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ORIGINAL RESEARCH ARTICLE

Alterations in the Levels of ACh and Associated AChE in the Tissues of Fresh Water Fish *Cirrhinus mrigala* Exposed to Deltamethrin

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ABSTRACT

The present investigation was undertaken to study the effect of deltamethrin on certain biochemical changes and neurotransmitter aspects in *Cirrhinus mrigala.* Fish were treated with deltamethrin and LC_{50} value for 96 hours was calculated following probit analysis methods. *Cirrhinus mrigala* was exposed to both lethal (8 µl/l) and sublethal (0.8 µl/l) concentration of Deltamethrin. Inhibition in the activity of acetylcholinesterase (AChE) suggested decrease in the cholinergic transmission and consequent accumulation of acetylcholine (ACh) in the tissues. This might have lead to behavioural changes and create widespread disturbance in the normal physiology, ultimately causing death of the fish. The biochemical analysis of tissues revealed a gradual increase in ACh and decrease in AChE during both lethal and sub-lethal periods of exposure. The results however clearly suggest that in environmental monitoring programme, AChE can be a good diagnostic tool for deltamethrin toxicity.

Key words: Acetylcholine, acetylcholinesterase, *Cirrhinus mrigala*, deltamethrin, pesticides.

INTRODUCTION

Recent evidence indicates that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life. The frequent uses of pesticides in agriculture practices as well as pest control pollute the soil and water bodies thus reaching the aquatic ecosystem and get enriched in the aquatic food chain organisms like fishes (Chebbi and David, 2009). And fish being intoxicated, it is more likely to affect humans at the top of food chain. Therefore it is desirable to study the effect of these pesticides on fish species. In this regard, possible hazard of Acetylcholinestrase (AChE) inhibiting pesticides in the aquatic environment should not be ignored, since these pesticides act as a nerve poison (Coppage and Braidech, 1976).

Acetylcholine (ACh) is the only classical neurotransmitter that after release into the synaptic cleft is inactivated by enzymatic hydrolysis, rather than by reuptake. As a consequence, it has a turnover rate *in vivo* that is much higher than that of any other transmitter, including catecholamines and amino acids (Chebbi and David, 2009). AChE was identified as the enzyme responsible for termination of cholinergic transmission by cleavage of ACh to acetate and choline. AChE, is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system (Soreq and Seidman, 2001; Silman and Sussman, 2005). ACh diffuses across the synaptic cleft, creating a delay of about 0.5 ms (milliseconds), and attaches to a specific receptor site (a protein) on the post synaptic membrane that recognizes the molecular structure of the acetylcholine molecules. The arrival of the ACh causes a change in the shape of the receptor site, which results in ion channels opening up in the postsynaptic membrane.

The pyrethroids can be neurotoxic and potent inhibitor of the AChE. Their primary effect is to inhibit the action of AChE, which have the function of breaking down ACh released into the synapse. It occurs from the ending of cholinergic nerves and it functions as a chemical messenger. When an impulse reaches a nerve ending, ACh is released and carries the signal across the synaptic

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cleft to a receptor on the postsynaptic membrane. When ACh interacts with its receptor, a signal is generated on the postsynaptic membrane, so that the impulse is carried on. To achieve this, ACh must be rapidly broken down by AChE in the vicinity of the receptor. Anticholinesterases have the effect of reducing or preventing altogether the breakdown of ACh. As a consequence, ACh builds up in the synapse leading to over stimulation of the receptor and the continued production of signal after this should have stopped. If this situation continues, the signalling systems will eventually rundown resulting in synaptic block. At this point, it will no longer be possible for ACh to relay signals across the synapse. In the case of neuromuscular junctions so affected, tetanus will result, with the muscle in a fixed state, unable to contract or relax in response to nerve stimulation (Fukuta, 1990). If AChE is inhibited, ACh accumulates and nerve impulses cannot be stopped, causing prolonged muscle contraction, as a consequence paralysis occurs and death may result.

Hence, the present study was undertaken to evaluate the toxic effect of pyrethroid deltamethrin on freshwater fish, *Cirrhinus mrigala*, and to study the relationship between concentration and the biochemical effects on the ACh and AChE activity.

MATERIALS AND METHODS

The commercial grade of deltamethrin (Decis, 30% EC) was obtained from Bayer Crop Science, India Ltd., Gujarat, India. Healthy fresh water fish, *Cirrhinus mrigala*, mean length 15-20 cm and weight 20-30 g were procured from Karnataka fisheries Board, Dharwad, India and brought to laboratory in 20 L plastic containers. Fishes free from any kind of infection were selected and treated with potassium permanganate solution (0.5% w/v) for 5 min to remove any dermal adherent. All the fishes were acclimated for seven days in a rectangular aquarium containing dechlorinated tap water at room temperature (26 ± 2^{o} C) with food ad libitum under standard laboratory condition. 12h photoperiod was maintained and the water was aerated twice a day.

The LC_{50} value for 96 hours of deltamethrin was determined by the procedure of Finney (1971). Fishes were exposed to both lethal (8 µl/l) for 1,2,3,4 days and sublethal concentration (0.8 µl/l) of deltamethrin for days 1, 5, 10, and 15. Simultaneously control group was also maintained.

Estimation of ACh content:

The tissue ACh content was estimated by the method of Hestrin as described by Augustinson (1957). After isolating and weighing, the tissue were teased and transferred in tubes already kept in boiling water bath for 10 minutes to inactivate enzyme AChE and to release bound ACh. The tubes were cooled and contents were homogenised in 2.0 ml of distilled water, 2.0 ml of alkaline hydroxylamine hydrochloride and 1.0 ml of 1:3 dilute hydrochloric acid and water was added. The contents were centrifuged and 1.0 ml of ferric chloride was added to the supernatant. The optical density of the sample was measured at 540 nm in a spectrophotometer against a blank. The blank consisted of 2.0 ml distilled water, 2.0 ml alkaline hydroxylamine hydrochloride, 1.0 ml diluted HCl and 1.0 ml of ferric chloride solution. A standard graph was prepared with ACh and the values were expressed as µM of ACh/g wet weight of tissue.

Estimation of AChE:

The activity was estimated by the method of Metcalf (1951). About 3% homogenate of brain, muscle, gill and liver tissues were prepared in cold 0.25 M sucrose solution and homogenates were used for the enzyme assay. The reaction mixture of 3.0 ml contained 12 µM of acetylcholine chloride, 100 µM of sodium phosphate buffer (pH 7.4) and 1ml of homogenate. After incubating at 37°C for 30 minutes the reaction was stopped by adding 2.0 ml of alkaline hydroxylamine hydrochloride solution followed by 1ml of 1:1 HCl. The un-incubated samples were treated with 2ml of alkaline hydroxylamine hydrochloride solution followed by 1ml of HCl, prior to the addition of the homogenate. The contents were thoroughly mixed and filtered. To the clear filtrate 1.0 ml of 0.37 M ferric chloride solution was added and the colour was read at 540nm in a spectrophotometer using blank. The blank preparation is same as homogenate. The values were expressed as µM of acetylcholine hydrolyzed/mg/protein/h.

Statistical analysis:

Data obtained from replicates were used to calculate mean values. The difference in mortality values was analyzed by chi-square test. The values were presented as mean ± standard deviation. Data were tested for normality and then analyzed by one-way analysis of variance (ANOVA) to test the significant differences among different parameters. All statistical analyses were carried out using SPSS 10.1 and $p < 0.05$ was considered statistically significant.

RESULTS

Lethal and sublethal toxicity of deltamethrin for the freshwater fish, *Cirrhinus mrigala* was found to be 8 µl/l and 0.8 µl/l respectively. It is evident from the results that the deltamethrin can be rated as highly toxic to fish. No significant mortality was observed during the sublethal experimental tenures, but the fish were under stress and showed symptoms of dullness, loss of equilibrium, loss of feeding, and erratic swimming.

ACh accumulation:

In the control fish tissue, maximum quantity of ACh was observed in muscle followed by gill and liver (**Table 1**). The accumulation of ACh under the median lethal concentration of deltamethrin increased gradually up to day 3 in all the tissues namely gill, muscle and liver. Liver recorded the lowest concentration 17.119 µM/g wet wt., which was 4.257 percent over control. A maximum increase of 58.621% was noted in the gill tissue on 3rd day of exposure. Day 4 in all the tissues recorded decreased ACh content under lethal concentration. During the median lethal concentration an overall maximum increase was observed in gill (58.621%) and a minimum was noted in liver (4.257%).

Table 1: ACh Activity In The Different Organs Of Fish *Cirrhinus mrigala* Exposed To Deltamethrin

Tissue	Control	exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	30.173 I	34.864 D	41.786 C	47.861 A	45.216 B	31.653 H	31.764 G	34.539 E	34.153 F
±SD	0.0254	0.0654	0.0569	0.0487	0.3254	0.0327	0.0331	0.0622	0.9331
%Change		15.547	38.488	58.621	49.855	4.905	5.272	14.469	13.19
Muscle	34.721 I	37.188 G	40.213 C	47.077 A	44.319 B	36.176 H	38.556 F	39.563 D	39.123 E
±SD	0.0564	0.6254	0.0125	0.3265	0.0054	0.0428	0.5037	0.0376	0.1415
%Change		7.105	15.817	35.586	27.643	4.19	11.045	13.945	12.678
Liver	16.420 I	17.119 G	17.971 C	18.564 A	18.100 B	17.189 H	17.621 E	17.897 D	17.265 F
±SD	0.2356	0.0854	0.0568	0.4796	0.0981	0.3298	0.6044	0.0552	0.1615
%Change		4.257	9.445	13.057	9.281	4.683	7.314	8.995	5.146

Means are ± SD (n=6) for a parameter in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

In the experimental fish under sublethal exposure very high quantity of ACh was recorded in gill on 10th day of exposure which showed 14.469% and lowest recorded on day 1 in liver which showed 4.905% increase over control. ACh content showed a continuous increase in gill, muscle and liver up to 10th day while the subsequent day 15 recorded a low percent of increase.

AChE activity:

The decrease in AChE activity was more pronounced in the liver tissue followed by gill and muscle in the fish exposed to lethal concentrations of deltamethrin. Maximum percent inhibition in the AChE activity was noted in liver on 3rd day (-29.736%) and minimum percent inhibition was observed in muscle as compared to control. While gill, muscle and liver exhibited continuous decrease in activity up to day 3, while day 4 witnessed a recovery with decrease in the inhibitory activity in the AChE. In sub lethal concentrations the data presented in (**Table 2**), revealed maximum percent inhibition of AChE activity in liver (-17.553%) followed by gill and muscle on day 15 in the whole experiment.

Table 2: AChE Activity in the Different Organs of Fish *Cirrhinus Mrigala* Exposed To Deltamethrin

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	4.716 A	4.909 C	4.153 G	3.817 H	4.006 I	4.549 B	5.054 D	5.218 E	5.246 F
±SD	0.3897	0.2498	0.4196	0.3861	0.5263	0.0432	0.0039	0.0122	0.0038
%Change	------	-4.092	-11.938	-19062	-15.055	-3.541	-7.167	-10.644	-11.238
Muscle	6.442 A	6.377 B	5.980 D	5.313 H	5.749 I	6.623 C	5.979 E	5.911 F	5.821 G
±SD	0.1856	0.2549	0.1974	0.0201	0.4361	0.0019	0.0033	0.0244	0.067
%Change	------	-1.009	-7.156	-17.525	-10.757	-2.809	-7.187	-8.242	-9.639
Liver	2.011 A	1.892 B	1.608 G	1.413 H	1.537 I	1.862 C	1.809 D	1.741 E	1.658 F
±SD	0.3842	0.2965	0.1472	0.0365	0.0584	0.6937	0.0029	0.5444	0.0439
%Change	------	-5.827	-20.039	-29.736	-23.57	-7.409	-10.044	-13.426	-17.553

Means are ± SD (n=6) for a parameter in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

DISCUSSION

AChE is an enzyme that modulates the amount of neurotransmitter substance at neuron junctions (Reddy *et al*, 1992), and it is also concerned with the ionic content. The inhibition of AChE and elevation of ACh content may be due to the decreased ionic composition in the tissues of *C.mrigala*. Reddy *et al*., (1992) was of the view that greater inhibition of AChE activity with a concomitant increase in ACh content in the tissues is an implication of greater inhibition in the integrator activity of the central nervous system and ACh accumulated in brain and other tissues. Damage to the central nervous system might have caused uncontrolled hormonal release and the toll of an animal may be possible by the degeneration of many biochemical and physiological functions (Corbett, 1974).

During the present study the level of AChE activity in gill, muscle and liver tissue of *C. mrigala* exposed to deltamethrin decreased suggesting the inhibitory effect of pesticide on the AChE system. Inconsonance with the decrease in the AChE activity there is a corresponding increase in the ACh content of the tissues suggesting decrease in the cholinergic transmission and consequent accumulation of ACh in the tissues. A similar corroborative increase in the ACh content consequent to a decrease in the tissue AChE levels was reported in fish, *Tilapia mossambica* exposed to malathion for 48h (Kabeer and Ramanarao, 1980). Singh and Kumar (2000) reported decrease in AChE activity in freshwater teleost, *Catla catla* subjected to sub chronic and acute exposure to malathion. Similar decrease in the AChE activity was reported by Parma et al., (2002) under acute toxicity of monocrotophos in a neotropical fish, *Prochilodus lineatus.* Rao et al., (2003) and Rao, (2006) observed similar inhibition of AChE activity in the fish, *Tilapia mossambica* exposed to chlorpyrifos and RPR-V respectively.

It is also known that pesticides and certain chemical compounds which inhibit AChE activity are known to disrupt the normal behavioural patterns in the affected animals (Mushigeri and David, 2005). The behavioural changes observed in the intoxicated animals like repeated opening and closing of opercular covering, hyper-extension of all fins, cock-screw swimming, S-jerks, coughing, burst-swimming can be directly related to the inhibition of peripheral and or central nervous system due to inhibition of cholinesterase activity (Kurtz, 1977). Tendency of restlessness and impaired behavioural activities of the fish during present study offers a strong support to this. The abnormalities in fish behaviour observed in this study could be related to the inhibitory action of deltamethrin on AChE and subsequent accumulation of ACh at the nerve endings.

Caudal bending was noticed in both lethal and sublethal concentrations and it persisted even under recovery tenures. The degree of caudal bending was intensified in higher toxicant concentration. This however largely affected the normal swimming pattern of the fish. Caudal bending may be a sort of paralysis, which is due to the inhibition of muscular AChE activity resulting in blockage of neural transmissions (Chebbi and David, 2009). Bending of caudal region is owing to the fact that caudal portion is the thinnest structure and hence can be conferred any sort of orientation due to paralysis of caudal musculature by inhibition of AChE activity as evidenced in the present study. Further inhibition of AChE activity resulted in a progressive accumulation of ACh, especially during periods of repetitive stimulation, leading to desensitization of nAChRs (nicotinic acetylcholine receptors) and consequent muscular weakness (Giniatullin *et al*., 1998). Thus deltamethrin reduced instinctive behavioural responses and affected morphological features by depression of AChE activity.

Hence, present findings offers an understanding that deltamethrin is a potent neurotoxin to *C. mrigala* and related fish species at a varied concentrations. Further, the activity of AChE is a possible measure to assess the health status of the fish and in environmental monitoring programme; it can be a good diagnostic tool for deltamethrin toxicity.

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