Short term effects of *Centella asiatica* on methylparathion induced toxicity on different regions of the brain in rat, *Rattus norvegicus*

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Abstract: *In the present study, Methyl parathion an organophosphate compound has been induced to the rats to study the activity of acetylcholinesterase on different regions of the brain for short term effects i.e., for 28 days and then an antidote Centella asiatica was given to the rats which were induced with methylparathion.*
Keywords: *AChE, Brain, Centella asiatica, Methyl parathion, Organophosphate compound,*

I.   Introduction

Organophosphorus (OP) compounds are a major component of many pesticides with widespread use in both agricultural and domestic situations (Kalender *et al*., 2006.) Organophosphate pesticides have increased in use, because they are less damaging to the environment and they are less persistent than organochlorine pesticides. OP insecticides elicit toxicity by inhibiting the enzyme acetylcholinesterase (Karanth *et al*., 2004, Pope, 1999, Ecobichon, 1996 and Gallo and Lawryk, 1991). With extensive AChE inhibition, the neurotransmitter acetylcholine (Ach) accumulates in the synapses of the central and peripheral nervous system, which in turn leads to over stimulation of postsynaptic cholinergic receptors and signs of cholinergic neurotoxicity (Karanth *et al*., 2004, Savolainen, 2001, Ecobiochon 1996 and Silver, 1974). Phosphorothioate parent molecules are non-toxic, but after undergoing metabolism they yield oxon (phosphates) derivatives (Norman *et al*., 1974 and Neal, 1967) that are capable of inhibiting AChE (Saunders and Harper, 1994 and Marrs, 1993). Inhibition of AChE results in accumulation of acetylcholine in certain synapses and neuromuscular plates, provoking a steady depolarization of the affected neurons; this might lead to death due to respiratory failure (Lima *et al*., 1996, Marrs, 1993, Koelle, 1992 and Rickett *et al*., 1986).

II.   Materials And Methods

Adult male albino rats *Rattus norvegicus* of Wistar strain weighing about 120-150 g were obtained from the King's Institute, Guindy, Chennai, India. The animals were housed in plastic cages, fed standard laboratory diet and water ad libitum. Rats were exposed to 12h light/12 h dark. Rat is the ideal animal model for laboratory observations. Adequate brain tissue is available for experimental studies. Animals were quarantined for 10 days before being randomized into experimental groups of 4 animals per cage. Animals were maintained according to the principle and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (IAEC No.945/c/01/01/06) under the supervision of Animal Ethical Committee.

CHEMICALS

Methyl parathion purity 99.8% was obtained from Bayer Science Crops, Mumbai, India. This was mixed with groundnut oil and given to the rats, because methyl parathion dissolves in groundnut oil.

PLANT MATERIALS

Centella asiatica was selected for the present study, the whole plant was collected, shade dried and finely ground with grinder. The finely powdered material was mixed with water and administered to the experimental rats.

EXPERIMENTAL DESIGN

The animals were separated into 5 groups and each group consisted of 6 animals.
Group I: served as control
Group II: Animals were fed with methyl parathion (MP) alone
Group III: Animals were fed with aqueous *Centella asiatica* (CA)
Group IV: Animals were fed with both methyl parathion (MP) and *Centella asiatica* (CA)
Group V: Animals were administered with groundnut oil. This served as negative control

The LD_{50} value for methyl parathion is 14mg/kg body weight according to Casarett, (1980). For acute toxicity study $1/3^{rd}$ of the LD_{50} value was taken, and mixed along with groundnut oil and administered to the rats. For short and long term study, care was taken so that the value didn't exceed the LD_{50} value.

COLLECTION OF TISSUE SAMPLES

The rats were starved prior to the experiment for a period of 24 h. Later they were fed with water. Both control and experimental rats were sacrificed by decapitation with anaesthetization. The tissues selected for the present study were the different regions of the brain (Olfactory lobes (OL), Optic lobes (OPL), Cerebrum (CB), Cerebellum (CL), Midbrain (MB) and Medulla oblongata (MO)), liver (Liv) and kidney (Kid) and in non-target organs like thymus(Thy), spleen (Spl), bone marrow (BM) and blood (Bld). The brain was removed as rapidly as possible and washed several times in ice cold saline and cleaned to remove blood clots and meninges following the procedure of Vijayan and Brownson, (1975).
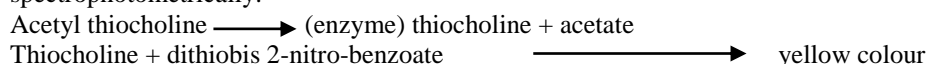
III. Methods
ESTIMATION OF ACTEYLCHOLINESTERASE (ACHE)

Different regions of the brain and blood sample were taken for the estimation of AChE as per the method of Ellman *et al.*, (1961).

PRINCIPLE

Measurement of the rate of production of the choline as acetylcholine is hydrolyzed. This is accomplished by the continuous reaction of thiol with 5, 5-dithio-bis-2 nitro-benzoate ion to produce the yellow anion of 5-thio-2-nitro benzoic acid; the rate of colour production is measured at 412 nm spectrophotometrically.

Acetyl thiocholine $\longrightarrow$ (enzyme) thiocholine + acetate

Thiocholine + dithiobis 2-nitro-benzoate $\longrightarrow$ yellow colour

REAGENTS

1. Phosphate buffer-(0.1M,pH 7 and pH 8)
2. Dithio-bis-nitrobenzoic acid (DTNB) was prepared by dissolving 36.9 mg of DTNB in 10 ml of 0.1M phosphate buffer of pH 7 and 15 mg of sodium carbonate was added.
3. Substrate - 21.67 mg of acetylcholine iodide was dissolved in 1ml of double distilled water
4. Inhibitors - Eserine sulphate (10^{-4} molarity for brain tissues) and quinidine sulphate (blood).

ESTIMATION OF ACHE IN DIFFERENT REGIONS OF THE BRAIN

After decapitation, the brain was removed following the procedure mentioned in 2.1.5. The regions were separated into OLB, CB, OPL, CL, MB and MO following the method of Vijayan and Brownson, (1975).The saline cleaned and weighed tissues of the different brain regions of *Rattus norvegicus* were homogenized in 0.1M phosphate buffer (pH.8) at a concentration of 20 mg/ml, by using a pre chilled glass homogenizer kept in a beaker containing ice-cubes. The homogenate was centrifuged at 1500 rpm for 5 minutes. The supernatant was used as the enzyme source.

PROCEDURE

0.4 ml of the sample was taken in a cuvette containing 2.6ml of 0.1M phosphate buffer (pH.8). To this, was added 0.1ml of DTNB reagent and mixed well. The absorbance was set to "zero' at 412nm in the spectrophotometer. The reaction was started by adding 0.02ml of the acetyl-thio choline iodide substrate to the mixture and mixed well. The readings were recorded for every minute upto 5 minutes. 0.1ml of eserine sulphate (10^{-4}M) a specific AChE inhibitor was added to another cuvette containing 0.4ml of homogenate, 2.5ml of 0.1M phosphate buffer (pH 8.0) and mixed well. Then 0.1ml of DTNB reagent was added and mixed thoroughly. The absorbance was set to "zero' at 412nm as stated earlier. Then 0.02ml of the substrate was added to the cuvette and mixed well. The readings were recorded for 5 minutes at an interval of 1 minute with inhibitor value being substracted from the recording without inhibitor. The enzyme activity was expressed as moles of substrate hydrolyzed/min/g tissue.

The enzyme activity was calculated using the formula given below:

$$R = \frac{\Delta A}{1.36(10^{-4})} \times \frac{1}{(400/3120)\,C_o} = \frac{5.74(10^{-4})\,\Delta A}{C_o}$$

Where,

R= rate of enzyme activity in moles of substrate hydrolyzed/min/g of tissue
A = change in absorbance /min
Co= Original concentration of tissue (mg/ml)
The enzyme activity was represented in moles of acetylthiocholine hydrolyzed /min/gm of tissue.

STATISTICAL ANALYSIS
The data obtained from various studies were subjected to statistical analysis using SPSS package. Student's t-test was applied for toxicity studies. The values are expressed as the mean $\pm$ SD and the difference between groups were statistically analyzed using one way analysis of variance (ANOVA)

IV. Results
In the present study short term oral toxicity of the organophosphate (OP), i.e., Methyl Parathion (MP) on rats *Rattus norvegicus* were analyzed and the effect of C*entella asiatica* (CA) an antidote was studied.
LD$_{50}$ value of Methyl Parathion =14mg/kg body weight.
Centella asiatica = 200mg/kg body weight.
The doses of MP and CA administered orally were well within the LD$_{50}$value.

SHORT TERM ORAL TOXICITY STUDY
Acetylcholinesterase (AChE) activity in the different regions of the brain of *Rattus norvegicus* due to short term oral toxicity on 7th, 14th, 21st and 28th day is shown in table - 1, 2, 3 and 4 and figure - 1, 2, 3 and 4.
The change in the level of AChE activity in the OL of *Rattus norvegicus* on 7th day is illustrated in table - 1 and figure - 1. Group II animals showed an increase in the enzyme activity (0.57×10^{-6} moles min^{-1} g^{-1}) and the increase is significant at $p<0.001$ level while group III and IV did not show enzyme activity. Group V remains the same as that of Group I (0.25×10^{-6} moles min^{-1} g^{-1}) when group IV was compared with group II, it showed a decrease and the decrease is significant at $p<0.001$ level. The rate of AChE activity in the OP of group II showed a decrease (0.28×10^{-6} moles min^{-1} g^{-1}) and is significant at $p<0.001$ level. Group V remains the same as that of group I (table - 1, figure - 1).
There was no AChE activity in CB of Group II and III. Group IV and V did not show any enzymatic change when compared with group I while group IV showed a significant increase at $p<0.001$ level when compared with group II (table - 1, figure - 1).
CL of group II, III and IV did not show any activity. In group V the AChE activity remains the same as that of group I (table - 1, figure - 1).
MB of group II and III did not show any enzymatic changes. Group IV and V did not show any significant change when compared with group I. group IV showed a significant increase at $p<0.001$ level when compared with group II (table - 1, figure – 1). The rate of AChE activity in MO of group II and V did not show significant change. There was no enzyme activity group III and IV when compared with group I (table -1, figure - 1).AChE activity in OL of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 2. Group II and III the activity increases (0.28×10^{-6} moles min^{-1} g^{-1}and 0.28×10^{-6} moles min^{-1} g^{-1}) and the increase is significant at $p<0.001$ level. Group V remains the same (0.25×10^{-6} moles min^{-1} g^{-1}) as that of group I (0.25×10^{-6} moles min^{-1} g^{-1}).
AChE activity in OP of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 2. Group II, III and IV showed a decrease in the AChE activity and the decrease is significant at $p<0.001$ level. Group V did not show any significant change (0.83×10^{-5} moles min^{-1} g^{-1}) when compared with group I (0.83×10^{-5} moles min^{-1} g^{-1}). Group IV did not show any significant change when compared with group II.
AChE activity in CB of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 2. Group II showed significant decrease (0.27×10^{-7} moles min^{-1} g^{-1}) at $p<0.001$ level. Group III and IV did not show any activity. Group V did not show any significant change when compared with group I.
AChE activity in CL of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 2. Group II showed a significant increase (0.57×10^{-6} moles min^{-1} g^{-1}) at $p<0/001$ level. Group III and IV did not show any activity. Group V did not show any significant change when compared with group I.
AChE activity in MB of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 2. Group II, IV and V remains the same when compared with group I. Group IV did not show any change when compared with group II.
The change in the level of AChE activity in MO of *Rattus norvegicus* on 14th day is illustrated in table - 2 and figure - 20 Group II increases (0.34×10^{-6} moles min^{-1} g^{-1}) and the increase is significant at $p<0.001$ level. Group III and IV showed no enzyme activity. In group V the activity remains the same as that of Group I.
The change in the level of AChE activity of OL of *Rattus norvegicus* on 21st day is illustrated in table - 3 and figure - 3. Group III and IV did not show any activity. Group II and V remains the same when compared with group I.

The change in the level of AChE activity in OP of *Rattus norvegicus* on 21st day is illustrated in table - 3 and figure - 3. Group II and IV did not show any enzyme activity. Group III showed a significant decrease (0.28x10^{-6} moles min^{-1} g^{-1}) at p<0.001 level. Group V did not show any significant change when compared with group I.

The rate of AChE activity in CB of *Rattus norvegicus* on 21st day is illustrated in table - 3 and figure - 3. Group II, IV and V did not show any significant change, Group III showed no activity when compared with group I (0.28x10^{-6} moles min^{-1} g^{-1}). Group IV did not show any changes when compared with group II.

The change in the level of AChE activity of CL in *Rattus norvegicus* on 21st day is illustrated in table - 3 and figure - 3. Group II increases (0.57x10^{-6} moles min^{-1} g^{-1}) and is statistically significant at p<0.001 level. Group III showed no activity. Group IV and V did not show any significant change when compared with group I. Group IV showed significant decrease at p<0.001 level when compared with group II.

The change in the level of AChE activity in MB of *Rattus norvegicus* on 21st day is illustrated in table – 3 and figure - 3. Group II increases (0.57x10^{-6} moles min^{-1} g^{-1}) and is statistically significant at p<0.001 level. Group III showed no activity. Group IV and V did not show any significant change when compared with group I. Group IV showed significant decrease at p<0.001 level when compared with group II.

The change in the level of AChE activity in MO of *Rattus norvegicus* on 21st day is illustrated in table - 3 and figure - 3. Group II increases (0.57x10^{-6} moles min^{-1} g^{-1}) and is statistically significant at p<0.001 level. Group III and IV did not show any enzyme activity. Group V did not show any significant change when compared with group I. Group IV showed a significant change at p<0.001 level when compared with group II.

The change in the level of AChE activity in OL of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group II did not show any enzyme activity. Group III and IV showed an increase (0.28x10^{-6} moles min^{-1} g^{-1}) and is statistically significant at p<0.001 level. Group V remains the same as that of Group I. Group IV showed a significant increase at p<0.001 level when compared with group II.

The rate of AChE activity in OP of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group II, III and IV showed a decrease and is statistically significant at p<0.001 level. Group V did not show any significant change when compared with group I. Group IV did not show any significant change when compared with group II.

The change in the level of AChE activity in CB of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group II, III, IV and V did not show any significant change when compared with group I. Group II and IV did not show any significant change when compared with group III.

The change in the level of AChE activity in CL of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group III, IV and V did not show any significant change. Group II showed no activity when compared with group I. Group IV showed no significant change when compared with group II.

The rate of AChE activity in MB of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group III, IV and V did not show any significant change. Group II showed no activity when compared with group I (0.28x10^{-6} moles min^{-1} g^{-1}). Group IV showed significant increase at p<0.001 level when compared with group II.

The rate of AChE activity in MO of *Rattus norvegicus* on 28th day is illustrated in table - 4 and figure - 4. Group II, III, IV and V did not show any significant change when compared with group I (0.28x10^{-6} moles min^{-1} g^{-1}). Group IV did not show any significant change when compared with group II.

V. Discussion:

The results of the short term study (28 days) has revealed that there is a significant decrease in group II of OL , group II,III of OP, group II of CB on 14th day, group III of OP, and group IV of CL on 21st day and group II,III and IV of OP on 28th day. The decrease in the AChE activity might be due to the effect of MP thereby leading to inhibition of AChE activity. The present study was in accordance with Dikshith *et al*., (1982) who reported that inhibition of AChE might decrease the activity, which was also noted in goats treated with quinalphos and a significant increase is noted in group II of OL on 7th day, II and III of OL, II of MO on 14th day, group II of CL on 21st day group II of MB and MO on 28th day. It may be pointed out that there exists a significant increase in the activity of AChE in brain tissue in toto (Sayim *et al*., 2005 and Aziz *et al*, 2001 and Husain *et al*., 1996) which is also supported by the studies of Rao *et al*., (2005) who reported that oral administration of *C.asiatica* also increased the Ach content and Acetylcholinesterase activity in the rat brain.

Table - 1 Acetylcholinesterase (AChE) activity in the different regions of the brain Of *Rattus norvegicus* due to short term oral toxicity at 7th day

Parameter	OL	OP	CB	CL	MB	MO
Group I	0.25×10^{-6} $\pm 0.25 \times 10^{-08}$	0.83×10^{-5} $\pm 0.26 \times 10^{-07}$	0.28×10^{-6} $\pm 0.2 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	0.28×10^{-6} $\pm 0.2 \times 10^{-08}$	0.28×10^{-6} $\pm 0.2 \times 10^{-08}$
Group II	0.57×10^{-6} $\pm 0.2 \times 10^{-08}$a***	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***	0 0	0 0	0 0	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS
Group III	0 0	0 0	0 0	0 0	0 0	0 0
Group IV	0 0	0 0	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNSb***	0 0	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNSb***	0 0
Group V	2.5×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	0.83×10^{-5} $\pm 0.2 \times 10^{-08}$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	2.8×10^{-6} $0.25 \times 10^{-08} \pm$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS

Values represent mean ± SD of 6 animals
*** p<0.001; ** p<0.01; * p<0.05; ^{NS} Non-significant
 a when compared with group I b when compared with group II

Table - 2 Acetylcholinesterase (AChE) activity in the different regions of the brain Of *Rattus norvegicus* due to short term oral toxicity at 14th day

Parameter	OL	OP	CB	CL	MB	MO
Group I	0.25×10^{-6} $\pm 0.25 \times 10^{-08}$	0.83×10^{-5} $\pm 0.26 \times 10^{-07}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$
Group II	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***	2.7×10^{-6} $\pm 0.25 \times 10^{-08}$a***	5.7×10^{-6} $\pm 0.25 \times 10^{-08}$a***	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	0.34×10^{-06} $\pm 0.2 \times 10^{-08}$a***
Group III	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Group IV	0 ± 0	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a***b^{NS}	0 ± 0	0 ± 0	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS b^{NS}	0 ± 0a***b***
Group V	0.25×10^{-6} $\pm 0.25 \times 10^{-08}$ aNS	0.83×10^{-5} $\pm 0.26 \times 10^{-07}$ a NS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$aNS

Values represent mean ± SD of 6 animals, *** p<0.001; ** p<0.01; * p<0.05; ^{NS} Non-significant,   when compared with group I b when compared with group II

Table - 3 Acetylcholinesterase (AChE) activity in the different regions of the brain Of *Rattus norvegicus* due to short term oral toxicity at 21st day

Parameter	OL	OP	CB	CL	MB	MO
Group I	0.25×10^{-6} $\pm 0.25 \times 10^{-08}$	0.83×10^{-5} $\pm 0.26 \times 10^{-07}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$
Group II	0.25×10^{-6} $\pm 0.25 \times 10^{-08}$ a***	0 ± 0a***	2.8×10^{-6} $\pm 0.25 \times 10^{-08}$a^{NS}	0.57×10^{-6} $\pm 0.2 \times 10^{-08}$a***	0.57×10^{-6} $\pm 0.2 \times 10^{-08}$a***	0.57×10^{-6} $\pm 0.2 \times 10^{-08}$a***
Group III	0	2.8×10^{-6} $\pm 0.25 \times 10^{-}$	0	0	0	0

	±0a***	^{08}a***	±0a***	±0a***	±0a***	±0a***
Group IV	0 ±0a***	0 ±0a***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b***	0 ±0a***
Group V	0.25×10^{-6} ±0.25×10^{-08}a^{NS}	0.83×10^{-5} ±0.26×10^{-07}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}

Values represent mean ± SD of 6 animals
*** $p<0.001$; ** $p<0.01$; * $p<0.05$; ^{NS} Non-significant
 a when compared with group I b when compared with group II

Table - 4 Acetylcholinesterase (AChE) activity in the different regions of the brain Of *Rattus norvegicus*

Parameter	OL	OP	CB	CL	MB	MO
Group I	0.25×10^{-6} ±0.25×10^{-08}	0.83×10^{-5} ±0.26×10^{-07}	2.8×10^{-6} ±0.25×10^{-08}	2.8×10^{-6} ±0.25×10^{-08}	2.8×10^{-6} ±0.25×10^{-08}	2.8×10^{-6} ±0.25×10^{-08}
Group II	0 ±0a***	2.8×10^{-6} ±0.25×10^{-08}a***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	0 ±0a***	0 ±0a***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}
Group III	2.8×10^{-6} ±0.25×10^{-08}a***	2.8×10^{-6} ±0.25×10^{-08}a***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a***
Group IV	2.8×10^{-6} ±0.25×10^{-08}a***b***	2.8×10^{-6} ±0.25×10^{-08}a***b^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b***	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b**	2.8×10^{-6} ±0.25×10^{-08}a^{NS}b^{NS}
Group V	0.25×10^{-6} ±0.25×10^{-08}a^{NS}	0.83×10^{-5} ±0.26×10^{-07}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}	2.8×10^{-6} ±0.25×10^{-08}a^{NS}

Values represent mean ± SD of 6 animals
*** $p<0.001$; ** $p<0.01$; * $p<0.05$; ^{NS} Non-significant
 a when compared with group I b when compared with group II

Figure - 1 Acetylcholinesterase (AChE) activity in the different regions of the brain Of rat *Rattus norvegicus* due to short term oral toxicity on 7th day
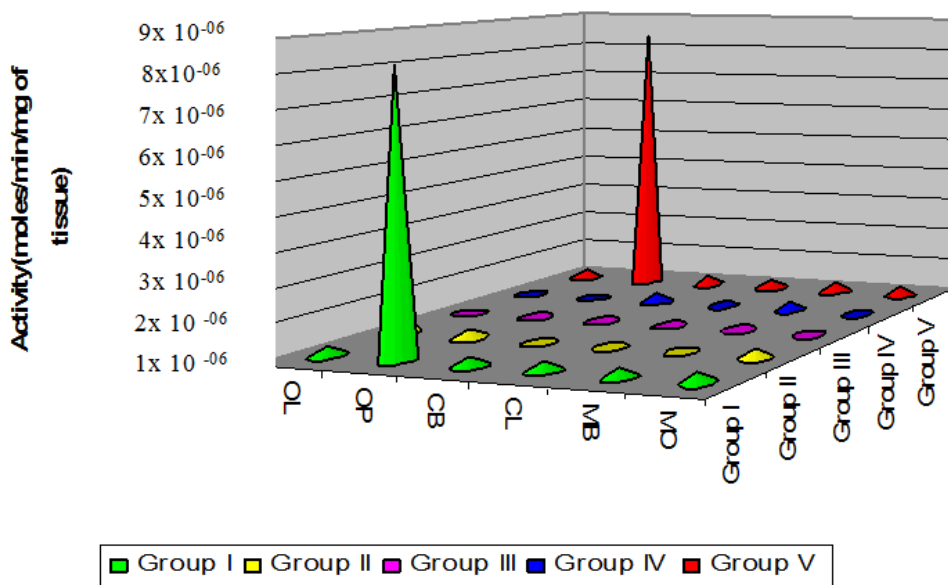
Figure - 2 Acetylcholinesterase (AChE) activity in the different regions of the brain Of rat *Rattus norvegicus* due to short term oral toxicity on 14th day
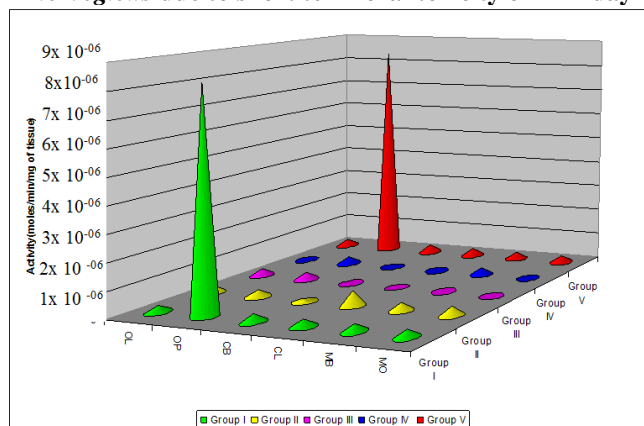

Figure - 3 Acetylcholinesterase (AChE) activity in the different regions of the brain Of rat *Rattus norvegicus* due to short term oral toxicity on 21st day
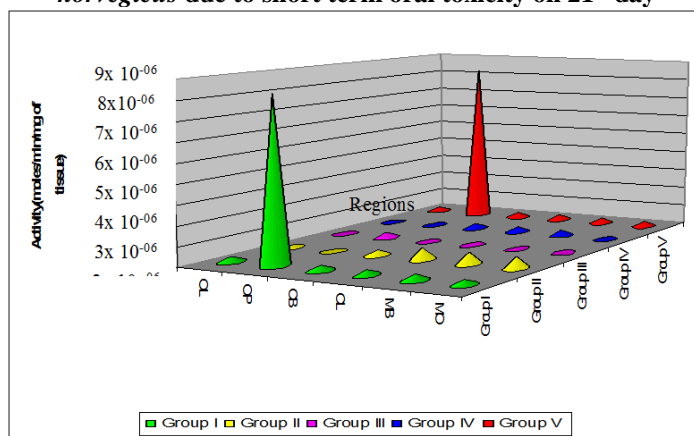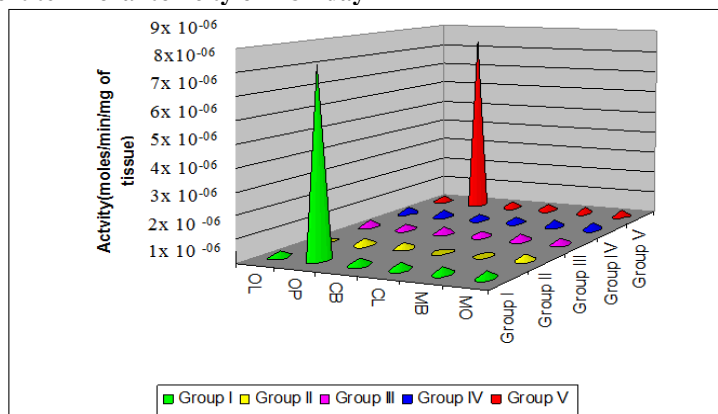

Figure - 4 Acetylcholinesterase (AChE) activity in the different regions of the brain Of rat *Rattus norvegicus* due to short term oral toxicity on 28th day

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