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Impact of Deltamethrin on Aetylcholinesterase (AChE) and Acetylcholine (Ach) in Fresh Water Fish *Labeo rohita* (Hamilton)

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Abstract: Today the task of preventing human exposure to toxic chemicals might appear impossible. Indiscriminate and wide use of pesticides to wipe out pests, careless handling, accidental spillage of pesticides and pesticides released from manufacturing industrial units are carried by wind or percolate through water and finally washed down and find their way into water bodies. This would equally affect non-target organisms like fish, which represent an important component of aquatic life and serves as a staple food for human beings. Hence, it is felt necessary to conduct toxicological studies which indicates the sequence of events in physiological and biochemical systems providing information on the nature and completion of compensatory mechanisms during toxic stress. The present investigation is aimed at estimating impact of Deltamethrin on the acetylcholinesterase and acetylcholine activity in the Indian major carp *Labeo rohita*. It is evident that the levels of AChE activity elevated at 24 h as compared to control in all the organs whereas the ACh content followed an opposite trend. The levels of AChE activity goes on decreasing through 7 day and continued up to 15 day; however, the levels of ACh are elevated. From 15 day onwards the levels of AChE activity gradually elevated and came nearer to control at 30 day exposure.

Keywords: Pesticides, Pyrethroid, Water bodies, Fish, Deltamethrin, *Labeo rohita*, Aetylcholinesterase activity, Acetylcholine

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Introduction

Water is considered as polluted when it is altered in physical, chemical and biological characteristics which may cause harmful effects on human and aquatic biota (Sharma and Kaur, 1994). The problem of chemical pollution is known to occur in several ways i.e., organic and inorganic chemical

wastes (Mc Kee and Wolf, 1963; Micheal, 1991), oil refineries (Dorris *et al.*, 1965), textile plant discharges (Dorn *et al.*, 1993; Lin and Peng, 1994), detergents, radioactive wastes and pesticides (Hiltbran, 1974). In addition to the agricultural practices together with pest control programmes,

the surface run-off and aerial spraying form the major source for translocating pesticides into aquatic ecosystem (Baumann, 1980; Glortfelly, 1990). Wide spread transport of pesticides by air and rain was also reported by Cohen and Pinkerton (1966). The aquatic environment becomes the ultimate sink for all pollutants (Nicholson, 1967; Kerr and Vas, 1973). Any compound that has been used in large quantities ultimately reaches the aquatic ecosystem (Zitko *et al.*, 1975). Pesticides easily reach adjacent vegetation, wildlife, soil, water, air and sometimes humans. Thus the impact of pesticides is felt through the environment and public health (Pimental *et al.*, 1992). The greatest pools or sinks of chemicals in the aquatic environment occur in the sediment (Ahsanullah *et al.*, 1984, Adams *et al.*, 1992). One of the largest risk to the aquatic environment is sediment contamination considered by many regulatory agencies (Burton, 1992).

The widespread use of pesticides in agriculture has detrimental effects on lakes and rivers due to surface runoff from fields, posing risks to both animals and humans owing to their ability to bioaccumulate and disrupt the food chain (Lushchak *et al.*, 2018; Bodnar *et al.*, 2022). Fish, is the integral part of fresh water and marine ecosystems, as they play a crucial role in maintaining ecological balance (Okwuosa *et al.*, 2019). Due to their agility and relatively long lifespan, fish are considered good bioindicator of long-term toxic effects and various habitat conditions (Falfushynska *et al.*, 2014; Falfushynska *et al.*, 2019). Certain reports suggest that few teleost and zebrafish species exhibit metabolic traits resembling those of humans, making them potential alternative animal model for mechanistic research into cellular events triggered by physical and chemical stimuli (Hahn and Sadler, 2020).

Insecticides such as pyrethroids are the synthetic analogs of naturally occurring pyrethrins from the flowers of *Chrysanthemum* species. These are considered as effective insecticides due to

their high insecticidal toxicity with low mammalian toxicity (Elliott *et al.*, 1974). Synthetic pyrethroids, including the newly synthesized insecticide called Cypermethrin and Deltamethrin, are types of pesticide that has been widely used. The use of these insecticides has raised concerns as they not only affect target pests but also affect the biology of non-target species (Elliot and Janes, 1978; Reddy and Yellamma, 1991). Particularly, they are highly toxic to fish and aquatic invertebrates even at very low concentrations. While these insecticides exhibit low mammalian toxicity, they pose a significant threat to invertebrates, fish, and amphibians (Robert Edwards, 1986). This enhanced toxicity has notable consequences on the health of ecosystems and their biodiversity (Madara Ranatunga *et al.*, 2023).

Acetylcholinesterase (AChE) is an enzyme that regulates neurotransmitter Acetylcholine (Ach) at neuron junctions. The inhibitory effects of insecticides on Acetylcholine esterase activity of fish had been reported by various authors--metacid-50 and carbaryl in *Channa punctatus* (Gosh and Bhattacharya, 1992) and cypermethrin in *Channa striatus* (Ahamed *et al.*, 2015). The present investigation is aimed at estimating impact of Deltamethrin (a pyrethroid) on the Acetylcholinesterase and Acetylcholine activity in the Indian major carp *Labeo rohita*.

Materials and Methods

Animal Selected

The Indian major carp, *Labeo rohita* (Hamilton) is an economically important edible fish having great commercial value. It is abundantly available in the fresh water tanks in and around Ananthapuramu, India. Besides its wide availability and commercial importance, this carp is known to have adaptability to laboratory conditions and appear to be suitable experimental animal for toxicity studies (Sreenivasan and Swaminath, 1967; Nair and Sherief, 1998). Hence, this fish has been selected as the ideal animal for the present investigation of toxicity studies.

Pesticide Selected

The pesticide selected for the present investigation is synthetic pyrethroid-Deltamethrin, belonging to "third generation pesticides". This pesticide is widely used in and around Anantapur district on diverse agriculture crops to control pests of crops, flies and mosquitoes. It has been widely used because of its high photostability, degradability, non-persistent nature and low mammalian toxicity. Deltamethrin has commercial name Decis. The commercial grade Deltamethrin (EC 2.8%) of liquid formulation was procured from local agrochemical stores.

Experimental design

Fresh water fish *Labeo rohita*, weighing 10 ± 2 g were procured from local fisheries department and stored in spacious aquaria. The temperature in aquaria was 28 ± 2 °C and the same is maintained as normal temperature throughout the course of this investigation. The fish were fed daily with groundnut cake as well as with rice bran. Before the experiments have been executed the fish were adapted to the laboratory conditions for a period of one week. After determination of LC_{50} for 96 h ($0.01 \mu\text{g/l}$), the fish were exposed to sublethal concentration of Deltamethrin ($1/10^{\text{th}}$ of 96 h LC_{50} i.e. $0.01 \mu\text{g/l}$) for five exposure periods i.e 1, 7, 15, 20 and 30 days.

Estimation of Acetylcholinesterase (AChE) activity

1% homogenate of the brain and 5% homogenate of the other tissues were separately prepared in 0.25M ice cold sucrose solution. The AChE activity in these extracts was estimated by the method of Augustinsson (1957).

Estimation of Acetylcholine Content

Acetylcholine content (ACh) is separately estimated in the gill, kidney, brain, liver and muscle in both Deltamethrin and control fish *Labeo rohita* by the Hestrin method modified by Augustinsson (1957).

Results

The data on the levels of AChE activity and ACh

content in brain, liver, kidney and muscle of *Labeo rohita* at 1, 7, 15, 20 and 30 days after exposure to sublethal concentration of Deltamethrin are presented Tables 1 and 2. For comparison, the differences in the levels of each parameter obtained between the controls and experimental were converted as percentages of the corresponding control and these per cent values are also presented in the same Tables.

From the data presented in the Tables 1 and 2 it is seen that the levels of AChE activity elevated at 24 h relative to control in all the organs whereas the ACh content followed an opposite trend. The levels of AChE activity goes on decreasing through 7 day and continued up to 15 day whereas the levels of ACh goes on elevation. From 15 day onwards the levels of AChE activity gradually elevated and became close to control at 30 day exposure, whereas the levels of ACh content followed an opposite trend and came nearer to control at the end of 30 day exposure period and the values were found to be significant ($P < 0.001$).

Discussion

Acetylcholine neurotransmitter substance at nerve cell junction will be acted upon by a substrate enzyme acetylcholinesterase (O'Brien, 1967; Rainsford, 1978). AChE is one of the hydrolytic enzymes disturbing the normal nervous functions finally resulting in the death of animals (Eto, 1974).

The organophosphorus (OP) pesticides inhibit the cholinesterase activity in almost all animal tissues (O'Brien, 1969; Goodman *et al.*, 1979). Inhibition of AChE activity was regarded as a significant parameter in assessing complex toxicogenic effects of various toxicants, including pesticides (Mukherjee and Bhattacharya, 1974; Olson and Christesen, 1980; Bhattacharya and Jash, 1981; Bhagylakshmi *et al.*, 1984b, 1985). In normal conditions, the enzyme AChE initially forms a complex with the substrate ACh, which then acetylates the enzyme with the release of choline. Deacetylation occurs by the reaction of

Table 1: Acetyl cholinesterase (AChE) activity in Deltamethrin treated *Labeo rohita* at different sublethal exposure periods. Mean is a pool of six individual measurements. The percent change in AChE activity at different periods is calculated in relation to the AChE activity in control medium (fresh water without Deltamethrin). The per cent recovery in AChE activity at 30-day exposure period is calculated relative to the level of the control medium which is fixed at 100%

S. No.	Organ	Control	Exposure period in days				
			1 day	7 day	15 day	20 Day	30 Day
I	Brain						
	Mean	1462.8	1832.4	1098.6	848.2	1002.6	1178.6
	±SD	2.16	1.82	1.96	0.94	1.54	1.2
	% Change	-	25.26	-24.89	-42.01	-31.46	-19.42
	% Recovery	-	-	-	-	-	68.53
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
II	Gill						
	Mean	572.2	680.4	462.6	392.8	432.6	441.2
	±SD	2.82	1.98	1.12	1.26	0.82	0.96
	% Change	-	18.90	-19.15	-31.35	-24.39	-22.89
	% Recovery	-	-	-	-	-	7.10
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
III	Kidney						
	Mean	182.4	214.6	150.8	127.4	132.6	140.2
	±SD	0.42	0.36	0.78	0.62	0.28	0.32
	% Change	-	17.65	-17.32	-30.15	-27.30	-23.13
	% Recovery	-	-	-	-	-	76.86
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
IV	Liver						
	Mean	241.4	289.2	216.6	160.4	172.6	191.8
	±SD	0.62	0.36	0.28	0.76	0.42	0.22
	% Change	-	19.80	-10.27	-3.69	-28.50	-20.54
	% Recovery	-	--	-	-	-	79.45
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
V	Muscle						
	Mean	974.2	1158.4	886.6	674.2	744.2	760.2
	±SD	1.12	1.36	0.95	1.04	0.96	0.74
	% Change	-	18.90	-8.97	-30.79	-23.56	-21.96
	% Recovery	-	-	-	-	-	78.03
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01

water with the acetylase enzyme and form acetic acid and the original free enzyme, whereas in the presence of pesticides, particularly OP compounds, the enzyme reacts with them in a way precisely analogous to that of the normal substrate and forms a pesticide-enzyme complex, instead of acetylated enzyme (Corbett *et al.*, 1984).

In the present investigation, the changes in AChE activity and ACh content during synthetic pyrethroid, Deltamethrin exposure was undertaken. The data on AChE activity and ACh content revealed the inhibition in the enzyme

activity followed by a concomitant increase of ACh content in all the tissues like gill, kidney, brain, liver and muscle of *Labeo rohita* exposed to Deltamethrin (Tables 1, 2). From the data, it is evident that the AChE activity suddenly elevated at 24 h exposure. The enhancement in activity of AChE might be due to the pollution stress.

Satyadevan *et al.* (1993) reported an initial elevation in acetylcholinesterase activity of the brain of *Cyprinus carpio* exposed to an organophosphorus dimethoate. Kufesak *et al.* (1994) reported an initial elevation in

Table 2: Acetylcholine (ACh) content in Deltamethrin treated *Labeo rohita* in different sublethal exposure periods. Mean is a pool of six individual measurements. The percent change in ACh content at different periods is calculated in relation to the ACh content in control medium (fresh water without Deltamethrin). The percent recovery in ACh activity at 30-day exposure period is calculated relative to the level of the control medium which is fixed at 100%

S. No.	Organ	Control	Exposure period in days				
			1 day	7 day	15 day	20 Day	30 Day
I	Brain						
	Mean	846.8	620.2	820.6	985.2	876.7	830.5
	±SD	0.96	1.16	1.32	1.08	0.86	0.42
	% Change	-	30.40	12.28	26.51	7.01	4.14
	% Recovery	-	-	-	-	-	96.04
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
II	Gill						
	Mean	312.6	248.2	368.6	426.4	416.2	398.2
	±SD	1.198	0.96	1.12	0.98	1.86	1.32
	% Change	-	-18.64	5.08	26.80	12.79	-3.55
	% Recovery	-	-	-	-	-	96.44
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
III	Kidney						
	Mean	804.6	662.4	780.2	890.5	836.6	798.5
	±SD	2.12	1.08	0.86	0.98	1.16	1.32
	% Change	-	-17.6	3.03	10.67	3.97	0.75
	% Recovery	-	-	-	-	-	99.24
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
IV	Liver						
	Mean	432.2	300.8	485.3	546.8	462.5	413.3
	±SD	1.18	1.32	0.86	2.10	1.10	0.97
	% Change	-	26.75	3.09	16.34	3.53	1.92
	% Recovery	-	--	-	-	-	95.85
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
V	Muscle						
	Mean	286.7	222.4	310.4	360.8	323.6	270.4
	±SD	0.32	0.64	0.74	0.96	0.82	0.56
	% Change	-	22.42	8.33	25.84	12.87	5.68
	% Recovery	-	-	-	-	-	94.31
	t- Test	-	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01

acetylcholinesterase activity in carp after exposure to pesticides. Nisar Ahmed (1994) reported an initial elevation and further inhibition of AChE activity in the major carp *Catla catla* after exposure to Deltamethrin. Abdul Subhan (2000) reported an initial elevation and further inhibition of AChE activity in *Catla catla* exposed to synthetic pyrethroid fenvalerate. An initial elevation in AChE activity was reported in the snail *Stagnicola species* after exposure to malathion (Martinez-Tabche *et al.*, 2002). These studies are in conformity with the present elevation in AChE

activity. Similarly Venkateswara Rao *et al.* (2005) observed inhibition of AChE enzyme activity in the brain of *Gambusia affinis* on exposure to chlorpyrifos. Mushigeri and David (2005) and Patil and David (2008) observed darting movements and loss of equilibrium of fish which may be due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyper-stimulation.

In the present study, at later periods like 7th day and 15th day exposure the AChE activity was reduced, with maximal reduction at 15th day

exposure period of Deltamethrin. The inhibition in AChE activity resulted in the accumulation of acetylcholine (ACh) at different sublethal exposure periods of Deltamethrin. Similarly Health (1961) and O'Brein (1967) have reported that OP insecticides react with AChE to form phosphorylated enzyme, this phosphorylated enzyme inhibits the AChE for several weeks (Coppage and Duke, 1971; Post and Leasure, 1974). These reports provide strength to the present study regarding inhibition of AChE.

In addition Bhagyalakshmi and Ramamurthy (1980) reported inhibition of AChE activity in *O. senex senex* exposed to fenitrothion. Gosh and Bhattacharya (1992) reported significant inhibition of brain AChE activity accompanied by a concurrent increase in Ach content in *Channa punctatus* exposed to carbaryl and metacid-50. A significant inhibition in AChE activity was reported in prawn, *Macrobrachium malcolmsonii* on exposure to carbaryl (Bhavan and Geraldine, 2002). These studies support the present finding regarding the inhibition in AChE activity. These findings i.e. activation of enzyme during early period and inhibition during later exposure period depend on the concentration of the pesticides and coincides with that of findings of various authors (Joseph and Raj, 2011; and Jindal and Kaur, 2014).

The measurement of brain AChE activity in the aquatic animals indicates the extent of pollution of that environment which they inhabit. The inhibition of AChE activity is directly related to the concentration of pesticides and the length of exposure (Coppage, 1972; Macek *et al.*, 1972). Koundinya and Ramamurthy (1979) also reported a greater inhibition in AChE activity, with higher elevation in Ach content in the brain than in the muscle of *Tilapia mossambica* exposed to sumithion.

According to Coppage *et al.*, (1975) inhibition of brain AChE activity to the level of 70-80% is critical to the fishes. The inhibition in AChE activity may result in the accumulation of Ach content and this led to consequent loss of muscular and nervous coordination. Bradbury

(1973) has observed the prostration and massive muscular tetanus followed by death in *Carcinus maenus* after parathion administration. Ramana Rao (1978) reported inhibition of muscle AChE activity in snails exposed to pesticide toxicity.

In the current study however, at 30th day exposure the inhibitory activity was decreased and came nearer to normal level. Thus, the fish *Labeo rohita* fairly recovered from the inhibitory activity in tissues of fish. The concomitant recovery in AChE activity during 30th day exposure period might be due to active metabolism of Deltamethrin which is being removed from the site of action and thus enabling the enzyme to resume unhindered hydrolysis of ACh.

Conclusion

Indiscriminate and wide use of pesticides to wipe out pests, careless handling, accidental spillage of pesticides and pesticides released from manufacturing industrial units are carried by wind or percolate through water and finally washed down and find their way into water bodies. This would equally affect non-target organisms like fish, which represent an important component of aquatic life and serves as a staple food for human beings. The present investigation is aimed at estimating impact of Deltamethrin on the acetylcholinesterase and acetylcholine activity in the Indian major carp *Labeo rohita*. It is evident that the levels of AChE activity elevated at 24 h as compared to control in all the organs whereas the ACh content followed an opposite trend. The levels of AChE activity goes on decreasing through 7 day and continued up to 15 day; however, the levels of ACh are elevated. From 15 day onwards the levels of AChE activity gradually elevated and came nearer to control at 30 day exposure. In the present investigation the shifts in enzymatic studies such as Acetylcholinesterase(AChE) and Acetylcholne (Ach) metabolism might be to compensate with situation shown by the fish for its survival to the imposed toxic stress.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the ¹Department of Zoology, Govt. College (A), Ananthapuramu, Andhra Pradesh, India

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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