Full Length Research Paper

Sub-lethal effect of cypermethrin on acetylcholinesterase (AChE) activity and acetylcholine (Ach) content in selected tissues of *Channa striatus* (Bloch.)

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This study was carried out to investigate the sub-lethal effect of cypermethrin on acetylcholinesterase (AChE) activity and acetylcholine (ACh) content in various organ tissues of *Channa striatus*. Activity of AChE and ACh content was estimated in the tissues of brain, gills, liver, kidney, intestine and muscle of the exposed fish at regular intervals of 1, 7, 15 and 30 days. The study revealed that the inhibition of the enzyme activity followed by a concomitant increase of ACh content was observed in all the tissues of *C. striatus* during the exposed period. It was also observed that there was a sudden increase in the AChE activity during 24 h exposure. Enhancement in the activity of AChE might be due to the stress experienced by the fish during the experimental period.

**Key words:** Cypermethrin, acetylcholinesterase (AChE), acetylcholine (Ach), *Channa striatus*.

**INTRODUCTION**

A large number of insecticides have been used to boost the agriculture yield. The run-offs from treated lands are known to interfere with nutria-economically important animals and growth in water bodies by altering and disrupting the different physiological function of aquatic animals. In fishes, nervous system shows profound influence on the functional activity. Pesticides are generally neurotoxins and thereby paralyzing the insect leading to their death (Casida et al., 1983; Clark and Matsumura, 1987). Recently emerged synthetic pyrethroids have increased its utilization in agriculture and horticulture. In insects, their principal effect is to induce a continuous series of nerve impulses referred to as repetitive activity which completely upsets proper functioning of the entire nervous system and eventually results in death (Vijverberg et al., 1982). The synthetic pyrethroids have distinct action depending on the presence or absence of Alfa-cyano group. Cypermethrin causes toxicity similar to that of DDT, while other pyrethroids show toxicity by inhibiting both ATPase and
acetylcholinesterase (AChE) systems. AChE is an enzyme that regulates neurotransmitter acetylcholine (Ach) at neuron junctions. The combination of enzyme (AChE) and substrate (ACh) results in the formation of acetylated enzyme, and is rapidly hydrolyzed to choline and acetic acid leaving the enzyme with both active sites. The inhibitory effects of synthetic pyrethroids on cholinesterase are generally considered as the basis for this biological activity. Hence, AChE estimation in fishes has proved valuable in detecting pollution of fresh and marine fishes (Tabassum et al., 2003; Shailendra et al., 2010; Rajini and Revathy, 2015).

Gosh and Bhattacharya (1992) reported inhibition of AChE by metacid-50 and carbaryl in *Channa punctatus*. Rao et al. (1985) reported the inhibition of AChE in the fish, *C. punctatus* on exposure to elsan. Malla et al. (1991) reported regulation of AChE system in *Cyprinus carpio* under fenvalerate toxicity. AChE activity was inhibited in ventral nerve cord of *P. americana* under fenvalerate toxicity. Bashamohideen and Sailabala (1988) studied inhibition of AChE activity and elevation in ACh content in *C. carpio* on exposure to sub-lethal concentration of marathon.

Furthermore, Koundinya and Ramamurthy (1979) reported decline in AChE activity and accumulation of ACh content in different fish; *Tilapia mossambica* exposed to sumithion and sevin. The inhibition of AChE activity is directly related to the concentration of the pesticide and period of exposure (Sambasiva Rao et al., 1985). The inhibition of AChE activity was regarded as a significant parameter in assessing complex toxicogenic effects of various toxicants including pesticides (Bradbury et al., 1986). Sudha Tiwari et al., (2012) studied the toxic effect of cypermethrin in *Labeo rohita*. Great deal of work has been turned out on these lines with reference to AChE inhibition by the synthetic pyrethroid, but no attempt has been made on *Charina striatus* with reference to sub-lethal concentration of cypermethrin. Hence, an attempt has been made to study the AChE activity-ACh content in economically important edible fish, *C. striatus* on exposure to sub-lethal concentration of cypermethrin.

**MATERIALS AND METHODS**

**Procurement of test fishes**

Live, healthy and disease free *C. striatus* (110 weights between 8 and 10 g and length, 7 and 8 cm) were collected from Department of Fisheries, Kurnool, A.P. and brought to the laboratory. Fishes were acclimatized in the laboratory condition for one week in plastic pools. The water in the plastic pool was aerated daily for 2 h and water in plastic pool was replaced thrice a week. The fish were fed daily with groundnut oil cake and frog muscles twice in a week. Experiments of the present study are conducted in static waters as suggested by Doudoroff et al. (1951). Fish were starved for 24 h prior to the starting of the experiment. The recorded LC50 value was 0.3802 ppm/96 h (Finney, 1971). The sub lethal concentration was reported to be taken approximately one-fifth of the LC50 value, that is, 0.0760 ppm (Reed and Muench, 1938). The experimental fishes were separated into six batches of 10 fishes in each aquarium (15 L capacity), containing 10 L water. First batch run as control without cypermethrin exposure and remaining five batches were exposed to sub lethal doses of cypermethrin for 24 h, 7, 15, 20 and 30 days period. Each experiment was conducted in triplicate. Control and exposed fishes were sacrificed at the end of each time interval. Gills, kidney, intestine, brain, liver and muscles were isolated and immediately transferred to deep freezer prior to analysis. Technical grade cypermethrin (95%) was obtained from United Phosphorus Ltd., Mumbai.

**Estimation of AChE activity**

1% homogenate of the brain tissue and 5% homogenate of the other tissues were separately prepared in 0.25 M ice cold sucrose solution. The AChE activity in these extracts was estimated by the method suggested by Ellman (1958). The reaction mixtures contained 1 ml of 0.1 M phosphate buffer (pH 7.2) and 0.5 ml of the tissue homogenate. After 30 min of incubation at 37°C, the reaction was stopped by the addition of 2 ml of alkaline hydroxylamine hydrochloride and 1 ml of HCl (1:1 HCl:H2O). The contents were thoroughly mixed and centrifuged at 100 rpm for 15 min. To the supernatant, 0.5 ml solution of 10% ferric chloride solution was added to deepen the colour. Colour of the solution was measured against the blank at 540 nm in a spectrophotometer. The blank contained 1 ml of the buffer solution. Enzyme activity was expressed as µmoles of ACh hydrolysed/ml of protein/h.

**Estimation of ACh content**

ACh content is separately estimated in the gill, kidney, intestine, brain, liver and muscle tissues in both cypermethrin; and in control fish, *C. striatus* was estimated. Tissues were isolated, weighed and kept in hot water bath for 5 min to inactivate AChE activity and to release abundant ACh. The samples were allowed to cool down at room temperature and homogenated in 2 ml of distilled water, 2 ml of alkaline hydroxylamine hydrochloride and 1 ml of HCl were added to the homogenate and centrifuged at 1000 rpm for 10 min. 0.5 ml of ferric chloride was added to the 2.5 ml of clear supernatant and ACh content was estimated by spectrophotometer at 540 nm. The ACh content was expressed as µ moles of ACh/g wet weight of the tissue.

**RESULTS AND DISCUSSION**

The effect of cypermethrin on activity of AChE and ACh content in gills, kidney, intestine, brain, liver and muscles of fish after its chronic exposure caused significant inhibition and recovery in its activity and content of ACh has been observed. The results are shown in Table 1 and Figures 1 and 2. For comparison, differences in the levels of each parameter mentioned earlier recorded in the control and experimental fish were converted as percentages of the corresponding control and the percent values are shown in Table 1. The data were plotted against exposure period in Figures 1 and 2. The percentage recovery in the levels is calculated in relation to the level in the control medium which is fixed at 100%.

From the data presented in Table 1 and Figures 1 and 2, it is observed that the levels of AChE activity were
Table 1. Effect of cypermethrin on AChE activity (µmoles/min/mg protein) in gills, kidney, intestine, brain, liver and muscles of *C. striatus*.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Exposure period (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>(I) Gill</td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t-Test</td>
</tr>
<tr>
<td>(II) Kidney</td>
<td>Mean</td>
<td>604.60</td>
</tr>
<tr>
<td></td>
<td>SD±</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-Test</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(III) Intestine</td>
<td>Mean</td>
<td>37.60</td>
</tr>
<tr>
<td></td>
<td>SD±</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-Test</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(IV) Brain</td>
<td>Mean</td>
<td>232.20</td>
</tr>
<tr>
<td></td>
<td>SD±</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-Test</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(V) Liver</td>
<td>Mean</td>
<td>646.80</td>
</tr>
<tr>
<td></td>
<td>SD±</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-Test</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(VI) Muscle</td>
<td>Mean</td>
<td>86.70</td>
</tr>
<tr>
<td></td>
<td>SD±</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-Test</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed in Mean±SD of six replicates. Values are significant at P<0.001.

Elevated at 24 h relative to control in all the organs whereas the ACh content followed an opposite trend. The levels of AChE activity decreased through the 7th day and continued up to 15th day corresponding to the levels of ACh activity that gradually elevates and come nearer to the control at 30 day exposure period, whereas the levels of ACh content followed an opposite trend and came nearer to the control at the end of 30 day exposure.
Figure 1. Variation in AChE activity in gills, kidney, intestine, brain, liver and muscle of *C. straitus* on exposed to cypermethrin.

Figure 2. Effect of cypermethrin on ACh content in different organ tissues of *C. striatus* on exposed to cypermethrin.

period and the values were found to be significant (P<0.001). The maximal percentage change in AChE activity and ACh content was recorded for 15 days period of cypermethrin exposed fishes. The decrease in AChE
activity with a concomitant increase in ACh content was noticed in these organs, with maximum inhibitory changes in ACh at 15 days exposure period and the fish recovered almost completely at 30 day exposure.

ACh neurotransmitter substance at nerve cell junction will be acted upon by a substrate enzyme AChE. AChE is one of the hydrolytic enzymes disturbing the normal nervous functions finally resulting in the death of animals (Eto, 1974). The decreased brain profiles under cypermethrin stress have been documented by Bashamohideen and Malla (1987). These insecticides effected significant inhibition of brain AChE activity accompanied by a concurrent increase in ACh. Sambasiva et al. (1985) studied the effect of elsan on the AChE activity in the fish C. punctatus and found that the AChE activity was inhibited in all the tissues. The AChE activity revealed the highest inhibition in the brain followed by liver and muscle. Bandhyopadhyay (1982) observed the inhibition of AChE in rat brain by cypermethrin and found that cypermethrin significantly inhibited the AChE activity under in vivo conditions. Reddy et al. (1991) reported that cypermethrin at sub-lethal concentrations induced significant changes in AChE activity and ACh content in the brain tissue of both juvenile and adult-fish. Maximum inhibition of AChE activity is noticed at 6 and 12 h after exposure to cypermethrin in juvenile and adult fish, respectively. In contrast, the ACh levels registered an increase in both cases. During subsequent periods, the rate of recovery in AChE activity and ACh content is variable in both groups. Rajini and Revathy (2015) have studied the effect of combination of pesticide on AChE activity in freshwater, Danio rerio. They reported that the effect of chlorpyrifos 50% and cypermethrin 5% EC on AChE activity in tissue of D. rerio. The inhibition of acetylcholinesterase activity increased with pesticide concentration and exposure period. Jindal and Kaur (2014) reported AChE inhibition and assessment of its recovery response in some organs of Ctenopharyngodon idella induced by chlorpyrifos.

The organophosphorus (OP) pesticides inhibit the cholinesterase activity in almost all animal tissues. Inhibition of AChE activity was regarded as a significant parameter in assessing complex toxicogenic effects of various toxicants, including pesticides (Mukherjee and Bhattacharya, 1974; Bhattacharya and Gosh, 1981; Bhagyalakshmi et al., 1984, 1985). Under normal conditions, the enzyme AChE initially forms a complex with the substrate ACh, which then acetylates the enzyme with the release of choline. Cypermethrin occurs by the reaction of water with the acetylated 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).

Organochlorides also disrupt the nervous activity by prolonging the inward Na+ current and also by suppressing the increase in K+ permeability thereby leading to the accumulation of ACh at the synaptic junctions (Narahashi, 1976). The suppression in AChE activity corresponded with the proportionate elevation in ACh content in the nervous and muscular tissue of the fish exposed to cypermethrin.

In the present investigation, changes in AChE activity and ACh content during synthetic pyrethroid, cypermethrin exposure was undertaken. Data on AChE activity and ACh content revealed inhibition in the enzyme activity followed by a concomitant increase of ACh content in all the tissues of C. striatus exposed to cypermethrin (Table 1 and Figures 1 and 2). From the data, it is evident that the AChE activity suddenly becomes activated during 24 h exposure. The enhancement in activity of AChE might be due to the pollution stress. Similarly, inhibition in its activity in various organ tissues of fish has been reported by various workers (Jindal and Kaur, 2009; Kumar and Champman, 2001; Rao et al., 2003; Joseph and Raj, 2011). Gupta (1994) reported that the inhibition of AChE consequently leads to excessive ACh accumulation at the synapses and neuromuscular junctions, resulting in overstimulation of Ach receptors.

Similarly Sathyadevan et al. (1983) reported an initial elevation in AChE activity of the brain of C. carpio exposed to an organophosphorus dimethoate. Kufesak et al. (1994) reported an initial elevation in AChE activity in carp on exposure to pesticides. In the major carp Catla catla on exposure to deltamethrin, Nissar Ahmed (1994) reported an initial elevation and further inhibition of AChE activity. Subhan (2000) reported an initial elevation and further inhibition of AChE activity in C. catla exposed to synthetic pyrethroid fenvalerate. An initial elevation in AChE activity was reported in the small, Stagnicola species on exposure to malathion and were in consonance with the present elevation in AChE activity. Das and Mukherji (2009) observed that cypermethrin inhibits AChE activity in the brain of L. rohita fingerlings.

But at later periods like 7 and 15th day exposure the AChE activity was reduced, with maximal reduction at 15th day exposure period of cypermethrin. The inhibition in AChE activity resulted in the accumulation of ACh at different sub-lethal exposure periods of cypermethrin. Similarly, Health and Prichard (1965) reported that OP insecticides react with AChE to form phosphorylated enzyme, this phosphorylated enzyme inhibits the AChE for several weeks (Coppage and Duke, 1971), and such inhibition is observed in the present study.

In addition, Bhagyalakshmi and Ramamurthy (1980) reported inhibition of AChE activity in Ozietelphusa sensex sensex exposed to fenithrothion. Gosh and Bhattacharya (1992) reported significant inhibition of brain AChE activity accompanied by a concurrent increase in ACh content in C. 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, 1997) which is in consonance with the present inhibition in AChE activity.

According to the environmental biologists and chemists, the measurement of brain AChE activity in the aquatic animals, indicates the extent of pollution of the environment in 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 *M. mossambica* exposed to sumithion.

According to Coppage et al. (1975), inhibition of brain AChE activity to the level of 70 to 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. There are also reports on the inhibition of muscle AChE activity in snails exposed to pesticide toxicity.

However, at 30th day exposure, the inhibitory activity was decreased and came nearer to normal level. Thus, the fish *C. striatus* fairly recovered from the inhibitory activity in tissues of fish. The concomitant recovery in AChE activity during the 30th day exposure period might be due to active metabolism of cypermethrin which is being removed from the site of action and thus enabling the enzyme to resume unhindered hydrolysis of ACh. The recovery of inhibited AChE was reported early by Hobbiger (1951). Similar reports were also observed by Coppage and Duke (1972) in fish brain AChE inhibition in Louisiana Lake exposed to malathion.

**Conclusion**

It is concluded that exposure to sub-lethal concentrations of cypermethrin for a period of 30 days, affected adversely the AChE activity and ACh content in different organ tissues and thus the behavior of the fish. The parameters studied could be biomarkers of toxicological effects of the pesticides on the fish and help in diagnosis of the pollution and control on indiscriminate use of the pesticides is suggested.

**Conflict of interest**

Authors have none to declare

**REFERENCES**


Sevin (Carbamate) and Sumithion (Organophosphate). Curr. Sci. 48:832.