

Effect of Cypermethrin, A Pyrethroid Compound on the Neurosecretory Cells in a Freshwater Field Crab, *Spiralothelphusa hydrodroma* (Herbst)

¹R.S. Sreenivasan, ²M. Arivalagan, ³S. Vijayananth, ⁴P.K. Praveen Kumar,
⁵P. Krishna Moorthy and ⁶M. Deecaraman

¹Department of Biotechnology, St. Peter's Engineering College, Chennai - 600 054, India

²Department of Biochemistry, C.T.M. College of Arts & Science, Chennai - 602 107, India

³Department of Biochemistry, Chennai National College of Arts & Science, Chennai- 602 107, India

⁴Department of Biotechnology, Sri Vengateshwara college of Engineering, Chennai - 602 105, India

⁵Department of Bioinformatics, Bharath University, Chennai - 600 073, India

⁶Department of Biochemistry, Dr.M.G.R. University, Chennai - 600 095, India

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 neurosecretory cells such as brain, thoracic ganglia and eyestalk was observed. Quantitative study of biochemical changes of lactate hydrogenase (LDH), succinate dehydrogenase (SDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the neurosecretory cells was undertaken.

Key words: Neurosecretory cells, brain, thoracic ganglia, eyestalk, cypermethrin, *Spiralothelphusa hydrodroma*

INTRODUCTION

The toxic substances include in insecticides, herbicides, fungicides, molluscides and nematocides^[15]. These pesticides are non-biodegradable and accumulate in the food chain. Mostly they are prone to 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^[19,23]. The trace metal concentration in Queensland estuarine crabs, *Australoplax tridentate* and *Scylla serrata* has been observed^[20]. The present work was that the effect of cypermethrin on the neurosecretory cells of *Spiralothelphusa hydrodroma*.

MATERIALS AND METHODS

The fresh water field crabs were collected from, in and around the irrigating channels and paddy fields. The crabs were 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 3 cm to 4.5 cm and breadth ranging from 5

cm 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 hr, 48 hr, 72 hr and 96 hr exposure to cypermethrin; were corrected for natural response by Abbott's formula^[11]. The LC50 values for 24 hr, 48 hr, 72 hr and 96 hr of exposure periods were estimated as 2.027, 1.698, 1.452 and 1.315 ppm respectively.

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^[30], 1/3rd and 1/10th 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 and the neurosecretory cells were collected for biochemical studies.

Table 1: The LC₅₀ values and regression equations for *S. hydrodroma* treated with cypermethrin

Exposure periods (hours)	LC50 (ppm)	Upper Confidence limits (ppm)	Lower Confidence limits (ppm)	Regression results	Slope function(SF)	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

Biochemical Analysis: Lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) were estimated following the techniques adopted^[18,21,33].

Statistical Analysis: One-way Analysis of Variance (ANOVA) was performed based on the methods of^[35].

RESULTS AND DISCUSSION

The lactate dehydrogenase (LDH) activity in the brain of the control crab was 2.43 and 2.25 mg / 100 mg wet tissue for 20 and 40 days respectively. In the experimental crabs, the LDH activity in the lower sublethal concentration was 2.89 and 3.03 mg /100 mg wet tissue and for higher sublethal level, it was 3.32 and 3.52 mg / 100 mg wet tissue for 20 and 40 days exposure periods. The increase in LDH activity of the brain calculated was found to be statistically insignificant.

In the thoracic ganglia of the control crab the LDH activity was found to be 3.87 and 3.81 mg / 100 mg wet tissue for 20 and 40 days of exposure periods. In the experimental crabs, the LDH activity of the lower sublethal concentration was 4.02 and 4.23 mg / 100 mg wet tissue and in the crabs treated with higher sublethal concentration, it was 4.67 and 4.74 mg / 100 mg wet tissue for 20 and 40 days of treatment. In the 40 days of exposure, maximum LDH activity in the thoracic ganglia was observed in both the sublethal concentrations. The readings were found to be statistically insignificant in both the lower and higher sublethal concentrations of cypermethrin.

In the control crabs, the LDH activity in the eyestalk was 2.97 and 2.77 mg / 100 mg wet tissue for 20 and 40 days of experimental periods respectively. In the experimental crabs treated with lower sublethal level, the LDH activity increased and it was found to be 3.15 and 3.27 mg / 100 mg wet tissue for 20 and 40 days of treatment. In higher sublethal level, the LDH activity further increased to 3.57 and 3.68 mg / 100 mg wet tissue for 20 and 40 days of exposure periods. The maximum increase in the enzyme activity was found in the 40 days of treatment period in both the sublethal concentrations of cypermethrin and the analyzed values were found to be statistically insignificant.

The succinate dehydrogenase (SDH) activity in the brain of the control crab was 6.54 and 6.66 MIU/min/mg protein for 20 and 40 days of treatment respectively. In the experimental crabs, the SDH activity was decreased for both the sublethal concentrations. The succinate dehydrogenase (SDH) activity for lower sublethal concentration was found to be 6.39 and 5.28 MIU/min/mg protein and for higher sublethal concentration, it was 6.12 and 4.23 MIU/min/mg protein for 20 and 40 days of exposure periods. Maximum decrease in SDH activity of the brain was observed in 40 days of exposure period and the decrease in SDH activity of the brain was statistically insignificant in both 20 and 40 days of treatment periods in both lower and higher sublethal concentration.

The SDH activity in the thoracic ganglia of the control crab was 7.34 and 7.22 MIU/min/mg protein for 20 and 40 days of treatment periods. In the experimental crabs, the SDH activity in the lower sublethal concentration was found to be 6.27 and 6.04 MIU/min/mg protein and in the crabs treated with higher sublethal concentration was 5.75 and 4.99 MIU/min/mg protein for 20 and 40 days of experimental periods respectively. The decline in SDH activity of the thoracic ganglia was found to be statistically significant in both the days of exposure and in both the sublethal concentrations of cypermethrin.

The SDH activity of eyestalk in the control crab was found to be 4.85 and 4.69 MIU/min/mg protein for 20 and 40 days of treatment respectively. In the experimental crabs, the SDH activity reduced to 4.23 and 3.88 MIU/min/mg protein in the lower sublethal concentration and in the higher sublethal concentration, the activity was further reduced to 3.67 and 3.12 MIU/min/mg protein for 20 and 40 days of exposure times respectively. The decline in the SDH activity of the eyestalk was statistically not significant for 20 days and significant for 40 days of experimental periods.

The acid phosphatase (ACP) activity in the brain of the control crab was 3.67 and 3.58 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment respectively. In the experimental crabs, the ACP activity increased for both the sublethal concentrations of cypermethrin. The ACP activity in the lower sublethal concentration was 3.87 and 4.33 mg PNPP to PNP/100mg wet tissue and for higher sublethal level, it was found

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

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days of exposure	Brain	2.43 ± 0.61	2.89 ± 0.49	3.32 ± 0.54	2.21 *	<0.05
	Thoracic ganglia	3.87 ± 0.61	4.02 ± 0.38	4.67 ± 1.01	1.02 *	<0.05
	Eyestalk	2.97 ± 0.51	3.15 ± 0.32	3.57 ± 0.60	2.26 *	<0.05
40 days of exposure	Brain	2.25 ± 0.61	3.03 ± 0.42	3.52 ± 0.71	2.76 *	<0.05
	Thoracic ganglia	3.81 ± 0.58	4.23 ± 0.31	4.74 ± 0.40	3.12 *	<0.05
	Eyestalk	2.77 ± 0.42	3.27 ± 0.71	3.68 ± 0.66	1.71 *	<0.05

Mean ± SD of six individual observations

Values are expressed mg / 100 mg wet 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 (SDH)

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days of exposure	Brain	6.54 ± 0.32	6.39 ± 0.64	6.12 ± 0.54	0.56*	<0.05
	Thoracic ganglia	7.34 ± 0.28	6.27 ± 0.56	5.75 ± 0.58	6.27*	<0.05
	Eyestalk	4.85 ± 0.41	4.23 ± 0.52	3.67 ± 0.49	4.23*	<0.05
40 days of exposure	Brain	6.66 ± 0.51	5.28 ± 0.75	4.23 ± 0.77	10.27**	<0.01
	Thoracic ganglia	7.22 ± 0.41	6.04 ± 0.72	4.99 ± 0.74	6.88*	<0.05
	Eyestalk	4.69 ± 0.43	3.88 ± 0.53	3.12 ± 0.83	4.11*	<0.05

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

Table 4: Effect of lower and higher sublethal concentrations of cypermethrin on acid phosphatase (ACP)

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days of exposure	Brain	3.67 ± 0.21	3.87 ± 0.58	4.06 ± 0.46	0.88 *	<0.05
	Thoracic ganglia	4.03 ± 0.32	4.37 ± 0.51	4.65 ± 0.44	0.46 *	<0.05
	Eyestalk	3.17 ± 0.51	3.79 ± 0.62	4.41 ± 1.03	1.94 *	<0.05
40 days of exposure	Brain	3.58 ± 0.22	4.33 ± 1.26	5.09 ± 1.08	2.28 *	<0.05
	Thoracic ganglia	4.08 ± 0.34	4.96 ± 0.42	5.54 ± 0.98	3.76 *	<0.05
	Eyestalk	3.21 ± 0.44	3.42 ± 0.81	4.07 ± 0.43	1.02 *	<0.05

Mean ± SD of six individual observations.

Values are expressed mg PNPP to PNP/100 mg wet tissue

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

Table 5: Effect of lower and higher sublethal concentrations of cypermethrin on alkaline phosphatase (ALP)

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days of exposure	Brain	7.35 ± 0.71	6.62 ± 0.72	5.88 ± 0.51	5.13**	<0.01
	Thoracic ganglia	5.27 ± 0.53	4.83 ± 0.26	4.45 ± 0.57	2.08*	<0.05
	Eyestalk	5.89 ± 0.32	5.38 ± 0.71	4.94 ± 0.43	1.92*	<0.05
40 days of exposure	Brain	7.28 ± 0.63	6.07 ± 0.54	5.70 ± 0.58	8.12**	<0.01
	Thoracic ganglia	5.23 ± 0.51	4.55 ± 0.82	4.17 ± 0.61	2.94*	<0.05
	Eyestalk	5.77 ± 0.36	5.13 ± 0.51	4.81 ± 0.59	1.97*	<0.05

Mean ± SD of six individual observations.

Values are expressed mg PNPP to PNP/100 mg wet tissue

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

to be 4.06 and 5.09 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure. The increase in enzyme activity of the brain was statistically significant. The ACP activity in the thoracic ganglia of the control crabs was analyzed as 4.03 and 4.08 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. In the experimental crabs, the ACP activity at the lower sublethal concentration was 4.37 and 4.96 mg PNPP to PNP/100 mg wet tissue and in higher sublethal concentration, it was found to be 4.65 and 5.54 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of experimental periods. The ACP activity was found to be statistically significant in the 20 days of exposure period and statistically insignificant in 40 days of exposure period. In the eyestalk, the ACP activity was found to be 3.17 and 3.21 mg PNPP to PNP/100 mg wet tissue in control. In the experimental crabs, the acid phosphatase (ACP) activity in the eyestalk was increased to 3.79 and 3.42 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods in lower sublethal concentration. In the higher sublethal level, the ACP activity was further increased to 4.41 and 4.07 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period. The increase in enzyme activity of eyestalk was statistically significant on both exposure periods.

The alkaline phosphatase (ALP) activity in the brain of the control crab was 7.35 and 7.28 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days respectively. The ALP of brain in the lower sublethal concentration was 6.62 and 6.07 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods and in the higher sublethal concentration was 5.88 and 5.70 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. The decrease ALP activity in the brain was statistically significant at 20 day and 40 day of exposure periods. The thoracic ganglia of crabs exposed to lower sublethal concentration expressed 4.83 and 4.55 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment periods. When the crabs treated with higher sublethal concentration, it was 4.45 and 4.17 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. In the control crabs, the enzyme activity was found to be 5.27 and 5.23 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. The values were found to be statistically significant on 20 and 40 days of exposure period in both the lower and higher sublethal concentrations of cypermethrin. In control crabs, the ALP activity in the eyestalk was 5.89 and 5.77 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. In the experimental crabs treated with lower sublethal concentration, the ALP activity was decreased to 5.38 and 5.13 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment periods. When the crabs were

treated with higher sublethal concentration, the ALP activity further decreased to 4.94 and 4.81 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. The decrease in ALP activity in the eyestalk was statistically insignificant on both 20 days and 40 days of treatment periods.

The results obtained in the present study of the effect of cypermethrin, a pyrethroid compound on a fresh water crab, *Spiralothelphusa hydrodroma* at two different sublethal concentrations and two different exposure periods showed interesting results. Enzymatic investigations of succinate dehydrogenase, lactate dehydrogenase, acid phosphatase and alkaline phosphatase at lower (0.1315ppm) and higher (0.4383) sublethal concentrations of cypermethrin on the brain, thoracic ganglia and eyestalk revealed highly fascinating informations and also study of bioaccumulation.

Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden^[14]. In the present study, the enzyme activity in succinate dehydrogenase, lactate dehydrogenase, acid phosphatase and alkaline phosphatase were estimated in both control crabs and the crabs treated with lower (0.1315ppm) and higher (0.4383) sublethal concentrations of cypermethrin.

The decline in the activity of respiratory oxidative enzyme, the succinate dehydrogenase in brain, thoracic ganglia and eyestalk indicates decline in enzyme synthesis, since cypermethrin disrupt the membrane bound enzyme. Mitochondrial damage leads to decreased respiration and partial coupling of oxidative phosphorylation^[3,4]. Suppression of succinate dehydrogenase activity indicates anoxic or hypoxic conditions when exposed to toxicants and was possibly due to mitochondrial disruption, leading to decrease in enzyme activity. Succinate dehydrogenase enzyme plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism^[4]. The results of the present study are also in conformity with those of the earlier observations.

Decreased succinate dehydrogenase activity and increased lactate dehydrogenase activity was reported by many workers namely in *O. senex* in response to sumithion^[28]. On the contrary lactate dehydrogenase activity increased in the hepatopancreas in the fielder crab, *U. pugilator* and decreased in the abdominal muscle when exposed to cadmium^[9] and in *S. serrata* in response to cadmium^[27].^[6] 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. The lactate dehydrogenase activity increased in the *U. annulipes* treated with sublethal concentrations of cadmium and

mercury^[32] and in *S.hydrodroma* in response to copper and zinc^[17]. 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.

Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes, due to the disruption of the membrane^[7]. Phosphatases play an important role in carbohydrate metabolism^[12].^[22] reported increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas^[24].^[2] were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase. It was concluded that both induction and inhibition of phosphatase takes place depending on the concentration of metals.^[26] concluded that sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatases. Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphate to release energy in view of impaired ATPase system during metal stress^[27].

Alkaline phosphatase is a brush border enzyme that splits various phosphorus esters at an alkaline pH and mediates membrane transport^[11]. It is also involved in synthesis of certain enzymes^[31], active transport^[8], protein synthesis^[25], glycogen metabolism^[13] and secretory activity^[16]. Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways.^[5] studied the effect of pyrethroid and mortality on the fish *Clarias batrachus* and found that alkaline phosphatase decreased in response to the toxicant.^[2] studied the effect of copper on oxygen consumption and phosphatase in *S.serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph. Similar observations were noted by^[10] in the same crab in response to naphthalene. In the present investigation, the activity of alkaline phosphatase was found to decrease in the experimental crabs when compared with that of the control crabs.

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