

ACETYLCHOLINESTERASE INHIBITION AND ASSESSMENT OF ITS RECOVERY RESPONSE IN SOME ORGANS OF *CTENOPHARYNGODON IDELLUS* INDUCED BY CHLORPYRIFOS

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Abstract: *Ctenopharyngodon idellus* was exposed to sublethal concentration (1.44 µg/l and 2.41µg/l) of an organophosphate insecticide chlorpyrifos (CPF). Activity of acetylcholinesterase (AChE) was estimated in liver, kidney and gills of the fish at regular intervals of 15, 30 and 60 days of chronic exposure, and at the same intervals recovery response was also evaluated. A direct relationship was observed in the inhibition of acetylcholinesterase activity with increase in the pesticide concentration and exposure period. Inhibition of AChE activity after intoxication suggested an increase in the acetylcholine content and its consequent accumulation at the synapses of neurons. Excessive accumulation of acetylcholine led to prolonged excitatory postsynaptic potentials, thus causing hyperstimulation of the receptor. The study revealed that the exposure to the pesticide influenced the behavioural activities of the fish. The effect on AChE activity in the organs of the fish can be used as an early biomarker of toxicity of chlorpyrifos. Almost total recovery upto 95-99% was found after 2 months.

Keywords: toxicity, chlorpyrifos, acetylcholinesterase, *Ctenopharyngodon idellus*.

Introduction

Organophosphates used during intensive agricultural practices can reach natural waters either via seepage of toxicant from the soil or through surface runoff of rain. Fishes are particularly sensitive to wide range of pesticides, chemicals and toxic conditions. Repeated exposures to sub-lethal concentrations of pesticides can cause physiological and behavioural changes in fish. Among organophosphates, chlorpyrifos is one of the most frequently used pesticide throughout the world. It is a broad-spectrum, organophosphate with wide application in the field of agriculture and has wide variation of toxicity among different species. The toxicity of this pesticide is primarily attributed to its ability to inhibit acetylcholinesterase, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Soreq and Zakut, 1993). The inhibitory effects of OP insecticides depend on their binding to the enzyme active site and by their rate of phosphorylation (Dutta *et al.*, 1995). Acetylcholinesterase (AChE) activity is routinely used as a biomarker of the exposure to

certain groups of contaminants (Grue *et al.*, 1997). Even low concentration of the toxicant can inhibit AChE (Varó *et al.*, 2003). The inhibition of the acetylcholinesterase by pesticides can affect locomotion and equilibrium of exposed organisms (Saglio & Trijasse, 1998; Bretaud *et al.*, 2000; Jindal & Jha, 2005) and adversely affect various metabolic activities (Pant and Singh, 1983). This enzyme is extremely important for many behavioural activities like prey location, predator evasion and orientation towards food (Miron *et al.*, 2005). AChE is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Arends, 2003). In the present study, investigations have been made on activity of AChE in the liver, kidney and gills of the *Ctenopharyngodon idellus* and behaviour of the fish on exposure to chlorpyrifos.

Materials and Methods

20% EC chlorpyrifos (O, O diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), a light yellow liquid was selected for the present study. *Ctenopharyngodon idellus* (weight: 10±2 gm, length: 10±2 cm) were collected from Nanoke Fish Seed Farm located at Nanoke village, Distt. Patiala, Punjab, India and were acclimatized to the laboratory conditions for 15 days in glass aquarium. They were fed with palletized supplementary feed once a day. Water in the aquarium was renewed daily. For acute toxicity tests, the physico-chemical characteristics of water were determined (APHA, 2012) and were: temperature 25±2 °C, pH 7.2±0.1, dissolved oxygen 8.0±0.3 mg/L, total alkalinity 175±10 mg/L and total hardness 18±0.5 mg/L. 96 h LC₅₀ of chlorpyrifos was determined using Probit analysis (Finney, 1980) and was found to be 7.24 µg/l.

For chronic toxicity tests, 10 healthy fish were introduced into three experimental tanks with one-third (2.41 µg/l) and one-fifth (1.44 µg/l) of the LC₅₀ of chlorpyrifos as sub-lethal concentrations. Two replicates along with control were also maintained simultaneously. Experiment was conducted for a period of 15, 30 and 60 days and recovery was assessed at similar intervals. At the end of each exposure period, the fish were sacrificed by cervical dislocation and liver, kidney and gills were excised & immediately kept in saline. 10% homogenate of tissue was prepared in 0.1M Tris-HCl buffer (pH-7.4) using a Potter-Elvehjum homogenizer at 0-4°C. The homogenate was centrifuged at 9200 rpm for 30 min and the supernatant was taken as source for the estimation of enzyme activity. For its determination, 2.8 ml of phosphate buffer was added to 25 ml of enzyme preparation and incubated at 37°C for 10 min and was followed by addition of 0.1 ml Ellman's reagent (DNTB). The reaction was initiated by addition of 0.1 ml AChI (acetylcholine iodide). The

absorbance was noted for 2 min at 412 nm (Ellman *et al.*, 1961). Protein was estimated (Lowry *et al.*, 1951). The behaviour of the fish during the experiment was observed. The significance was calculated at ($p < 0.05$).

Results and Discussion

Effect of chlorpyrifos on the activity of AChE ($\mu\text{moles}/\text{min}/\text{mg}$ protein) in liver, kidney and gills of the fish after its chronic exposure caused significant inhibition and recovery in its activity has been observed (Table 1, Fig. 1). After exposure to the toxicant, AChE activity decreased at both the concentrations in liver, kidney and gills of *C. idellus* as compared to the control. A significant inhibition in the activity of AChE was observed and it increased with the increase in the exposure to chlorpyrifos in the organs of the fish studied. In the liver, AChE activity decreased significantly by 5.01% & 9.6% (15 days), 14.8% & 19.50% (30 days) at 1.44 $\mu\text{g}/\text{l}$ and at 2.41 $\mu\text{g}/\text{l}$ of CPF (Fig 1a). Similarly, on 60th day exposure, there was significant decrease in AChE activity and this decrease was more at higher concentration (33.5%) as compared to the lower concentration (30.7%). Similarly, inhibition in its activity in various tissues of the fish has been reported by various workers (Kumar & Chapman, 2001; Rao *et al.*, 2003; Joseph & Raj, 2011). The inhibition of AChE consequently leads to excessive ACh accumulation at the synapses and neuromuscular junctions, resulting in overstimulation of ACh receptors (Gupta, 1994). In the kidney its activity was found to be inhibited by 11.2% & 17.34% (15 days), 19.1% and 23.46% (30 days) and further inhibited by 37.6% and 49.5% (60 days) at 1.44 $\mu\text{g}/\text{l}$ and at 2.41 $\mu\text{g}/\text{l}$ of chlorpyrifos respectively as compared to control (Fig. 1b). The higher the concentration of the pesticide and exposure time, the greater is the negative impact. Similarly in the gills, its activity was found to be inhibited by 4.9% and 18.8% (15th day), 12.7% and 26.47% (30 days) and 34.6% and 49.5% (60 days) at 1.44 $\mu\text{g}/\text{l}$ and 2.41 $\mu\text{g}/\text{l}$ of CPF respectively (Fig 1c). The inhibition observed in the activity of AChE, is in agreement with the findings of other workers (Das & Mukherjee, 2003; Rao, 2006; Crestani *et al.*, 2007; Joseph & Raj, 2011).

The toxicant exposed fish showed erratic, speedy and jerky movements at lower concentration (1.44 $\mu\text{g}/\text{l}$) and at the higher concentration (2.41 $\mu\text{g}/\text{l}$) fish exhibited hyperactivity, violent behaviour and jumping out of the tanks violently (escape behaviour). The intensity of altered behaviour was found to be related to toxicant concentration and time dependent. Prolonged exposure i.e. 30 days and 60 days, the fish became hypoactive, struggled for breathing, restricted swimming movements finally led to lethargic condition and loss of equilibrium. Normal behaviour was observed during the recovery period of the

experiment. When AChE activity decreases, ACh is not broken and accumulates within synapses and cannot function in a normal way (Dutta & Arends, 2003). Hence, the altered locomotor behaviour of fish could be attributed to the accumulation of acetylcholine which interrupted coordination between the nervous and muscular junctions (Rao *et al.*, 2005; Rao, 2006). Considering the role of AChE in neurotransmission in both central nervous system and at neuromuscular junctions, the inhibition of AChE activity could be correlated to behavioural changes observed in *C. idellus* exposed to chlorpyrifos (Brewer *et al.*, 2001; Kavitha and Rao, 2008).

Present findings demonstrate that AChE activity in liver, kidney and gills of *C. idellus* showed recovery response in toxicant free water (Table 1). This is in concurrence with the findings of Rath & Mishra (1981) and Oruc (2012). The variation in the recovery of AChE activity in different organs might be because of different molecular forms of this enzyme can hydrolyze ACh as well as other esters (Rao, 2004). A maximum recovery of 99.54 % has been found in the liver followed by kidney and gills, as gills are more sensitive to the toxicant and its detoxification system is not robust.

The present results as well as previous findings (Sancho *et al.*, 1997; Oruc & Usta, 2007; Patil & David, 2009) reflected the affinity of chlorpyrifos for fish AChE. AChE recovery

Table 1. Effect of chlorpyrifos on AChE activity ($\mu\text{moles}/\text{min}/\text{mg}$ protein) in liver, kidney and gills of *Ctenopharyngodon idellus*.

Exposure	Organ	Control	15 days		Control	30 days		Control	60 days	
			2.41 $\mu\text{g}/\text{l}$	1.44 $\mu\text{g}/\text{l}$		2.41 $\mu\text{g}/\text{l}$	1.44 $\mu\text{g}/\text{l}$		2.41 $\mu\text{g}/\text{l}$	1.44 $\mu\text{g}/\text{l}$
With toxicant	Liver	0.279 \pm 0.001	0.252 \pm 0.001	0.265 \pm 0.003	0.282 \pm 0.001	0.227 \pm 0.002	0.240 \pm 0.002	2.80 \pm 0.005	0.186 \pm 0.001	0.194 \pm 0.004
	Kidney	0.098 \pm 0.001	0.081 \pm 0.002	.087 \pm 0.001	0.098 \pm 0.001	0.075 \pm 0.003	0.089 \pm 0.002	0.101 \pm 0.004	0.051 \pm 0.003	0.063 \pm 0.002
	Gills	0.101 \pm 0.0005	0.082 \pm 0.002	0.096 \pm 0.001	0.102 \pm 0.001	0.075 \pm 0.003	0.089 \pm 0.002	0.101 \pm 0.002	0.051 \pm 0.006	0.066 \pm .003
Without toxicant	Liver	0.276 \pm 0.002	0.203 \pm 0.005	0.213 \pm 0.002	0.275 \pm 0.003	0.228 \pm 0.001	0.26 \pm 0.007	0.276 \pm 0.002	0.238 \pm 0.003	0.275 \pm 0.003
	Kidney	0.097 \pm 0.001	0.084 \pm 0.001	0.089 \pm 0.004	0.096 \pm 0.001	0.090 \pm 0.001	0.094 \pm 0.001	0.097 \pm 0.001	0.095 \pm 0.0005	0.096 \pm 0.006
	Gills	0.101 \pm 0.001	0.072 \pm 0.002	0.079 \pm 0.002	0.099 \pm 0.001	0.084 \pm 0.003	0.087 \pm 0.003	0.097 \pm 0.001	0.090 \pm 0.001	0.096 \pm 0.001

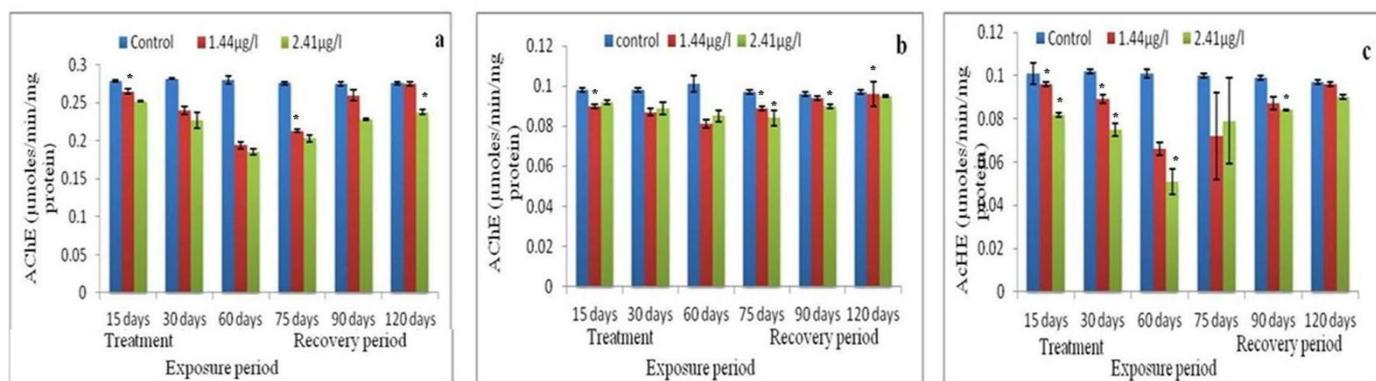


Fig 1. Variation in AChE activity in liver (a), kidney (b) and gills (c) of *C. idellus* on exposure to chlorpyrifos.

was reported also in fishes exposed to organophosphates (Dembélé *et al.*, 1999; Dutta and Arends, 2003; Rao, 2004; Rao, 2008; Oruc, 2012).

Conclusion

It is concluded that exposure to sublethal concentrations of chlorpyrifos for a prolonged period of 60 days, affected adversely the AChE activity in different organs and thus the locomotory behaviour of the fish *C. idellus*. The parameter measured could be biomarker of toxicological effects of the pesticide on the fish and help in the diagnosis of the pollution and control on indiscriminate use of the pesticide is suggested.

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