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AN OBSERVATIONAL STUDY ON NEUROBEHAVIOURAL EFFECTS OF ACUTE OXYDEMETON-METHYL (INSECTICIDE AND ACARICIDE) EXPOSURE IN RATS

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Abstract : Oxydemeton-methyl, an organophosphate insecticide and acaricide produced decrease in the exploratory behaviour and prolongation of barbitone sodium-induced hypnosis after intermittent aerosol spray inhalational exposure for 1 h in rats compared to the saline control group. Further, CD50± S.E.M. value for pentylenetetrazole (PTZ) and CI50±S.E.M. value for electroshock (i.e. the dose of PTZ and intensity of electroshock producing positive seizure response in 50% of rats) were significantly decreased by acute exposure to oxydemeton-methyl compared to that of saline control group. The study has established the central nervous system depressant effect and proconvulsant potential of oxydemeton-methyl which is widely used by the agricultural workers in the form of field spray.

Key words : Oxydemeton-methyl electroshock seizure
pentylenetetrazole (PTZ) rats

INTRODUCTION

Oxydemeton-methyl is a systemic insecticide and acaricide for sucking pests. It belongs to organophosphate group of compounds. Organophosphate compounds produce characteristic pharmacological and toxicological effects through irreversible inhibition of acetylcholinesterase enzyme at sites of cholinergic transmission in the body (1, 2).

No published document is available on the effects of acute exposure to oxydemeton-methyl on neurobehavioural parameters in mammals. It is imperative that such compounds be screened through a battery of neurobehavioural models of experimentation in animals for the purpose of their safety evaluation. Hence, the present study was designed to ascertain the effects of acute exposure to oxydemeton-methyl on certain selected experimental models namely, exploratory behaviour, barbitone sodium induced hypnosis, electroshock induced seizures and pentylenetetrazole (PTZ) induced convulsions in rats.

METHODS

Animals :

Experiments were performed on adult (either gender) Sprague-Dawley rats, 100-

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150 g. The animals were kept in colony cages at an ambient temperature of 25±1°C, a relative humidity of 60±2%, and were exposed to a 12-h light-dark cycle. They had free access to food (rat chow and bengal grams) and water. The experimental groups consisted of 10 rats. Concurrent saline control groups were maintained for each of the studied parameters.

Drugs and chemicals

Pentylenetetrazole (Sigma, M.O.,U.S.A.) Barbitone sodium (Fluka Chemie AG, Switzerland); Oxydemeton-methyl (Bayer, India Limited).

Exposure to oxydemeton-methyl

Rats were sprayed for 1 h with 1% solution of oxydemeton-methyl in normal saline at a pressure of 80-100 mm of Hg. An intermittent regimen of aerosol spray was adopted with spraying time 2 min followed by nonspraying time for 1 min. Aerosol spray was undertaken with the help of a histamine chamber (Swastika Bioremedies Limited, Ambala) with attached atomiser and a manometer. Rats were rested for 1 h following oxydemeton-methyl spray in order to allow for the systemic effects of oxydemeton-methyl to attain its peak. Subsequently, the rats were subjected to further experimental procedures.

Hole board exploratory behaviour

The board made of plywood has the size (60 cm x 60 cm, 3 mm thick). The matt finishing of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 10 uniformly distributed holes (each of 5 cm in diameter). Each rat was acclimatised for 4 minutes and then the number of holes explored through head plunging acts during the total observation time period of 1 min (recorded with stop watch) were counted. Care was taken to avoid multiple events i.e. 2 or more head plungings in quick succession. A 'fresh exploration' was considered when the animal had neatly plunged its head once and did something else in between like grooming, taking a short walk etc. before plungings its head for the next time. One animal at a time was tested (3).

Barbitone sodium-induced hypnosis

In this study, both the saline control group and oxydemeton-methyl exposed group of rats were administered with graded intraperitoneal (i.p.) doses of barbitone sodium ranging from 150 mg. kg^{-1} to 250 mg kg^{-1}(150, 175, 200 and 250 mg kg^{-1}). Sleeping time for each animal was recorded from the loss of righting reflex to the gain of righting reflex (4).

Graded electroshock induced seizure

In this study, both the saline control group and oxydemeton-methyl exposed group of rats were subjected to graded intensity of electroshock ranging from 30 mA to 150 mA (i.e. 30, 48, 60, 66, 72, 96, 105, 120 and 150 mA) applied for a period of 0.2s with the help of a pair of corneal electrodes. An electroconvulsiometer (Swastika Bioremedies Limited, Ambala) was used for this study. Rats developing tonic hind-limb extension to electroshock application were considered as positive responders (5).

Graded pentylenetetrazole (PTZ) induced seizure

In this study, both the saline control group and oxydemeton-methyl exposed group of rats were administered with graded intraperitoneal (i.p.) doses of PTZ ranging from 30 mg/kg^{-1} 125 mg/kg^{-1} (i.e. 30, 40, 50, 60, 75, 100 and 125 mg/kg^{-1}). Response to PTZ administration was scored as: 0-no response, 1-ear and facial twitchings, 2-one to twenty myoclonic jerks in 10 min, 3-more than 20 body jerks in 10 min, 4-clonic forelimb convulsions, 5-generalised clonic convulsions with rearing and falling down episodes, 6-generalised convulsions with tonic extension episode and status epilepticus. Each PTZ treated rat was placed within perspex chamber and observed for 1 h for recording of the seizure score. A score of equal to or greater than 3 was taken as positive (6).

Data presentation and statistical analysis

Data were expressed in terms of mean± S.E.M. Data for positive responders and lethality were compared by Fisher's exact probability test. Kruskal-Wallis analysis of ranks was applied for the seizure score data in the PTZ model of seizures. Pentylenetetrazole CD50 values (i.e. the dose of PTZ causing positive seizure response in 50% of rats) and electroshock CI50 values (i.e. the electroshock intensity causing positive seizure response in 50% of rats) were determined by the graphic method. Log-dose probit lines were obtained by least squares regression analysis. The standard error (S.E.M.) of CD50 and CI50 values were calculated using the formula (Log CD84 - Log CD16)/2N$^{-1/2}$ for the S.E.M. of CD50 and (Log CI84 - log CI16)/2N$^{-1/2}$ for the S.E.M. of CI50, where N is the total number of animals in the groups which from the best fitting line would be expected to show effects (seizures) between the probits 3.5 and 6.5. The Log values were obtained from the line on the graph corresponding to probits 6 and 4 (7,8). CI50± S.E.M., CD50 ± S.E.M. and the mean ± S.E.M. values for barbitone sodium sleeping time and exploratory behaviour were compared by Student's t-test. P values less than 0.05 were considered statistically significant.

RESULTS

Table I shows the effect of acute exposure to oxydemeton-methyl on the exploratory behaviour in rats. The mean ± S.E.M. exploratory values were 23.4±1.51 and 19.3± 1.66 respectively for the saline and oxydemeton-methyl exposed group of rats. However, the decrease in the exploratory value in the oxydemeton-methyl group was not statistically significant compared to the saline control group.

TABLE I: Effect of acute exposure to oxydemeton methyl on exploratory behaviour in rats.

Treatment groups	*Exploratory value (Mean ± S.E.M.)*	*P value*
Saline Control	23.4±1.51	N.S.
Oxydemeton-methyl	19.3±1.66	–

Exploratory value refers to number of holes explored in 4 minutes by rats.
N.S. = Not statistically significant.

Table II shows the effect of exposure to oxydemeton-methyl on barbitone sodium induced hypnosis in rats. The sleeping time to barbitone sodium was significantly increased (P<0.05) at doses of 150, 175, 200

TABLE II: Effect of acute exposure to oxydemeton-methyl on barbitone sodium-induced hypnosis and lethality in rats.

Treatment groups	*Dose of Barbitone Sodium (mg/kg^{-1}, i.p.)*	*Sleeping Time (Mean±S.E.M.) (min)*	*Loss of righting reflex/total*	*Death/ Total*
Saline Control	150	108.7±10.23	8/10	0/10
	175	136.2±12.13	9/10	0/10
	200	139.4±15.54	9/10	0/10
	250	235.5±20.00	9/10	0/10
Oxydemeton-methyl	150	117.7±9.36	8/10	0/10
	175	198.4±14.72*	9/10	0/10
	200	211.8±18.67*	10/10	0/10
	250	340.0±22.46*	10/10	3/10*

*Significant difference compared to the correseponding dose of babritone sodium in the saline control group at P<0.05.

Sleeping time was recorded from loss of righting reflex to the gain of righting reflex.

TABLE III: Effect of exposure to oxydemeton-methyl on electroshock induced seizure and lethality in rats.

Treatment groups	*Intensity of Electroshock (mA)*	*Positive response/ Total*	*CI50 ± S.E.M. (mA)*	*Death/ Total*
Saline Control	30	0/10		0/10
	48	0/10		0/10
	60	2/10		0/10
	66	3/10		0/10
	72	4/10	79.43±1.09	0/10
	96	6/10	(50)	0/10
	105	7/10		0/10
	120	8/10		0/10
	150	10/10		0/10
Single Exposure	30	0/10		0/10
(Oxydemeton-methyl)	48	3/10*		0/10
	60	5/10*		0/10
	72	6/10	58.21±1.10*	0/10
	96	8/10	(40)	0/10
	105	10/10		2/10

*Indicate significant difference compared to the saline control group at P<0.005.
Figures in parenthesis indicate the number of rats considered for the determination of S.E.M. value of CI50.

Positive response refers to animals showing tonic hind limb extension to electroshock. CI50±S.E.M. indicates the electroshock intensity that elicits positive response in 50% of rats ± standard error of mean.

and 250 mg/kg^{-1}, i.p. in the oxydemeton-methyl exposed group compared to the saline control group of rats. However, there was no significant difference in the number of rats showing loss of righting reflex between the two groups. Three rats succumbed to death at 250 mg. kg^{-1}, i.p. dose of barbitone-sodium in the oxydemeton-methyl exposed group while none had died at any other dose level in either group of rats. There was profound respiratory depression and cyanosis in the rats which succumbed to death. These three rats were excluded from the calculation of sleeping time.

Table III shows the effects of electroshock application to saline and oxydemeton-methyl exposed groups of rats. Number of rats showing positive response (i.e. tonic hind limb extension) to electroshock application was significantly more in the oxydemeton-methyl exposed group compared to the saline control group at 48 and 60 mA current intensities while it was insignificant at other electroshock intensity levels. The CI50 ± S.E.M. value was significantly less at 58.21 ±1.10mA in the oxydemeton-methyl exposed group compared to 79.43 ± 1.09 mA in the saline control group. Two out of ten rats died after severe convulsions at 105 mA current intensity in the oxydemeton-methyl exposed group while there was no other lenthality in any of the two groups at other intensity levels of electroshock application.

Pentylenetetrazole produced graded seizure score response in both saline and oxydemeton-methyl exposed groups of rats. Seizure score was significantly higher in the oxydemeton-methyl exposed group compared to the saline treated group of rats at 30, 40, and 60 mg/kg^{-1}, i.p. dose levels while it was insignificant at 75 and 100 mg/kg^{-1} i.p. doses. Number of rats showing positive seizure

TABLE IV: Effect of acute exposure to oxydemeton-methyl on pentyleneterazole (PTZ) induced seizure and lethality in rats.

Treatment groups	*Dose of PTZ (mg/kg^{-1}, i.p.)*	*Seizure score (mean ± S.E.M.)*	*+VE response/ Total*	*CD 50 ± S.E.M. (mg/kg^{-1}, i.p.)*	*Death/ Total*
Saline Control	30	1.0±0.33	1/10		0/10
	40	1.9±0.32	2/10		0/10
	50	2.5±0.27	3/10		0/10
	60	3.5±0.52	5/10	57.54±1.10	1/10
	75	4.7±0.50	8/10	(40)	3/10
	100	5.8±0.13	10/10		8/10
	125	6.0±0.00	10/10		10/10
Single Exposure	30	2.2±0.45*	4/10*		0/10
(Oxydemeton-methyl)	40	4.0±0.36*	8/10*	33.11±1.15*	0/10
	60	5.2±0.13*	10/10*	(30)2/10	
	75	5.6±0.16	10/10		6/10*
	100	6.0±0.00	10/10		9/10

*Indicates significant difference compared to the saline control group at P<0.05.
Figures in parenthesis indicate the number of rats considered for the determination of S.E.M. of CD50.
+ve response refers to seizure score ≥3 (Scale:0-6) (See text)
CD50 ± S.E.M. indicate the dose of PTZ that elicits positive seizure response in 50% of ± standard error of mean.

score response was significantly higher in the oxydemeton-methyl exposed group compared to the saline treated group at 30, 40 and 60 mg/kg^{-1}, i.p. doses of PTZ. While all the rats showed positive seizure score at 60 mg/kg^{-1}, i.p. dose of PTZ in oxydemeton-methyl exposed group, it was 100 mg/kg^{-1}, i.p dose of PTZ in the saline control group which produced positive seizure score in 100% of the animals. CD50±S.E.M. value was significantly less in oxydemeton-methyl exposed group at 33.11±1.15 mg/kg^{-1}, compared to 57.54±1.10 mg/kg^{-1} in the saline control group. Lethality to PTZ administration was significantly more in oxydemeton-methyl exposed group at 75 mg/kg^{-1}, i.p. dose of PTZ compared to that in the saline control group at similar dose level.

DISCUSSION

Oxydemeton-methyl, an insecticide and acaricide belongs to organophosphate group of compounds. Organophosphates are known to cause irreversible inhibition of true and pseudocholinesterase both in the central and peripheral nervous system (1). In the present study, the method of exposure of rats to oxydemeton-methyl was done by regulated spraying as is the pratice amongst the agricultural and horticultural workers in the field situation. Acute exposure to oxydemeton-methyl resulted in piloerection, frequent urination and defaecation, fasciculation of skeletal muscles, excessive sweating, hyperventilation, decreased locomotor activity and dozing in the exposed group of rats. The peripheral and central features to oxydemeton-methyl exposure in the rats became noticeable within 30 minutes after beginning the spray and attained its peak by 1 h after stopping the spray and lasted for several hours thereafter. Barbitone sodium induced hynopsis in rats was found to be significantly potentiated by oxydemeton-methyl. Sedative barbiturates are reported to cause area specific (9) as well as whole brain content of acetylcholine (10) to increase. Further, the resting and stimulated release of acetylcholine from nervous is also reported to be augmented by barbiturates (11). Oxydemeton-methyl, being an anticholinesterase agent, would certainly facilitate the augmented cholinergic neurotransmission caused by barbiturates through its ability to prevent degradation of acetylcholine in the synaptic cleft after its release from presynaptic neurons. Thus, excess and prolonged availability of acetycholine might contribute to the potentiated barbitone exposed group of rats, since brain cholinergic system has been implicated in the anaesthetic action of barbiturates (12).

A general bahavioural depression was observed in the rats exposed to oxydemeton-methyl. The rats exhibited decreased locomotion, dozing and decreased exploratory behaviour. Similar behavioural depression with organophosphates was also reported earlier (13). Behavioural depression with organophosphate was reported to be well correlated to decreased brain cholinestrase activity and increase in brain acetylcholine levels (11). However, it was paradoxical to note that oxydemeton-methyl exposure resulted in heightened seizure susceptibility to both PTZ and electroshock induced seizures in rats despite general central nervous system depression. Data for CD50±S.E.M. to PTZ and CI50±S.E.M. to electroshock were significantly reduced by acute exposure to oxydemeton-methyl.

Enhanced seizure susceptibility to oxydemeton-methyl exposure might, again, be related to augmented cholinergic neurotransmission in the brain. Both neostigmine bromide, a reversible anticholinestrase and pilocarpine, a muscarinic cholinomimetic alkaloid reduced the seizure threshold to PTZ in rats (15). The facilitated convulsions with organophosphate insecticide, chlorpyrifos was attributed to increased acetylcholine levels in the brain (16).

Thus it was evident from the present study that oxydemeton-methyl, an irreversible anticholinesterase agent, caused general behavioural depression concomitant to enhanced seizure susceptibility through augmented central cholinergic neurotransmission after administration. The study bears considerable clinical relevance for such agriculture or horticultural workers who are prone to seizure disorders since they might get breakthrough seizure attacks on exposure to this insecticide.

REFERENCES

1. Aldridge WN. Survey of major points of interest about reactions of cholinerterases. *Croat Chem Acta* 1976; 47 : 225–233.
2. Albuquerque EY, Aracava Y, Idris M, Schonenberger B, Brassi A, Deshpande SS. Activation and blockade of the nicotinic and glutaminergic synopses by reversible and irreversible cholinesterase inhibitors. In: Neurobiology of acetycholine (Dun NJ, Pestman RL eds.) Plenum Press, New York, 1987; 301–328.
3. Boissier JR, Simon P. Dissociation de deux compasantes dansle compartments D-investigation delat eaixis. *Int Pharmacodyn Ther* 1961; 147 : 372.
4. Kuhn WL, Van Maanen EF. *J Pharmacol Exp Ther* 1961; 134 : 60–64.
5. Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. *J Pharmacol Exp Ther* 1952; 106 : 319–330.
6. Giorgi O, Orland M, Lecca D, Corda MG. MK-801 prevents chemical kindling induced by pentylenetetrazole in rats. *Eur J Pharmacol* 1991; 193 : 363–365.
7. Ghosh MN. Statistical analysis. In: Fundamentals of Experimental Pharmocology (Ghosh MN ed.) 2nd Edition. Scientific Book Agency; 1984; 177–190.
8. Chakrabarti A, Saini HK, Garg SK. A comparative study of aminophylline and acepifylline-induced seizures and death in the chemoconvulsion model in rats. *J Pharm Pharmacol* 1997; 49 : 812–815.
9. Trabucchi M, Cheney DL, Racagni G, Costa E. Pentobarbital and *in vivo* turner rate of acetylcholine in mouse brain and in regions of rat brain. *Pharmacol Res Commun* 1975; 7 : 81–94.
10. Consolo S, Ladinsky H, Peri G, Garattini S. Effects of central stimulants and depressions on mouse brain acetylcholine and choline levels. *Eur J Pharmacol* 1972; 19 : 226–234.
11. Richter JA, Waller MB. Effects on pentobarbital on regulation of acetylcholine content and release in different regions of rat brain. *Biochem Pharmocol* 1977; 26 : 609–615.
12. Cheney DL, Costa E. Pharmacological implications of brain acetylcholine turnover measurement in rat nuclei. *Ann Rev Pharmacol Toxicol* 1977; 17 : 369–386.
13. Feldberg W, Fleischhauer K. A new experimental appraoch to the physiology and pharmacology of the brain. *Br Med Bull* 1956; 21 : 36–43.
14. Bignami G, Rosic N, Michalek H, Milosevic M, Gatti GL. Behavioural toxicity of anticholinesterase agents: Methodological, Neurochemical and neuropsychological aspects. In: Behavioural Pharmacology. (Weiss B and Laties VG eds.) Plenum Press, New York, 1975; 155–215.
15. Miller JW, Gray BC, Bardgett ME. Effect of selective toxic lesions of cholinergic nervous of the laterodorsal tegmental nucleus on experimental seizures. *Brain Res* 1992; 579 (1): 161–164.
16. Wurpel JND, Hirt PC, Bidanest JH. Amygdala kindling in immature rats: Proconvulsant effect of the organophosphate insecticide chlorpyrifos. *Neurotoxicol* 1993; 14 : 429–436.