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Impact of Heavy Metal Copper on the Neurosecretory Cells in a Freshwater Field Crab, *Spiralothelphusa hydrodroma*

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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 copper on the experimental crab for the LC_{50} value was carried out. Effect of copper 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.

Keywords: Neurosecretory cells, brain, thoracic ganglia, eyestalk, Copper, *Spiralothelphusa hydrodroma*

INTRODUCTION

The heavy metals include in insecticides, herbicides, fungicides, molluscides and nematicides[8]. 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[17,14]. The trace metal concentration in Queensland estuarine crabs, *Australoplax tridentate* and *Scylla serrata* has been observed[15]. The present work was that the effect of heavy metal, copper as copper sulphate 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 3.27 cm to 4.86 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 copper for static but renewal type of bioassay was adopted in the present investigation to estimate the LC_{50} values. The heavy metal, copper was used as commercial preparation of copper sulphate. 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 copper; were corrected for natural response by Abbott's formula [1]. The LC_{50} values for 24 hr, 48 hr, 72 hr and 96 hr of exposure periods were estimated as 271.01, 265.46, 257.03 and 254.68 ppm respectively (Table: 1).

Design of Sublethal Toxic Study: Chronic time course study on the effect of copper on the crab was conducted by exposing to two sublethal, safe concentrations for 15 days and 30 days. According to[12,23], $1/3^{rd}$ and $1/10^{th}$ of the 96 hr LC_{50} value represent higher and lower sublethal concentrations respectively. Hence lower (25.46 ppm) and higher (84.66 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.

Biochemical Analysis: Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) were estimated following the techniques adopted[16,11,26].

Statistical Analysis: One-way Analysis of Variance (ANOVA) was performed based on the methods of[29].

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

Exposure periods (hours)	LC_{50} (ppm)	Upper Confidence limits (ppm)	Lower Confidence limits (ppm)	Regression results	Slope function (SF)	r^2
24	271.01	297.29	248.24	Y = - 143.77 X + 61.06	1.040	0.99
48	265.46	288.60	244.17	Y = - 116.02 X + 49.91	1.042	0.99
72	257.03	281.06	235.06	Y = - 143.36 X + 61.42	1.044	0.98
96	254.68	277.12	234.25	Y = - 121.88 X + 52.72	1.050	0.98

RESULTS AND DISCUSSIONS

Effect of Copper on Lactate Dehydrogenase (LDH) (Table: 2): The lactate dehydrogenase (LDH) activity in the brain of the control crab was 4.11 and 4.04 mg / 100 mg wet tissue for 15 and 30 days respectively. In the experimental crabs, the LDH activity in the lower sublethal concentration was 4.56 and 4.69 mg /100 mg wet tissue and for higher sublethal level, it was 4.90 and 5.03 mg / 100 mg wet tissue for 15 and 30 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 4.40 and 4.35 mg / 100 mg wet tissue for 15 and 30 days of exposure periods. In the experimental crabs, the LDH activity of the lower sublethal concentration was 4.55 and 4.72 mg / 100 mg wet tissue and in the crabs treated with higher sublethal concentration, it was 5.10 and 5.15 mg / 10 mg wet tissue for 15 and 30 days of treatment. In the 30 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 copper.

In the control crabs, the LDH activity in the eyestalk was 4.03 and 3.98 mg / 100 mg wet tissue for 15 and 30 days of experimental periods respectively. In the experimental crabs treated with lower sublethal level, the LDH activity increased and it was found to be 4.27 and 4.55 mg / 100 mg wet tissue for 15 and 30 days of treatment. In higher sublethal level, the LDH activity further increased to 4.84 and 4.83 mg / 100 mg wet tissue for 15 and 30 days of exposure periods. The maximum increase in the enzyme activity was found in the 30 days of treatment period in both the sublethal concentrations of copper, and the analyzed values were found to be statistically insignificant.

Effect of Copper on Succinate Dehydrogenase (SDH) (Table: 3): The succinate dehydrogenase (SDH) activity in the brain of the control crab was 7.84 and 7.87 MIU/min/mg protein for 15 and 30 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 7.72 and 6.72 MIU/min/mg protein and for higher sublethal concentration, it was 7.48 and 5.86 MIU/min/mg protein for 15 and 30 days of exposure periods. Maximum decrease in SDH activity of the brain was observed in 30 days of exposure period, and the decrease in SDH activity of the brain was statistically insignificant in both 15 and 30 days of treatment periods in both lower and higher sublethal concentration.

The SDH activity in the thoracic ganglia of the control crab was 8.41 and 8.33 MIU/min/mg protein for 15 and 30 days of treatment periods. In the experimental crabs, the SDH activity in the lower sublethal concentration was found to be 7.49 and 7.22 MIU/min/mg protein, and in the crabs treated with higher sublethal concentration was 7.28 and 6.68 MIU/min/mg protein for 15 and 30 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 copper.

The SDH activity of eyestalk in the control crab was found to be 5.82 and 5.71 MIU/min/mg protein for 15 and 30 days of treatment respectively. In the experimental crabs, the SDH activity reduced to 5.07 and 5.06 MIU/min/mg protein in the lower sublethal concentration and in the higher sublethal concentration, the activity was further reduced to 4.92 and 4.41 MIU/min/mg protein for 15 and 30 days of exposure times respectively The decline in the SDH activity of the eyestalk was statistically not significant for 15 days and significant for 30 days of experimental periods.

Effect of Copper on Acid Phosphatase (ACP) (Table: 4): The acid phosphatase (ACP) activity in the brain of the control crab was 4.72 and 4.64 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of treatment respectively. In the experimental crabs, the ACP activity increased for both the sublethal concentrations of copper. The ACP activity in the lower sublethal concentration was 4.98 and 5.45 mg PNPP to PNP/100mg wet tissue and for higher sublethal level, it was found to be 5.13 and 6.15 mg PNPP to PNP/100 mg wet tissue for 15 and 30

Table 2: Effect of sublethal concentrations on LDH

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15 days of exposure	Brain	4.11 ± 0.63	4.56 ± 0.52	4.90 ± 0.59	2.70	0.0996NS
	Thoracic ganglia	4.40 ± 0.72	4.55 ± 0.46	5.10 ± 1.08	1.26	0.3123 NS
	Eyestalk	4.03 ± 0.62	4.27 ± 0.49	4.84 ± 0.66	2.93	0.0845 NS
30 days of exposure	Brain	4.04 ± 0.68	4.69 ± 0.44	5.03 ± 0.78	3.53	0.556 NS
	Thoracic ganglia	4.35 ± 0.67	4.72 ± 0.38	5.15 ± 0.44	3.51	0.562 NS
	Eyestalk	3.98 ± 0.59	4.55 ± 0.81	4.83 ± 0.76	2.15	0.1513 NS

Mean ± SD of six individual observations
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test). NS- Not significant

Table 3: Effect of sublethal concentrations of on SDH

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15 days of exposure	Brain	7.84 ± 0.39	7.72 ± 0.68	7.48 ± 0.56	0.67	0.5287 NS
	Thoracic ganglia	8.41 ± 0.32	7.49 ± 0.61	7.28 ± 0.65	7.09	0.0068 *
	Eyestalk	5.82 ± 0.44	5.07 ± 0.55	4.92 ± 0.53	5.37	0.0174 *
30 days of exposure	Brain	7.87 ± 0.62	6.72 ± 0.76	5.86 ± 0.80	11.19	0.0011 *
	Thoracic ganglia	8.33 ± 0.48	7.22 ± 0.77	6.68 ± 0.81	8.55	0.0033 *
	Eyestalk	5.71 ± 0.50	5.06 ± 0.57	4.41 ± 0.91	5.26	0.0186 *

Mean ± SD of six individual observations.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test).
NS – Not Significant.

Table 4: Effect of sublethal concentrations of on ACP

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15 days of exposure	Brain	4.72 ± 0.25	4.98 ± 0.67	5.13 ± 0.54	0.96	0.4037 NS
	Thoracic ganglia	4.89 ± 0.40	5.14 ± 0.60	5.18 ± 0.53	0.55	0.5862 NS
	Eyestalk	4.21 ± 0.56	4.91 ± 0.78	5.27 ± 1.16	2.26	0.0296 NS
30 days of exposure	Brain	4.64 ± 0.28	5.45 ± 1.37	6.15 ± 1.17	3.08	0.076 NS
	Thoracic ganglia	4.86 ± 0.43	5.77 ± 0.51	6.01 ± 1.02	4.49	0.296 *
	Eyestalk	4.23 ± 0.58	4.45 ± 0.96	4.88 ± 0.52	1.25	0.3143 NS

Mean ± SD of six individual observations.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison
NS- Not significant

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 4.89 and 4.86 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. In the experimental crabs, the ACP activity at the lower sublethal concentration was 5.14 and 5.77 mg PNPP to PNP/100 mg wet tissue and in higher sublethal concentration, it was found to be 5.18 and 6.01 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of experimental periods. The ACP activity was found to be statistically significant in the 15 days of exposure period and statistically insignificant in 30 days of exposure period. In the eyestalk, the ACP activity was

found to be 4.21 and 4.23 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 4.91 and 4.45 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods in lower sublethal concentration. In the higher sublethal level, the ACP activity was further increased to 5.27 and 4.88 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure period. The increase in enzyme activity of eyestalk was statistically significant on both exposure periods.

Effect of Copper on Alkaline Phosphatase (ALP) (Table: 5): The alkaline phosphatase (ALP) activity in the brain of the control crab was 8.28 and 8.23 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days respectively. The ALP of brain in the lower sublethal concentration was 7.53 and 6.90 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods and in the higher sublethal concentration was 6.82 and 6.73 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. The decrease ALP activity in the brain was statistically significant at 15 day and 30 day of exposure periods. The thoracic ganglia of crabs exposed to lower sublethal concentration expressed 6.44 and 6.42 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of treatment periods. When the crabs treated with higher sublethal concentration, it was 6.06 and 5.75 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. In the control crabs, the enzyme activity was found to be 5.67 and 5.34 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. The values were found to be statistically significant on 15 and 30 days of exposure period in both the lower and higher sublethal concentrations of copper.In control crabs, the ALP activity in the eyestalk was 6.04 and 5.96 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. In the experimental crabs treated with lower sublethal concentration, the ALP activity was decreased to 5.55 and 5.35 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of treatment periods. When the crabs were treated with higher sublethal concentration, the ALP activity further decreased to 5.26 and 5.17 mg PNPP to PNP/100 mg wet tissue for 15 and 30 days of exposure periods. The decrease in ALP activity in the eyestalk was statistically insignificant on both 15 days and 30 days of treatment periods.The inhibition of succinate dehydrogenase in the fish, *Tilapia mossambica* due to the exposure of the pesticide sevin was reported [13]. Decrease in succinate dehydrogenase (SDH) activity in the tissues of the same fish during methyl parathion exposurewas reported[22]. The reduction of succinate dehydrogenase activity in the fish, *Sacobranchus fossilis* in response to thiomidon toxicity was reported[27]. The reduced activity of the enzyme succinate dehydrogenase activity in different tissues in the fish, *Hereropneustes fossilis* in response todimethoate stress[28]. The effects of metal mixtures in the fish, *Oreochromis mossambicus* and the suppression of succinate dehydrogenase activity indicated anoxic hypoxic conditions when the fish was exposed to toxicant and it was possibly due to mitochondrial disruption, leading to decrease in the activities of oxidative enzymes and an increase in glycolic enzymes[4,10].Present findings showed decrease in the activity of the respiratory oxidative enzyme, succinate dehydrogenase (SDH) activity in the brain, thoracic ganglia and eyestalk. This clearly shows the disturbance in enzyme synthesis, since the pesticides are known to disrupt the membrane bound enzymes. The inhibition of SDH suggests that the metabolic pathway might have turned anaerobic to meet the increased energy demand during pollution stress. The present observations tally with the earlier reports made by[25] in the brackish water crab *Uca annulipes*.The increase in lactate dehydrogenase (LDH) activity has been reported by[5] in the fish *Channa punctatus* treated with the pesticides, quinalphos, dichlorvos and suquin. The elevation of lactate dehydrogenase activity in the muscle and gill of the freshwater fish, *Heteropneustes fossilis* was due to the pesticide rogor exposure[19]. The sublethal effects of the pesticide cypermethrin on enzyme activities in the freshwater fish *Cyprinus carpio* and observed that the lactate dehydrogenase activity increased after and 8 and 12 days of treatment[21]. In the present study the elevation in the lactate dehydrogenase (LDH) activity of the crab *Spiralothelphusa hydrodroma* treated with chlorpyrifos might have been increased depending on anaerobic carbohydrate metabolism, cumulative effect or possibly to meet the increased energy demands under sustained and prolonged toxic stress of chlorpyrifos.The intracellular distribution patterns of enzymes in the rat liver tissue and reported that generally the increased activity of acid phosphatase (ACP) activity attributed to the activation of enzyme, which was kept in latent state inside the membrane of lysosomes[2]. The effect of manganese in the cerebellum of rabbit increased the acid phosphatase activity[30]. The increased acid phosphatase activity in *Cavia porcellus* was because of the pesticide chlorpyrifos exposure[20]. In the present study, the increased acid phosphatase (ACP) activity was observed in both the lower (25.46 ppm) and higher (84.66 ppm) sublethal concentrations of copper in both 15 and 30 days of treatment. The increase in acid phosphatase activity was high in the higher (0.04 ppm) sublethal concentration in the 30 days of treatment. The increase

Table 5: Effect of sublethal concentrations of on ALP

	Tissues	Control	LSC	HSC	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15 days of exposure	Brain	8.28 ± 0.76	7.53 ± 0.80	6.82 ± 0.58	6.07	0.0117 *
	Thoracic ganglia	6.44 ± 0.59	6.06 ± 0.32	5.67 ± 0.64	3.00	0.0799 NS
	Eyestalk	6.04 ± 0.40	5.55 ± 0.79	5.26 ± 0.51	2.59	0.1081 NS
30 days of exposure	Brain	8.23 ± 0.69	6.90 ± 0.55	6.73 ± 0.72	9.24	0.0024 *
	Thoracic ganglia	6.42 ± 0.56	5.75 ± 0.89	5.34 ± 0.66	3.81	0.0458 NS
	Eyestalk	5.96 ± 0.43	5.35 ± 0.58	5.17 ± 0.67	3.08	0.0757 NS

Mean ± SD of six individual observations.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test).
NS – Not Significant.

in acid phosphatase activity may be inferred as a response to altered metabolism due to copper stress. The effect of the pollutants in aquatic animals and stated that alkaline phosphatase (ALP) is a brush border enzymes, which splits various phosphorous esters at an alkaline pH and mediated transport [6]. The involvement of alkaline phosphatase in active transport[3], glycogen metabolism[7], protein synthesis[18], synthesis of some enzymes[24] and secretory activity[9] were reported. Thus, any alteration in the activity of alkaline phosphatase affects the organisms. In the present investigation, the activity of alkaline phosphatase (ALP) was found to decrease in the tissues of the test crabs when compared with control crabs. The maximum decrease was seen in higher (84.66 ppm) sublethal concentration of copper for 30 days.

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