

Concentration related differences in the inhibition of acetylcholinesterase activity in the common carp *Cyprinus carpio* by Phorate, an organophosphorous insecticide

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Abstract

The activity of acetylcholinesterase (AChE) in the vital organs of fish such as gill, liver, muscle, kidney and brain of the freshwater common carp *Cyprinus carpio* (*C. carpio*) was investigated after exposing to acute lethal toxicity (ALT) and chronic sublethal toxicity (CST) of phorate. *C. carpio* fish were exposed to ALT (LC₅₀/96 hours - 0.71 ppm/l) of Phorate for one day and 4 days and CST (one-tenth of the LC₅₀/96 hours - 0.071 ppm/l) of Phorate for 1, 7, 15 and 30 days and the concentration related differences in the inhibition and recovery of the AChE enzyme activity was evaluated in the target organs of the fish. Relative to controls, the activity of AChE in all the organs of the fish exposed to ATP gradually decreased significantly ((P<0.05)) at 1 and 4 days of exposure period whereas in the fish exposed to CTP the AChE activity was elevated at day 1 and gradually decreased from day 7 to day 15 followed by an increase at day 30 in all the organs significantly (P<0.05). Based on the percent values obtained the inhibition of AChE activity was predominantly more in the organs of the fish exposed to ATP in a concentration-dependent manner. On prolonged exposure for 30 days in CTP the AChE activity was restored gradually depending on the initial pesticide exposure concentration. Findings from this study have demonstrated that inhibition of AChE activity in *C. carpio* is a useful biomarker for assessment of anticholinesterase pesticide contaminations in water.

Keywords: Acetylcholinesterase, *Cyprinus carpio*, Acute lethal toxicity, Chronic sublethal toxicity, Phorate.

1. Introduction

Neurological and behavioural activities of animals can be extremely sensitive to environmental contamination [1-4]. Organophosphate (OP) pesticides are competitive inhibitors of AChE, the key enzyme in the transmission of nerve impulse. AChE is readily phosphorylated by the OP pesticides at the active site serine [5]. OPs cause inhibition of AChE and accumulation of acetylcholine at the synapse which leads to over stimulating the post synaptic cells. It is well accepted that 20% or greater inhibition of AChE in birds, fishes and invertebrates indicates the exposure to organophosphate insecticides [6].

The OP pesticides inhibit the cholinesterase activity in almost all animal tissues like fishes. The AChE activity differs from one species to the other in fishes. Chuiko [7] worked on comparative study of AChE and butyrylcholinesterase (BChE) in brain and serum of several fresh water fish by DDVP, an organophosphorus pesticide-reported that brain AChE activity varied among fish species approximately 10-fold, ranging from 192.6 to 1353.2 μ mol/g/h

respectively in perch and white fish. All cyprinids had higher brain AChE activity than those of other fish families. Serum AChE activity was 100-fold lower than in brain. Duration of exposure, type of OP, as well as species of fish has an effect on the extent of AChE activity. It was well documented that highly purified phosphorothionates are not direct inhibitors of cholinesterase but when they are metabolized to their corresponding oxygen analogues become highly potent inhibitors [8, 9]. The susceptibility of animals to poisoning by organophosphorus insecticides (OPI) will be dependent upon the rate at which the analogues are made available to inhibit cholinesterase at critical site in nerve tissue or organs innervated by cholinergic nerves.

The AChE activity is vital to normal behaviour and muscular function in animals and represents a prime target on which some toxicants can exert a detrimental effect. Inhibition of the AChE activity results in a buildup of acetylcholine causing prolonged excitatory postsynaptic potential. This results in repeated, uncontrolled firing of neurons

leading to hyper stimulation of the nerve or muscle fibres, which leads paralysis, and eventual death. AChE activity is routinely used as a biomarker of the exposure to certain groups of contaminants, such as OPI [10]. The inhibition of the AChE by pesticides can affect locomotion and equilibrium of exposed organisms [11, 12].

The measurement of AChE activity can provide useful information on toxicant impact [13, 14] on fishes and also to correlate between behaviour of fish and cholinesterase inhibition. It also provides the species-specific differences in the relationship between AChE inhibition and mortality and other physiological disturbances associated with the AChE inhibition [15, 16]. Fishes are aquatic vertebrates that are members of the largest and most diverse vertebrate taxon which are the trophic level connection in aquatic ecosystems. Hence, fish bioassay experiments are indices to determine the acute toxicity and possible effect on metabolisms of the animals like fish due to the toxicant stress [17, 18]. Hence in the present investigation, the inhibition and recovery of the AChE enzyme activity was studied in the vital organs of fish *C. carpio*, a representative of the aquatic environment, on exposure to ALT and CST of phorate, an OPI, which is widely used in the local area to combat pests.

2. Materials And Methods

2.1. Test Species

The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fish, having great commercial value. The animals were starved for 24 hours prior to each estimation to avoid any influence of differential feeding.

2.2 Test Chemical

Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an OPI which is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops. Commercial names of phorate are Thimet, Rampart, Granutox, Agrimet etc and its molecular formula is $C_7H_{17}O_2PS_3$.

2.3 Acute and Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by Probit method of Finney [19]. $LC_{50}/96$ hours (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity and one-tenth of the $LC_{50}/96$ hours (0.071 ppm/l) concentration of phorate was taken as the sub-lethal concentration for chronic toxicity study.

2.4 Experimental Design

160 fishes were divided into two batches, again batch I was divided into 3 groups and batch II into 5 groups comprising of 20 fishes each. Batch I

was exposed for ATP (exposed to LC_{50} of Phorate) and batch II was exposed for CTP (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm/l). In batch I, group 1 was considered as normal control, group 2 and 3 were experimental groups. The fishes of group 2 were exposed for 1 day and group 3 for 4 days. In batch II, group 1 was considered as normal control group, group 2, 3, 4 and 5 were experimental groups. The fishes of group 2 were exposed for 1 day, group 3 for 7 days, group 4 for 15 days and group 5 for 30 days.

2.5 Estimation of Acetylcholinesterase (Acetylcholine hydrolase, EC: 3.1.1.7) activity

AChE activity in the organs of the fish was estimated by the method of Ellman *et al* [20] and the enzyme activity was expressed as μ moles of Ach hydrolysed /mg protein/hr.

2.6 Statistical analysis

Duncan's Multiple Range (DMR) test had been employed for the statistical analysis of the AChE activity data. P value (level of significance) is significant at < 0.05 .

3. Results and Discussion

3.1. Results

The data on the activity of AChE in the organs of the fish such as gills, liver, muscle, kidney and brain of *C. carpio* at 1 and 4 days on exposure to ATP and 1, 7, 15 and 30 days on exposure to CTP, besides controls, are presented in the Table 1. For comparison, the differences obtained in relation to the controls of each organ of the fish at the above said exposure periods in acute and chronic toxicity study of phorate, were converted as percentages of the corresponding controls and those percent values are also presented in the same table and was plotted a graph of percent changes against exposure periods in Figure1.

3.2 Activity of Acetylcholinesterase

From the data presented in the Table-1 and Figure-1 relative to controls, the levels of AChE activity in all the organs of the fish exposed to phorate gradually decreased at 1 and 4 days of exposure in acute toxicity in the order of day 1>4 and the differences in the activity between controls and experimental were also found to be statistically significant ($P<0.05$). In the fish exposed to CTP relative to controls the levels of AChE activity elevated at day 1 and gradually decreased from day 7 to day 15 followed by an increase at day 30 in all the organs of the fish in the order of day 1>7>15<30 and the values were found to be statistically significant ($P<0.05$). However, based on the percent values obtained (Table-1 and Figure-1) the decrease in AChE activity was found to be predominantly more in the organs of the fish exposed to ATP.

Table-1: AChE activity (μ moles of Ach hydrolysed /mg protein/hr) in different organs of the fish *C. carpio* at different periods of exposure to ACTP. The values below the mean are percent changes over the respective control.

Organ		Exposure period in days							
		Acute toxicity			Chronic toxicity				
		Control	1	4	Control	1	7	15	30
Gill	Mean \pm	328.760 ^c	168.430 \pm	85.556 \pm	328.760 ^d	386.775 \pm	265.407 \pm	220.532 \pm	257.221 \pm
	S.D. (% change)	0.490	0.397 ^b (-48.76)	0.490 ^a (-73.97)	0.490	0.249 ^e (+17.64)	0.216 ^c (-19.27)	0.353 ^a (-32.92)	0.401 ^b (-21.76)
Liver	Mean \pm	152.083 ^c	91.468 \pm	46.055 \pm	152.083 ^d	188.461 \pm	116.206 \pm	88.968 \pm	122.548 \pm
	S.D. (% change)	0.610	0.434 ^b (-39.85)	0.275 ^a (-69.71)	0.610	0.296 ^e (+23.92)	0.165 ^b (-23.59)	0.289 ^a (-41.50)	0.194 ^c (-19.42)
Muscle	Mean \pm	706.148 ^c	359.803 \pm	118.081 \pm	706.148 ^d	846.530 \pm	583.560 \pm	513.440 \pm	561.175 \pm
	S.D. (% change)	0.517	0.324 ^b (-49.04)	0.508 ^a (-83.27)	0.517	0.415 ^e (+19.88)	0.102 ^c (-17.36)	0.310 ^a (-27.29)	0.050 ^b (-20.53)
Kidney	Mean \pm	204.950 ^c	123.750 \pm	68.106 \pm	204.950 ^d	236.122 \pm	175.929 \pm	133.094 \pm	156.130 \pm
	S.D. (% change)	0.469	0.531 ^b (-39.61)	0.590 ^a (-66.76)	0.469	0.328 ^e (+15.21)	0.244 ^c (-14.16)	0.205 ^a (-35.06)	0.247 ^b (-23.82)
Brain	Mean \pm	1080.418 ^c	408.850 \pm	117.435 \pm	1080.418 ^d	1398.060 \pm	807.720 \pm	567.651 \pm	928.403 \pm
	S.D. (% change)	1.246	0.526 ^b (-62.15)	0.451 ^a (-89.13)	1.246	0.295 ^e (+29.40)	0.356 ^b (-25.24)	0.279 ^a (-47.46)	0.465 ^c (-14.07)

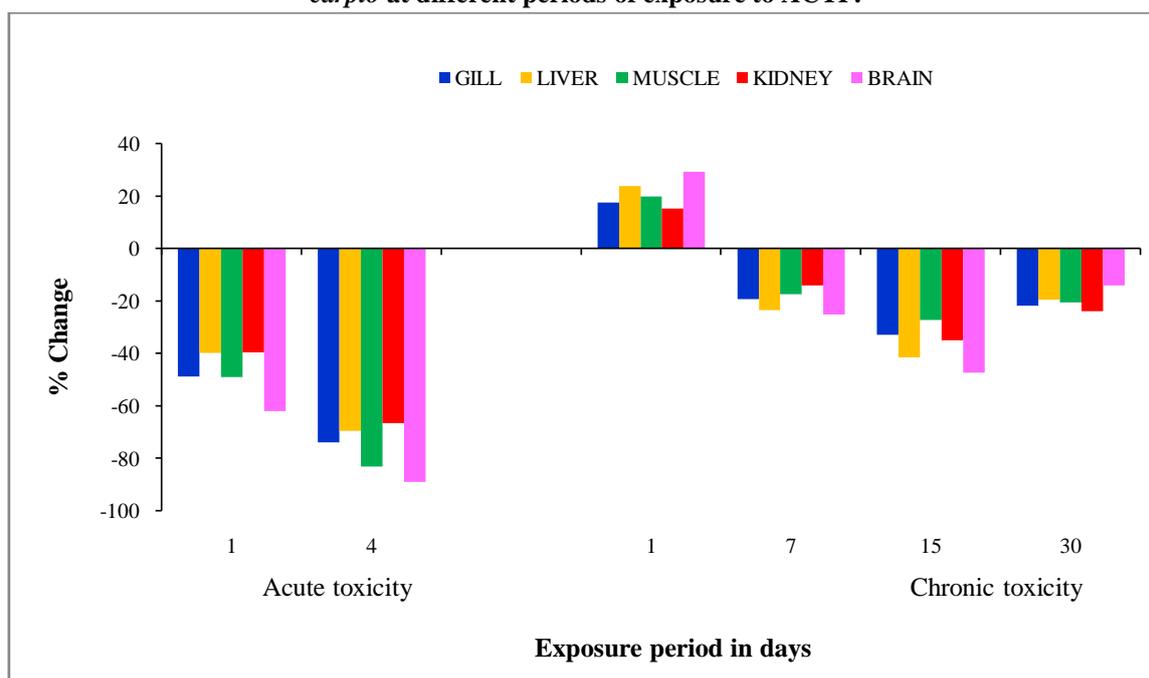
All the values are mean \pm SD of six individual observations. Values with different superscripts within the column are significantly different from each other at $P < 0.05$ according to DMR test.

3.2. Discussion

In the present investigation the data on AChE activity revealed a decrease in the enzyme activity in both the nervous (brain) and non-nervous (gill, liver, muscle and kidney) organs of the fish *C. carpio* exposed to acute and chronic toxicity of phorate (ACTP) except at day 1 in chronic toxicity exposure (Table-1 and Figure-1). A decrease in AChE activity was observed earlier by Vijayaendrababu and Vasudev [21] when treated with Roger and Dimecron in fresh water mussel, *Lamellidens marginalis*. Similar observations were made in snails exposed to different

OP compounds [22, 23] and in fishes [24, 25]. Pan and Dutta [26] studied the inhibition of brain AChE activity of juvenile largemouth bass, *Pterus salmoides* by sublethal concentrations of diazinon and reported that juvenile brain AChE activities were significantly inhibited by sublethal doses of diazinon. Erwin *et al* [27] studied the effects of chronic exposure of parathion on AChE inhibition and increased food consumption rate in the zebra fish, *Danio rerio* and reported that inhibition rate was significant above 0.9 μ g/l after 144 days and above 4.3 μ g/l after 250 days of exposure.

Figure-1: AChE activity (μ moles of Ach hydrolysed /mg protein/hr) in different organs of the fish *C. carpio* at different periods of exposure to ACTP.



All the values are mean \pm SD of six individual observations.

Kristen *et al* [28] studied the effects of diazinon exposure in hybrid striped bass on biochemical and behavioural aspects and reported that the sublethal exposure to diazinon, an OP pesticide, may lead to feeding behavior abnormalities in hybrid striped bass through inhibition of brain AChE activity. Venkateswara *et al* [29] studied sublethal effects of profenofos on locomotive behaviour and gill architecture of the mosquito fish, *Gambusia affinis* and reported that the sublethal concentration of profenofos altered locomotive behaviour such as distance travelled and swimming speed in fish due to inhibition in the activity of AChE and caused for the deformities in the primary and secondary lamellae of gill. Khalid *et al* [30] studied the ethological response, haematological and biochemical profiles of carp, *C. carpio* exposed to trichlorfos, and reported a significant reduction in the AChE activity in the brain tissue of the fish. Pavlov *et al* [31] studied the effect of DDVP, an OPI on feeding behaviour and brain AChE activity in bream, *Abramis brama* (L.) and reported that DDVP exposure resulted in the inhibition of brain AChE activity. Vineet *et al* [32] studied the behaviour and respiratory dysfunction as an index of malathion toxicity in the fresh water fish, *Labeo rohita* and reported that the carp in toxic media exhibited irregular, erratic and darting, swimming movements, hyper excitability, and loss of equilibrium and sinking to the bottom which might be due to inactivation of AChE activity.

In the fish exposed to ATP in the present study the suppression in AChE activity has increased in all the organs with the increase in exposure period. Jaqueline *et al* [33] also reported a decrease in AChE activity in the brain and muscle of fingerlings of the common carp (*C. carpio*), grass carp (*Ctenopharyngodon idella*) and bighead carp (*Aristichthys nobilis*) on exposure to lethal concentration (LC₅₀) of diafuran. Similar results obtained, like in the fish exposed to CTP in the present study by Sailabala [34] in major carp, *Catla catla* on exposure to an OPI malathion and Prasada Charyulu [35] in common carp *C. carpio* on exposure to Phosphamidin with regarding to AChE activity. The AChE activity suddenly activated during 24 hours of exposure in CTP. The enhancement might be due to the pesticide stress. But at later periods of exposure like 7th day and 15th day the AChE activity was reduced, with maximal reduction at 15th day exposure period of phorate. Heath [36] and O' Brein [37] reported that OP insecticides react with AChE to form phosphorylated enzyme. This phosphorylated enzyme inhibits AChE activity for several weeks [38] and such inhibition is observed here in the present study. However at day 30 the inhibitory activity was

decreased and came nearer to normal level. Thus, the fish *C. carpio* fairly recovered from the inhibitory activity in the tissues. The concomitant recovery in AChE activity at day 30 might be due to active metabolism of phorate which is being removed from the site of action and thus enabling the enzyme to resume unhindered hydrolysis of Ach. Similar reports were also observed by Coppage and Duke [39] in fish brain exposed to malathion.

Maximum decrease in AChE activity in the brain of *C. carpio* on exposure to phorate toxicity may indicate disruption in the integratory activity of central nervous system. The reports of Coppage [40] revealed that death occurs in fishes when AChE activity falls below a critical level and according to Coppage *et al* [41] inhibition of brain AChE to the level of 70 to 80% is critical to fishes. In the present study this critical situation was observed in the fish at 4 days of exposure to ATP. Probably the inhibition of respiratory centre of the brain, and the inhibitory nature of pesticide may be responsible for the decrease in this enzyme activity. The rate of inhibition of AChE activity in the organs of animals exposed to pollutants can be correlated to the concentration of pollutant and length of exposure [40]. The inhibition of brain AChE activity is directly proportional to the concentration of the pollutant [39, 40, 42]. Hence in the present study, inhibition in the AChE activity is more in the organs of the fish exposed to ATP than to CTP. Further within acute toxicity, the suppression in the enzyme activity is more at 4 days of exposure than at 1 day and it may be due to the availability of more pesticide for enzyme inhibition. This ultimately leads to the suppression in nervous activity, osmo and ion regulatory activity as well as cellular enzyme metabolic activity.

4. Conclusion

The results obtained in the present study show the sensitivity of AChE activities in gill, liver, muscle, kidney and brain of the *C. carpio* on exposure to ALT and CST of phorate an OPI. The pesticide phorate is interfering with the nervous system of the fish by inhibiting the enzyme AChE activity and it can be used as a useful biomarker for assessment of anticholinesterase pesticide contaminations in water.

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