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Effect of Endosulfan 35 EC on Some Biochemical Changes in the Tissues and Haemolymph of a Freshwater Field Crab, *Barytelphusa guerini*

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Endosulfan is a broad-spectrum organochlorine insecticide, widely used and is known for its potency against insects. The deleterious effects of endosulfan on mammalian models have been well documented (Garg et al 1980). Various aspects of toxic effects of endosulfan pollution on fishes have been extensively reported (Rao et al 1981 ; Mathiessen and Roberts 1982). However, information on freshwater crustaceans, particularly on freshwater field crabs that inhabit paddy fields, is very scanty (Nagender Reddy et al 1991). Barytelphusa guerini, is a fresh water field crab and is of importance as food to rural populations. This crab forms one of the important components of the paddy field ecosystem and is constantly exposed to endosulfan during the commercial insecticide operations in paddy fields. Hence the present investigation is aimed to understand the effect of an emulsifiable concentrate, 35 EC of Endosulfan on some biochemical aspects related to oxidative metabolism in the tissues of a freshwater field crab, Barytelphusa guerini, in a short-term exposure.

MATERIALS AND METHODS

Healthy, male crabs Barytelphusa guerini weighing 24±0.5 g collected around Hyderabad from an unpolluted river belt were acclimated to laboratory conditions in 50-L plastic containers at room temperature 27±0.5° C for a period of 15 d. The animals were fed fish meat ad libitum. A density of 10 crabs per 8 L of tap water was used with 10 individuals in each test container. The physicochemical characteristics of the test water were as follows : pH 7.2 - 7.4; dissolved oxygen 7.8 - 8.0 mg/L; salinity 0.19%; chlorinity 0.110 g/L; alkalinity 102 mg/L; hardness of water 112 mg/L (as Ca CO3) and carbon dioxide 2.08 mg/L. Endosulfan commercial grade, 35%. (V/W 35 EC, Hoechest) 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

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in each concentration at the end of 96 hr. The LC50 value (17.78 mg/L) for 96 hr was calculated by using probit curve 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 4 d. The toxicant water and control water were renewed every 24 hr 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 d. The tissues, chelate leg muscle, hepatopancreas, heart, gills, and thoracic ganglion were isolated from both control and toxicant-treated animals and were immediately transfered to a deep-freezer at -10°C for biochemical assays.

Lactate of the tissues was estimated according to Barker and Summerson (1941). One mL supernatant of 5% trichloroacetic acid tissue extract was processed with copper sulphate and calcium hydroxide. The resultant extract was treated with 0.1 mL of p-hydroxybiphenyl reagent and color development was read at 540 nm in spekol against reagant blank. Values were expressed as mg lactate/gm wet weight of the tissue. For haemolymph lactate, 2 mL of haemolymph was taken and 2 mL of 5% TCA added and centrifuged at 3000 rpm for 15 min and from this 2 mL of clear supernatant was processed as done for tissues. Values were expressed as mg lactate per 100 mL of haemolymph. Pyruvate was estimated by the method of Friedmann and Hanghen (1942). 10% homogenates of tissues were prepared in 10% TCA and then centrifuged at 2500 rpm for 15 min. To 1 mL of TCA filtrate 1 mL of 2,4-Dinitrophenyl hydrazine (0.001 N HCL) was added and tubes were kept for incubation at room temperature, and color was extracted with 5 mL of 4N NaOH. After 10 min. the color was read at 545 nm in spekol against reagant blank. Values were expressed as mg of sodium pyruvate/gm weight of the tissue. For haemolymph pyruvate 2 mL of haemolymph was taken and to this 2 mL of 5% TCA was added and centrifuged at 3000 rpm. From this 2 mL of clear supernatant was taken and the procedure as for tissues was followed. Values were expressed as mg pyruvate/100 mL of haemolymph. Succinate dehydrogenase [Succinate (Acceptor) Oxidoreductase,E.C. 1.3.99.1) and lactate dehydrogenase (L-Lactate NAD Oxidoreductase, E.C. 1.1.1.27) enzymes were assayed by the modified method of Nachlas et al (1960). The tissues were homogenized in 0.25 M ice-cold sucrose solution and centrifuged at 2500 rpm for 15 min. The clear supernatant was used for enzyme assay. The reaction mixture contained in 2 mL volume of 100 µmoles (0.5 mL) of phosphate buffer (pH 7.4), 2 µmoles of Ido-phenolnitrophenol tetrazolium (INT), 100 µmoles (0.5 mL) of sodium succinate (pH 7.4) or

sodium lactate (pH 7.4) and 0.5 mL of homogenate. For LDH 0.1 µmole of NAD also was added. The reaction mixture was incubated for 30 min at 37°C and the reaction was stopped with 5 mL of glacial acetic acid and the color was extracted by adding 5 mL of toluene. After keeping overnight in the refrigerator, the color of extracted formazan was measured using reagent blank in spekol at 495 nm. The enzyme activity as expressed as µmoles of formazan/mg protein/hour. The protein content in the enzyme source was estimated according to Lowry et al (1951) using bovine serum albumin (Sigma Chemical Co., St.Louis,USA) as a standard. Students `t' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

The results of the present investigation (Tables 1,2 and 3), clearly demonstrate an altered oxidative metabolism characterized by tissue-specific and time-dependent alterations in the metabolites and associated enzyme activities. Pyruvate levels in the tissues, exhibited a progressive depletion throughout the exposure. Lactate levels in the tissues exhibited an exactly opposite relationship to that of pyruvate levels. Lactate levels progressively accumulated in all the tissues and haemolymph throughout the exposure. Lactate and pyruvate levels form meaningful biochemical indices of oxygen debt (Huckabee 1958) and also indicate the possibility of gluconeogenesis (Story and Freedland 1978). Pesticide toxicity induces motor activity and physiological disturbance and the onset of these two factors in animal lead to the accumulation of lactic acid. Organochlorine pesticide induced lactate accumulation was reported in various animal models (Bhatia et al 1973; Sastry and Siddiqui 1983). The results of the present investigation, in the light of the above cited literature, indicate the crabs' dependency on the anaerobic metabolism during endosulfan toxicosis. Loss in the pyruvate levels throughout the exposure indicate its possible conversion to lactate under anoxic conditions and also partial oxidation of pyruvate with available oxygen even in hypoxic state. However, inhibition of pyruvate dehydrogenase (PDH) in the tissues of Channa punctatus exposed to endosulfan (Sastry and Siddiqui 1983), rules out the possible depletion of pyruvate through direct oxidation. Pyruvate is considered to be one of the links between carbohydrate and protein metabolism. Reduction in the pyruvate levels due to its conversion to amino acid (alanine) through transamination may also be the causative factor for the depletion in pyruvate levels in the tissues of the crab in the present study. The anoxic conditions in the tissues favor the conversion of pyruvate to lactate and this reaction is reversibly catalyzed by lactate dehydrogenase.

Table 1. Pyruvate levels in the tissues of Barytelphusa guerini exposed to a sublethal concentration (6 mg/L) of endosulfan. No. of specimens = 6.

Tissues	24 hr			48 hr			72 hr			96 hr		
	Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D
Gills	0.154 ±0.026	0.148 ±0.012	-3.89	0.137 ±0.018	0.128 ±0.004	-6.56	0.142 ±0.002	0.122* ±0.008	-14.08	0.150 ±0.008	0.124[b] ±0.014	-17.33
Muscle	0.152 ±0.024	0.144 ±0.002	-5.26	0.149 ±0.012	0.138[a] ±0.008	-7.38	0.136 ±0.003	0.018[b] ±0.006	-13.23	0.142 ±0.008	0.112[b] ±0.016	-21.12
Hepato-pancreas	0.280 ±0.016	0.258* ±0.006	-7.85	0.277 ±0.016	0.252[a] ±0.002	-9.02	0.269 ±0.006	0.238[a] ±0.018	-11.52	0.278 ±0.008	0.212[b] ±0.014	-23.74
Heart	0.242 ±0.016	0.228 ±0.012	-5.78	0.248 ±0.002	0.218[b] ±0.014	-12.09	0.233 ±0.006	0.212* ±0.014	-9.01	0.258 ±0.008	0.206[b] ±0.004	-20.15
Thoracic ganglion	0.186 ±0.017	0.172 ±0.008	-7.52	0.192 ±0.008	0.176[a] ±0.005	-8.33	0.170 ±0.004	0.148* ±0.016	-12.94	0.184 ±0.008	0.136[b] ±0.018	-26.08
Haemol-ymph	3.58 ±0.122	3.42 ±0.076	-4.46	3.69 ±0.046	3.38[b] ±0.118	-8.40	3.28 ±0.082	2.92 ±0.166	-11.28	3.66 ±0.058	3.08[b] ±0.132	-15.84

Values for pyruvate expressed as mg pyruvate per gm wet weight of the tissue. Each value is a mean of 6 observations ± S.D. Experimental values significantly different from controls with statistical significance at *P < 0.01; [a]P < 0.005; [b]P < 0.001, and %D = percent difference

Table 2. Lactate levels in the tissues of Barytelphusa guerini exposed to a sublethal concentration (6 mg/L) of endosulfan. No. of specimens = 6.

Tissues	24 hr			48 hr			72 hr			96 hr		
	Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D
Gills	0.317 ±0.026	0.324 ±0.018	+2.20	0.332 ±0.021	0.366 ±0.032	+10.24	0.312 ±0.018	0.358* ±0.026	-14.74	0.305 ±0.042	0.362* ±0.028	+18.68
Muscle	0.785 ±0.048	0.812 ±0.026	+3.43	0.798 ±0.035	0.844[a] ±0.078	+5.76	0.782 ±0.023	0.856* ±0.048	+9.46	0.768 ±0.057	0.902* ±0.084	+17.44
Hepato-pancreas	0.452 ±0.029	0.470 ±0.052	+3.98	0.478 ±0.052	0.509 ±0.012	+6.48	0.462 ±0.026	0.504 ±0.022	+9.09	0.428 ±0.028	0.492[a] ±0.026	+14.95
Heart	0.810 ±0.053	0.834 ±0.042	+2.96	0.796 ±0.032	0.852[a] ±0.018	+7.439	0.814 ±0.028	0.892[a] ±0.034	+9.58	0.789 ±0.047	0.912[a] ±0.028	+15.58
Thoracic ganglion	0.582 ±0.062	0.618 ±0.088	+6.18	0.560 ±0.027	0.625[a] ±0.034	+11.42	0.525 ±0.026	0.160* ±0.062	+14.66	0.539 ±0.019	0.626[b] ±0.028	+16.14
Haemol-ymph	0.372 ±0.022	0.384 ±0.018	+3.22	0.348 ±0.012	0.362 ±0.026	+9.77	0.352 ±0.020	0.396* ±0.018	+12.50	0.346 ±0.026	0.412[b] ±0.014	+19.07

Values for lactate expressed as mg lactate per gm wet weight of the tissue. Each value is a mean of 6 observations ± S.D. Experimental values significantly different from controls with statistical significance at *P < 0.01; [a]P < 0.005; [b]P < 0.001, and %D = percent difference

Table 3. Lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activites in the tissues of Barytelphusa guerini exposed to a sublethal concentration (6 mg/L) of endosulfan. No. of specimens = 6.

Tissues		24 hr			48 hr			72 hr			96 hr		
		Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D	Control	Exp.	%D
Gills	LDH	1.762 ±0.104	1.698 ±0.122	-9.30	1.824 ±0.219	0.532[b] ±0.181	-16.00	1.852 ±0.133	1.485[b] ±0.098	-19.81	1.847 ±0.186	0.216[b] ±0.164	-34.21
	SDH	0.938 ±0.022	0.986[a] ±0.014	+5.11	0.890 ±0.011	0.832 ±0.013	-6.29	0.897 ±0.014	0.808[b] ±0.017	-9.92	0.924 ±0.018	0.742[b] ±0.006	-19.69
Muscle	LDH	3.396 ±0.246	3.109* ±0.152	-8.46	3.471 ±0.152	2.832[a] ±0.091	-18.40	3.246 ±0.191	2.378[b] ±0.218	26.74	3.416 ±0.203	2.382[b] ±0.178	-30.24
	SDH	0.378 ±0.018	0.422[a] ±0.088	+11.64	0.394 ±0.016	0.328 ±0.016	-16.75	0.383 ±0.022	0.239[b] ±0.016	-37.59	0.412 ±0.019	0.146 ±0.016	-64.32
Hepato-pancreas	LDH	0.852 ±0.048	0.748* ±0.082	-12.20	0.811 ±0.066	0.656[a] ±0.078	-18.11	0.793 ±0.092	0.612[a] ±0.084	-22.82	0.826 ±0.069	0.498[b] ±0.052	-39.70
	SDH	0.242 ±0.019	0.286[a] ±0.012	+18.18	0.261 ±0.021	0.228* ±0.016	-12.64	0.268 ±0.027	0.196[b] ±0.024	-26.86	0.278 ±0.017	0.170[b] ±0.016	-38.84
Heart	LDH	2.804 ±0.150	2.712 ±0.088	-3.28	2.752 ±0.096	2.508 ±0.148	-6.68	2.766 ±0.078	2.398[b] ±0.122	-13.30	2.632 ±0.149	2.366[b] ±0.072	-10.10
	SDH	0.784 ±0.026	0.808 ±0.008	+3.06	0.788 ±0.018	0.720 ±0.012	-7.19	0.794 ±0.024	0.702[b] ±0.018	-11.58	0.788 ±0.014	0.688[b] ±0.012	-13.45
Thoracic ganglion	LDH	1.241 ±0.033	0.156[b] ±0.076	-6.84	1.136 ±0.045	1.024[b] ±0.052	-9.86	1.232 ±0.057	0.986[b] ±0.108	19.96	1.116 ±0.068	0.842[b] ±0.094	-24.55
	SDH	0.508 ±0.016	0.532* ±0.006	+4.72	0.517 ±0.022	0.474[b] ±0.016	-8.31	0.514 ±0.018	0.452[a] ±0.008	-12.06	0.522 ±0.028	0.436[b] ±0.028	-16.47

Values for LDH & SDH expressed as µmoles of Formazan/mg protein/hour. Each value is a mean of 6 observations ± S.D. Experimental values significantly different from controls with statistical significance at *P < 0.01; [a]P < 0.005; [b]P < 0.001, and %D = percent difference

The levels of lactate are reflected in the activity levels of lactate dehydrogenase of the tissues of crabs in the present study. Endosulfan induced inhibition of LDH in the tissues of Channa punctatus (Sastry and Siddiqui 1983) supports the LDH profile in the present study. It has been reported that organochlorine compounds could directly inhibit LDH activity (Hendrickson and Bowden 1973). Succinate dehydrogenase, a mitochondrial enzyme of Kreb's cycle, was elevated on early exposure of 24 hrs. On subsequent exposure periods, a progressive inhibition was observed in all tissues. Elevation in SDH activity thereafter suggests depressed respiratory rate which might be due to the close association of pesticide with mitochondrial membrane. Such an impairment of mitochondrial enzymes was reported during pesticidal toxicosis (Pardini et al 1980). In conclusion, the alterations in metabolite levels and inhibition in the enzyme activities as observed in the present investigation indicate altered glycolytic mechanism as a consequence of endosulfan induced stress.

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