Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(2): 123-126

Research Article ISSN 0975-248X

Effect of Phosphatases Activity in the Hepatopancreas and Muscle of the Fresh Water Female Field Crab, *Spiralothelphusa hydrodroma* (Herbst) Treated with Cypermethrin

R. S. Sreenivasan[1*], P. Krishna Moorthy[2], M. Deecaraman[3]

[1]*Department of Chemistry, VELTECH MULTITECH Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai-62, India*
[2]*Department of Bioinformatics, Bharath University, Chennai-73, India*
[3]*Department of Industrial Biotechnology, Dr. MGR University, Chennai-95, 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 LC_{50} value was carried out. Effect of cypermethrin on the biochemical changes in the hepatopancreas and muscle was observed. Quantitative study of biochemical changes of acid phosphatase and alkaline phosphatase were undertaken.

Keywords: Hepatopancreas, muscle, acid phosphatase, alkaline phosphatase, cypermethrin, LC_{50}, *Spiralothelphusa hydrodroma.*

INTRODUCTION

The toxic substances include in insecticides, herbicides, fungicides, molluscides and nematicides. [1] 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. [2-3] The trace metal concentration in Queensland Estuarine crabs, *Australoplax tridentate* and *Scylla serrata* has been observed. [4] The present investigation is aimed to study the effect of cypermethrin on the *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 LC_{50} values (Table 1). 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 h exposure to cypermethrin was corrected for natural response by Abbott's formula. [5] The LC_{50} values for 24, 48, 72 and 96 h of exposure periods were estimated at 2.027, 1.698, 1.452 and 1.315 ppm respectively.

Design of Sub lethal Toxic Study

Chronic time course study on the effect of cypermethrin on the crab was conducted by exposing to two sub lethal, safe concentrations for 20 and 40 days. According to Sprague, 1971 [6], $1/10^{th}$ and $1/3^{rd}$ of the 96 h LC_{50} value represent lower and higher sub lethal concentrations respectively. Hence lower (0.1315 ppm) and higher (0.4383 ppm) sub lethal concentrations of the insecticide were arbitrarily used. At the end of the treatment period, the control and treated crabs were dissected and the hepatopancreas and muscle were collected for biochemical studies.

***Corresponding author: Dr. R. S. Sreenivasan,** Department of Chemistry, VELTECH MULTITECH Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai-600062, India; **Tel.:** +91-94441 20348; **E-mail:** rssvasan1973@yahoo.co.in, rssvasan1973@rediffmail.com

Biochemical Analysis: Acid phosphatase and alkaline phosphatase were estimated following the techniques adopted. [7]

Statistical Analysis: One-way Analysis of Variance (ANOVA) was performed based on the methods of devised by Winer. [8]

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

Table 2: Effect of Lower and Higher Sub lethal Concentrations of Cypermethrin on Acid Phosphatase Activity

Period of Exposure	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days	Hepatopancreas	5.46 ± 0.57	6.37 ± 0.68	6.85 ± 0.58	5.85 **	<0.01
	Muscle	3.87 ± 0.53	4.68 ± 0.52	5.07 ± 0.53	4.88 **	<0.01
40 days	Hepatopancreas	5.34 ± 0.71	6.29 ± 0.62	6.80 ± 0.86	4.14 **	<0.01
	Muscle	3.83 ± 0.52	4.30 ± 0.68	4.86 ± 0.33	4.58 **	<0.01

Mean ± SD of six individual observations
Values are expressed µg PNPP to PNP/100 mg wet tissue
* * indicates significance at 0.01 level
* indicates significance at 0.05 level

Table 3: Effect of Lower and Higher Sub lethal Concentrations of Cypermethrin on Alkaline Phosphatase Activity

Period of Exposure	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
20 days	Hepatopancreas	7.18 ± 0.52	6.43 ± 0.61	6.77 ± 0.58	4.76*	<0.05
	Muscle	4.36 ± 0.74	4.10 ± 0.52	3.35 ± 0.43	2.23*	<0.05
40 days	Hepatopancreas	7.12 ± 0.53	6.57 ± 0.72	5.68 ± 0.62	5.85**	<0.01
	Muscle	4.26 ± 0.68	3.68 ± 0.56	3.26 ± 0.54	2.12*	<0.05

Mean ± SD of six individual observations
Values are expressed µg PNPP to PNP/100 mg wet tissue
* * indicates significance at 0.01 level
* indicates significance at 0.05 level

RESULTS

The acid phosphatase (ACP) activity in the hepatopancreas (Table 2, Fig. 1) of the control crab was 5.46 and 5.34µg 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 sub lethal concentrations of cypermethrin. The ACP activity in the lower sub lethal concentration was 6.37 and 6.29 µg PNPP to PNP/100 mg wet tissue and for higher sub lethal level, it was found to be 6.85 and 6.80 µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure. The increase in enzyme activity of the hepatopancreas was statistically significant.

The ACP activity in the muscle (Table 2, Fig. 2) of the control crabs was analyzed as 3.87 and 3.83µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period. In the experimental crabs, the ACP activity at the lower sub lethal concentration was 4.68 and 4.30µg PNPP to PNP/100 mg wet tissue and in higher sub lethal concentration, it was found to be 5.07 and 4.86µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of experimental period. The ACP activity was found to be statistically significant in the both 20 and 40 days of exposure period.

The alkaline phosphatase (ALP) activity in the hepatopancreas (Table 3, Fig. 3) of the control crab was 7.18 and 7.12µg PNPP to PNP/100 mg wet tissue for 20 and 40 days respectively. The ALP of hepatopancreas in the lower sub lethal concentration was 6.43 and 6.57µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period and in the higher sub lethal concentration was 6.77 and 5.68µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period. The decrease ALP activity in the hepatopancreas was statistically insignificant at 20 days and statistically significant at 40 days of exposure period.

The muscle (Table 3, Fig. 4) of crabs exposed to lower sub lethal concentration expressed 4.10 and 3.68µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment period. When the crabs treated with higher sub lethal concentration, it was 3.35 and 3.26µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period. In the control crabs, the enzyme activity was found to be 4.36 and 4.26µg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure period. The values were found to be statistically insignificant on 20 and 40 days of exposure period in both the lower and higher sub lethal concentrations of cypermethrin.

DISCUSSION

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 sub lethal concentrations and two different exposure periods showed interesting results. Enzymatic investigations of acid phosphatase and alkaline phosphatase at lower and higher sub lethal concentrations of cypermethrin on the hepatopancreas and muscle revealed highly fascinating informations. Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden. [9] In the present study, the enzyme activity in acid phosphatase and alkaline phosphatase were estimated in both control crabs and the crabs treated with lower (0.1315 ppm) and higher (0.4383 ppm) sub lethal concentrations of cypermethrin.

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. [10] Phosphatases play an important role in

carbohydrate metabolism. [11] Norseth [12] 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. [13] Ahmed *et al* [14] pointed 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.

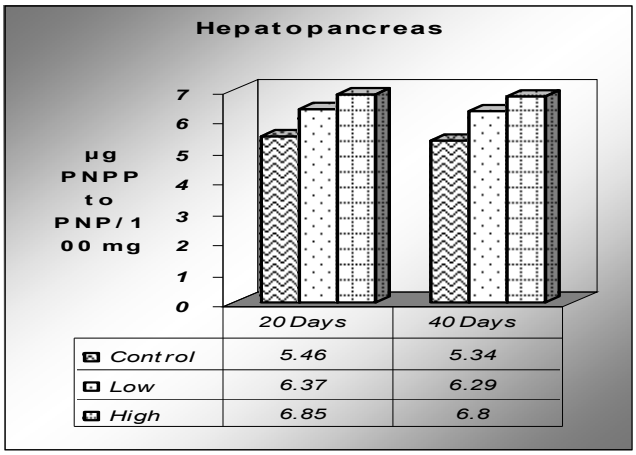

Fig. 1: Effect of Lower and Higher Sub lethal Concentrations of Cypermethrin on Acid Phosphatase Activity in Hepatopancreas

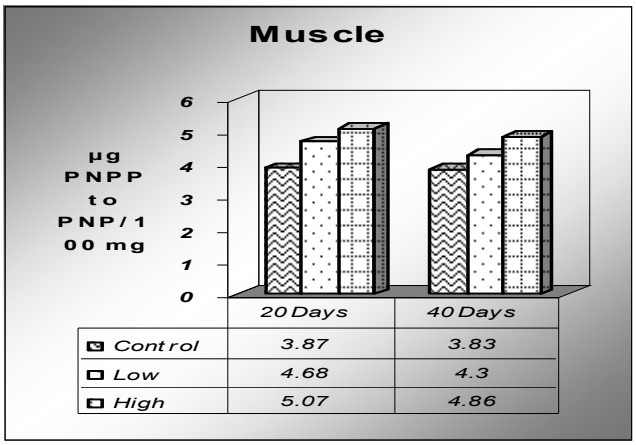

Fig. 2: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Acid Phosphatase Activity in Muscle

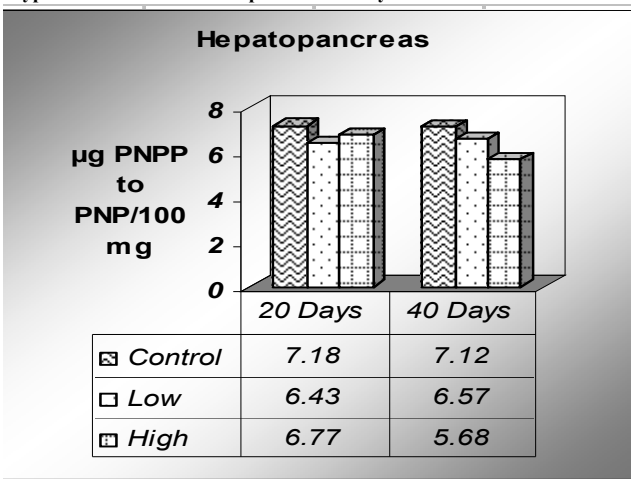

Fig. 3: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Alkaline Phosphatase Activity in Hepatopancreas

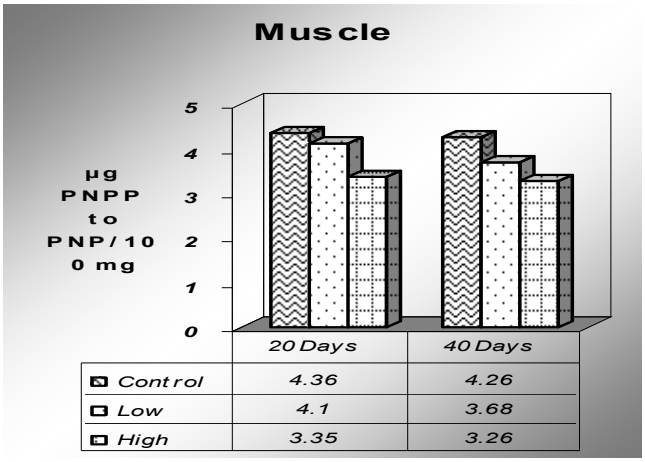

Fig. 4: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Alkaline Phosphatase Activity in Muscle

Reddy *et al* [15] 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. [16]

Any alterations in the activity of alkaline phosphatase affect the organisms in a verity of ways. Alkaline phosphatase is a brush border enzyme that splits various phosphorus esters at an alkaline pH and mediates membrane transport. [17] It is also involved in synthesis of certain enzymes [18], active transport [19], protein synthesis [20], glycogen metabolism [21] and secretory activity. [22] Bhatnagar *et al* [23] studied the effect of pyrethroid and mortaliry on the fish *Clarias bactrachus* and found that alkaline phosphatase decreased in response to the toxicant. Ahmed *et al* [14] 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 in the same crab in response to naphthalene. [24] 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.

ACKNOWLEDGEMENT

Authors sincerely thank Dr. R. Rangarajan, Chairman, Dr. Sakunthala Rangarajan, Vice-Chairman, VELTECH Group of Educational Institutions and Dr. K. Siddappa Naidu, Principal, VELTECH MULTITECH Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai–600062, India, for their unremitting encouragement for publishing this paper successfully.

REFERENCES

1. Hayes WJ. Toxicology of Pesticides. The Williams and Wilkins, Baltimore, 1975; 37-106.
2. Matsumura F. Toxicology of Insecticides. Plenom Press, New York, 1975.
3. O' Brien RD. Insecticides: Action and Metabolism. Academic Press, New York, 1967.
4. Mortimer MR. Pesticide and Trace Metal Concentrations in Queensland Estuarine Crabs. Marine Pollut. Bull. 2000; 41(7-12): 359-366.
5. Abbott WS. A Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomo. 1925; 18: 265-267.

6. Sprague JB. Measurement of Pollutant Toxicology of Fish III: Sub lethal Effects and Safe Concentrations. Wat. Res. 1971; 5: 245-266.
7. King J. In: Practical Clinical Enzymology. D.Van Norstrand Co., London, 1965.
8. Winer BJ. Statistical Principles in Experimental Design II. McGraw-Hill, New York, 1971.
9. Hansen JI, Mustafa T, Depledge M. Mechanism of Copper Toxicity in the Shore Crab, *Carcinus maenas:* Effects on Na, K-ATPase activity, Hemolymph Electrolyte Concentrations and Tissue Water Contents. Mar. Biol. 1992; 114(2): 253-257.
10. Deduve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Intracellular Distribution Patterns of Enzymes in Rat Liver Tissue. Biochem. J. 1985; 60: 604-617.
11. Goodman J, Rothstein A. The Active Transport of Phosphate into the Yeast Cell. J. Genet. Physiol. 1957; 40: 915-925.
12. Norseth T. The Intracellular Distribution of Mercury in Rat Liver after Single Injection of Mercuric Chloride. Biochem. Pharmacol. 1967; 17: 581-593.
13. O' Connor JD, Gilbert LI. Aspects of Lipid Metabolism in Crustaceans. Am. Zool. 1968; 8: 529-539.
14. Ahmed MR, Elumalai M, Balasubramanian SE, Balasubramanian MP. Individual and Combined Effect of Copper and Chromium on Oxygen Consumption and Phosphatases of a Marine Edible Crab, *Scylla seratta.* Biomed. Lett. 1997; 55: 147-152.
15. Reddy PS, Bhagyalakshmi A, Ramamurthi R. *In vivo* Subacute Physiological Stress Induced by Sumithion on the Hepatopancreatic Acid Phosphatase Activity in the Fresh Water Crab, *Oziotelphusa senex senex*. Water Air Soil Pollut. 1984; 22: 299-302.
16. Reddy PS, Bhagyalakshmi A. Changes in Oxidative Metabolism in Selected Tissues of the Crab, *Scylla serrata* in response to Cadmium Toxicity. Ecotoxicol. Environ. Saf. 1994; 29(3): 255-264.
17. Goldfisher SE, Esser E, Novikoff AB. In: Use of Histological and Histochemical Assessment in the Prognosis of the Effects of Aquatic Pollutants. Amer. Soc. Test. Mat. Philadelphia. 1964.
18. Sumner AT. The Cytology and Histochemistry of the Digestive Gland of *Helix aspersa.* Quart. J. Microsc. Sci. 1965; 106: 173-192.
19. Denielli JF. Structural Factors in Cell Permeability and Secretion. Symp. Soc. Exp. Biol. 1972; 6: 1-15.
20. Pilo B, Ansari MV, Shah RV. Studies of Wound Healing and Repair in Pigeon Liver III: Histochemical Studies on Acid and Alkaline Phosphatase Activities during the Process. J. Anim. Morphol. Physiol. 1972; 19: 205-212.
21. Gupta V, Rao G. Histological Studies on the Chloride Plexus of the Goat Embryos II: Histological Distribution of Acid and Alkaline Phosphatases. Acta. Histochem. 1974; 49: 253-257.
22. Ibrahim AM, Higazi MG, Demian ES. Histochemical Location of Alkaline Phosphatase Activity in the Alimentary Tract of the Snail, *Marisa carnvarietus*. Zool. Soc. Egypt. Bull. 1974; 26: 94-105.
23. Bhatnagar MC, Tyagi M, Tamata S. Pyrethroid Induced Toxicity to Phosphatases in *Clarias bactrachus*. J. Environ. Biol. 1995; 16(1): 11-14.
24. Elumalai M, Balasubramanian SE, Balasubramanian MP. Influence of Naphthalene on Protein, Carbohydrate and Phosphatases System during the Vitellogenesis Marine Edible Crab, *Scylla serrata*. Bull. Environ. Contam. Toxicol. 1998; 60: 25-29.