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ALTERATIONS IN THE ACETYLCHOLINESTERASE ACTIVITY IN THE BRAIN OF *ALBINO MICE* EXPOSED TO ACEPHATE

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ABSTRACT
Acephate (AP), a widely available organophosphorus (OP) insecticide, has low mammalian toxicity and is considered non-phytotoxic on many crop plants and therefore it is preferred in agricultural crops. In plants and insects, AP is metabolized extensively to methamidophos (MP), a more potent OP insecticide. The limited mammalian metabolism of AP to MP has been studied in laboratory rat models and suggests that initial formation of MP from AP may inhibit further formation. Hence in the present investigation we have studied the effect of an AP in cholinergic mechanisms in the different regions of brain. For the present study the male mice were exposed to 1/10th LD_{50} of AP via oral gavage (*i.e.* 40.5mg/kg body weight). Our results indicate a steady decline of AChE activity in all the regions of the brain of Acephate exposed animals. As expected an increase in ACh activity was noticed in all the regions of the AP exposed animals. We suggest that cholinergic system is seriously affected by the intoxication of Acephate and the effect was more effective in 30 days when compared to 10 days.

INTRODUCTION

Acephate (AP), an organophosphorus (OP) insecticide, is considered as one of the safest OP insecticides because of its low mammalian toxicity. AP is rapidly and extensively metabolized to a more potent OP insecticide methamidophos (MP), which has a lower LD_{50} in mice and rats, thus a higher toxicity than AP (Spassova *et al.,* 2000; Tanaka *et al.,* 2005). Earlier reports issued by the Food and Agriculture Organization of the United Nations have indicated that urinary levels of MP and other metabolites are measureable in human subjects exposed to AP in formulation plants. Reports of acute human exposures to AP are extremely limited. Other reports indicate that chronic exposure in workers in industrial AP manufacturing has resulted in measurable urinary levels of AP, but not MP and no depression of AChE activity (Maroni *et al.,* 1990). AP exposure in humans results in bradycardia/ tachycardia, central nervous system impairment, eye problems, gastrointestinal problems and respiratory problems and finally death due to respiratory failure.

AP toxicity studies were done mostly in rats. Nakuleswar Dut *et al* (2013) studied the effect of acephate on accessory sex organs in male rats. The toxic effects of AP are not extensively studied in mice. In this paper we report the toxic effect of acephate on cholinergic mechanisms in different regions of the brain in mice exposed to sub-lethal AP toxicity.

MATERIALS AND METHODS

Acephate (Technical grade of 97% purity) was obtained from Hyderabad chemical limited, Hyderabad A.P., India.

Animal and experimental design

The protocol was approved by Institutional Animal Ethics Committee, S.V. University (Regd. No. 438/01a/CPCSEA). Male adult Mice of 7 weeks old and weighing45 $\pm$ 5g. were obtained from Indian Institute of Science (II.Sc.), Bangalore. They were housed at an ambient temperature 28 $\pm$ 2ºC in a 12h light/dark cycle and a minimum humidity of 40%. The animals had free access to commercial pellet diet supplied by Sai Durga Feeds and Foods, Bangalore, India and water *ad libitium*. All the male healthy adult male mice were randomly divided into four groups having with six mice per group. The first group animals were considered as control animals. Second group of animals were treated with AP via oral gavage (40.5 mg/kg body weight) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively.

Determination of AChE

The AChE activity was determined according to the procedure of Ellman's colorimetric method (Ellman *et al.*, 1961). Briefly, a 30μL of aliquot of homogenate was added to 3mL phosphate buffer containing 5,5'-dithio-bis-nitrobenzoic acid (DTNB) and Thioacetyl-choline iodide (ACh) and incubated in 37ºC water bath for 6 min and then the activity was determined using a Hitachi Spectrophotometer. The AChE content was expressed as μmoles of Ach hydrolyzed/mg protein/h.

Determination of ACh

Table: 1: Changes in the acetylcholine activity in different regions of the brain of mice exposed to sub-lethal dose of Acephate

Name of the tissue	Control	10 days	20 days	30 days	F value
Cerebral Cortex ± SD% Change	29.935 ± 3.509	38.125[a] ± 2.121(27.35)	40.131 [a] ± 3.666(34.06)	48.131 ± 5.425(60.75)	15.162*
Hippocampus ± SD% Change	36.625 ± 3.928	40.018 ± 5.095(9.26)	44.536 ± 4.667(48.80)	53.475 ± 5.808(78.60)	10.373*
Cerebellum ± SD% Change	20.232 ± 1.802	24.865 ± 3.146(22.90)	30.261 ± 3.246(49.57)	36.095 ± 3.576(78.41)	12.535*
Medulla Oblongata ± SD% Change	21.651 ± 2.714	24.634 ± 3.103(13.78)	29.888 ± 2.766(38.04)	36.235 ± 3.607(67.36)	22.351*

Values expressed in μmoles of ACh per g. wet weight of tissue are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ significantly among themselves through S-N-K test; Significance level *P < 0.01

The ACh was estimated by the method of Metcalf (1951) as given by Augustinsson (1957). The different areas of the brain were quickly frozen in liquid nitrogen, weighed accurately and placed on Pyrex glass tubes. These tubes were placed in boiling water for 5minutes to terminate the AChE enzyme activity and also to release the bound ACh. The tissues were then homogenized in 1mL distilled water. To the homogenate 1mL of alkaline hydroxylamine hydrochloride followed by 1mL of 50% hydrochloric acid solution (1:1 HCl: H_2O) was added. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5mL of 0.37 M ferric chloride solution was added and the brown colour developed was read at 540nm against a reagent blank in a spectrophotometer. The acetylcholine content was expressed as μ moles of Ach /g.wet wt of tissue.

Statistical treatment

The data was subjected to statistical treatment. One way analysis of variance (ANOVA), two way ANOVA and S-N-K tests were performed using SPSS (ver. 20) in the personal computer and $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The results on the effect of ACh and AChE are presented in the Tables (1 and 2). Exposure to Acephate induced typical signs of OP toxicity in all the regions of the brain. The toxic effect was more in 30 days when compared to 20 days and 10 days. Cerebellum showed a maximum decrement in the AChE activity when compared to other regions. A steady decline in the AChE activity was observed in all the regions of the brain of AP exposed animals. As is observed with many OP compounds an increase in the ACh activity was seen in all the AP exposed mice.

AP administration induced typical signs of OP toxicity like weakness, salivation, fasciculations, tremor, facial movements. Animals which were exposed for longer duration showed more pronounced effects. The structural alterations in the brain tissues have a profuse effect on the functional integrity of the cholinergic neurons. Decrease in the AChE activity is due to the change in the lipid environment of various regions of the brain (Mason, 2000). Periera *et al.* (2013) reported that OP affects cholinesterases in *Folsomia candida* and their locomotion. The slow recovery of depressed AChE activity may mean that affected organisms in the natural system are unable to sustain physical activities. The motor function in mice under OP toxicity was studied by Valenzuela *et al.* (2012) and they reported that the motor function and performance is badly affected under OP stress. Mdegela *et al.* (2010) reported that inhibition of AChE activity in *C. gariepinus* is a useful biomarker in assessing aquatic environment contaminated by anticholinesterases.

Changes in the AChE activity is frequently used as a biomarker for OP induced toxicity. AChE is an enzyme that breaks down the neurotransmitter ACh at the synaptic cleft. Like AChE, butyrylcholinesterase inactivates the ACh neurotransmitter and is hence a viable therapeutic target in Alzheimer's disease characterized by a cholinergic deficit. In the present study, AChE was decreased in all the regions of the brain of Acephate exposed animals and the animals exposed for longer duration showed maximum decrement. A number of neurological disorders are observed on fall of AChE activity. Several OP compounds cause degeneration of long axons in the spinal cord and peripheral nerves, a syndrome known as OP-induced delayed neuropathy (Ehrich *et al.*, 1997). Kakani *et al.* (2008) reported that small deletion in the olive fly acetylcholinesterase gene is associated with high levels of organophosphate resistance. Yang *et al.* (2005) studied the mechanism underlying intermediate myasthenia syndrome following acute dimethoate poisoning in rats. They reported that specific nicotinic acetylcholine receptor binding activity in the gastrocnemius muscle and blood lymphocytes of myasthenia rats was significantly increased at 48h after OP poisoning. They reported that the functional changes of nicotinic acetylcholine receptor at neuromuscular junction might play an important role in the paralysis of skeletal muscle following acute OPs poisoning. Olmos *et al.* (2009) reported that dichlorvos enhances long-term potentiation through a postsynaptic mechanism that involves the inhibition of enzyme acylpeptide hydrolase and the modulation of alpha nicotinic receptors. Siraj Mohiyuddin *et al.* (2009) reported a decline in the AChE activity in different regions of the brain in Acephate exposed Albino rats.

Table 2: Changes in the AChE activity in different regions of the brain of mice exposed to sub-lethal dose of Acephate

Name of the tissue	Control	10 days	20 days	30 days	F value
Cerebral Cortex ± SD% Change	14.978 ± 2.211	11.861 ± 2.653(-20.81)	10.799 ± 2.451(-27.90)	7.567 ± 1.888(-49.50)	7.041*
Hippocampus ± SD% Change	16.281 ± 1.464	14.21 ± 1.497(-12.72)	12.819 ± 0.960(-21.26)	7.683 ± 1.013(-52.80)	34.805*
Cerebellum ± SD% Change	14.929 ± 1.814	9.971 ± 1.601(-33.21)	7.720 ± 1.951(-48.30)	5.377 ± 0.944(-64.00)	26.582*
Medulla Oblongata ± SD% Change	12.123[a] ± 2.657	11.698[a] ± 2.729(-3.51)	7.7378 ± 1.094(-36.17)	7.126 ± 1.514(-41.20)	6.224*

Values expressed in μmoles of Ach hydrolyzed/mg protein/h. are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ significantly among themselves through S-N-K test; Significance level level*P < 0.01

In conclusion, the present data shows that Acephate intoxication enhances ACh levels in all the regions of the brain and significantly inhibits AChE.

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