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In Vivo Effects of Cadmium Chloride on Certain Aspects of Protein Metabolism in Tissues of a Freshwater Field Crab *Barytelphusa guerini*

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Heavy metals discharged from industries are a major source of pollution which has become a threat to all forms of life. Among the various heavy metals, cadmium is known to be highly toxic even in low concentraations (Miettinen 1974). The harmful effects of cadmium is attributed to its effeccts on sulfhydryl groups of enzymes, especially dehydrogenases (Belies 1975). Various aspects of cadmium pollution on fishes have been extensively reviewed (Cornelius et al. 1983). A Survey of the literature reveals that few attempts have been made to study the various aspects of cadmium toxicity in crustaceans (Thurberg et al. 1973; Calabrese et al. 1977; Tucker and Matte 1980) and these studies were mainly devoted to marine forms. The freshwater crustaceans, particularly the freshwater field crab, Barytelphusa guerini, has received little attention (Reddy et al. 1989).

The present study reorts the influence of cadmium on certain aspects of protein metabolism in the tissues of a freshwater field crab, Barytelphusa guerini, an important component of the paddy field ecosystem, exposed to sublethal concentrations of cadmium chloride.

MATERIALS AND METHODS

Healthy, uniform-sized male crabs Barytelphusa guerini collecctd from and around Hyderabad were acclimated to laboratory conditions for a period of 15 days. The animlas were fed fish flesh ad libitum. To determine the LC_{50} value, the crabs were exposed to six serial concentrations of cadmium chloride. A density of 10 crabs per 8 litres of water was used with 10 individuals in each test container. The physico-chemical characteristics of the test water were as follows: pH 7.4; dissovled oxygen 4.6 ppm; total alkalinity expressed as HCO_3 16 ppm; and carbonates CO_3) 4 ppm. Free carbon dioxide was absent. The LC_{50} value (1.82 ppm) for 96 hours was determined

according to Finney (1964). The crabs were divided into two groups of twelve each. Group I served as a control and group II was subsequently exposed to a sublethal concentration of Cd (1/3 of LC_{50} i.e., 0.6 ppm) for a period of 15 days. The toxicant water and control water were renewed every 24 hours after feeding. The animals were starved a day prior to experimentation to avoid metabolic differences, if any, due to differential feeding and food reserves. Six crabs each from the experimetnal and control groups were sacrificed on the 4th and 15th day of exposure. 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 analysis of various biochemical parameters, i.e., total protein (Lowry et al. 1951), free amino acid content (Moore and Stein 1954), aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) (Reitman and Frankel 1957), and glutamate dehydrogenase (GDH) (Nachlas et al. 1960). The protein content in the enzyme source was estimated according to Lowry et al. (1951), using bovine serum albumin (Sigma) as a standard. The results were subjected to statistical analysis. Students 't' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

Biochemical parameters measured in the present investigation to assess the toxicological effects of a sublethal concentration of cadmium chloride on the physiological organization of individual tissues of functionally divergent nature show quite significant changes in the normal patterns of metabolites and related enzymes of protein metabolism, indicating a tissue-specificity (Tables 1-3).

Protein profiles of individual tissues of the crab show a considerable loss in protein content on the 15th day of exposure, with a maximum depletion in thoracic ganglion (Table 1). A similar trend was observed in the free amino acid content also, wherein hepatopancreaas and thoracic ganglion lost appreciable amounts of free amino acid pool while gills accumuleted free amino acid content on the 4th day of exposure. Significant loss of free amino acids was observed after 15 days of exposure in all tissues. Decrease in protein content and free amino acid levels in the tissues of crabs during the present investigation may be due to the higher rates of proteolysis by activating the protease activities and amino acid catabolism through transamination and oxidative deamination. Accumulation of amino acids in gills on the 4th day in comparision to other tissues

Table 1. Total protein content (A) and free amino acid levels (B) in the tissues of the freshwater field crab Barytelphusa guerini exposed to a sublethal concentration of cadmium chloride.

Tissues		4 days Control	4 days Experimental	% Difference	15 days Control	15 days Experimental	% Difference
Gills	A	52.960±1.259	48.450±2.154	-8.52	56.350±2.314	40.220±3.422 *	-28.62
	B	8.148±0.120	11.267±0.365	+38.37	7.167±0.211	5.275±0.067 *	-26.39
Muscle	A	72.570±2.355	70.000±2.958 @	-3.54	73.330±2.044	60.970±1.508 *	-16.86
	B	14.306±0.270	12.865±0.390	-10.08	12.450±0.201	8.594±0.222 *	-30.97
Hepato-pancreas	A	81.360±2.571	74.120±2.501 *	-8.90	80.500±3.052	59.600±2.497 *	-25.96
	B	13.833±0.401	9.580±0.158 **	-30.75	14.949±0.260	8.048±0.142 *	-46.16
Heart	A	58.580±1.875	52.330±1.926 @	-10.67	60.670±1.563	45.400±1.279 *	-25.00
	B	11.242±0.243	10.587±0.162	-5.82	10.292±0.216	6.896±0.258 *	-32.99
Thoracic Ganglion	A	49.330±1.382	46.170±3.042 *	-6.41	50.670±2.325	34.400±1.889 *	-32.11
	B	16.375±0.455	12.569±0.145	-23.25	14.100±0.305	8.976±0.227	-37.62

Values expressed as mg protein for A and mg amino acids for B per gm wet weight of the tissue:
*P<0.001; **P<0.01; @P<0.05.

Table 2. Aspartate amino transferase (A) and alanine amino transferase (B) activities in the tissues of the freshwater field crab Barytelphusa guerini exposed to a sublethal concentration of cadmium chloride.

Tissues		4 days Control	4 days Experimental	4 days % Difference	15 days Control	15 days Experimental	15 days % Difference
Gills	A	29.750±0.955	32.178±0.856 *	+8.16	32.750±0.300	42.248±0.317 *	+32.05
	B	10.317±0.518	13.965±0.260 *	+35.35	12.230±0.229	9.492±0.376 *	-22.38
Muscle	A	40.200±0.723	44.420±0.911 *	+10.50	44.173±0.250	54.225±0.350 *	+22.48
	B	14.667±0.430	17.895±0.290 *	+22.00	16.020±0.435	10.452±0.156 *	-34.75
Hepato-pancreas	A	20.750±0.522	26.260±0.622 *	+26.25	23.325±0.252	35.797±0.401 *	+53.47
	B	11.550±0.633	15.082±0.321 **	+30.58	12.533±0.404	9.833±0.300 *	-21.54
Heart	A	19.200±0.432	21.109±0.401 **	+ 9.94	21.366±0.201	27.015±0.269 *	+26.47
	B	9.893±0.441	8.476±0.137	-14.33	10.550±0.357	6.736±0.236 *	-36.15
Thoracic Ganglion	A	29.155±0.389	36.353±0.402 *	+24.69	27.055±0.266	36.177±0.190 *	+33.72
	B	11.250±0.626	10.266±0.247	-8.74	8.750±0.201	5.924±0.034	-39.24

Values expressed as μ moles of Sodium-Pyruvate/mg protein/hour; *P<0.001**P<0.01.

Table 3. Glutamate dehydrogenase (GDH) activity in the tissue of the freshwater field crab *Barytelphusa querini* exposed to a sublethal concentration of cadmium chloride.

Tissues	4 days			15 days		
	Control	Experimental	% Difference	Control	Experimental	% Difference
Gills	3.060±0.094	4.463±0.172*	+45.80	3.350±0.158	1.517±0.080*	-52.93
Muscle	2.417±0.126	3.721±0.169*	+53.95	2.651±0.120	1.314±0.041*	-50.42
Hepato-pancreas	5.267±0.121	6.225±0.124*	+18.21	5.565±0.129	3.207±0.136*	-42.37
Heart	2.619±0.112	3.620±0.225**	+38.30	2.885±0.165	4.178±0.149*	+44.82
Thoracic Ganglion	4.031±0.019	5.621±0.221*	+39.44	4.355±0.161	7.013±0.133*	+61.49

Values expressed as μ moles of Formazan/mg protein/hour; *P<0.001; **P<0.01.

indicates an imbalance between proteolysis and amino acid catabolism. The loss of amino acids could be accounted for incrased utilization through transaminases and oxidative deamination. The tissue - specific and time-dependent depletion in amino acid content and enhanced activities in the transinases and GDH was reported in crabs by Reddy et al. (1982) and in fish by Venugopal (1989) after metal intoxication supports the loss in the amino acid content in the present study. The protein loss in the present study can also be attributed to the impairment of protein synthesis.

Aminotransferses, key enzymes of nitrogen metabolism and also important in energy mobilization (Calabrese et al. 1977), were consideraably elevated in the crabs exposed to cadmium chloride (Table 2). Tissue - specific and time-dependent enhancement in the activities of transaminases recorded in Channa punctatus (Malla reddy 1979), Anabas scandens (Chandravathy et al. 1987) after heavy metal intoxication offers excellent support for the present observations. Elevated transaminases in the present study could be taken as a measure of compensatory mechanisms as a consequence to impaired carbohydrate metabolism (Reddy et al. 1989). Ketoacids formed in this reaction may sere as precursors in the synthesis of essential constituents, in addition to their utilization in the Kreb's cycle. The pyruvate and oxaloacetate precursors may be utilized in the synthesis of glucose by operating gluconeogenesis to provide additional levels of glucose for the generation of energy through anaerobic glycolysis under cadmium-induced hypoxic conditions. Heavy-metal induced hyppoxic conditions were recorded in the same crab (Reddy 1981). The ketoacids converted into aminoacids in this reaction may be utilized in the protein synthesis, thus regulating protein and carbohydrate metabolism (Knox and Greengard 1963). The decrease in AlAT activity in heart and thoracic ganglion on 4th day and in all tissues on 15th day of exposure presents, however, an interesting deviation in this study. The reports on enzymurea, a most sensitive indicator of earliest signs of cadmium injury in higher animals (Nomiyama et al. 1973), may be of doubtful relevance to crustaaceans, although the decapod antennal gland parallels that of glomerular nephrons (Smith 1951). Therefore, more investigations on the tissue - specific and time - dependent variations in this enzyme are warranted.

The responses of GDH activity in the tissues were found different in comparision to the AAT and AlAT activities (Table 3). The GDH activity was elevated in all the tissues on 4th day; however, the enzyme activity was

found inhibited in gills, muscle and hepatopancreaas on the 15th day of exposure. Increased glutamate oxidation through enhanced GDH may be to provide reduced potential (NADH) and α-ketoglutarate to provide energy and also to serve as precursor in the synthesis of essential organic molecules. The differential responses in GDH and AlAT activities could be attributed to the differential rates of distribution and elimination of cadmium in the tissues. The decrease in the GDH activity suggests less availability of ammonia. GDH is a regulatory enzyme, known to check the deamination process to minimize the ammonia level. The fate of ammonia through increased glutamate oxidation needs further investigation. However, crabs, being semi-aguatic animals, can eliminate ammonia through their gills.

In conclusion, alterations in the enzyme activities and metabolite profiles indicate the possibility of compensatory mechanisms as a consequence to impaired carbohydrate metabolism. The tissue-specific and time-dependent responses observed in this study need further probing on the bioavailability, distribution and elimination patterns of cadmium in this model animal.

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