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NEUROBEHAVIOURAL STUDY OF SUBCHRONIC ADMINISTRATION OF OXYDEMETON-METHYL (INSECTICIDE AND ACARICIDE) 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 in rats after intermittent aerosol spray inhalational exposure, for ½ hour daily for 7 consecutive days, compared to the saline control group. Further, ED50±SEM value for haloperidol induced catalepsy, CD50±SEM value for pentylenetetrazole induced seizure and CI50 ± SEM value for electroshock (i.e. the dose of haloperidol, PTZ and intensity of electroshock producing cátalepsy or positive seizure response in 50% of rats) were significantly decreased after 7 days exposure to oxydemeton-methyl compared to that of saline control group. The study has established the central nervous system depressant effect, extrapyramidal effect and proconvulsant potential of oxydemeton-methyl which is widely used by the agricultural workers in the form of field spray.

Key words : rats hypnosis catalepsy seizure oxydemeton-methyl

INTRODUCTION

Oxydemton-methyl, a systemic insecticide and acaricide for sucking pests belongs to organophosphate group of compounds.

Effects of single inhalational exposure to oxydemeton-methyl on neurobehavioural parameters in rats suggest its neurotoxicological potential (1). For the purpose of their safety evaluation after subchronic exposure the present study was designed to ascertain the effects of 7 days exposure to oxydemeton-methyl on selected experimental models namely, (1) exploratory behaviour, (2) barbitone sodium induced hypnosis, (3) haloperidol induced catalepsy, (4) maximal electroshock and (5) pentylenetetrazole (PTZ) induced convulsions in rats.

METHODS

Animals

Experiments were performed on adult (either sex) Sprague-Dawley rats, 100-150 g. The animals were housed in standard laboratory conditions under natural light dark cycle. They had free access to food and

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water. Each experimental group consisted of 10 rats.

Drugs and Chemicals

Haloperidol (Searle, India Ltd.) : Pentylenetetrazole (Sigma, M. O., U.S.A.); Barbitone sodium (Fluka Chemie AG, Switzerland); Oxydemeton-methyl (Bayer, India Limited) were used in the study.

Exposure to oxydemeton-methyl

Rats were sprayed for half an hour daily (between 9-9.30 AM) for 7 consecutive days with 1% solution of oxydemeton methyl in normal saline at a pressure of 80–100 mmHg. An intermittent regimen of aerosol spray was adopted with spraying time for 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. On the 8th day, the rats were subjected to further experimental procedures.

Hole board exploratory behaviour

The board made of plywood has the size (60 cm × 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 4 min (recorded with stop watch) were counted. Care was taken to avoid multiple events i.e. 2 or more head plunging 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 plunging its head for the next time. One animal at a time was tested (2).

Barbitone sodium-induced hypnosis

Both the saline control group and oxydemeton-methyl exposed group of rats were administered with graded doses (150, 175, 200 and 250 mg.kg^{-1}, i. p.) of barbitone sodium. Sleeping time for each animal was recorded from the loss of righting reflex, to the regain of righting reflex (3).

Haloperidol-induced catalepsy

Haloperidol was injected in graded doses i.e. 125, 250, 500, and 1000 µg/kg^{-1}, i.p. to different groups of rats. Catalepsy was graded as below.

Stage I: Animals maintain a quiet sitting posture without any attempt to move unless they were pushed gently.

Stage II: Rats sit quietly and did not move even when pushed gently.

Stage III: Forepaws of the animal were gently raised to a retort ring of the catalepsy stand. The ring was placed at a height of 5 cm from the base while hind limbs of the rats touched the base, rats maintain the posture for 30 s or more.

Stage IV: One of the forepaws of rat is removed from the retort ring and kept hanging in air while the other forelimb is still placed on the retort ring. Rat maintains abnormal posture for 30 s or more.

Stage IV rats were gently lifted by the tail and placed on a ring (2 inch diameter) at a height of 40 cm from the base of the catalepsy stand and observed for the duration of immobility. Any movement like snouting or whiskering, other than quiet respiratory excursions and typical sagging movements, marked the end point of immobility. Two stop watches having been matched and synchronized were run simultaneously. One recorded the total study period of 5 min while the other recorded duration of immobility to the nearest second possible (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 (30, 36, 42, 48, 60, 72, 96, 120 and 150 mA) applied for a period of 0.2 s 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 were taken as positive responders (5).

Graded pentylenetetrazole (PTZ) induced seizure

Both the saline control group and oxydemeton-methyl exposed group of rats were administered with graded intraperitioneal (ip) doses of PTZ (i.e. 30, 40, 50, 60, 75, 100 and 125 mg/kg^{-1}). Response to PTZ administration was scored as : O-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 a period of 1 hour for recording of the seizure score. A score of equal to or greater than 3 was taken as positive (6).

Statistical analysis

Data were expressed as mean ± SEM. Data for positive responders and lethality compared by Fisher's exact probability test. Kruskal-Wallis analysis of ranks was applied for the seizure score data in the PTZ model of seizure. Further haloperidol ED50 ± SEM values Pentylenetetrazole CD50 ± SEM values were determined by the graphic method. Log-dose probit lines were obtained by least squares regression analysis. The standard error (SEM) of ED50, CD50 and CI50 values were calculated using the formula (Log ED84 – Log ED16)/ $2N^{-1/2}$ for the SEM of ED50, (Log CD84 – Log CD16)/$2N^{-1/2}$ for the SEM of CD50 and (Log CI 84- CI 16)/$2N^{-1/2}$ for the SEM 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 (Catalepsy or seizure) 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 ± SEM and the mean ± SEM 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

Data of saline control have been taken from our previous study (1).

Table I shows the effect of 7 days exposure to oxydemeton-methyl on the cxploratory behaviour in rats. The mean ± SEM exploratory values were 23.4 ± 1.51 and 14.3 ± 1.92 respectively for the saline and oxydemeton-methyl exposed group of rats. The exploratory value in the oxydemeton-methyl exposed group was significantly less compared to that of the saline control group.

TABLE I : Effect of 7 days exposure to oxydemeton-methyl on exploratory behaviour in rats.

Treatment groups	*Exploratory value (mean± SEM)*
Saline control	23.4±1.51
7 days exposure (oxydemeton-methyl)	14.3±1.92*

*P<0.05.

Table II shows the effect of 7 days 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 175 and 200 mg/kg^{-1}, ip in the oxydemeton-methyl exposed group compared to the saline control group of rats. Two rats succumbed to death at 250 mg.kg^{-1}, ip 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 both these rats. These two rats were excluded from the calculation of sleeping time.

TABLE II : Effect of 7 days exposure to oxydemeton-methyl on barbitone sodium-induced hyphosis in rats.

Treatment groups	*Dose of barbitone sodium (mg/kg^{-1}, ip)*	*Sleeping time (Mean ± SEM) (min)*
Saline control	150	112.7±10.29
	175	132.2±16.13
	200	146.4±15.94
	250	245.5±22.09
7 Days exposure (oxydemeton-methyl)	150	146.25±16.21
	175	188.25±17.47*
	200	207.60±17.95*
	250	284.40±19.21

*P<0.05.

Table III shows the effect of exposure to oxydemeton-methyl on haloperidol induced catalepsy in rats. The percent catalepsy was significantly increased at doses of 125, 250 and 500 µg.kg^{-1}, ip in the oxydemeton-methyl exposed groups compared to the saline control group of rats. With further higher doses, there was no further increase. The ED50 ± SEM was significantly less at 89.12 ± 1.60 µg.kg^{-1}, ip in the oxydemeton-methyl exposed group compared to 158.83 ± 1.55 ug/kg^{-1}, ip in the saline control group.

Table IV shows the effect 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 groups compared to the saline control group at 48, 60 and 72 mA current intensities while it was nonsignificantly more at other electroshock intensity levels. The CI50 ± SEM value was significantly less at 43.15 ± 1.08 mA in the oxydemeton-methyl exposed group compared to 79.43 ±1.09 mA in the saline control group. Four out of ten rats died after severe convulsions at 72 mA current intensity in the oxydemeton methyl exposed group while there was no other lethality 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 50 mg.kg^{-1}, ip dose levels while it was non-significantly more at 60 and 75 mg.kg^{-1}, i.p. doses. Numbers of rats showing positive seizure score response was

TABLE III: Effect of 7 days exposure to oxydemeton-methyl on haloperidol-induced catalepsy in rats.

Treatment group	*Dose of haloperidol (μg/kg^{-1}, ip)*	*% Catalepsy (Mean ± SEM)*	*ED50± SEM (μ/kg^{-1}, ip)*
Saline control	125	34.00±0.82	158.83±1.55 (50)
	250	66.18±2.16	
	500	84.85±1.48	
	1000	94.48±1.09	
	2000	98.47±0.51	
7 Days exposure (Oxydemeton-methyl)	125	42.63±2.31*	89.12±1.60* (30)
	250	83.91±1.49*	
	500	91.93±2.13*	
	1000	97.86±1.30	
	2000	98.73±1.34	

*P<0.05.
Figures in parenthesis indicate the number of rats considered for the determination of SEM value of ED50.

TABLE IV : Effect of 7 days exposure to oxydemeton-methyl on electroshock induced seizure in rats.

Treatment group	*Intensity of electroshock (mA)*	*Positive response/total*	*CI50± SEM (mA)*
Saline control	30	0/10	79.43±1.09 (50)
	48	0/10	
	60	2/10	
	72	4/10	
	96	6/10	
	120	8/10	
	150	10/10	
7 Days exposure (Oxydemeton-methyl)	30	0/10	43.15±1.08* (30)
	36	3/10	
	42	5/10	
	48	7/10*	
	60	9/10*	
	72	10/10*	

*P<0.05.
Figures in parenthesis indicate the number of rats considered for the determination of SEM value of CI50.

TABLE V: Effect of 7 days exposure to oxydemeton-methyl on pentylenetetrazole (PTZ) induced seizure in rats.

Treatment groups	*Dose of PTZ (mg.kg^{-1}, ip)*	*Seizure score (mean ± SEM)*	*Positive response / total*	*CD50± SEM (mg.kg^{-1}, ip)*
Saline control	30	1.0±0.33	1/10	57.54±1.10 (40)
	40	1.9±0.32	2/10	
	50	2.5±0.27	3/10	
	60	3.5±0.52	5/10	
	75	4.7±0.50	8/10	
	100	5.8±0.13	10/10	
	125	6.0±0.00	10/10	
7 Days exposure (oxydemeton-methyl)	30	2.0±0.29*	2/10*	38.01±1.08* (30)
	40	2.8±0.32*	4/10*	
	50	3.6±0.30	8/10*	
	60	4.6±0.34*	10/10*	
	75	5.4±0.33	10/10	
	100	6.0±0.00	10/10	

*P<0.05.

Figures in parenthesis indicate the number of rats considered for the determination of SEM value of CD50.

significantly higher in the oxydemeton-methyl exposed group compared to the saline treated group at 30, 40, 50, and 60 mg.kg^{-1}, ip doses while all the rats showed positive seizure score at 60 mg.kg^{-1}, ip dose of PTZ in oxydemeton-methyl exposed positive group, it was 100 mg.kg^{-1}, ip dose of PTZ in the saline control group which produced positive seizure score in 100% of the animals. CD50 ± SEM value was significantly less in oxydemeton-methyl exposed group at 38.01±1.08 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 60 and 75 mg.kg^{-1}, ip dose of PTZ compared to that in the saline control group at similar dose level (Table V).

DISCUSSION

Oxydemeton-methyl, an insecticide and acaricide belongs to organophosphate group of compounds. In the present study, the method of exposure of rats to oxydemeton-methyl was done by regulated spraying as is the practice amongst the agricultural and horticultural workers in the apple orchards in Himachal Pradesh. 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 neurons 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 acetylcholine might contribute to the potentiated barbitone sodium induced hypnosis in the oxydemeton-methyl exposed group of rats, since brain cholinergic system has been implicated in the anaesthetic action of barbiturates (12).

A general behavioural depression was observed in the rats exposed oxydemeton-methyl. The rats exhibited decreased locomotion, dozing and decreased exploratory behaviour. Similar behavioural depression with organophosphate 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 (14). Rats exposed to oxydemeton-methyl demonstrated heightened haloperidol induced catalepsy compared to the saline control group of rats. The synergistic interaction was because of the fact that while haloperidol produced catalepsy through the blockade of D2 receptors in the striatum, oxydemeton-methyl aggravated catalepsy through increased acetylcholine levels in the synaptic cleft of the striatal neurons by inhibiting cholinestease enzymes. It was reported earlier that monocrotophos an organophosphate compound potentiated the catalepsy induced by haloperidol (15). 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 CD±SEM to PTZ and CI50± SEM to electroshock were significantly reduced by subchronic exposure to oxydemeton-methyl. Enhanced seizure susceptibility to oxydemeton-methyl exposure might, again be related to augmented cholinergic neurotransmissin in the brain. Both neostigmine bromide, a reversible anticholinestrase and pilocarpine, a muscarinic cholinomimetic alkaloid reduced the seizure threshold to PTZ in rats (16). The facilitated convulsions with organophosphate .insecticide, chlorphyrifos was attributed to increased acetylcholine levels in the brain (17).

Thus it is evident from the study that oxydemeton-methyl, caused general behavioural depression concomitant to enhanced seizure susceptibility through augmented central cholinergic neurotransmission after 7 days of exposure. The study bears relevance for such agricultural or horticultural workers who are susceptible to seizure disorders since they might get breakthrough seizure attacks on exposure to this insecticide.

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