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**EFFECT OF SYNTHETIC PYRETHROID PERMETHRIN ON CHOLINERGIC MECHANISMS IN DIFFERENT REGIONS OF THE BRAIN IN *ALBINO MICE*****¹C.OBULPATHI, ²S.V. RAVIKANTH AND ^{1*}P. JACOB DOSS***¹Dept. of Zoology, S.V. University, Tirupati**²Sree Vidyanikethan Degree College, A. Rangampet
(Affiliated to S.V. University, Tirupati)***ABSTRACT**

Latest pesticide monitoring results suggests that there is a shift in residential pesticide exposure from organophosphorous insecticides to pyrethroid insecticides. Although it is well known that pyrethroid insecticides are potential neurodevelopmental toxicants, research pertaining to their effect, especially permethrin, has not been carried out in mice. In the present study we report the effect of sublethal dose of permethrin (84 mg/kg body weight), on the cholinergic mechanisms in different regions of brain in the Albino mice. There is a steady decline in the AChE activity in different regions of the brain of permethrin, exposed mice. An increase in the ACh activity was noticed in all the regions of the brain of permethrin exposed animals. The present results suggest that mechanism of ACh synthesis is seriously affected by the intoxication of permethrin. The effect of permethrin was more in 30 days when compared to 10 days.

KEY WORDS: Pyrethroids, Permethrin, Mice, Brain, Toxicity**P. JACOB DOSS**

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INTRODUCTION

A significant percentage of world insecticide market is represented by pyrethroid insecticides¹. This usage results in an increased potential for human exposure. The residues of pyrethroids have been detected in sediments from agricultural runoff², residential dust samples³ etc. Pyrethroid metabolites have also been detected in human urine⁴. Pyrethroids disrupt nervous system function by interacting with membrane bound ion channels and altering their normal gating kinetics⁵. The primary molecular targets of pyrethroids are neuronal voltage-sensitive sodium channels. Pyrethroids affect nervous system function by producing hyperexcitability in neurons and changing neuronal firing rates⁶. Permethrin (3-Phenoxyphenyl methyl (+) *cis*, *trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate) is a synthetic, third generation, type I pyrethroid insecticide that is commonly used in household insect foggers and sprays, flea dips and sprays for cats and dogs, ornamental gardens, repellent/insecticide for clothing, mosquito abatement products, termite treatments, agricultural products and for scabies control. Pyrethroid insecticides are some of the most widely used pesticides in the world because they are believed to be less harmful to humans than other pesticides. Low doses of Permethrin cause mild neurological signs whereas higher doses cause several peripheral and central nervous system clinical signs including paralysis and even death⁷. Studies in mice and rats show that sub-lethal intoxication leads to aggression, hypersensitivity to external stimulation, whole-body tremor, convulsions, and paralysis⁸. As the use of pyrethroids is steadily rising, there is an urgent need to identify the possible adverse effects that may be associated with their use. The literature pertaining to the studies on the effects of pyrethroids in mice are scanty, hence the present study was taken up to assess the impact of sublethal dose of permethrin in the cholinergic mechanisms of male Albino mice.

MATERIALS AND METHODS

Permethrin (92.0% purity, isomer composition: 40% *cis*, 60% *trans*, 1:1 ratio of 1*R* & 1*S*) 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 weighing 45 ± 5 g. were obtained from Indian Institute of Science (I.I.Sc.), Bangalore. They were housed at an ambient temperature $28 \pm 2^\circ\text{C}$ in a 12-h 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 libitum*. 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 permethrin 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 method⁹. Briefly a 30 μl 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 37°C 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

The ACh was estimated by the method of Metcalf (1951) as given by Augustinsson¹⁰. 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 5 minutes to terminate the AChE enzyme activity and also to release the bound ACh. The tissues were then homogenized in 1 ml distilled water. To the homogenate 1 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 50% hydrochloric acid solution (1:1 HCl: H₂O) was added. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml of 0.37 M ferric chloride solution was added and the brown color developed was read at 540 nm 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. 12) in the personal computer and

$p < 0.05$ was considered as statistically significant.

RESULTS

The results on the effect of ACh and AChE are presented in the tables (1 & 2). Permethrin exposed mice showed an increase in the ACh content in different regions of the brain. The increase in the ACh content was more in medulla oblongata of permethrin exposed mice when compared to other regions. Permethrin at all exposures showed an increase in the ACh content and the increase was more prevalent in 30 days exposed mice. AChE activity in the brain of permethrin exposed mice showed a decrease when compared to control mice. The decrease was more in the animals which were exposed to 30 days.

Table 1
Changes in the AChE activity in different regions of the brain of Mice exposed to sub-lethal dose of Permethrin

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	10.473	9.582	8.457 ^a	8.020 ^a	14.127 ^{**}
± SD	0.898	0.402	0.721	0.389	
(% Change)		(-8.51)	(-19.25)	(-23.42)	
Hippocampus	11.047 ^b	11.172 ^b	9.889	9.073	5.11 [*]
± SD	1.491	0.766	0.518	0.876	
(% Change)		(1.13)	(-10.48)	(-17.87)	
Cerebellum	7.958	8.274	7.675	7.205	2.135 ^{ns}
± SD	0.621	0.658	0.539	0.813	
(% Change)		(3.97)	(-3.55)	(-9.46)	
Medulla Oblongata	9.426	8.574 ^c	8.371 ^c	7.171	7.117 [*]
± SD	0.449	1.241	0.966	0.640	
(% Change)		(-9.03)	(-11.19)	(-23.92)	

ANOVA

Source of Variation	df	SS	MS	F
Between different time intervals	3	84.56346	28.18782	43.57533
Between tissues	3	50.17975	16.72658	25.8575
Error	9	9.208703	1.023189	1.58174
Within groups	80	51.75005	0.646876	
Total	95	195.702		

Values expressed in μ moles of ACh hydrolyzed/mg protein/h. are Mean \pm SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. Significance level $P < 0.01$, $**P < 0.001$

Table 2
Changes in the ACh activity in different regions of the brain
of Mice exposed to sub-lethal dose of Permethrin

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	26.680	30.131	35.386 ^a	36.557 ^a	21.001
± SD	1.120	2.496	1.481	2.991	
(% Change)		(12.93)	(32.63)	(37.02)	
Hippocampus	32.108	37.237	39.632	42.232	17.707
± SD	2.618	1.958	1.831	2.645	
(% Change)		(15.97)	(23.43)	(31.53)	
Cerebellum	21.439	24.801	29.201	36.578	95.307
± SD	1.436	1.606	0.963	1.355	
(% Change)		(15.68)	(36.20)	(70.61)	
Medulla Oblongata	13.749	16.873	19.777	24.179	78.486
± SD	0.708	1.009	0.932	1.495	
(% Change)		(22.72)	(43.84)	(75.86)	

ANOVA

Source of Variation	df	SS	MS	F
Between different time intervals	3	4698.376	1566.125	484.7212 [*]
Between tissues	3	1725.35	575.1168	178.0007 [*]
Error	9	116.2759	12.91955	3.998644
Within groups	80	258.4785	3.230982	
Total	95	6798.481		

Values expressed in $\mu\text{moles of ACh per g. wet weight of tissue}$ are Mean \pm SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. Significance level $P < 0.001$

DISCUSSION

Permethrin is a member of one of the newest classes of synthetic type I pyrethroids. This compound has gained in popularity due to its photostability, high activity against insects and relatively low mammalian toxicity. Pesticide exposure resulted in high Weakness, salivation, fasciculations, tremors and alterations in the facial movements. The effect was more in animals which were exposed for longer duration. Loss of memory, fatigue, muscle and joint pains, ataxia, skin rash, respiratory difficulties and gastrointestinal disturbances are noticed in pyrethroid exposed animals¹¹. The AChE activity was significantly decreased in all the regions of the brain while a steady increase in the Ach levels were observed in different regions of the brain in permethrin exposed mice. Mason¹² reported that there is a significant decrease in the AChE activity in the cholinergic neurons and this is due to the change in the lipid environment. Organophosphorous compounds affect 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 activities¹³. Valenzuela¹⁴ reported that the motor function and performance is badly affected under Organophosphorous stress. Mdegela¹⁵ 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 toxicity studies. 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 permethrin exposed mice and the animals exposed for longer duration showed maximum decrement. Fall in the AChE activity leads to a series of neurological disorders. Degeneration of long axons in the spinal cord and peripheral nerves

are noticed in pesticide exposed animals¹⁶. Kakani¹⁷ reported that small deletion in the olive fly acetylcholinesterase gene is associated with high levels of organophosphate resistance. Mechanism underlying the myasthenia syndrome in Dimethoate poisoning in rats was studied by Yang¹⁸. 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. Dichlorvos enhances long-

term potentiation through a postsynaptic mechanism that involves the inhibition of enzyme acylpeptide hydrolase and the modulation of alpha nicotinic receptors¹⁹. Siraj Mohiyuddin²⁰ reported a decline in the AChE activity in different regions of the brain in Acephate exposed Albino rats. Use of permethrin-containing products as pediculicides and scabicides may result in considerably higher levels of human permethrin exposure especially in children²¹. Accurately determining the use of permethrin will therefore be an important step for estimating risk to human health as the present data shows that permethrin intoxication enhances ACh levels in all the regions of the brain and significantly inhibits AChE.

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