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Levels of Transaminases in Tissues of the Penaeid Prawn, *Metapenaeus monoceros* (Fabricius) Following Sublethal Kelthane Exposure

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Several pollutants find their way into the aquatic environment. Of them, insecticides constitute a major chemical group which cause stress to many nontarget organisms (Holden 1973; Addison 1976; Attri 1981). Among them, organochloride (OCl) compounds are very potent, because they are persistant with low biodegradability (Addison 1976). They are found to alter the physiological and metabolic conditions of organisms (Holden 1973) under chronic exposure to low concentrations of OCl insecticides. Many animals develop compensatory mechanisms to mitigate the toxic stress.

Aspartate (AST; EC 2.6.1.1) and alanine (ALT; EC 2.6.1.2) transaminases are widely present in all organisms. These enzymes not only function as link enzymes between the protein and carbohydrate metabolisms but also serve as an indicator of altered physiological or stress condition (Knox and Greengard 1965). Since these enzymes play a significant role under stress, the present work aims to ascertain their function at acute and chronic exposures of the prawn, Metapenaeus monoceros to kelthane.

MATERIAL AND METHODS

The tropical penaeid prawn, Metapenaeus monoceros of 75 ± 5 mm length and 2.5 ± 0.5 g weight were collected from the Buckingham Canal adjoining the Thummalapenta sea coast (lat. 14°55'N and long. 80°3'E), India. They were sorted in groups of 10 each and placed in large glass aquaria (20 L) containing filtered and diluted seawater obtained from Thummalapenta sea coast. They were acclimatized for 1 week in diluted seawater of 15 ± 2‰ salinity, 7.3 ± 0.1 pH and temperature 23 ± 2° C.

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They were fed with a mixture of groundnut cake and minced frog muscle. The water was changed once a day. The control and the experimental prawns were maintained under similar conditions, except that control prawns were kept in kelthane free water. Continuous aeration was provided for all sets of prawns. Only intermolt prawns were used. Technical grade kelthane (1,1-bis(chlorophenyl)-2,2,2-trichloro ethanol), an organochlorine insecticide obtained from Indofil chemical Company, Bombay (affliated with Rohm and Haas Co., Philadelphia, USA.) was employed as the test compound.

LC_{50} values were calculated using probit analysis (Finney 1964). Five concentrations of kelthane (0.02 to 0.32 mg/L) were used in log 2 proportions. For each concentration, 8 prawns were used every time, exposing 2 prawns in 15 L of test solution. Each experiment was repeated 5 times in the selected concentration of kelthane. The number of prawn kills in each concentration were recorded after 96 hours of exposure. LC_{50} values were calculated and it was 0.15 mg/L (Surendranath 1988). The sublethal concentration of 0.03 mg/L (1/5th of LC_{50}) was selected for the present study, as it closely approximated the levels found in the environment.

After the required exposure period, the prawns were sacrificed, the midgut gland, muscle, and gill tissues were isolated. Cell-free extracts prepared in ice-cold 0.25 M sucrose solution, were used for the enzyme assay. Aspartate (AST; EC 2.6.1.1) and alanine (ALT; EC 2.6.1.2) transaminase activities were assayed by the method of Reitman and Frankel (1957). Initial experiments were carried out for enzyme standardization, and the reactions were carried under linear conditions with substrate, incubation time and enzyme protein. The tissue protein content was estimated using Folin-phenol reagent (Lowry *et al*, 1951). Total free aminoacids (FAA) were estimated by using ninhydrin reagent (Moore and Stein 1954). The hemolymph glucose and the tissue glycogen contents were determined by the methods of Mendel et al (1954) and Carrol *et al* (1956) respectively. The statistical analysis were performed following the methods given in Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Our results indicated an increase in the tissue AST and ALT activities in prawns exposed to Kelthane under chronic and acute periods. Simultaneously an increase in the tissue FAA pool was also detected. Between acute and chronic exposure periods, the increase in AST

and ALT activities and the tissue FAA pool is greater at chronic exposure (Tables 1 and 2). The tissue glycogen content and hemolymph glucose level showed a reciprocal trend. The decrease in tissue glycogen content agrees with the increased hemolymph glucose levels at both exposure periods. But the trends obtained under chronic exposure are much greater when compared to acute exposure (Table 2). The above trends demonstrate kelthane to impose toxic stress on M. monoceros. The effect of kelthane was well maintained

Table 1. Aspartate (AST) and alanine (ALT) transaminase activities (umol pyruvate/mg protein/h) in the midgut gland, muscle, gill of control and kelthane exposed prawns.

Tissue	Control	Kelthane exposure	
		Acute	Chronic
	Aspartate Transaminase (AST)		
Midgut gland	0.945 ± 0.029	1.229 ± 0.029 (+30)	1.813 ± 0.037 (+92)
Muscle	1.282 ± 0.028	1.511 ± 0.031 (+18)	2.153 ± 0.054 (+68)
Gill	0.853 ± 0.028	0.992 ± 0.017 (+16)	1.370 ± 0.030 (+61)
	Alanine Transaminase (ALT)		
Midgut gland	2.916 ± 0.076	3.747 ± 0.114 (+29)	5.767 ± 0.308 (+98)
Muscle	1.547 ± 0.060	1.863 ± 0.049 (+20)	2.897 ± 0.308 (+87)
Gill	1.108 ± 0.022	1.356 ± 0.030 (+22)	2.006 ± 0.049 (+81)
	AST/ALT Ratios		
Midgut gland	0.324	0.328	0.314
Muscle	0.829	0.811	0.743
Gill	0.770	0.730	0.683

Each value is mean ± SD of six individual observations. Values in parentheses indicate percent change over control. All values from kelthane exposure are significantly different from control at $p < 0.001$.

at chronic exposure period even at low concentration of kelthane. Such a mode of action is only exhibited by the OCl insecticides, because they are persistent pesticides, having low biodegradability (Bhatia 1973; Addison 1976). Thus kelthane posses the basic property of the organochlorine insecticides.

At the tissue level, kelthane toxic impact is highly felt on the midgut gland, to a lesser extent on the muscle closely followed by the gill tissue, a trend observed with transaminase activity levels (Table 1),

Table 2. Free aminoacid and glycogen contents in midgut gland, muscle and gill tissues and glucose levels in haemolymph of control and kelthane exposed prawns.

Tissue	Control	Kelthane exposure	
		Acute	Chronic
Free Amino Acids (umol/g wet wt. of tissue)			
Midgut gland	66.15 ± 2.19	97.71 ± 2.42 (+48)	147.26 ± 4.82 (+123)
Muscle	51.14 ± 1.19	66.15 ± 2.19 (+29)	100.42 ± 2.42 (+96)
Gill	43.33 ± 0.92	54.17 ± 1.49 (+25)	80.31 ± 1.63 (+85)
Glycogen (mg/g wet wt. of tissue)			
Midgut gland	12.92 ± 0.21	9.53 ± 0.27 (-26)	1.65 ± 0.07 (-87)
Muscle	3.55 ± 0.10	2.90 ± 0.05 (-18)	0.74 ± 0.07 (-79)
Gill	2.08 ± 0.08	1.73 ± 0.07 (-17)	0.70 ± 0.04 (-66)
Glucose (mg/100 mo of haemolymph)			
Haemolymph	51.51 ± 0.29	72.47 ± 1.59 (+41)	100.89 ± 2.88 (+96)

Each value is mean ± SD of six individual observations. Values in parentheses indicate percent change over control. All values from kelthane exposure are significantly different from control at $p < 0.001$.

because they are stress mediated enzymes (Knox and Greengard 1965; Prasada Rao and Ramana Rao 1985). But at acute and chronic exposure periods, the increase in the activities of AST and ALT, and FAA levels clearly demonstrate that the hepatic tissue is highly susceptible to kelthane toxic stress compared to the non-hepatic tissues at both exposure periods (Tables 1 and 2). This trend is acceptable because the midgut gland of crustaceans is homologous to the liver of vertebrates and serves as a metabolic center (Florkin and Scheer 1970).

Since organochlorine compounds are lipophilic having high affinity for lipids and fats (Reinert 1970; Johnson 1973), it was observed that kelthane being an OCl insecticide should account for the highest toxic stress on the midgut gland which has high lipid content. The muscle tissue was also reasonably rich in total lipids (Surendranath 1988) and this showed vulnerability to kelthane toxic stress. The effect of kelthane on the gill tissue was greater, because of the continuous exposure of kelthane molecules by the medium. In aquatic animals like fish, crabs, etc., the entry of pesticide was mostly through gills (Holden 1962). Therefore, this could be the reason for an increase in the gill AST and ALT activities.

Since AST and ALT not only function as link enzymes but also serve as indicators of stress (Knox and Greengard 1965), the increase in the activity levels of these enzymes at both exposure periods clearly demonstrates kethane induced toxic stress and this is greater under chronic exposure.

The AST/ALT ratios give an assessment of the nature of the carbohydrate metabolism being favored by the tissues (Prasada Rao and Ramana Rao 1984). Values less than 1.0 show more of ALT activity, thus favoring pyruvate formation, indicating preponderance of anaerobic nature of carbohydrate metabolism. The utilization rate of glycogen and glucose is usually much higher during anaerobic metabolism (Sreenivasulu Reddy et al 1985). Therefore to conserve rapid depletion of the tissue glycogen content and to utilize the increased FAA pool, the intermediary metabolic enzyme systems, namely the transaminases, are accelerated to promote gluconeogenesis, possibly to meet the increased energy demands under sustained and prolonged toxic stress. The AST/ALT ratios are very low in the hepatic tissue compared to non-hepatic tissues (Table 1) indicating that the midgut gland favored more of ALT activity. It is established that ALT activity levels also indicate the degree of

adaptability (Chaplin et al 1967). Since the effect of kelthane was high in the midgut gland at both exposure periods either due to more entry of kethane molecules or accumulation (acute) or residual formation (chronic), the increase in the midgut gland ALT activity can be considered as conferring protection and adaptability against kelthane toxic stress particularly under chronic exposure.

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