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RESEARCH ARTICLE

Attenuating Property OF *Delonix elata* Plant Against Inhibition of Acetylcholinesterase Due to Cypermethrin Toxicity on Fresh Water Fish *Cyprinus carpio* (Linn)

M.Meenambal, K.Pugazhendy*, C.Vasantharaja, S.Venkatesan and K.Jayachandran

Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India

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ABSTRACT

The toxic effect of cypermethrin on acetylcholinesterase and acetylcholine activity in the brain, gill and liver of *Cyprinus carpio* fresh water fish was investigated.120 hour's sublethal concentration (50µg/l) was used for experimental study. In the entire experiment acetylcholinesterase activity was inhibited in brain>gill>liver, where as acetylcholine activity was increased significantly P<0.05% level due to the toxic effect of cypermethrin. A markable inhibition in the activity of AChE recorded by the rapid accumulation of acetylcholine at nerve endings, leading to distruption of nervous activity by over stimulating the acetylcholine receptors leads to neurotoxicity. Present investigation indicates that brain was the main target organ for cypermethrin insecticide. *Delonix elata* leaves used as supplementary feed, to observe the attenuating property of the plant.

Key words: Cypermethrin, *Delonix elata*, Acetylcholinesterase, Acetylcholine, *Cyprinus carpi*.

1. INTRODUCTION

Cypermethrin is a class II synthetic pyrethroids insecticide, it is extensively used an agriculture and forestry because of high activity against a broad spectrum of insect pests [1]. Cypermethrin is less toxic to mammals, birds and highly toxic to fish. Due to their lipophilicity it have high rate of gill absorption even at very low concentrations in the water. Fishes are unable to metabolize the pyrethroids efficiently [2] . Which can affecting the Na^+ channels, delayed the activation and inactivation process. Cypermethrin also affecting the biochemical enzyme by mode of neurotransmitters like acetylcholine (Ach) and acetylcholinesterase (AChE) activities. Acetylcholine (Ach) is important cholinergic neurotransmitter released in the packaged form of vesicles from the synapse, the enzyme acetylcholinesterase (AChE) found in the synaptic cleft in bound form in proteoglycan matrix during neurotransmission enzyme substrate complex is formed. Acetylcholinesterase (AChE) splitting the acetylcholine into acetate and choline. This kind of biochemical interaction is very important for normal function of nerve signal transmission and regulating the acetylcholine concentration in the synapses. There are several studies indicating inhibition of acetylcholinesterase (AChE) as biomarker to monitoring and asses the pesticide pollution on aquatic environment [3,4]. Inhibition of AChE activity in the fish altered behavioral patterns in the laboratory. In the wild AChE inhibition could drastically affect growth, survival, feedings and reproductive behavior of any living fish [5]. In the present study to investigate the acetylcholinesterase and acetylcholine activity in the cypermethrin treated fresh water fish *Cyprinus carpio* and also monitoring the attenuating property of *Delonix elata* plant leaves against cypermethrin.

2. MATERIALS AND METHODS

2.1. Rearing of fish

The fish *Cyprinus carpio* 75g±5 of weight 15±5cm length were obtained from the Navarathna fish farm nearby Pinnaloor fish were safely transferred to the laboratory. They were kept in the cement tank filled with dechlorinated water and continuous aeration. Acclimatization to experimental condition for 15 days at room temperature fish were feed artificial libitam during acclimatization and tank water was renewed every day after feeding, food was withheld from before 24 hours to the experiment.

***Corresponding Author:** K. Pugazhendy**, Email:** pugalendy@rediffmail.com

2.2. Cypermethrin toxicity

Cypermethrin technical trade 98.8% pure were obtained from Gharda chemicals Mumbai, 1g of cypermethrin dissolved into 100 ml technical grade acetone, this stoke is used for daily requirement. The toxicant used for determining the sublethal concentration of cypermethrin. Toxicity of cypermethrin was evaluated by static bioassay method of Finney (1971) [6] and the LC_{50} value of 96 hour to be 250µg/l. One fifth of LC_{50} was selected as nominal sublethal concentration (50µg/l) and used in the present investigation to analyze the sub acute effect at exposure period of 24,48,72,96 and 120 hours respectively.

Group I -Fish reared in cypermethrin free water.

Group II -Fish exposed to 50 µg /l cypermethrin for five days.

Group III -Exposed to 50 µg/l cypermethrin for five days, fed with supplementary feed *Delonix elata.*

Group IV -Exposed to cypermethrin free water and feed with *Delonix elata* supplementary feed alone.

Supplementary feed:

Delonix elata leaves are collected around university campus. Leaves were washed with water and dried at room temperature; dried leaves were powdered then mixed with rice bran powder1:1 ratio and add some ml of distilled water to make small pellets. Then dried inside the room with better aeration. Dried pellet feed with Group III and IV were given at 10g daily during the experimental period. Aquaria, water renewed cypermethrin was added every day for Group II and III exposed to 120 hours. Six fishes of each group were sacrificing and removed the organs immediately within an hour's for the assay of AChE and ACh activity using brain, gill and liver tissues of the fish.

2.3. Assay method

Acetylcholinesterase and acetylcholine were determined by the method of Metacalf (1951) [7]. 2% homogenate prepared in 0.25M aliquot filter was taken and 0.5 ml of ferric chloride solution was added the intensity of the colour developed was measured at 545nm in a double beam spectrophotometer against reagent blank.

The statistical analysis of data was done using SPSS11.5, Mean ± SEM standard error and T value was calculated. The significance of the test result was observed at P<0.05%level. Percentage changes were calculated.

3. RESULTS

Although animals were slower its activity and no mortality were observed at the time of cypermethrin treated period.

3.1. Acetylcholinesterase activity

Acetylcholinesterase activity (AChE) was inhibited in the treated group II like brain, gill and followed by liver at the period of 24hour to 120 hours. At the end of 120 hours the treated group percentage changes decreased over the control group, group II brain (59%), gill (32%) and liver (27%). More inhibitory activity was observed in the brain tissues. Group III acetylcholinesterase activity in the various organs like brain, gill and liver were increased slowly up to 72 hours at the end of the 96 hours onwards acetylcholinesterase activity increased considerably. Group III at 120 hours percentage increased over the treated group brain (105%), gill (36%) and liver (21%). Supplementary feed group IV acetylcholinesterase activity was increased slightly due to the supplementary feed (**Table 1**).

Table 1: Changes in the level of acetylcholinesterase (µmoles acetylcholine hydrolysed /mg of protein/hr) activity in the freshwater fish *Cyprinus carpio* on the effect of cypermethrin and *D.elata* exposed at 120 hrs sublethal concentrations

Tissues	Groups	Hours of exposure				
		24	48	72	96	120
Brine	Group I	7.30 ± 0.01	7.30 ± 0.02	7.31 ± 0.02	7.32 ± 0.05	7.34 ± 0.05
	Group II	6.92 ± 0.02**	6.57 ± 0.09**	5.32 ± 0.03**	4.21 ± 0.02**	2.99 ± 0.02**
	COC	- 5.16	- 10.05	- 27.14	- 42.47	- 59.20
	Group III	7.11 ± 0.09*	6.81 ± 0.17*	6.41 ± 0.02**	6.54 ± 0.03**	6.56± 0.05**
	COC	- 2.64	- 6.81	- 12.21	- 10.76	- 9.36
	COT	+ 2.65	+ 3.60	+ 20.48	+ 55.12	+ 122.03
	Group IV	7.31 ± 0.01	7.33 ± 0.03	7.35 ± 0.03	7.41 ± 0.03	7.45 ± 0.08
	COC	+ 0.16	+ 0.31	+ 0.54	+1.10	+1.44
Gill	Group I	3.66 ± 0.01	3.67 ± 0.02	3.69 ± 0.03	3.72 ± 0.07	3.73 ± 0.08
	Group II	3.56 ± 0.04*	3.31 ± 0.05*	3.10 ± 0.03**	2.81 ± 0.07**	2.54 ± 0.05**
	COC	- 3.66	- 9.75	- 15.93	- 24.39	- 32.10
	Group III	3.60 ± 0.02	3.47± 0.07**	3.25 ± 0.15**	3.27 ±0.07**	3.47 ± 0.03**
	COC	- 1.44	-5.44	- 11.92	+12.07	+ 6.91
	COT	+ 12.09	+ 4.77	+ 4.77	- 16.29	- 36.37
	Group IV	3.68 ± 0.03	3.71 ± 0.08	3.73 ± 0.10	3.78 ± 0.06	3.80 ± 0.03
	COC	+ 0.68	+ 1.08	+ 1.10	+ 1.63	+ 1.87
Liver	Group I	2.32 ± 0.01	2.32 ± 0.06	2.33 ± 0.09	2.33 ± 0.04	2.34 ± 0.04
	Group II	2.15 ± 0.07*	2.02 ± 0.04*	1.82 ± 0.04**	1.74 ± 0.08**	1.62± 0.06**

COC	- 7.52	- 13.04	- 21.88	- 25.32	- 30.76
Group III	2.27 ± 0.15**	2.20± 0.12**	1.89 ± 0.01**	1.97 ± 0.01**	2.11 ± 0.03**
COC	- 2.36	- 5.41	- 18.88	- 15.55	- 9.65
COT	+ 5.58	+ 8.91	+ 3.84	+ 8.21	+ 21.28
Group IV	2.33 ± 0.07	2.34 ± 0.02	2.35 ± 0.04	2.36 ± 0.02	2.37 ± 0.04
COC	+ 0.21	+ 0.60	+ 0.8	+1.28	+ 1.28

*Mean± S.E - mean of six individual observation; *Significant at P<0.05% level. **Significant at P<0.01% level. (+,-) denotes increased and decreased. COC (percent change over to control) COT (percent change over to treated).*

3.2. Acetylcholine activity

Acetylcholine (ACh) activity was rising at the time of experiment in the treated group II. Acetylcholine activity in the 120 hours, organs like brain (71%), gill (52%) and liver (47%) percentage increased over the control group. In the group III acetylcholine activity diminished remarkably at the end of the 120 hours. Various organs like brain (26%), gill (31%) and liver (23%) percentage decreased over the treated group. Group IV acetylcholine activity slightly decreased over the control group due to the supplementary feed (**Table 2**).

Table 2: Changes in the level of acetylcholine (µmoles acetylcholine hydrolysed /mg of protein/hr) activity in the freshwater fish *Cyprinus carpio* on the effect of cypermethrin and *D.elata* exposed at 120 hrs sublethal concentrations.

Tissues	Groups	Hours of exposure				
		24	48	72	96	120
Brine	Group I	52.32±0.08	52.35±0.06	2.39±0.03	52.41±0.03	52.45±0.03
	Group II COC	63.32±0.06**	68.22±0.11**	75.96±0.03**	83.75±0.04**	89.96±0.07**
		+ 21.02	+30.50	+44.98	+59.79	+71.51
	Group III	54.86±0.13**	56.21±0.05**	63.86±0.09**	69.86±0.04**	65.75±0.09**
	COC	+4.85	+7.37	+21.89	+33.29	+25.35
	COT	- 13.36	- 17.60	- 15.92	- 14.54	- 26.91
	Group IV COC	52.20±0.08	52.25±0.06	51.92±0.09	51.80±0.04	51.49±0.03
		- 0.21	- 0.22	- 0.81	-1.16	-1.83
Gill	Group I	23.20±0.08	23.24±0.05	23.26±0.03	23.28±0.06	23.29±0.02
	Group II	26.42±0.06**	28.92±0.05**	32.54±0.07**	36.09±0.11**	38.10±0.02**
	COC	+13.87	+24.44	+39.89	+55.02	+63.58
	Group III	25.62±0.03*	27.35±0.02*	29.78±0.06**	29.54±0.10**	27.93±0.03**
	COC	+10.43	+26.29	+28.03	+26.80	+19.92
	COT	- 3.02	- 5.49	- 8.48	- 18.20	- 26.69
	Group IV COC	23.11±0.07	22.82±0.03	22.71±0.04	22.65±0.07	22.63±0.02
		- 0.38	-1.80	- 2.36	-2.70	-2.83
Liver	Group I	16.36±0.06	16.39±0.03	16.41±0.03	16.45±0.03	16.47±0.04
	Group II	19.37±0.07**	21.29±0.02**	23.29±0.07**	24.91±0.04**	26.72±0.02**
	COC	+18.39	+29.89	+41.92	+ 54.48	+ 63.23
	Group III	16.99±0.02**	19.37±0.03**	21.32±0.07**	21.53±0.10**	18.96±0.02**
	COC	+ 3.85	+18.18	+29.92	+30.88	+ 15.11
	COT	- 12.28	- 9.01	- 8.45	- 22.85	- 40.22
	Group IV	16.25±0.05	16.19±0.03	16.10±0.02	16.09±0.01	15.98±0.02
	COC	- 0.6	- 1.22	- 1.88	- 2.18	- 2.97

*Mean± S.E - mean of six individual observation; *Significant at P<0.05% level. **Significant at P<0.01% level. (+,-) denotes increased and decreased. COC (percent change over to control) COT (percent change over to treated).*

4. DISCUSSION

In this study, sublethal concentration of cypermethrin inhibiting the acetylcholinesterase (AChE) activity in the group II. Because cypermethrin is a neurotoxic substance, it interfere with neurotransmitter activity. Inhibition of acetylcholinesterase preventing enzyme substrate complex formation finally acetylcholine (Ach) not reduced into acetate and choline. So, nerve cell signaling process is disturbed and normal function is altered. [8] has been reported as inhibition of acetylcholinesterase (AChE) is responsible for the degradation of acetylcholine will result in the excessive stimulation of cholinergic nerves. This will result in tumors, convulsions and finally the death of the aquatic organism. The inhibition of acetylcholinesterase (AChE) activity in fish can be dangerous since it will affect feeding capability, swimming activity, identification, avoidance of predators and spatial orientation of the species [9]. The inhibition of acetylcholinesterase (AChE) activity decreases the cellular metabolism, induces deformities of cell membrane, and disturbs metabolic and nervous activity [10]. Inhibition of acetylcholinesterase (AChE) in the brain, was quite high compared to gill and liver, this similar observation as noticed by [11]. Acetylcholinesterase (AChE) activity inhibition is well known as a biomarker indicating the effect of neurotoxic substances [12]. Acetylcholine (ACh) activity was increased in the treated group II, the inhibition of AChE consequently leads to excessive Ach accumulation at the synapses and neuromuscular junctions resulting in over stimulation of Ach receptors which could ultimately end in death due to respiratory failure [13]. Group III acetylcholinesterase (AChE) activity was considerably recovered and diminishing the acetylcholine (Ach) this may due to the supplementary feed. *Delonix elata* leaves containing Sodium, Magnesium, Calcium,

Potassium, and trace minerals like Iron, Zinc, Copper, Chromium, and Manganese. Rich protein and Vitamins like vitamin C, Thiamine and carotenes also found in the leaves of *Delonix elata* [14]. These minerals or its salts may be detoxifying the cypermethrin toxicity. Vitamin C act as a non enzymatic antioxidant reduced cypermethrin toxicity [15, 16, 17]. Group IV observed slight changes in AChE and ACh activity.

5. CONCLUSION

In the present investigation indicating that cypermethrin inhibiting the acetylcholinesterase enzyme during the experimental period than recovered acetylcholinesterase enzyme this may due to supplementary feed. Supplementary feed *Delonix elata* leaves may have capability to enhancing the cholinergic neurotransmitter enzyme (AChE). Further study will carried to know the detoxifying property of *Delonix elata* and isolation the bioactive compounds.

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