214 ± 0.084	2.258 ± 0.042
	Experimental	2.138 ± 0.046	2.144 ± 0.014**	1.964 ± 0.106***	2.008 ± 0.086***
	% Difference	- 3.43	- 4.37	-11.29	-12.232

Value expressed as umoles on Pi/mg protein/hr. Each value is a mean of six observations ± S.D. Experimental value significantly different from control with statistical significance at *P<0.01; **<P 0.005. *** P<0.001.

Table 2. Mg^{+2} ATPase activity in the tissues of Barytelphusa guerini during endosulfan toxicity toxicity.

	Tissues	24hr	48hr	72hr	96hr
Gills	Control	1.602 ± 0.058	1.622 ± 0.082	1.648 ± 0.062	1.634 ± 0.028
	Experimental	1.548 ± 0.072	1.524 ± 0.064	1.462 ± 0.108**	1.308 ± 0.084***
	% Difference	- 3.37	- 6.04	-11.28	-19.95
Muscle	Control	2.908 ± 0.078	2.956 ± 0.036	2.942 ± 0.102	2.968 ± 0.112
	Experimental	2.822 ± 0.056	2.788 ± 0.092***	2.656 ± 0.054***	2.512 ± 0.054***
	% Difference	- 2.95	- 5.68	- 9.72	-15.36
Hapato-pancreas	Control	1.112 ± 0.064	1.154 ± 0.088	1.134 ± 0.122	1.148 ± 0.146
	Experimental	1.078 ± 0.098	1.056 ± 0.104	1.012 ± 0.074***	0.998 ± 0.058***
	% Difference	- 3.05	- 8.49	-10.75	-13.06
Heart	Control	2.602 ± 0.116	2.634 ± 0.084	2.656 ± 0.068	2.664 ± 0.142
	Experimental	2.558 ± 0.098	2.522 ± 0.052*	2.488 ± 0.104*	2.352 ± 0.064***
	% Difference	- 1.69	- 4.25	- 6.32	-11.04
Thoracic-ganglion	Control	1.326 ± 0.158	1.358 ± 0.086	1.344 ± 0.046	1.372 ± 0.034
	Experimental	1.288 ± 0.084	1.252 ± 0.062	1.228 ± 0.054**	1.204 ± 0.078**
	% Difference	- 2.862	- 7.80	- 8.63	-12.242

Values expressed as μmoles of Pi/mg protein/hr. Each value is a mean of six observations ± SD. Experimental values significantly different from control with statistical significance at * P<0.001; ** P< 0.005; ***P < 0.001.

mitochondria through oxidative phosphorylation. Evidences also indicate implicating OS (mitochondrial) Mg^{++} ATPases in the terminal step of oxidative phosphorylation result in the formation of ATP. Mitochondrial disorganization resulted during pesticidal toxicosis (Pardini et al. 1980) may cause inhibition of Mg^{++} -ATPase in this study. It may be concluded that endosulfan induced inhibition in ATPases not only disrupt various aspects of cell function and also energy production necessary for maintenance of physiological processes. Further analysis of adenine nucleotides which are reliable estimates of adenylate energy charge (AEC) will provide needed insight into metabolic energy state of cells.

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