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Effect of Sublethal Concentration of Copper Sulphate on Glutathione-s-transferase Enzyme Activity in Various Tissues of Freshwater Crab, *Barytelphusa cunicularis* (Westwood)

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Abstract: Enzymatic studies would be useful and form a type of meaningful biochemical indices of toxicant action and chemical intoxication is reflected through changes in the activity pattern of enzymes. In the present study crabs *Barytelphusa cunicularis* were exposed to sublethal concentration [$1/10^{th}$: 28.2 ppm] of copper sulphate for 1, 2, 3, 4, 5, 7 & 10 days. The crabs were sacrificed and tissues like ovary, gills, hepatopancreas, thoracic muscles and spermathecae were dissected out. Wet tissue was weighed from both the control and treated crabs to assess the activity of Glutathion-S-Transferase [GST]. GST activities in the control crab were found 0.171, 0.393, 0.205, 0.231 and 0.163 μmoles of thioether formed/100 mg wet tissue/min. in ovary, hepatopancreas, gills, thoracic muscle and spermatheca respectively.

Key words: *Barytelphusa cunicularis* · Copper Sulphate · GST

INTRODUCTION

The distribution and partitioning with discharging industrial and urban effluents containing heavy metals into ambient water system as well as an adjoining fields without any pre treatment had been conducted worldwide [1]. Aquatic organisms are characterized by the uptake and retention of heavy metals and the rate of accumulation is affected by chemical form of metal [2, 3]. There is substantial evidence that crustaceans accumulate a range of heavy metals at various rates from their environment and have bodily, anatomical and physiological deleterious effects [4-7].

The freshwater female crab, *Barytelphusa cunicularis*, is a commercially important decapod crustacean consumed extensively in Marathwada region. Glutathione S-Transferase is an important detoxifying enzyme that is found in many organisms and protects organisms from toxic chemicals once they are ingested or absorbed from the environment and protects cells from reactive epoxides and oxygen species [8]. As an antioxidant enzyme it prevents oxidative damage and participates in the defense mechanisms against oxidative products [9, 10].

Toxicants bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activity of various enzymes [11]. The *Barytelphusa cunicularis* is commonly available crab around Aurangabad region. An attempt has been made to investigate copper sulphate induced alterations in activities of enzymes Glutathione-S-Transferase [GST] in various tissues of this crab. The increase or decrease in their level may be sufficient to provide information of diagnostic value.

MATERIALS AND METHODS

The freshwater female crabs, *Barytelphusa cunicularis* were collected from outskirt of Aurangabad region. They were acclimatized to laboratory conditions under normal day/night illumination of 11 L: 13 D and temperature 27±1º C for about one week in plastic troughs [18 cm diameter] was containing sufficient tap water so that crabs are submerged. Before experimentation intermoult (stage C_3) [12] female crabs of approximately equal carapace width [45 to 50 mm] and body weight [50 to 55 g] were sorted into two groups as experimental and control.

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The crabs were exposed to sublethal concentration [1/10th of 24 h LC_{50}: 28.2 ppm] of copper sulphate for 1, 2, 3, 4, 5, 7 & 10 days. The crabs were sacrificed and tissues like ovary, gills, hepatopancreas, thoracic muscles and spermathecae were dissected out. Wet tissue was weighed from both the control and experimental crabs to assess the activity of Glutathione-S-Transferase [GST, E.C: 1.1.137.] using 1 chloro 2, 4 dinitro benzene [CDNB] as the substrate and estimated by using the method of Habig [13]. The activity of GST was expressed as μmoles of thioether formed/100 mg/min.

All the observed results were expressed as mean of three replicates and the data obtained are statistical evaluated using student "t" test [14].

RESULTS

In the crab *Barytelphusa cunicularis* a significant [P<0.05] decrease in the rate of Glutathione-S-Transferase [GST] activity was observed after 1, 2, 3, 4, 5, 7 and 10 days of exposure respectively to sublethal concentration [1/10: 28.2 ppm]of copper sulphate (Table 1).

Ovary: The percent decrease in GST activity found in experimental crab exposed to copper sulphate was 2.3%, 4.7%, 8.8%, 10.5%, 14.0%, 17.5% and 19.8%.

Hepatopancreas: The percent decrease in GST activity found in experimental crab exposed to copper sulphate was 1.5%, 3.6%, 5.8%, 7.9%, 9.9%, 11.4% and 13.7%.

Gill: The percent decrease in GST activity found in experimental crab exposed to copper sulphate was 5.4%, 10.2%, 13.2%, 18.0%, 22.0%, 25.8% and 31.2%.

Thoracic Muscles: The percent decrease in GST activity found in experimental crab exposed to copper sulphate was 2.2%, 6.1%, 10.8%, 15.5%, 18.6%, 22.0% and 26.4 %.

Spermatheca: The percent decrease in GST activity found in experimental crab exposed to copper sulphate was 4.3%, 9.2%, 15.3%, 19.6%, 25.1%, 29.4% and 34.9%.

GST activity was found to be time dependent, as the period of exposure increased there was more decrease in GST activity.

DISCUSSION

Decrease or increase in the enzymes activities represent the stress in any organism that results in metabolic burden which normally indicates the occurrence of greater energy demands, normally associated with synthetic activity of cell [15].

The percent decrease in GST activity in the tissue of the crab is in the order of: hepatopancreas > ovary > thoracic muscle > gills > spermatheca.

Earlier studies show change in GST activity in other aquatic invertebrates exposed to metals with potential oxidative stress potential [16]. Cadmium significantly decreases GST activity with increasing concentration and period of exposure in the freshwater crab, *Sinopotamon yangtsekiense* [17].

Table 1: Glutathione-S-Transferase [E.C: 2.5.1.18] activity in different tissues of freshwater crab, *Barytelphusa cunicularis* exposed to sublethal concentration [1/10th of 24 h LC_{50}: 28.2 ppm] of copper sulphate

	Glutathione-S-Transferase [GST]							
		Exposure period in days						
TISSUE	CONTROL	1 Day	2 Days	3 Days	4 Days	5 Days	7 Days	10 Days
OVARY	0.171±0.0026	0.164±0.0015 [-2.3 %]	0.160±0.005 [-4.7 %]	0.154±0.0057 [-8.8 %]	0.152±0.0040 [-10.5 %]	0.144±0.0015 [-14.0%]	0.138±0.005 [-17.5%]	0.130±0.0040 [-19.8%]
HEPATOPANCREAS	0.393±0.0015	0.380±0.0049 [-1.5%]	0.372±0.0034 [-3.6%]	0.366±0.0025 [-5.8%]	0.358±0.0036 [-7.9%]	0.351±0.004 [-9.9%]	0.342±0.025 [-11.4%]	0.334±0.002 [-13.7%]
GILL	0.205±0.0025	0.189±0.0021 [-5.4%]	0.180±0.0028 [-10.2%]	0.173±0.0026 [-13.2%]	0.161±0.0040 [-18.0%]	0.155±0.0020 [-22.0%]	0.147±0.0025 [-25.8%]	0.137±0.0026 [-32.2%]
THORACIC MUSCLE	0.231±0.0042	0.222±0.0030 [-2.2%]	0.208±0.0040 [-6.1%]	0.200±0.0058 [-10.8%]	0.193±0.037 [-15.5%]	0.183±0.0032 [-18.6%]	0.174±0.0025 [-22.0%]	0.164±0.0021 [-26.4%]
SPERMATHECA	0.163±0.003	0.152±0.0035 [-4.3%]	0.142±0.0036 [-9.2%]	0.135±0.0031 [-15.3%]	0.126±0.0030 [-19.6%]	0.118±0.005 [-25.1%]	0.108±0.0047 [-29.4%]	0.101±0.010 [-34.9%]

Enzyme activity unit: - expressed in μmoles of thioether /100 mg wet tissue/min.

Values are presented as mean± SD (X ± SD), n =3, Values [-] are % decreased in enzyme activity.

Glutathione S-transferase activity is high in blue crab hepatopancreas and gills [18]. A portion of these metabolites is bound to cellular lipoproteins and glutathione-S-Transferase, one of the major proteins in the cytosol of blue crab hepatopancreas [19].

In the present study decrease in the enzyme activity represent the stress in crab *Barytelphusa cunicularis* that result the disturbance in the activity of animal during chemical toxicities will be reflected through changes in the activity pattern of enzymes. Therefore enzymatic studies would be useful and form a type of meaningful biochemical indices of toxicant action from tissues of freshwater female crab, *Barytelphusa cunicularis*.

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