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Enzyme Inhibition (AChE) in Brain of *Oreochromis mossambicus* due to Pesticidal Pollution of Herbicide "Pursuit"

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ABSTRACT

In the present investigation, the effect of three sublethal concentrations of Pursuit, that is, 63.7 ppm, 85 ppm, and 127.5 ppm in *Oreochromis mossambicus* was studied. Pursuit inhibited acetyl cholinesterase in the brain of *Oreochromis mossambicus* by increasing the K_m and V_{max}, thereby acting as a mixed inhibitor. The assay of brain AChE is thus useful for monitoring pesticide toxicity of fish.

Keywords: Acetyl cholinesterase, *Oreochromis mossambicus*, Pursuit and Mixed inhibitor.

INTRODUCTION

The unscrupulous use of pesticides, approximately 19,000 to 20,000 pesticides, which broadly include; herbicide, insecticide, and fungicide are, currently approved for release by the U.S. Environmental Protection Agency (EPA), with the advent of "Green Revolution" (Boon and Bridge 2003). Acetylcholine is released from pre-ganglion neurons of parasympathetic division of autonomic nervous system. It is a unanimously accepted fact that hydrolysis of Acetylcholine (ACh) to choline and acetic acid is catalyzed by enzyme cholinesterase in animal system. The enzyme prevents accumulation of excessive acetylcholine at cholinergic synapse and at neuromuscular junction (Konar, 1979; Kollberg, 1976). Quantitative estimation of acetyl cholinesterase (AChE) is taken as a good indicator of the extent of pesticide pollution in animals. Enhanced ACh accumulation results in affecting metabolism, muscle coordination, and irregular transmission of impulse and ultimate death of the animal. The test pesticide pursuit (10% st) is a herbicide (a carbamate compound) used extensively for effective control of annual grasses, sludge, and broad leaf weeds in soyabean and groundnut crops, and so on. Its main chemical IMAZETHAPYR ($C_{15}H_{22}N_4O_3$) blocks protein synthesis. Therefore, the present study was undertaken to investigate long term exposure of pesticides pursuit on brain AChE enzyme kinetics of an exotic carp *Oreochromis mossambicus* which may be used as a diagnostic tool to assay toxicity of carbamate compounds to vertebrates and as a controlling measure to check the growth of *Oreochromis mossambicus* which is commonly known as a neuscence fish, as it destroys the indigenous fauna.

MATERIAL AND METHOD

Healthy fingerlings of *Oreochromis mossambicus* of 5 cm length of both sexes were kept in glass aquaria and acclimatized to laboratory conditions for two weeks. They were fed daily, until two days prior to acute and chronic exposure of pursuit (American Cynamide Co. USA). LC_{50} value of pursuit for 96 hrs was determined by Doudroff *et al.*, (1951) and estimated to be 0.51 ml/L. Three sublethal concentrations were taken from 2/3rd of LC_{50} value and were 63.7 ppm, 85 ppm, and 127.5 ppm. Group of 10 fingerlings was exposed to 3 sublethal concentrations for 96 hrs and control was also maintained for the same duration. At the end of the experiment,

the control and experimental fishes were dissected and brain was removed. 5% tissue homogenate was prepared in ice-cold 0.25 M sucrose solution and centrifuged at 12000 rpm for 7 minutes. AChE activity was measured spectrophotometrically at 540 nm by the method of Metcalf (1951) using AChI as substrate. Protein estimation was done according to Lowry's *et al.* method (1951) using Bovine serum albumin as standard. K_m and V_{max} were calculated by applying Line weaver Burk plot for enzyme kinetic study.

RESULTS

The kinetic parameters K_m and V_{max} of enzyme AChE were undertaken for pursuit toxicity (acute exposure of 96 hrs). The three sublethal concentrations were 63.7 ppm, 85 ppm, and 127.5 ppm of LC_{50}, 96 hrs. The calibration standard graph was made to access K_m values .In Control fingerlings, the K_m value of brain was observed as 4.78 x 10^{-3} at 63.7 ppm, and the K_m x 10^{-3} M was 6.24 x 10^{-3} M, further increased to 12.47 x 10^{-3} M at 85 ppm and was maximum at 127.5 ppm, i.e., 18.34 x 10^{-3} M. The V_{max} was found to be highest at 127.5 ppm as 14.3 Absorbance/mg proteins/30 min which decreased to 9.10 A/mg protein/30 min at 85 ppm. The V_{max} at 63.7 ppm was observed as 5.0 A/mg protein/30 min and V_{max} for control fingerlings were observed as 1.22 A/mg protein/30 min (Table1 and Fig. 1).

Table 1: Acute effect of different concentrations of Pursuit on kinetic parameters K_m and V_{max} of AChE of Brain of *O. mossambicus* (Substrate used was AChI).

Pursuit			**KINETIC PARAMETERS**
Concentration (ppm)	K_m x 10^{-3}M		V_{max} Absorbance/mg protein/ 30 min.
96 hrs.			
Control	4.78 x 10^{-3}	± 0.69	1.22
63.7 ppm	6.24 x 10^{-3}	± 0.269	5.0
85.0 ppm	12.47 x 10^{-3}	± 0.48	9.10
127.5 ppm	18.34 x 10^{-3}	± 0.16	14.31

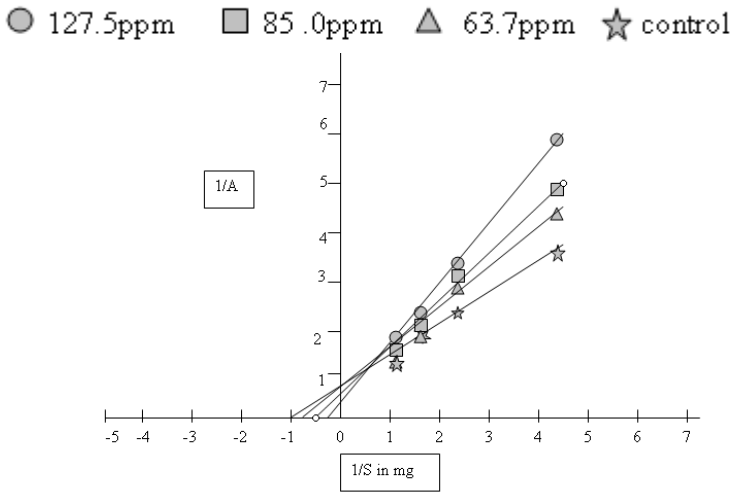

Fig. 1: Line weaver burk plot of inhibitory effect of 63.7; 85.0; and 127.5 ppm PURSUIT on AChE on brain of *O.mossambica* treated for 96 hrs. (S is the concentration of AChI).

The slopes obtained from uninhibited (controlled) and inhibited (treated) enzymes intersected at different ordinates of Michaels menten constant, showed a significant increase in toxic conditions at 96 hrs.

The fingerlings were exposed for 15 days and 30 days, for chronic exposure, the fingerlings were exposed to minimum concentration of pursuit, i.e., 63.7 ppm (1/8th of LC_{50} for 96 hrs).The fingerlings were exposed to 63.7 ppm pursuit for 15 days, the K_m in brain tissues was observed as 7.48 x 10^{-3} M against a control value of 4.76 x 10^{-3} M. The K_m x 10^{-3} M reached 8.9 x 10^{-3} M after 30 days at 63.7 ppm concentration against a control value of 4.76 x 10^{-3} M on the 30th day. Fingerlings exposed to 63.7 ppm values gave a V_{max} of 4.5 A/mg protein/30 min for 15 days and 30 days of chronic exposure to pursuit. While the V_{max} in control fingerlings was observed as 1.25 A/mg protein/30 min (Table 2 and Figure 2) on the 15th and the 30th day.

Table 2: Chronic effect of different concentrations of Pursuit on kinetic parameters K_m and V_{max} of AChE of Brain of *O. mossambicus* (The Substrate use was AChI).

Pursuit Concentration (ppm)	**KINETIC PARAMETERS**	
	K_m x 10^{-3}M	V_{max} Absorbance/mg Protein/30min
15 Days		
Control	4.76 x 10^{-3} ± 0.28	1.25
63.7 ppm	7.48 x 10^{-3} ± 0.52	4.5
30 Days		
Control	4.76 x 10^{-3} ± 0.46	1.25
63.7 ppm	8.9 x 10^{-3} ±0.52	4.5
Control	4.76 x 10^{-3} ± 0.28	1.25

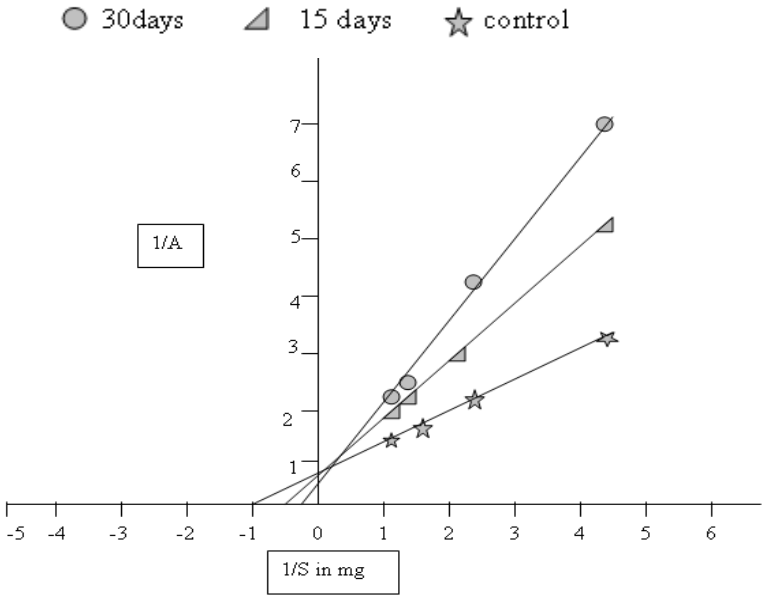

Fig. 2: Line weaver burk plot of inhibitory effect of 63.7 ppm Pursuit on AChE on brain of *O.mossambica* treated for 15 and 30 days. (S is the concentration of AChI).

DISCUSSION

The Line weaver Burk plots of the present investigations revealed the acute exposure of pursuit for 96 hrs at three sublethal concentrations, i.e., 63.7 ppm, 85 ppm, and 127.5 ppm, and reported an increase in K_m x 10^{-3} M from 4.78 x 10^{-3} M in control to 63.7 ppm, 85ppm, and 127.5 ppm concentrations where K_m x 10^{-3} M increased to 6.24 x 10^{-3}, 12.47 x 10^{-3} M and 18.34 x 10^{-3} M, respectively, in Brain. The V_{max} at control 22 A/mg protein/30 min and at 63.7 ppm, 85 ppm, and 127.5 ppm as 5.0 A/mg protein/30 min, 9.10 A/mg protein/30 min, and 14.3 A/mg protein/30 min, respectively. The slope obtained from uninhibited and inhibited enzymes intersect at different ordinates of Michaelis Menten constant showed a significant increase in toxic conditions at 96 hrs .Our results are in conformity with Basha Mohideen and Sailbala (1989), and Coppage and Mathews (1974), although with different fish species and with Rao *et al.*, (1984) and Rao and Rao (1989), Tembhre and Kumar (1995), who also reported similar trends of mixed inhibition. Moreover *Oreochromis mossambicus* (Tilapia), which has created a serious problem for survival of indigenous fauna, can be controlled to certain extent by this carbamate compound pursuit as the fish is hardy and resistant to majority of toxicants pursuit being a carbamate and a mixed inhibitor is strong toxicant and its recovery is far more difficult.

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