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Inhibitory efficacy of Chlorpyriphos and *Datura stramonium* on Acetylcholinesterase activity, Kinetics and Histology of Brain of *Catla catla*

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Abstract : Influence of ethanol leaf extract of *Datura stramonium,* an indigenous plant used in ayurvedic medicine in India and commonly used organophosphorus pesticide; chlorpyriphos on acetylcholinesterase (AChE) activity, kinetics and histology were investigated in the brain of *Catla catla.* The recovery of inhibited AChE by both compounds was also determined. It was observed that 96 hrs exposure of *Datura stramonium* leaf extract (100 mg/L) produces 47.2% AChE inhibition and chlorpyriphos (0.00073 mg/L) elicits 33% AChE inhibition. A considerable synergistic inhibitory effect was found in the brain AChE with pre-treatment of *Datura stramonium* leaf extract followed by chlorpyriphos exposure. This treatment could yield 60% inhibition. The recovery of *Datura stramonium* extract-induced AChE inhibition was ranged from 1.6% (48 hrs) to 11.7% (120 hrs) while chlorpyriphos-induced AChE inhibition was recovered to 8.1% (48 hrs) and 23.8% (120 hrs). Kinetic study of AChE also showed inhibitory potential of these compounds. The Km of control group was 0.41 x 10^{-3} M. This was increased to 0.9 x 10^{-3} M (*Datura stramonium* extract), 0.76 x 10^{-3} M (chlorpyriphos), and 1.1 x 10^{-3} M (pre-treated with *Datura stramonium* extract followed by chlorpyriphos exposure). The Vmax values were constant i.e. 0.11 activity/min/mg protein in control and treated groups showing competitive AChE inhibition. The cyto-architectural profile of treated brain shows histological alterations indicated by varying degree of necrosis and vacuolation in the molecular and granular layers.

Keywords: Acetylcholinesterase, Brain, *Catla catla*, Chlorpyriphos, *Datura stramonium* Inhibition, Kinetics, Histology.

Introduction

The acetylcholinesterase enzyme (AChE; E.C 3.1.1.7) is most acceptable target for the assessment of inhibitory action of various organophosphate (OP) pesticides. Hence, determination of inhibition of AChE is widely employed in bio-monitoring studies and considered as most reliable biomarker of pesticide pollution (Carr *et al.,* 1995; Somnuek *et al.,* 2007, Kumar and Tembhre, 2010 and Tembhre *et al*., 2012). The primary mode of action of organophosphorus pesticide is inhibition of AChE activity, the enzyme that degrades the neurotransmitter acetylcholine in cholinergic synapses in the vertebrate nervous system (Silver, 1974). The use of large number of new generation pesticides is still in progress, which leads to adverse synergistic implications on non-target species (Susan *et al.,* 2010). Many of these pesticides may linger on in the environment and lead to detrimental effects. Inhibitory effects of dimethoate on AChE activity of fish have been reported by Satyadevan *et al.* (1993), Tembhre and Kumar (1994), Singh and Kumar (2000). Methylamine is also known to produce significant inhibition in fish AChE. (Tembhre and Kumar, 1995 & 1997). The chronic exposure to sub lethal concentration of Diazinon shows significant inhibitory effect on AChE in the brain of *Clarius gariepinus* (Adedeji, 2011). Chlorpyriphos (CPF), an organophosphate pesticide is widely used for pest control on cotton, corn, almond and fruit trees including oranges and apples. CPF binds with acetylcholinesterase in cholinergic nerves at synaptic vesicles. This results in inhibition of AChE causing impairment of hydrolysis of neurotransmitter acetylcholine (Tembhre *et al.,* 2006; Somnuek, 2007). Chlorpyriphos have been reported to inhibit acetylcholinesterase activity in the brain of fish (Rao *et al.,* 2005; Halappa and David 2009; Wang *et al.,* 2010).

Study of recovery of pesticide-induced acetylcholinesterase inhibition is important as it reveals status of pesticide-AChE complex. Carr *et al.* (1995) reported that after 60 days, the brain AChE of mosquito fish was almost fully recovered from inhibition by chlorpyriphos. Tembhre *et al.* (2006) observed recovery of chlorpyriphos-induced AChE inhibition in *Cyprinus carpio*. They reported 83%, 88% and 90% recovery in fore-, hind- and mid brain on seventh day after chlorpyriphos exposure. Complete recovery of inhibited brain AChE was observed in mosquito fish after 60 days exposure of chlorpyriphos (Russel *et al.,* 1997)

The kinetic study on the brain AChE inhibited by malathion in *Tilapia mossambica* reveals that malathion competitively inhibits AChE (Sahib *et al.,* 1980). Dembele *et al.* (1999) investigated AChE kinetics in the brain of *Cyprinus carpio* exposed to chlorfenvinphos and carbofuran. They reported that both the pesticide induced competitive inhibition in the brain AChE of the fish.

Scientists have investigated a large number of medicinal plants for screening their pesticides properties (Tiwari and Singh, 2004; Crowch and Okello, 2009). Some

medicinal plant extract contain various phyto-chemical known as alkaloids, tannin, saponin, nicotine etc. These active ingredients are toxic to fish (Murthy *et al.,* 2010; Kumar and Sikarwar, 2003). Since, synthetic pesticides create various environmental problems because of slow rate of their degradation. The plant based insecticides are considered to be eco friendly because of their easy degradability. *Datura stramonium* has been reported to be used as medicine in reducing pain and as a narcotic and local anaesthetic drug in many places (Abena *et al.,* 2003). Some findings of investigation revealed that *Datura stramonium* extract predominately contained alkaloids *viz*. atropine, hyoscyamine, and scopolamine (Adekomi *et al.,* 2011). In all species of genus *Datura* the concentration of alkaloid varies depending on species and on the part of the plant (Duez *et al.,* 1985). Friedman and Levin, (1989) reported *Datura stramonium* seeds as neuroinhibitor. Several investigators have discussed the effect of ethanol leaf extract of *Datura stramonium* on animals (Gidado *et al.,* 2000 & 2007).

Organophosphate pesticides are well known to adversely affect the histological components of various organs including brain of fish (Gupta and Guha, 2006; Butchiram *et al.,* 2009). Exposure of sub lethal concentrations of malathion and dimethoate to *Catla catla* showed vacuolation in brain with eccentric nuclei, necrosis in molecular and granular layers (Singh M, 1998). Sarma *et al.* (2010) also reported mild necrosis in the apical lobe of cerebrum of brain of *Channa punctatus* intoxicated with endosulfan.

The present study was undertaken to investigate that whether the use of synthetic pesticides can have a botanical alternative which may proved to be more effective and less persistent in the environment due to its rapid rate of degradation. Nevertheless, *Datura stramonium (D.s)* has never been tried for anticholinesterase activity in fish. Therefore, in the present study, we compared the effects of efficacy of an organophosphorus pesticide, chlorpyriphos and a medicinal herb *Datura stramonium* leaf extract on AChE activity, kinetics, and recovery of inhibited AChE and histology of brain of *Catla catla*.

Materials and Methods

Chemicals

Chlorpyriphos (O,O,Diethyl-O-(3,5,6 trichloro-2-pyridyl) phosphorothioate) 94% purity technical grade, Acetylthiocholine Iodide (ATChI) and DTNB (5-5'dithiobis-2-nitrobenzoic acid) (Himedia, India), Bovine Serum Albumin (BSA) (Loba Ceremic), Folin's reagent (Merck).

Plant Material

Fresh leaves of *Datura stramonium* were collected from botanical garden, authenticated, thoroughly washed in water and shade dried. Leaves were powdered and extracted in Soxhlet apparatus with 90% ethanol as solvent. The extract was kept at room temperature for evaporation of ethanol till semi solid mass left. This was kept stored at 4°C for further use.

Experimental Animal

Fingerlings of *Catla catla* collected from the fishpond near Kolua village, Raisen road, Bhopal were used in this experiment. They were acclimatized for 15 days prior to the experiment. Fishes were fed daily with commercial dry feed pellets (Tokyu, Spirulina, Japan). The fishes had median weight 100 ± 10 gm. The fishes were stocked in glass aquaria of 60 liters supplied with tap water (temperature 22.7 ± 0.61^0C, hardness as $CaCO_3$ 212 ± 4.8 ppm, pH 7.3 ± 0.05, chlorides 87.62 ± 2.39, total alkalinity as $CaCO_3$ 165 ± 1.15 ppm). A Physico-chemical property of water was constantly checked according to APHA/AWWA/WEF (2005). Oxygen content was maintained with the help of aerator. The feeding was stopped before 24 hrs prior to and during the exposure period, which extended 96 hrs.

Fishes were divided into four groups of thirty each. Group-I: served as Control; Group-II: exposure to sub lethal concentration 0.00073 mg/ L was based on the 96 h LC_{50} value (0.0034 mg/ L) of CPF for *Catla catla* was selected for test. The fishes were exposed to this concentration daily for 96 hrs with replenishment of water at every 24 hrs. Group-III: Fishes were exposed to 100 mg/L of *Datura stramonium* leaf extract for 96 hrs. ; Group- IV: The fishes were pre-treated with 100 mg/L of *Datura stramonium* extract for 96 hrs followed by the exposure to 0.00073 mg/L chlorpyriphos for 96 hrs. At the end of the experiment, five fish were removed from each group to study the effect of CPF and *D. s* extract. However, remaining fishes were transferred to toxicant free water to study recovery of AChE. Water was changed after every 24 hrs. Five fishes from this stock were removed and dissected at the end of 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs.

Sample Preparation

Treated fish were euthanized, dissected and the brains were removed quickly and washed in 0.9% saline. A 10% (w/v) tissue homogenate was prepared in Elvehjem-potter homogenizer and centrifuged at 5000 rpm for 20 min in cooling centrifuge (Remi) at 4^0C. The supernatants were kept in deep freeze for AChE assay, kinetics and Protein content.

Enzyme Assay

AChE activity was measured spectrophotometrically according to the method of Ellman's *et al.* (1961) in the Brain of *Catla catla*. Samples of homogenate were diluted with 2.6 ml 0.1 M Sodium phosphate buffer (pH 7.4) to which 100 mM DTNB and 75 mM ATChI was added. The rate of color production was measured at 412 nm in SL 164 UV-VIS spectrophotometer. All measurements were done in duplicate. Specific activity was expressed in nmol/min/mg protein.

Protein estimation

Protein was estimated by the method of Lowry *et al.* (1951) using BSA as the standard. Samples of homogenate were diluted with reagents then 0.5 ml Folin's reagent was added and after 20 min read at 620 nm against a reagent blank. Measurements were done in duplicate.

AChE Kinetics

Kinetic parameters (Km & Vmax) were determined by using four different concentrations of substrate i.e 0.66 mM, 0.44 mM, 0.33 mM, 0.26 mM. Lineweaver-Burk plot was made by plotting the reciprocals of velocity and substrate concentration.

Histology

Brain was removed from three fishes from each group after treatment. Tissues were fixed in freshly prepared aqueous Bouin's fluid in glass vials for 24 hrs, washed in running tap water, dehydrated in graded series of alcohol, cleared in xylene, infiltrated and embedded in paraffin wax. Multiple sections were cut at 5-6 micron thickness and stained with Haematoxylin and Eosin. Slides were examined using binocular microscope (Olympus) and the selected fields were microphotographed at 100X and 400X with computer-aided microscope (Leica).

Statistical analysis

Graphs of results were prepared by applying Excel 2007 software. For the data of statistical comparison between different treatments and control, data were analyzed by Student's t-test to determine the effect of the treatment. The level for the accepted statistical significance was $p>0.05$.

Results and Discussion

The effect of sub lethal concentration of CPF (0.0034 mg/ L) for 96 hrs demonstrated significant ($p > 0.01$) reduction in acetylcholinesterase activity in the brain of *Catla catla.* The brain AChE activity was declined to 9.45 ± 2.69 nmole/ min/ mg protein against the control fishes in which AChE activity was 14.14 ± 8.41 nmole/ min/ mg protein. CPF produced -33% inhibition (Table-1: Fig-1). The treatment of ethanol leaf extract of *Datura stramonium* (100 mg/L) also caused depletion in AChE activity to 7.46 ± 3.68 nmole/ min/ mg protein showing 47.2% brain AChE inhibition. However, 96 hrs pretreatment of fish to *D.s* extract (100 mg/L) followed by (0.0034 mg/ L) CPF exposure for 96 hrs produced significant ($p > 0.01$) inhibition in AChE activity to 5.65 ± 2.11 nmole/ min/ mg protein indicating 60% inhibition (Table-1: Fig-1).

Our previous study reported that the AChE activity of *Cyprinus carpio* intoxicated with CPF displayed differential inhibition i.e. 66.6% in fore- , 40% in mid- and 50% in hind brain (Tembhre *et al.,* 2006). Balint *et al.* (1995) reported that the exposure of 2 mg/L methidathion for 5 days caused 90-92% decrease in AChE activity in *Cyprinus carpio.* A dose dependent reduction in AChE activity in the brain of *Clarius gariepinus* was demonstrated up to 85% (Adedeji, 2011). The decrease in brain AChE activity in *Cyprinus carpio* exposed to sub lethal concentration 7.5 µg/L of quinalphos was recorded with 75.2% AChE inhibition (Chebbi and David, 2009). The present study revealed that CPF produced significant brain AChE inhibition in *Catla catla*. The neurotoxic effect of CPF is potentiated by its biotransformation to a more potent oxon metabolite that inhibits AChE substantially (Fukuto, 1990).

It has been reported that various parts of *Datura stramonium* being medicinal plant, were observed to be poisonous (Devi *et al.,* 2012). There is a little information in the literature regarding its anticholinergic property to the animals. The present investigation is a first attempt towards the understanding of the neurotoxic effect of *Datura stramonium* leaf extract by observing brain AChE inhibition in *Catla catla.* The exposure of plant extract at 100mg/L concentration was found to induce significant 47.2% AChE inhibition.

Suganthy *et al.* (2009) reported 50% AChE inhibition by methanolic leaf extract of *Rhizophora lamarkii*, *Suaeda monica*, *Avicennia officinalis* and *Sesuvium portulacastrum*. An active compound mahanimbine, a carbazole alkaloid isolated from *Murraya koengii* displayed a dose-dependent AChE inhibition (Kumar *et al.,* 2010). Ethanol extract of *Bacopa monnieri* and *Ginkgo biloba* possess AChE inhibitory power as suggested by Das *et al.* (2002). They reported that 100 mg/kg and 300 mg/kg extract of *Bacopa monnieri* and *Ginkgo biloba* respectively showed dose-dependent *in vitro* AChE inhibition in brain.

Recently it has been revealed that ethanol extract of *Bacopa monnieri* noticeably inhibit AChE activity in various regions of the brain (Ahirwar *et al.,* 2012). They reported 72% AChE inhibition in hippocampus, 58% in brain stem, 50% in pons, 46.5% in cerebellum, 44% in thalamus, 40% in Cerebral cortex and 33.3% straitum. The preliminary phytochemical study showed the presence of alkaloids including atropine, hyoscyamine and scopolamine that can all elicit anticholinergic poisoning (Ertekin *et al.,* 2005). The analysis also showed that these anticholinergic alkaloids are responsible for blocking of AChE activity in cholinergic nerves (Friedman, 2004). We observed that pre-treatment of *D.s* leaf extract followed by CPF exposure substantiate AChE inhibition in brain further to 60%. It might be causing synergistic physiological effect when used in continuous exposures.

Our result showed that AChE activity was recovered gradually to 8.1% at 48 hrs; 17.7% at 72 hrs; 21.6% at 96 hrs & 23.8 % at 120 hrs after exposure to chlorpyriphos. Recovery of inhibited AChE after exposure to leaf extract of *Datura stramonium* was found to be 1.6% at 48 hrs; 2.4% at 72 hrs; 5.2% at 96 hrs & 11.7% at 120 hrs. Our findings revealed that there is considerable difference in the extent of recovery of chlorpyriphos and *Datura*

stramonium treated fish (Table-2: Fig-2). Brain AChE inhibition in *Cyprinus carpio* exposed to sub lethal concentration of quinalphos was recovered up to 35% after 14th day (Chebbi and David, 2009). Parathion-induced brain AChE inhibition in *Gasterosteus aculeatus* was significantly recovered after 48 hrs of exposure (Wogram *et al.,* 2000). It is evident from observation of various investigations that recovery period vary with tenure after exposure, degradability of inhibitor and degeneration of acetylcholinesterase (Russel *et al.,* 1997). Tembhre *et al.,* (2006) reported 83%, 88% and 90% recovery of AChE in fore-, hind- and mid brain respectively after 168 hrs of exposure of chlorpyriphos in *Cyprinus carpio.* Our findings indicate that leaching of *D. s* extract is slow than CPF in the brain.

We have compared AChE Kinetics also to study the nature of inhibition. The Km value of control brain was 0.41 x 10^{-3} M. Our study showed that Km increased in chlorpyriphos treated Brain to 0.76 x 10^{-3} M and in *D. s* extract treated to 0.9 x 10^{-3} M. However, in group IV it was further enhanced to 1.1 x 10^{-3} M. However, Vmax remained constant i.e. 0.11 activity/min/mg protein in control and treated fishes (Table-1). Therefore, increasing pattern of Km and constant Vmax showed that both the CPF and *D.s* extract inhibit the Brain AChE in a competitive manner (Fig-3). Similar findings were reported with malathion in *Catla catla* (Singh and Kumar, 2000), with methyl paraoxon in *Prochilodus lineatus* (Silva Filho *et al.,* 2004) and with monocrotophos in *Channa punctatus* (Rahman *et al.,* 2004). There is lack of information on kinetics of enzyme inhibition in fish by herbal extracts. Our study has demonstrated for the first time that *Datura stramonium* extract is able to display brain AChE inhibition kinetics similar to those of OP pesticides. Therefore, we reported for the first time that it has competitive inhibitory property of AChE. *Acacia niloticus* and *Rhamnus prinoides* showed mixed inhibitory effects *viz.* non competitive-uncompetitive type (Crowch and Okello, 2009).

The histological observations of the control brain (Fig-4, 5) showed the molecular layer, granular layer and purkinje cell layer. The components of these layers were architecturally normal. CPF treatment mildly affected molecular and granular layers causing necrosis, which is evidenced by the appearance of narrow spaces and vacuolation (Fig-6, 7). These changes were moderately increased in the brain treated with leaf extract of *Datura stramonium* (Fig-8, 9). However, it was slightly greater in the brain of fish pre-treated with *Datura stramonium* leaf extract followed by CPF exposure (Fig-10, 11). Intoxication of 0.35 ppm hexachlorocyclohexane to *Labeo rohita* produced mild vacuolar changes with small spaces in brain, whereas 1.73 ppm exposure showed severe necrosis (Das and Mukherjee, 2000). Chronic exposure of 0.6 and 1.3 µg/L endosulfan to rainbow trout did not cause lesions in brain (Altinok and Capkin, 2007). Severe damage in brain cells and neural cells with broken neural bundles were observed in 100 ppm malathion treated *Ophiocephalus punctatus* (Pugazhvendan *et al.,* 2009). Recently, above mentioned alterations were found in common carp exposed to atrazin and chlorpyriphos (Houjuan *et al.,* 2012).

Thus, present investigation leads us to conclude that leaf extract of *Datura stramonium* induced more neurotoxicity in the brain as compared to chlorpyriphos. The study revealed that *Catla catla* brain AChE is more sensitive to herbal extract. Thus, leaf extract of *Datura stramonium* may be considered as potential herbal pesticide for the control of predatory fishes. However, further isolation and purification of phytochemicals are necessary to investigate the presence of active ingredient.

Table-1: Effect of 96 hrs exposure of chlorpyriphos and *Datura stramonium* on acetylcholinesterase activity, inhibition, Km and Vmax of the **Brain** of *Catla catla.* The Specific activity is expressed in nmole/ min/ mg protein. Each value is mean ± SD of five individual observations. Significant: P > 0.05*; Highly significant: P > 0.01**

Parameters / **Groups**	**Specifi c activity of AChE**	**% inhibition of AChE**	**Km x 10^{-3} M**	**Vmax (activity/min/mg protein)**
Control	14.14 ± 8.41	-	0.41 x 10^{-3} M	0.11
CPF	9.45 ± 2.69**	-33%	0.76 x 10^{-3} M	0.11
D. s	7.46 ± 3.68*	-47.2%	0.9 x 10^{-3} M	0.11
Pre -treatment of *D. s* followed by exposure of CPF	5.65 ± 2.11**	-60%	1.1 x 10^{-3} M	0.11

Table-2: Recovery of inhibited AChE due to chlorpyriphos and *Datura stramonium* exposure in the **Brain** of *Catla catla.* Each value is mean ± SD of five individual observations. Significant: P > 0.05*; Highly significant: P > 0.01**; Very highly significant: P > 0.001***

Group / Parameter	Control	CPF (0.00073 mg/ L)	Recovery of inhibited AChE				*D.s* (100 mg/L)	Recovery of inhibited AChE			
			48 hrs	72 hrs	96 hrs	120 hrs		48 hrs	72 hrs	96 hrs	120 hrs
AChE activity (nmole/min/ mg protein)	14.14± 8.41	9.45± 2.69 **	10.2± 4.0**	11.1±2 .3 ***	11.5± 0.68 ***	11.7± 0.86 ***	7.46± 3.68*	7.58± 0.50 ***	7.6± 0.81 ***	7.85± 0.48 ***	8.34± 0.26 ***
% inhibition of AChE	-	33%	27.7	21.2	18.6	17.2	47.2%	46.3	45.9	44.4	41
% recovery of AChE	-	-	8.1	17.7	21.6	23.8		1.6	2.4	5.2	11.7

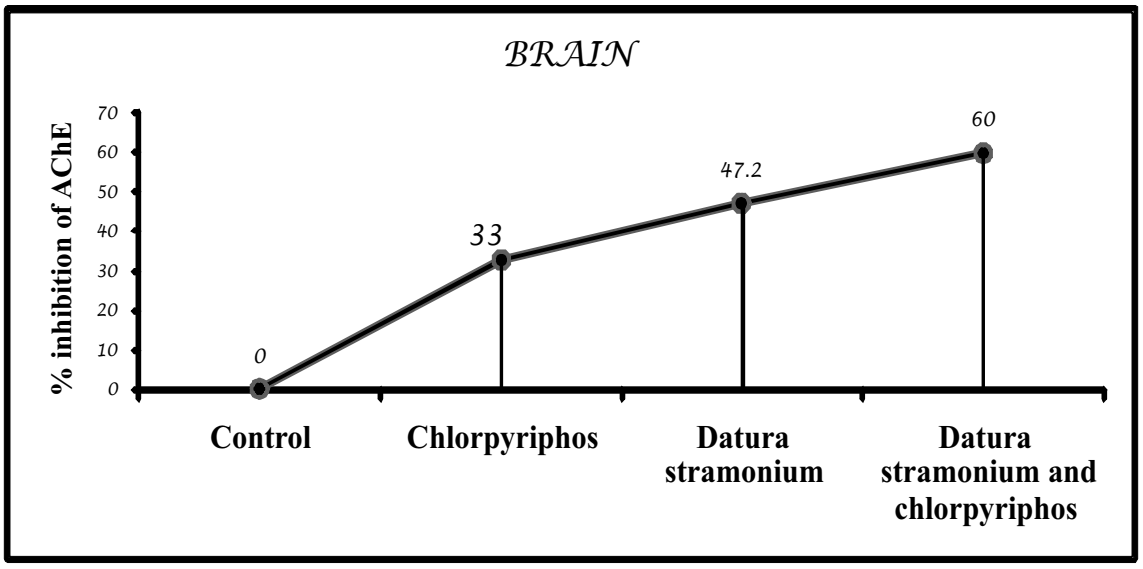

Fig-1: Percentage inhibition of AChE activity in the Brain of *Catla catla* of control group, CPF group (0.00073 mg/ L), *D.s* group (100 mg/L) and pre-treatment of *D.s* leaf extract followed by CPF.

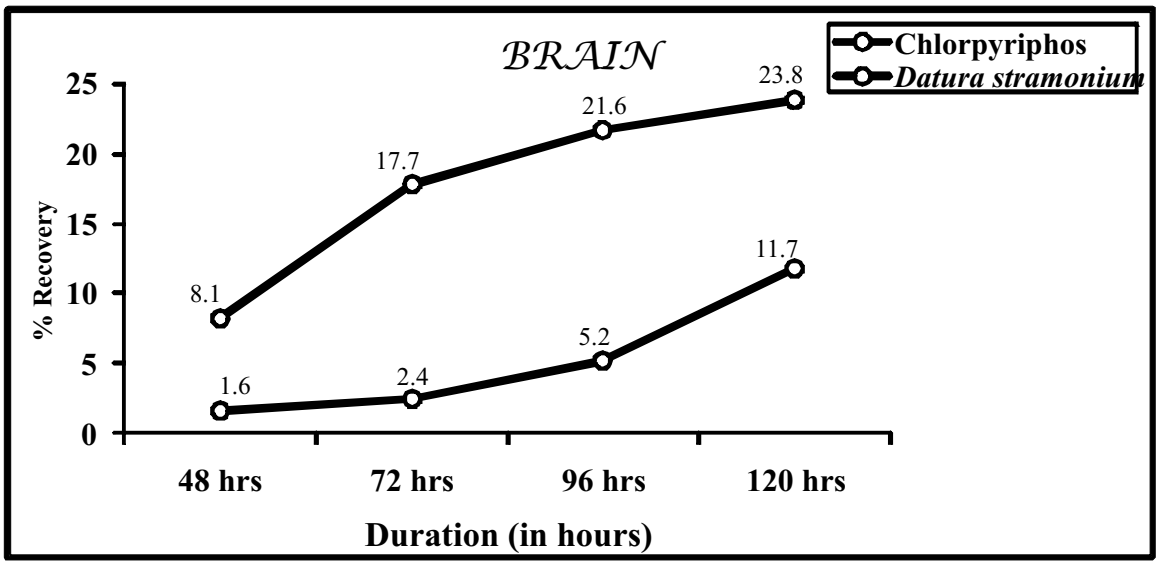

Fig: 2 - Recovery of inhibited AChE after 48,72, 96 and 120 hours in the Brain of *Catla catla* exposed to chlorpyriphos (0.00073 mg/ L) and *Datura stramonium* (100 mg/L) for 96 hours.

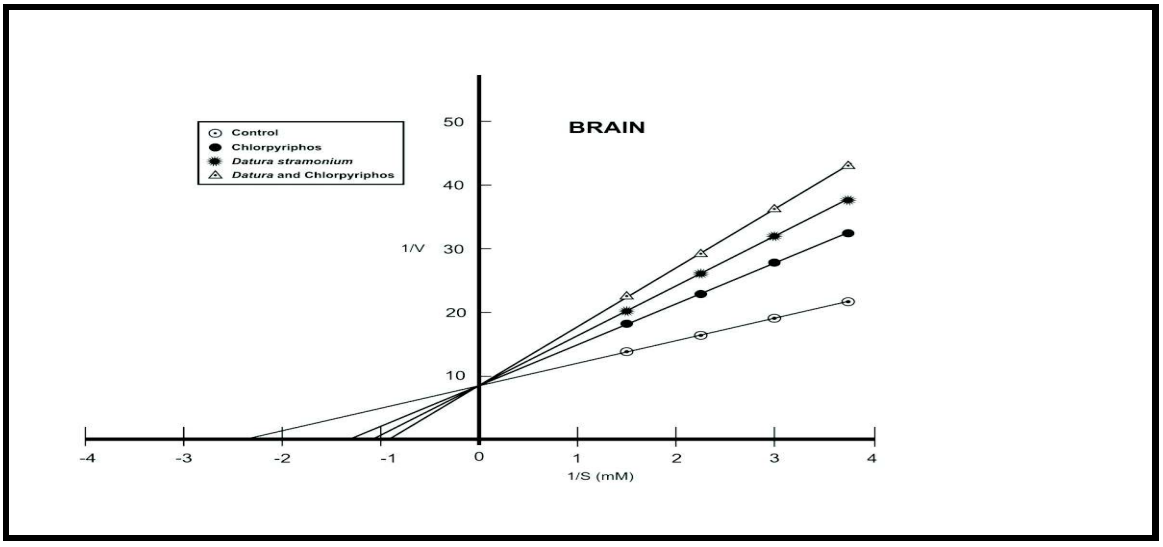

Fig-3: Lineweaver-Burk plot showing competitive inhibition in the Brain AChE of *Catla catla* exposed to CPF (0.00073 mg/ L), *D. s* leaf extract (100 mg/L) and pre-treatment of *D.s* leaf extract followed by CPF for 96 hrs.

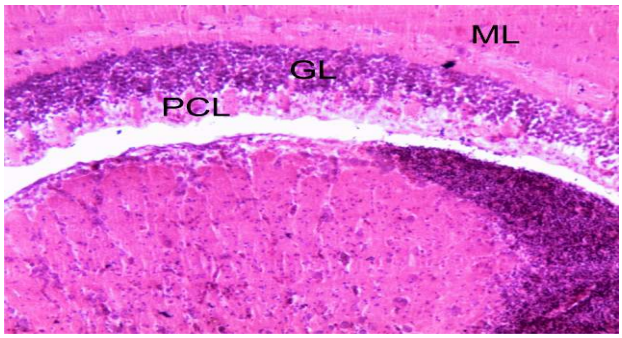

Fig-4: Microphotograph of T. S. of the Brain of *Catla catla* (Control group) showing molecular layer (ML); granular layer (GL); purkinje cell layer (PCL) (H & E; 100 X)

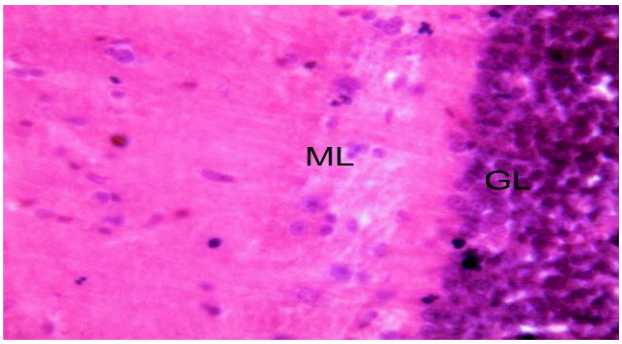

Fig-5: Microphotograph of T. S. of the Brain of *Catla catla* (Control group) showing molecular layer (ML); granular layer (GL) (H & E; 400 X)

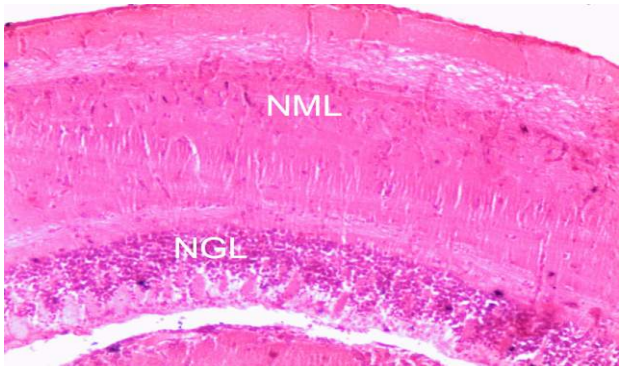

Fig-6: Microphotograph of T. S. of the Brain of *Catla catla* exposed to CPF (0.00073 mg/L) for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL) (H & E; 100 X)

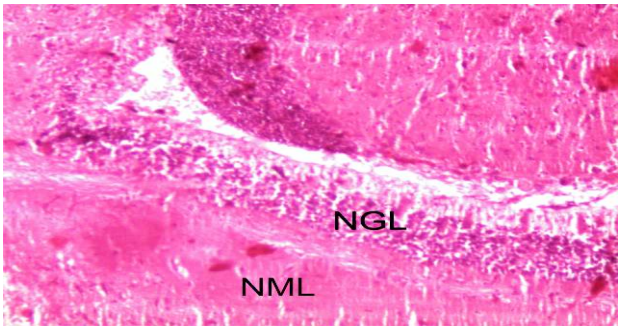

Fig-7: Microphotograph of T. S. of the Brain of *Catla catla* exposed to CPF (0.00073 mg/L) for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL) (H & E; 400 X)

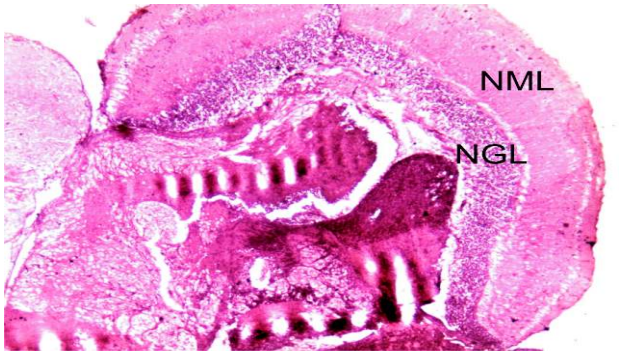

Fig-8: Microphotograph of T. S. of the Brain of *Catla catla* exposed to 100 mg/L *D.s* extract for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL) (H & E; 40 X)

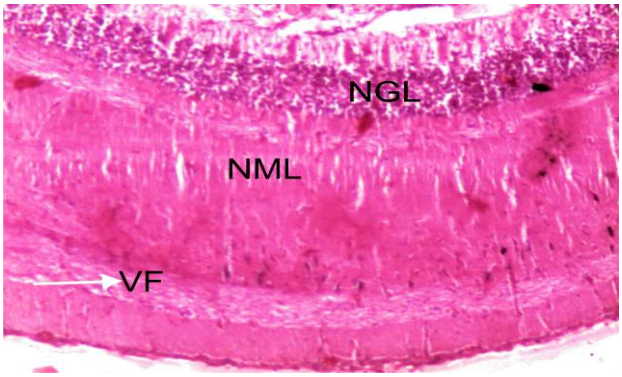

Fig-9: Microphotograph of T. S. of the Brain of *Catla catla* exposed to 100 mg/L *D.s* extract for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL); vacuole formation (VF) (H & E; 100 X)

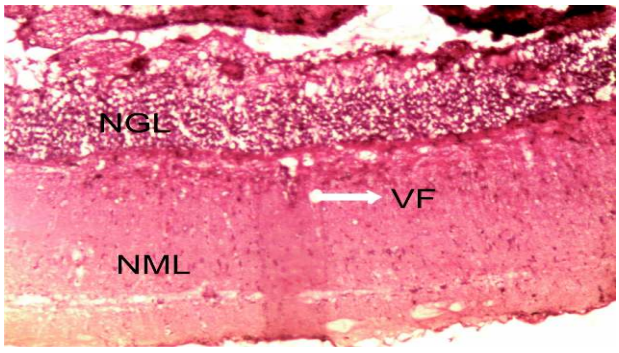

Figure-10: Microphotograph of T. S. of the Brain of *Catla catla* pretreated with 100 mg/L ethanolic leaf extract of *D.s* followed by CPF (0.00073 mg/L) for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL); vacuole formation (VF) (H & E; 100 X)

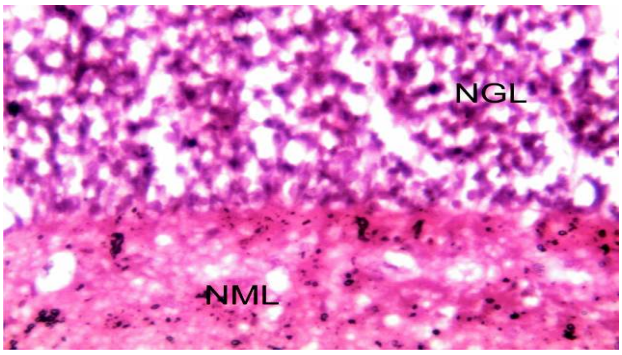

Figure: 11 - Microphotograph of T. S. of the Brain of *Catla catla* pretreated with 100 mg/L *D.s* extract followed by CPF (0.00073 mg/L) for 96 hrs showing necrosis in molecular layer (NML); necrosis in granular layer (NGL); vacuole formation (VF) (H & E; 400 X)

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References

Abena A.A., Miguel L.M., Mouanga A. and Hondi A.T. (2003): Evaluation of analgesic effect of *Datura fastuosa* leaves and seed extracts. *Fitoterapia, 486-488.*

Adedeji O.B. (2011): Response of acetylcholinesterase activity in the brain of *Clarias gariepinus* to sub-lethal concentration of diazinon. *Journal of Applied Sciences in Environmental Sanitation,* **6(2),**13-14

Adekomi D.A., Musa A.A.,Tijani A.A., Adeniyi T.D. and Usman, B. (2011): Exposure to smoke extract of *Datura stramonium* leaf: Some of its effect s on the heart, liver, lungs, kidneys and testes of male Sprague Dawley rats. *Journal of Pharmacognosy and phytotherapy,* **3(5),**67-75.

Ahirwar S., Tembhre M., Gour S. and Namdeo A. (2012): Anticholinesterase efficacy of *Bacopa monnieri* against the brain regions of rat-a novel approach to therapy for Alzheimer's disease. *Asian J. Exp. Sci.,* **26(1)**,65-70.

Altinok I. and Capkin E. (2007): Histopathology of rainbow trout exposed to sub-lethal concentrations of methiocarb or endosulfan. *Toxicologic pathology,* **35** , 405-410.

APHA/AWWA/WEF (2005) – Standard methods for the examination of water and waste water. *21st edition, Andrew D. Eaton, Lenore S. Clesceri, Eugene, W. Rice and Arnold E. Greenberg editors.*

Balint, T., Szegletes, T., Szegletes, Zs., Halasy, K. and Nemcsok J. (1995): Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and the pyrethroid deltamethrin. *Aquat. Toxicol.* 33, 279.

Butchiram M.S., Tilak K.S. and Raju P.W. (2009): Studies on histopathological changes in the gills, liver and kidney of *Channa punctatus* (Bloch) exposed to alachlor. J. *Environ. Biol.* **30(2)**, 303-306.

Carr R.L., Straus D.L. and Chambers J.E. (1995): Inhibition and aging of channel catfish brain acetylcholinesterase following exposure to two phosphorothionate insecticides and their active metabolites. *Journal of Toxicology and Environmental Health, Part A,* **45(3)**, 325-336.

Dembele K., Haubruge E. and Gaspar Ch. (1999): Recovery of acetylcholinesterase activity in the common carp, *Cyprinus carpio L.* after inhibition by organophosphate and carbamate compounds. *Bull. Environ. Contam. Toxicol.* 62: 731- 742.

Chebbi S.G. and David M. (2009) : Neurobehavioural responses of the freshwater teleost, *Cyprinus carpio* (Linn) under quinalphos intoxication. *Biotechnology in Animal Husbandary* **25(3-4)**, 241-249.

Crowch C.M. and Okello E.J. (2009): Kinetics of acetylcholinesterase inhibitory activities by aqueous extract of *Acacia nilotica* (L.) and *Rhamnus prinoides* (L' Her). *African Journal of Pharmacy and Pharmacology. Vol. 3(10), 469-475, October.*

Das B.K. and Mukherjee S.C. (2000): A histopathological study of carp, *Labeo rohita* exposed to hexachlorocyclohexane. *Veterinarski Arhiv* **70(4)**, 169-180.

Das A., Shanker G., Nath C., Pal R., Singh S. and Singh

K.H. (2002): A comparative study in rodents of standardized extracts of *Bacopa monnieri* and *Ginkgo biloba*. Anticholinesterase and cognitive enhancing activities. *Pharmacology, Biochemistry and Behaviour,* 73, 893-900.

Devi M.R., Bawari M., Paul S.B. and Sharma G.D. (2012) : Characterization of the toxic effects induced by *Datura stramonium* L. leaves on mice: A behavioral, biochemical and ultrastructural approach. *Asian J Pharm Clin Res, Vol 5, Suppl* 3, 143-146.

Duez P., Chamart S., Hanocq J., Molle L., Vanhaelen M. and Vanhalen F. (1985) : Comparison between thin chromatography, densitometry and HPLC for the determination of hyoscyamine and hyoscine in leaves, fruits and seeds of *Datura* species. *Chromatography,* 329: 501-510.

Ellman G. L., Courtney K.D., Andres V. Jr. and Featherstone R.M. (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**, 88-95.

Ertekin V., Selimoglu MA., Altinkaynak S. (2005): A combination of unusual presentations of *Datura stramonium* intoxication in a child. Rhabdomylysis and fulminant hepatitius. *J Emerg Med* **28**:227-228.

Friedman M. and Levin C. (1989) – Composition of Jimson weed (*Datura stramonium*) seeds. *Agric. Food Chem.,* **37**:998-1005.

Friedman M. (2004) : Analysis of biologically active compounds in potatoes (*Solanum tuberosum*), Tomatoes (*Lycopersicon esculentum*) and Jimson weed (*Datura stramonium*) seeds. *J chromatogram,* 1054:143-155.

Fukuto T.R. (1990): Mechanism of action of organophosphorus and carbamate insecticides. *Environ Health Perspect.* **87**, 245.

Gidado A., Okoli R.M., Ambi A.A. and Umar I.Y. (2000) : Effects of aqueous extracts of the leaves of *Datura stramonium* (Jimson weed) on some serum biochemical parameters in rats. *Nigeria Journal of Experimental and Applied Biology.* **1(2)** 149-152.

Gidado A., Zainab A.A., Hadiza M.U., Serah D.P., Anas H.Y. and Milala M.A. (2007) : Toxicity studies of ethanol extract of the leaves of *Datura stramonium* in rats. *African Journal of Biotechnology.* **6(8)**, 1012-1015.

Gour S. (2010): Comparative study of sensitivity of chick and rat acetylcholinesterase to organophosphorous pesticide and effects of pretreatment of herbal extract. Ph.D Thesis, Barkatullah University, Bhopal, Madhya Pradesh.

Gupta U.S. and Guha S. (2006): Microcystin toxicity in a freshwater fish, *Heteropneustes fossilis* (Bloch). *Current Science,* **91(9)**.

Halappa R. and David M. (2009): Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linn.) following sub-lethal exposure to chlorpyriphos. *Turkish Journal of Fisheries and Aquatic Sciences* **9**,233-238.

Houjuan X., Li. S., Wang Z., Gao X., Xu S., and Wang X. (2012): Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazin and chlorphyrifos. *Chemosphere*, **88**, 377-383.

Kumar V. and Sikarwar R.L.S. (2003): Plants used as fish poison by tribals of Surguja district in Chhattisgarh, India. *Ethnobotany.* **15**, 87-89.

Kumar N.S., Mukherjee P.K. Bhadra S., Saha B.P. and Pal, B.C. (2010): Acetylcholinesterase inhibitory potential of a carbazole alkaloid, mahanimbine, from *Murraya koenigii*. *Phytotherapy Research,* **24(4)**; 629-31.

Kumar S. and Tenbhre M. (2010) : Fish and Fisheries. (New Central Book Agency Pvt. Ltd., London, Hyderabad, Ernakulam, Bhubneswar, Pune, Kolkata).

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951) : Protein measurement with folin phenol reagent. *J. Bol. Chem.* **193**, 265-275.

Murthy, E.N., Pattanaik, C., Reddy, C.S. and Raju, V.S. (2010): Piscicidal plants used by Gond tribe of Kawal wildlife sanctuary, Andhra Pradesh, India. *Indian Journal of Natural Products and Resources.* **1(1)**, 97-101.

Pugazhvendan S.R., Narendiran N.J., Kumaran R.G., Kumaran S. and Alagappan K.M. (2009): Effect of malathion toxicity in the freshwater fish *Ophiocephalus punctatus* – A histological and histochemical study. *World Journal of Fish and Marine Sciences* **1(3)**: 218-224.

Rao J.V., Begum G., Pallela R., Usman P.K. and Nageshwara Rao R. (2005): Changes in behaviour and brain acetylcholinesterase activity in mosquitofish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyriphos. *Int. J. Environ. Res. Public Health,* **2(3)**, 478-483.

Rahman M.F., Mahboob M. and Grover P. (2004): *In vitro* acetylcholinesterase inhibition by novel OP compounds in various tissues of the fish *Channa punctatus*. *Bull Environ. Contam. Toxicol.* **72**: 38-44.

Russell L.C. Ho L.L. and Chambers J.E. (1997) : Selective toxicity of chlorpyriphos to several species of fish during an environmental exposure: biochemical mechanisms. *Environmental Toxicology and Chemistry.* 2369-2374.

Russel A. Hardin J., Grand L. and Fraser A. (1997): Poisonous plants of North Carolina. *Charlotte: North Carolina State University.*

Sahib, K.A., Sailatha, D. and Ramana Rao, K.V. (1980) : Impact of malathion on acetylcholinesterase in the tissues of the fish *Tilapia mossambica* (Peters) – time course study. *J. Biosci. Vol. 2, No.1, 37-41.*

Sarma, K., Pal, A.K., Sahu, N.P., Mukherjee, S.C. and Baruah, K. (2010) – Biochemical and histological changes in the brain tissue of spotted murrel, *Channa punctatus* (Bloch) exposed to endosulfan. *Fish Physiol. Biochem. 36 (3): 597- 603.*

Satyadevan, S., Kumar, S. and Tembhre, M. (1993) – Acetylcholinesterase activity and enzyme kinectics in the brain of common carp, *Cyprinus carpio* subjected to sub lethal exposure to dimethoate. *Biosci. Biotech. Biochem. 57 (9): 1566-1567.*

Silver, A. (1974) - The biology of cholinesterases. *North-Holland Publishing Company Amsterdam. The Netherlands.*

Silva Filho, M.V., Oliveira, M.M., Salles, J.B., Bastos, V.L., Cassano, V.P. and Bastos, J.C. (2004) – Methyl-paraoxon comparative inhibition kinetics for acetylcholinesterases from brain of neotropical fishes. *Toxicol Lett., Nov 2; 153(2): 247-54.*

Singh, M. and Kumar, S. (2000) - Effects of sublethal concentrations of dimethoate and malathion on AChE activity and enzyme kinetics of liver of *Catla catla* (Ham.). *Uttar Pradesh Journal of Zoology 2000 Vol. 20 No. 2, 131-135*

Singh, M. (1998) – The histopathological study of Brain and Liver of *Catla catla* due to effect of some pesticides with special reference to acetylcholinesterase kinetics. (*Ph.D thesis, Barkatullah University*).

Somnuek, C., Cheevaporn, V., Saengkul, C. and Beamish, F.W.H. (2007) – Variability in acetylcholinesterase upon exposure to chlorpyriphos and carbaryl in hybrid catfish. *Science Asia 33: 301-305.*

Suganthy, N., Pandian, S.K. and Devi, K.P. (2009) – Cholinesterase inhibitory effects of *Rhizophora lamarckii, Avicennia officinalis, Sesuvium portulacastrum* and *Suaeda monica*; Mangroves inhabiting an Indian coastal area (Vellar Estuary). *Journal of Enzyme inhibition and Medicinal Chemistry, 24 (3): 702-7.*

Susan, T.A., Sobha, K. and Tilak, K.S. (2010) - A study on acute toxicity, oxygen consumption and behavioural changes in the three major carps, *Labeo rohita* (Ham.), *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.) exposed to fenvalerate. *Bioresearch Bulletin 1: 33-40.*

Tiwari, S. and Singh, A. (2004) – Piscicidal activity of alcoholic extract of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. *African Journal Traditional, Complementary and Alternative medicines, Vol. 1, Num. 1, 15-29.*

Tembhre, M. and Kumar, S. (1994) – Effect of acute and chronic exposure to sub-lethal dose of dimethoate in the gut of *Cyprinus carpio*. *J. Ecotoxicol. Environ. Monit. 4(3) 205-210.*

Tembhre, M. and Kumar, S. (1995) – Effect of sub-lethal concentration of methylamine on AChE activity and enzyme kinetics of alimentary canal of *Cyprinus carpio*. *Ind. J.Z. Spect., Vol. 6, No.2, (39-42).*

Tembhre, M. and Kumar, S. (1997) – Acetylcholinesterase inhibition in the fish gut as an indicator of environmental poisoning by methylamine. *Proc. Acad. Environ. Biol. 6 (1), 29-33.*

Tembhre, M., Ahirwar, S., Gour, S. and Gaur, M. (2006) – Chlorpyriphos induced inhibition of AChE in *Cyprinus carpio* and recovery during drug leaching. *Journal of cell and tissue research. Vol. 6 (2) 793-796.*

Tembhre, M., Gour, G., Ahirwar, S. and Namdeo, A. (2012) – Diacetylemonoxime reactivation of Acetylecholinestrase and butyrylcholinesterase inhibited by dichlorovos in central and peripheral nervous system of Rat. IPCBB Vol. 48, IACIT press Singapore, 126-133.

Wang, C., Lu, G. and Cui, J. (2010) – Response of acetylcholinesterase and GST activities to insecticide co-exposure in *Carassius auratus*. *Wiley Periodicals, Inc. Environmental Toxicology*

Wogram, J., Sturm, A., Segner, H. and Liess, M. (2000) - Effects of parathion on acetylcholinesterase, butyrlcholinesterase and carboxylesterase in three-spined stickleback, *Gasterosteus aculeatus* following short-term exposure. *Environmental Toxicology and Chemistry, 1528-1531.*