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Significance of Glucose-6-Phosphate Dehydrogenase Activity in Tissues of Penaeid Prawn, *Metapenaeus monoceros* (Fabricius) under Acute Kelthane Exposure

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Organochlorine insecticides are used to control a wide variety of agricultural pests due to their broad spectrum and long lasting effective action.But because of widespread and indiscriminate usage of these insecticides several nontarget organisms are effected . The prawns were selected for the present study due to their abundant availability and since prawns also serve as sensitive indicators of pollution (Butler 1966). Besides, the prawns are commercially and nutritionally important. Literature survey revealed that only few reports are available on detoxification of organochlorines in crustaceans. So the present study aims to explore the possible role of glucose-6-phosphate dehydrogenase, a key enzyme of hexose monophosphate shunt, in the detoxification of kelthane.

MATERIAL AND METHODS

Penaeid prawn, Metapenaeus monoceros (Fabricius) were collected from Buckingham canal, near Kavali sea coast A.P. India. Only intermolt, healthy prawns of size 75±5 mm long and 2.5±0.5 g were selected and acclimatised for 1 Wk at constant salinity of 15±1 ppt, pH 7.1±0.2 and temperature of 23±2°C. They were fed ad lib diet of groundnut oil cake powder and rice bran. The water was changed daily. Technical grade kelthane (1,1-bis(chlorophenyl)-2,2,2-trichloroethanol) of 85% purity obtained from Indofil Chemicals Ltd, Bombay , was used as test chemical. Toxicity studies were conducted by following the static bioassay method (Doudoroff et al 1951) and LC_{50} was computed as per probit method (Finney 1961). The LC_{50}/48h to Metapenaeus

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monoceros was found to be 0.151 mg/L and a sublethal concentration of 0.05 mg/L (1/3 LC_{50}) was chosen and prawns were exposed to this concentration for 48 h. After 48 h exposure, the hepatopancreas, muscle and gill tissues were isolated quickly and were subjected to biochemical analysis. The protein content (both sucrose soluble and insoluble fractions) was estimated using the Folin phenol reagent (Lowry et al 1951). Hemolymph glucose and tissue glycogen levels were estimated by the method of Kemp and Mayers (1954) and Carrol et al (1956) respectively. Total lipid content was determined as per Folch et al (1957).

5% homogenates were prepared in 0.25M sucrose solution and centrifuged at 1000 g for 15 min. supernatant form the enzyme source for SDH and G-6-PD assay. SDH activity was assayed following the method of Nachlas et al (1960). The reaction mixture consists of 100 μ moles of phosphate buffer (pH 7.4), 40 μ moles of Sodium succinate, 4 μ moles of Iodo-phenyl Neotetrazolium salt, 0.6 ml of distilled water and 0.5 ml of supernatant . Incubation is carried at 37°C for 15 min. The reaction is arrested by adding 5 ml of glacial acetic acid.The color is extracted with 5 ml of Toluene by keeping it overnight at 5°C. The color is read at 545 nm against blank in a spectrophotometer. The G-6-PD was assayed by the method of Bergmeyer and Bernt (1965). The reaction mixture consists of 100μmoles of triethanolamine buffer (pH 6.8), 4 μmoles of iodophenyl neotetrazolium salt, 20 μmoles of Glucose-6-phosphate (disodium salt), 1 μmole of NADP and 0.5 ml of homogenate. The reaction mixture is incubated at 37°C for 15 min. 5 ml of glacial acetic acid is added to arrest the reaction and color is extracted by adding 5 ml of toluene and kept at overnight at 5°C . The color is read at 545 nm against blank in a spectrophotometer. Cytochrome-c-oxidase was assayed as per Oda et al (1958). 5% homogenate is prepared in 0.17M cold phosphate buffer (pH 7.4) and centrifuged at 1500 g for 15 min. The supernatant is used as enzyme source. The reaction mixture consists of 0.2 ml of 0.2M p-phenylene diamine, 0.2 ml of 0.2% neotetrazolium chloride, 0.2 ml of 10^{-4}M cytochrome-c and 0.4 ml of the enzyme source. The reaction mixture is incubated at 37°C for 15 min. The reaction was stopped by the addition of 0.4 ml of 1N H_2SO_4. The color is extracted with 5 ml of 1:1 ether-acetone mixture and the color is read at 520 nm in spectrophotometer against blank.

RESULTS AND DISCUSSION

Sucrose soluble and insoluble proteins and total lipids showed significant increment in selected tissues of kelthane exposed prawns, whereas hemolymph glucose levels and tissue glycogen levels decreased considerably under

kelthane toxic stress (Table 1). Succinate dehydrogenase activity (SDH) and cytochrome-c-oxidase activity levels showed inhibition while Glucose-6-phosphate dehydrogenase (G-6-PD) activity levels indicated a significant elevation in hepatopancreas, muscle and gill tissue during sublethal kelthane toxic stress (Table 2).

The decreased glucose and glycogen levels under kelthane exposure signified their utilisation for energy requirements to face the toxic stress. Since there are reports about tissue damage and energy crisis during organochlorine pesticide exposure (Mary 1984), the present decrease might also be due to the energy demand warranted by the toxic environment. This decrease in carbohydrate content might also be due to the prevalence of hypoxic conditions due to kelthane toxic stress. The increase in carbohydrate utilisation under hypoxic conditions is also reported (Dezwan and Zandee 1972).

A small decrease in Krebs cycle enzyme and electron transport system enzyme particularly SDH and cytochrome-c-oxidase activity levels demonstrate a sharp decrease in oxidative metabolism. Similar occurence of reduced oxidative metabolism under stress condition have been reported in invertebrates (Hochachka 1973). But a significant increment in G-6-PD activity levels in selected tissues suggests the higher operation of the hexose monophosphate pathway as an alternative pathway to mitigate kelthane toxic stress. The increased levels of G-6-PD may be to generate NADPH and pentose sugars. The NADPH is required for fatty acid synthesis and also plays an important role in detoxification of insecticides (O'Brien 1967).

The pentose sugars are essential for the synthesis of nucleic acids. Increment in DNA and RNA contents of same species under pesticide stress is also reported (Vijayalakshmi 1987). The increased nucleic acids causes increase in turnover of proteins. Thus it might be due to this reason, both the soluble and insoluble protein contents were significantly increased in selected tissues of KE prawns (Table 1). Since soluble proteins represent the enzymes, hormones, the increase in soluble proteins should represent an increase in the synthesis of enzymes that detoxify kelthane. NADPH produced in HMP pathway may be utilized to increase the synthesis of total lipids in the present study. The increment in total lipids of selected tissues suggests that this may be utilized for the synthesis of structural elements to rectify tissue damage and as an alternative source of energy to meet the extra energy demand to mitigate kelthane toxic stress. It can be inferred that G-6-PD plays a significant role in kelthane exposed prawn tissues to mitigate toxic stress condition by

Table 1: Levels of hemolymph glucose, tissue glycogen, sucrose soluble and insoluble proteins and total lipids in selected tissues of control and kelthane exposed (KE) prawn.

Organic constituent	Hepatopancreas/ Hemolymph		Muscle		Gill	
	Control	KE	Control	KE	Control	KE
Glucose (mg/100ml hemolymph)	50.32 ±2.53	30.44[b] ±1.64 (-39.51)	-	-	-	-
Glycogen (mg/g wet wt. tissue)	11.43 ±1.20	8.08[b] ±0.65 (-29.31)	2.51 ±0.83	2.05 ±0.62 (-18.33)	1.98 ±0.56	1.67 ±0.64 (-15.66)
Soluble proteins (mg/g wet wt. tissue)	82.68 ±3.26	104.70[b] ±1.35 (+26.63)	56.25 ±1.34	67.34[b] ±1.22 (+19.72)	52.87 ±1.76	60.35[b] ±1.85 (+14.15)
Insoluble proteins (mg/g wet wt. tissue)	32.57 ±1.38	37.76[b] ±1.22 (+15.94)	67.43 ±1.62	75.70[b] ±1.74 (+12.27)	56.28 ±1.53	62.15[b] ±1.36 (+10.43)
Total lipids (mg/g wet wt. tissue)	46.18 ±1.13	53.67[b] ±1.08 (+16.22)	22.56 ±0.84	24.74[b] ±0.92 (+9.65)	12.18 ±0.73	13.00[a] ±0.86 (+6.73)

Each value is mean ± SD of six individual observations. Values in parentheses indicate percent change over control. All differences are significant at a = $P<0.05$, b = $P<0.001$ No symbol = Not significantly different.

Table 2. Activity levels of succinate dehydrogenase (SDH), Cytochrome-c-oxidase and glucose-6-phosphate dehydrogenase (G-6-PD) in selected tissues of control and kelthane exposed (KE) prawn.

Enzyme	Hepatopancreas		Muscle		Gill	
	Control	KE	Control	KE	Control	KE
SDH (µmoles of formazan formed/mg protein/h)	0.831 ±0.022	0.654[c] ±0.030 (-21.30)	0.164 ±0.019	0.145 ±0.032 (-11.59)	0.413 ±0.024	0.375[b] ±0.028 (-9.20)
Cytochrome-c-oxidase (µg of diformazan formed/mg protein/h)	52.48 ±0.36	38.37[c] ±0.52 (-26.89)	18.97 ±0.65	16.42[c] ±0.85 (-13.44)	29.58 ±0.26	26.48[c] ±0.14 (-10.48)
G-6-PD (µmoles of formazan formed/mg protein/h)	2.63 ±0.14	3.53[c] ±0.16 (+34.22)	1.28 ±0.08	1.66[c] ±0.20 (+29.69)	0.096 ±0.015	0.119[a] ±0.022 (+23.96)

Each value is mean ± SD of six individual observations. Values in parentheses indicate percent change over control. All differences are significant at a = $P<0.05$, b= $P<0.01$, c= $P<0.001$ and No symbol = Not significant.

providing more NADPH and pentose sugars by higher G-6-PD activity levels for the synthetic and detoxification purposes as an adaptive response to survive well under the kelthane toxic stress condition.

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