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Effects of Cypermethrin Exposure on Dehydrogenases Activity in the Hepatopancreas and Abdominal Muscle of the Fresh Water Female Field Crab, *Spiralothelphusa hydrodroma* (Herbst)

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Abstract: The fresh water field crab, *Spiralothelphusa hydrodroma* is an important human food source in parts of South India and the crab is constantly exposed to pesticides, which are used extensively to control agricultural pests. Evaluation of the toxic effect of cypermethrin on the experimental crab for the LC50 value was carried out. Effect of cypermethrin on the biochemical changes in the hepatopancreas and muscle was observed. Quantitative study of biochemical changes of lactate dehydrogenase and succinate dehydrogenase was undertaken and the results are reported in this paper.

Key words: Hepatopancreas % Muscle % Cypermethrin % *Spiralothelphusa hydrodroma*

INTRODUCTION

The pesticides include in insecticides, herbicides, fungicides, molluscides and nematicides [1]. These pesticides are non-biodegradable and accumulate in the food chain. Mostly they affect the nervous system causing tumors in living organisms. They are not only neurotoxic, but also affect other systems and have shown a high degree of impact on metabolism by inhibiting enzymes like acetyl cholinesterase. In biological systems, the biocatalysts play a vital role in the metabolic pathways. Animals exposed to stress conditions alter their physiological status with the help of enzymes. Toxicants like pesticides are known to secrete hyper or hypo level of enzymes. The trace metal concentration in Queensland estuarine crabs, *Australoplax tridentate* has been observed. Various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels. The pesticide derivatives are known to alter the physico-chemical characteristics of water; these in turn interfere and interact with various physiological activities of organisms. Changes in metabolic rate among organisms exposed to pollution stress have been used as indicator of stress condition. When any aquatic animal is exposed to polluted medium, a sudden stress is developed. The animals should meet more energy demand to overcome this toxic stress. Verma *et al*. [6] reported on the toxic effects of sublethal concentration of copper sulphate, on certain biologically important enzymes in *Saccobranchus fossilis*. The present work aims to bring outthe effect of cypermethrin on the hepatopancreas and muscle of *Spiralothelphusa hydrodroma*.

MATERIALS AND METHODS

Spiralothelphusa hydrodroma were collected from fresh water ponds and paddy fields on the outskirts of Walajapet, Vellore District, Tamil Nadu, India. The crabs were brought to the laboratory in large plastic trough and maintained in normal daylight illumination in the laboratory thereby providing normal acclimatization. The crabs were fed with uncooked oats. For all experiments, the crabs were used with carapace length ranging from

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Table 1: The LC_{50} values and regression equations for *S. hydrodroma* treated with cypermethrin

Exposure periods (hours)	LC_{50} (ppm)	Upper confidence limits (ppm)	Lower confidence limits (ppm)	Regression results	Slope function	r^2
24	2.027	2.561	1.739	Y = - 0.932 X + 0.468	2.973	0.99
48	1.698	1.938	1.345	Y = - 0.658 X + 0.281	3.265	0.98
72	1.452	1.883	1.136	Y = - 0.724 X + 0.391	4.121	0.99
96	1.315	1.763	1.118	Y = - 0.611 X + 0.324	4.973	0.99

3 to 4.5 cm and breadth ranging from 5 to 6.5 cm. The water level was maintained carefully so that the crabs were partially immersed. Acute toxicity study was carried out to determine the potency of cypermethrin for static but renewal type of bioassay was adopted in the present investigation to estimate the LC50 values. The cypermethrin was used as commercial preparation. The experiment was carried out to find the range of concentrations for confirmatory evaluation. The mortality was recorded for the crab at 24, 48, 72 and 96 hr exposure to cypermethrin; the LC50 values were estimated as 2.027, 1.698, 1.452 and 1.315 ppm respectively; were corrected for natural response by Abbott's formula [7].

Table 1 depicts the LC50 values with regression analysis for *S. hydrodroma* treated with cypermethrin.

Design of Sublethal Toxic Study: Chronic time course study on the effect of cypermethrin on the crab was conducted by exposing to two sublethal, safe concentrations for 20 days and 40 days. According to Sprague [8], $1/3^{rd}$ and $1/10^{th}$ of the 96 hr LC50 value represent higher and lower sublethal concentrations respectively. Hence lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of the insecticide were arbitrarily used. At the end of the treatment period, the control and treated crabs were dissected. The hepatopancreas and muscle were collected for enzyme assays.

Biochemical Analysis: Lactate dehydrogenase and succinate dehydrogenase were estimated by adopting the the techniques reported in [9, 10].

Statistical Analysis: One-way Analysis of Variance (ANOVA) was performed based on the methods of devised by Winer [11].

RESULTS

It is seen from the Table 2 that the lactate dehydrogenase (LDH) activity in the hepatopancreas of the control crab was 5.58 and 5.51 µg / 100 mg tissue for 20 and 40 days respectively. In the experimental crabs, the LDH activity in the lower sublethal concentration was 6.42 and 6.02 µg /100 mg tissue and for higher sublethal level, it was 7.37 and 7.00 µg / 100 mg tissue for 20 and 40 days exposure period. The maximum increase in LDH activity of the hepatopancreas calculated was found to be statistically significant in both exposure periods.

In the muscle of the control crab the LDH activity was found to be 7.75 and 7.84 µg / 100 mg tissue for 20 and 40 days of exposure period. In the experimental crab, the LDH activity of the lower sublethal concentration was 8.37 and 8.73 µg / 100 mg tissue and in the crab treated with higher sublethal concentration, it was 9.06 and 9.88 µg / 100 mg tissue for 20 and 40 days of treatment. The readings were found to be statistically insignificant in both the lower and higher sublethal concentrations of cypermethrin in both exposure periods.

It is seen from the Table 3 that the succinate dehydrogenase (SDH) activity in the hepatopancreas of the control crab was 6.96 and 7.05 MIU/min/mg protein for 20 and 40 days of treatment respectively. In the experimental crabs, the SDH activity was decreased for

Table 2: Effect of lower and higher sublethal concentrations of cypermethrin on lactate dehydrogenase

		Control	LSC	HSC		
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Exposure period	Tissues	Mean ± SD	Mean ± SD	Mean ± SD	F-Value	P-Value
20 days	Hepatopancreas	5.58±0.49	6.42±0.97	7.37±0.48	10.26**	<0.01
	Muscle	7.75±0.72	8.37±0.63	9.06±0.94	2.12*	<0.05
40 days	Hepatopancreas	5.51±0.49	6.02±0.57	7.00±0.68	12.41**	<0.01
	Muscle	7.84±0.77	8.73±0.73	9.88±0.63	7.27*	<0.05

Mean ± SD of six individual observations

Values are expressed µg / 100 mg tissue

* * indicates significance at 0.01 level * indicates significance at 0.05 level

Table 3: Effect of lower and higher sublethal concentrations of cypermethrin on succinate dehydrogenase

		Control	LSC	HSC		
Exposure period	Tissues	Mean ± SD	Mean ± SD	Mean ± SD	F-Value	P-Value
20 days	Hepatopancreas	6.96±0.74	6.22±0.47	5.56±0.64	3.86*	<0.05
	Muscle	11.86±0.48	10.65±0.36	9.66±1.10	5.16*	<0.05
40 days	Hepatopancreas	7.05±0.61	6.08±0.53	5.13±0.22	14.77**	<0.01
	Muscle	11.92±0.71	10.24±0.83	9.26±0.77	8.38**	<0.01

Mean ± SD of six individual observations

Values are expressed MIU/min/mg protein

* * indicates significance at 0.01 level * indicates significance at 0.05 level

both the sublethal concentrations. The succinate dehydrogenase activity for lower sublethal concentration was found to be 6.22 and 6.08 MIU/min/mg protein and for higher sublethal concentration, it was 5.56 and 5.13 MIU/min/mg protein for 20 and 40 days of exposure periods. Maximum decrease in SDH activity of the hepatopancreas was observed in 40 days of exposure period was statistically significant and the decrease in SDH activity of the hepatopancreas was statistically insignificant in 20 days of treatment period in both lower and higher sublethal concentrations.

The SDH activity in the muscle of the control crab was 11.86 and 11.92 MIU/min/mg protein for 20 and 40 days of treatment period. In the experimental crabs, the SDH activity in the lower sublethal concentration was found to be 10.65 and 10.24 MIU/min/mg protein and in the crabs treated with higher sublethal concentration was 9.66 and 9.26 MIU/min/mg protein for 20 and 40 days of experimental periods respectively. The decline in SDH activity of the muscle was found to be statistically insignificant in 20 days of exposure and statistically significant in 40 days of exposure in both the sublethal concentrations of cypermethrin.

DISCUSSION

Aquatic systems are contaminated by disposal of various abiotic factors. Water is one of the basic requirements of all aquatic as well as terrestrial lives for growth and survival. Pesticides by virtue of their design and application induce a broad spectrum biocidal effect influencing most of the organisms [12]. These pollutants also destroy the quality of aquatic ecosystems and render it unfit for various aquatic organisms, particularly fresh water crabs. In the present study, the strategy of energy production adopted by *Spiralothelphusa hydrodroma* were assayed by tracking changes in the activities of lactate dehydrogenase and succinate dehydrogenase in its hepatopancreas and muscle under stress at lower (0.1315ppm) and higher(0.4383ppm) sublethal concentrations of cypermethrin revealed highly fascinating informations.

The activity of lactate dehydrogenase, which is a cytoplasmic enzyme, shows a marked elevation in activity in the hepatopancreas and muscle. Lactate dehydrogenase activity is generally associated with cellular metabolic activity which acts as a pivotal enzyme between the glycolysis and the citric acid cycle. Thus, the elevation of lactate dehydrogenase may suggest a bias towards the anaerobic glycolytic pathway. The lactate dehydrogenase activity increased in the *U.annulipes* treated with sublethal concentrations of cadmium and mercury [13] and in *S.hydrodroma* in response to copper and zinc [14]. Increased lactate dehydrogenase activity and decreased succinate dehydrogenase activity were reported in [15]. Chandravathy and Reddy, [16] studied the effect of lead on *Anabas scandens* and found that there was increase in the activity of lactate dehydrogenase and decrease in the succinate dehydrogenase activity. Lactate dehydrogenase activity increased in the hepatopancreas in the fielder crab, *U.pugilator* in response to cadmium [17]. On the contrary, lactate dehydrogenase activity decreased in the abdominal muscle in *U. pugilator*[17] and in *S. serrata* [18] when exposed to cadmium. Significantly decreased lactate dehydrogenase activity was reported in accordance with increase in Cu^{2+} concentration in giant fresh water prawn *Macrobrachium rosenbergii*[19]. The results of the present study are well in accordance with that of previous investigations in the increased activity of lactate dehydrogenase in cypermethrin treated crabs.

The decline in the activity of respiratory oxidative enzyme, the succinate dehydrogenase in hepatopancreas and muscle indicates decline in enzyme synthesis, since cypermethrin disrupt the membrane bound enzyme. Decrease or increase in the enzyme activity represents the metabolic stress in any organism. Mitochondrial damage

leads to decreased respiration and partial coupling of oxidative phosphorylation [20]. Suppression of succinate dehydrogenase activity disrupts mitochondria in anoxic or hypoxic conditions when exposed to toxicants. Succinate dehydrogenase enzyme plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism [21]. The results of the present study are also in conformity with those of the earlier observations.

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