

Research Article



Acute 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 acute effects and then an antidote *Centella asiatica* was given to the rats which were induced with methylparathion.

Keywords: AChE, Acute studies, Brain, *Centella asiatica*, Methyl parathion, Organophosphate compound.

INTRODUCTION

The organophosphorus pesticides are of particular importance, because of the increased application as insecticides to replace the more environmentally and biologically persistent organochlorine pesticides. The use of organophosphorus compounds is alarmingly on the increasing side in view of its non-persistent nature. The organophosphate compounds are important agrochemicals which contain one or more phosphorous atoms and are relatively short lived in biological system. The primary mode of action for OP pesticides is initiated through inhibition of acetylcholinesterase, the enzyme responsible for degrading the neurotransmitter acetylcholine.¹⁻³ Organophosphorus (OP) insecticides elicit toxicity through inhibition of acetylcholinesterase, leading to accumulation of acetylcholine in the nervous system and consequent signs of cholinergic toxicity.^{1,4} The inhibition of acetylcholinesterase (AChE) by most of the organophosphorus compounds can be easily detected by considering AChE as biological indicator and the levels of the activity of the enzyme can be used for monitoring the magnitude of organophosphate poisoning.⁵

Methyl parathion (C₈H₁₀NO₅PS), (O, O-dimethyl O-4-nitrophenylphosphorothioate) is an OP insecticide with a broad range of activity which inhibits acetylcholinesterase activity.^{6,7}

To study the toxic effects of methyl parathion on rat (*Rattus norvegicus*) for acute (24 h, 48 h, 72 h and 96 h).

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.

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.

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 LD₅₀ value for methyl parathion is 14mg/kg body weight according to 8. For acute toxicity study 1/3rd of the LD₅₀ value was taken, and mixed along with groundnut oil and administered to the rats.

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 9. 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 up to 5 minutes. 0.1ml of eserinesulphate (10^{-4} M) a specific AChE inhibitor was added to another cuvette containing 0.4ml of homogenate, 2.5ml of 0.1M phosphate buffer (pH8.0) 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 subtracted 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:

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

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. *Centella asiatica* of 300mg/kg body weight was given to the rats as an antidote. Statistical analysis were done using SPSS package.

RESULTS

Acetylcholinesterase (AChE) activity in the different regions of the brain of *Rattus norvegicus* due to acute oral toxicity during 24 h, 48 h, 72 h and 96 h.

The change in the level of the AChE activity in the olfactory lobes (OL) of *Rattus norvegicus* during 24 h is illustrated in table-1 and figure-1. Group I serves as control and group II animals did not show any significant change in the AChE activity (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group III and IV animals showed a slight increase in the AChE activity (0.289×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and this increase is significant at $p < 0.001$ level. Group V did not show any changes in the values when compared with group I

(0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group V serves as negative control. Group IV did not show any significant change (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group II (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Acetylcholinesterase activity in the optic lobe (OP) of *Rattus norvegicus* during 24 h is illustrated in table - 1 and figure-1. Group II and III animals exhibited a decrease in the AChE activity (0.34×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$, 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively and the decrease is significant at $p < 0.001$ level. Group IV showed an increase in the AChE activity (0.56×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) which is statistically significant at $p < 0.001$ level. Group V animals did not show significant change (0.83×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.83×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and group IV showed a significant increase (0.56×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group II at $p < 0.001$ level (0.34×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Acetylcholinesterase activity in the cerebrum (CB) of *Rattus norvegicus* during 24 h is illustrated in table - 1 and figure-1. Group II animals showed a decrease in the AChE activity (0.11×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and it is significant at $p < 0.001$ level. Group III, IV and V did not show any significant change when compared with group I. Group IV showed no significant change (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group II. Acetylcholinesterase activity in the cerebellum (CL) of *Rattus norvegicus* during 24 h is illustrated in table 1 and figure 1. Group II animals showed a decrease in the AChE activity (0.14×10^{-8} moles $\text{min}^{-1} \text{g}^{-1}$) and the decrease was not significant. Group III showed no activity. Group IV did not exhibit any significant change in the activity (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed an increase but was not statistically significant (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group II (0.14×10^{-8} moles $\text{min}^{-1} \text{g}^{-1}$).

The changes in the level of AChE activity in the midbrain (MB) of *Rattus norvegicus* is illustrated in table 1 and figure 1. Group II animals showed a decrease in the AChE activity (0.17×10^{-8} moles $\text{min}^{-1} \text{g}^{-1}$) and the decrease is significant at $p < 0.001$ level. Group III and group V did not show any changes. Group IV showed an increase in the AChE activity (0.43×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and the increase is significant at $p < 0.001$ level When compared with group I (0.29×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and group II.

The change in the level of AChE activity in the medulla oblongata (MO) of *Rattus norvegicus* during 24 h is illustrated in table 1 and figure 1. Group II, III, IV and V did not show any significant change in the activity when compared with group I and group II Acetylcholinesterase activity in the olfactory lobes (OL) of *Rattus norvegicus* during 48 h is illustrated in table 2 and figure 2. Group II animals showed a decrease in the AChE activity (0.25×10^{-8} moles $\text{min}^{-1} \text{g}^{-1}$) and the decrease is significant at $p < 0.001$ level Group III and IV showed no activity. Group V did not show any significant change (0.26×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$).

Acetylcholinesterase activity in the optic lobe (OP) of *Rattus norvegicus* during 48 h is illustrated in table 2 and



figure 2. Group II, III and IV animals showed a marked decrease in the AChE activity (0.28×10^{-6} , 0.28×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively and the decrease is statistically significant at $p < 0.001$ level. Group V animals did not show any changes when compared with group I. Group IV did not show any change when compared with group II. The change in the level of the AChE activity in the cerebrum (CB) of *Rattus norvegicus* at 48 h is illustrated in table 2 and figure 2. Group II the activity increased (0.45×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and the increase is significant at $p < 0.001$ level. Group III, IV and V did not show any changes when compared with group I and group II. The change in the level of the AChE activity in cerebellum (CL) of *Rattus norvegicus* during 48 h is illustrated in table - 2 and figure-2. Group II animals showed decrease in the AChE activity (0.26×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and the decrease is significant at $p < 0.001$ level. Group III and V did not show any changes (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed no activity when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and group II.

The change in the AChE activity in midbrain (MB) of *Rattus norvegicus* during 48 h is illustrated in table - 2 and figure - 2. Group II, III showed an increase in the AChE activity (0.34×10^{-6} , 0.86×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively which is significant at $p < 0.001$ level. Group IV showed no activity. Group V did not show any significant change (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.29×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Acetylcholinesterase activity in medulla oblongata of *Rattus norvegicus* during 48 h is illustrated in table 2 and figure 2. Group II, III and V did not show any significant change. Group IV showed no activity when compared with group I. Group IV showed a decrease but was not statistically significant when compared with group II. Acetylcholinesterase activity in olfactory lobes (OL) of *Rattus norvegicus* at 72 h is illustrated in table 3 and figure 3. Animals belonging to Groups II and IV showed an increase in the AChE activity (0.34×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively and the increase is significant at $p < 0.001$ level. Group III showed no activity. Group V did not show any significant change (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed significant decrease at $p < 0.001$ level when compared with group II. Acetylcholinesterase activity in optic lobe (OP) of *Rattus norvegicus* during 72 h is illustrated in table 3 and figure 3. Animals belonging to Group II, III and IV showed a significant decrease in the AChE activity (0.44×10^{-6} , 0.57×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively and the increase is statistically significant at $p < 0.001$ level. Group V did not show any significant change (0.83×10^{-5} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.83×10^{-5} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed significant decrease at $p < 0.001$ level when compared with group II.

The change in the level of AChE activity in cerebrum (CB) and in cerebellum (CL) of *Rattus norvegicus* during 72 h is illustrated in table 3 and figure 3. Animals belonging to Group II, III, IV and V did not show any significant change

(0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I and group II (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). The change in the level of AChE activity in midbrain (MB) of *Rattus norvegicus* during 72 h is illustrated in table - 3 and figure-3. Group II showed an increase in the AChE activity (0.34×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Animals belonging to III, IV and V did not show any significant change (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed significant decrease at $p < 0.001$ level when compared with group II. Acetylcholinesterase activity in the medulla oblongata (MO) of *Rattus norvegicus* during 72 h is illustrated in table 3 and figure 3. Animals belonging to Group II and III showed a significant increase in the AChE activity (0.31×10^{-6} , 0.57×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Group IV and V did not show any changes in the activity (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed significant decrease at $p < 0.001$ level when compared with group II. Acetylcholinesterase activity in the olfactory lobes (OL) of *Rattus norvegicus* during 96 h is illustrated in table 4 and figure 4. Group II animals showed a significant increase in the AChE activity (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) and the increase is statistically significant at $p < 0.001$ level. Group III and IV did not show any activity. Group V did not show any significant change (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.25×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$).

The changes in the AChE activity in optic lobe (OP) of *Rattus norvegicus* during 96 h is illustrated in table - 4 and figure-4. Animals belonging to Group II, III and IV showed a significant decrease in the AChE activity (0.31×10^{-6} , 0.28×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Group V did not show any significant change (0.83×10^{-5} moles $\text{min}^{-1} \text{g}^{-1}$) when compared with group I (0.83×10^{-5} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV did not show any significant change when compared with group II. The changes in the AChE activity in cerebrum (CB) of *Rattus norvegicus* during 96 h is illustrated in table 4 and figure 4. Group II animals showed a significant increase in the AChE activity (0.40×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Animals belonging to Group III and V did not show any significant change in the activity (0.28×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively. Group IV exhibited no activity when compared with group I. Acetylcholinesterase activity in cerebellum of *Rattus norvegicus* during 96 h is illustrated in table 4 and figure 4. Group II animals showed a significant increase in the AChE activity (0.34×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Group III and V animals did not show any changes in the activity (0.28×10^{-6} , 0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) respectively. Group IV showed no activity when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). The changes in the AChE activity in midbrain (MB) of *Rattus norvegicus* during 96 h is illustrated in table 4 and figure 4. Group II animals exhibited a significant increase in the AChE activity (0.51×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.001$ level. Animals belonging to Group III, IV and V did not show any significant change (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$)

when compared with group I (0.28×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$). Group IV showed significant decrease at $p < 0.001$ level when compared with group II.

The changes in the AChE activity in medulla oblongata (MO) of *Rattus norvegicus* during 96 h is illustrated in table 4 and figure 4. Group II animals showed a significant decrease in the AChE activity (0.23×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$) at $p < 0.05$ level. Group III animals showed a significant increase in the AChE activity (0.57×10^{-6} moles $\text{min}^{-1} \text{g}^{-1}$)

DISCUSSION

The nervous system has the most sensitive toxic chemicals which interrupt the electrophysiological mechanisms of neurons. Brain extracts provides a useful tool to study the activation mechanisms of organophosphorus compounds (OP) since they contain both the activating enzymes and the molecular target for OP toxicity (10). AChE is considered as a tool to quantify the effects caused by the OP.⁵ Inhibition of AChE is regarded as a significant parameter to assess the toxicogenetic effects of various toxicants. AChE is considered as excellent biomarkers of organophosphate poisoning.¹¹⁻¹³ Though there are several methods for gathering the information about the toxicity of OP compounds the present study is aimed to see the effect of *C.asiatica* and in combination using the biomarker

enzyme (AChE) as the end point. The results of the acute oral toxicity study (96 h) has revealed that there has been a significant decrease in the activity of group II of OP, CB, CL and MB of the brain, during 24 h, group II of OL, II, III and IV of OP, II of CL during 48 h, group II, III and IV of OP during 72 h and group II, III and IV of OP, MO of group II after 96 h indicates the effects of methyl parathion and the inhibition of acetylcholinesterase (AChE) activity in the CNS resulted in the suppression of the immune response. It seems that ACh in the brain plays an immunoinhibitory role. It is suggested that a functional connection is present in the ACh of the brain and the immune system and significant increase in the activity of group III and IV of OL, IV of OP, IV of MB during 24 h, group II of CB, II and III of MB during 48 h, group II and IV of OL, II of MB, II and III of MO during 72 h and II of OL, II of CB, II of CL, II of MB and IV of MO may be due to the effects of *Centella asiatica*. Increased AChE levels might reflect the enhancement of ACh release, which could facilitate the synaptic transmission of CA3 pyramidal neurons¹⁴, suggested that increase in the AChE activity might modulate the dendritic branching pattern in specific brain regions. According to¹⁵ *C. asiatica* acts as a potent antioxidant which also exerts significant neuroprotective effect, which is in agreement with the present study.

Table 1: Acetylcholinesterase (AChE) activity in the different regions of the brain of *Rattus norvegicus* due to acute oral toxicity during 24 h

0	OL	OP	CB	CL	MB	MO
Group I	$0.25 \times 10^{-06} \pm 1 \times 10^{-08}$	$0.83 \times 10^{-05} \pm 2 \times 10^{-07}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-07}$	$0.29 \times 10^{-06} \pm 30 \times 10^{-08}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08}$
Group II	$0.25 \times 10^{-06} \pm 5 \times 10^{-07} \text{a}^{\text{NS}}$	$0.34 \times 10^{-06} \pm 3 \times 10^{-08} \text{a}^{***}$	$0.11 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{***}$	$0.14 \times 10^{-08} \pm 28 \times 10^{-06} \text{a}^{\text{NS}}$	$0.17 \times 10^{-08} \pm 34 \times 10^{-06} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$
Group III	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 1 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$	0.0 ± 0	$0.29 \times 10^{-06} \pm 30 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$
Group IV	$0.28 \times 10^{-06} \pm 15 \times 10^{-08} \text{a}^{***} \text{b}^{***}$	$0.56 \times 10^{-05} \pm 44 \times 10^{-07} \text{a}^{***} \text{b}^{***}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}} \text{b}^{***}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}} \text{b}^{\text{NS}}$	$0.43 \times 10^{-06} \pm 12 \times 10^{-07} \text{a}^{***} \text{b}^{***}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}} \text{b}^{\text{NS}}$
Group V	$0.25 \times 10^{-06} \pm 15 \times 10^{-08} \text{a}^{\text{NS}}$	$0.83 \times 10^{-05} \pm 2 \times 10^{-07} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 25 \times 10^{-08} \text{a}^{\text{NS}}$

Values represent mean \pm 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 acute oral toxicity during 48 h

	OL	OP	CB	CL	MB	MO
Group I	$0.28 \times 10^{-06} \pm 25 \times 10^{-08}$	$0.83 \times 10^{-05} \pm 2 \times 10^{-07}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08}$	$0.29 \times 10^{-06} \pm 3 \times 10^{-08}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$
Group II	$0.25 \times 10^{-08} \pm 10 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{***}$	$0.45 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{***}$	$0.26 \times 10^{-06} \pm 1 \times 10^{-08} \text{a}^*$	$0.34 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$
Group III	0.0 ± 0	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$	$0.86 \times 10^{-06} \pm 1 \times 10^{-08} \text{a}^{***}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$
Group IV	0.0 ± 0	$0.28 \times 10^{-06} \pm 1 \times 10^{-08}$	$0.45 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}} \text{b}^{\text{NS}}$	0.0 ± 0	0.0 ± 0	0.0 ± 0
Group V	$0.26 \times 10^{-06} \pm 20 \times 10^{-08} \text{a}^{\text{NS}}$	$0.83 \times 10^{-05} \pm 12 \times 10^{-07} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$	$0.28 \times 10^{-06} \pm 2 \times 10^{-08} \text{a}^{\text{NS}}$

Values represent mean \pm 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 3: Acetylcholinesterase (AChE) activity in the different regions of the brain of *Rattus norvegicus* due to acute oral toxicity during 72 h

	OL	OP	CB	CL	MB	MO
Group I	0.25x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸	0.83x10 ⁻⁰⁵ ±.2x10 ⁻⁰⁷	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸	0.29x10 ⁻⁰⁶ ±.30x10 ⁻⁰⁸	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸
Group II	0.34x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸ a***	0.44x10 ⁻⁰⁶ ±.5x10 ⁻⁰⁸ a***	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ 2a ^{NS}	0.34x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸ a***	0.31x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***
Group III	0.0 ±.0	0.57x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.57x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸ a***
Group IV	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***b***	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***b***	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b***
Group V	0.25x10 ⁻⁰⁶ ±.25x10 ⁻⁰⁸ a ^{NS}	0.83x10 ⁻⁰⁵ ±.00a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻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* due to acute oral toxicity during 96 h

	OL	OP	CB	CL	MB	MO
Group I	0.25x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸	0.83x10 ⁻⁰⁵ ±.2x10 ⁻⁰⁷	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸	0.29x10 ⁻⁰⁶ ±.3x10 ⁻⁰⁸	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸
Group II	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***	0.31x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***	0.40x10 ⁻⁰⁶ ±.5x10 ⁻⁰⁸ a***	0.34x10 ⁻⁰⁶ ±.01x10 ⁻⁰⁸ a***	0.51x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸ a***	0.23x10 ⁻⁰⁶ ±.3x10 ⁻⁰⁸ a*
Group III	0.0 ±.0	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.57x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***
Group IV	0.0 ±.0	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a***b ^{NS}	0.0 ±.0	0.0 ±.0	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS} b***
Group V	0.25x10 ⁻⁰⁶ ±.1x10 ⁻⁰⁸ a ^{NS}	0.83x10 ⁻⁰⁵ ±.2x10 ⁻⁰⁷ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻⁰⁸ a ^{NS}	0.28x10 ⁻⁰⁶ ±.2x10 ⁻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

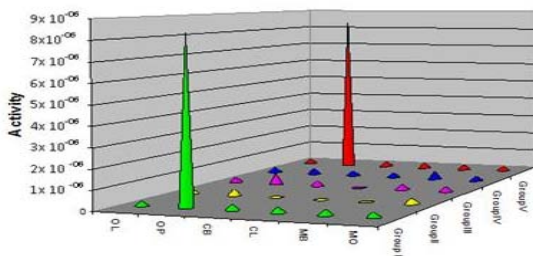


Figure 1: Acetylcholinesterase (AChE) activity in the different regions of the brain of rat *Rattus norvegicus* due to acute oral toxicity during 24h.

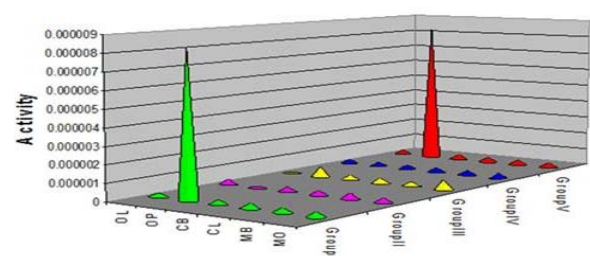


Figure 3: Acetylcholinesterase (AChE) activity in the different regions of the brain of rat *Rattus norvegicus* due to acute oral toxicity during 72 h.

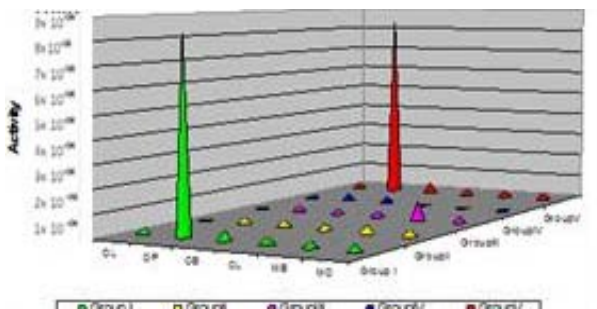


Figure 2: Acetylcholinesterase (AChE) activity in the different regions of the brain of rat *Rattus norvegicus* due to acute oral toxicity during 48h.

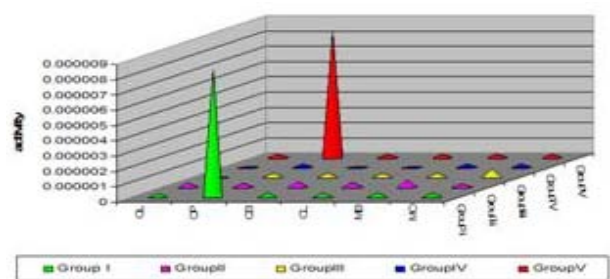


Figure 4: Acetylcholinesterase (AChE) activity in the different regions of the brain of rat *Rattus norvegicus* due to acute oral toxicity during 96h.

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