PHYTOTHERAPY RESEARCH, VOL. 10, 402–405 (1996)

Studies on Some Psychopharmacological Actions of *Moringa oleifera* Lam. (Moringaceae) Leaf Extract

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The methanol extract of the leaves of *Moringa oleifera* Lam. was investigated for some psychopharmacological actions in animals. The extract was found to produce a significant alteration in general behavioural pattern by head dip test, Y-maze test, evasion test, and reduction in muscle relaxant activity by rotarod test, chimney test and traction test. Beside these, the extract also potentiated pentobarbitone induced sleeping time and lowered body temperature in experimental animals.

Keywords: *Moringa oleifera*; general behaviour; muscle relaxant activity; pentobarbitone sleeping time; body temperature; MOE.

INTRODUCTION

Moringa oleifera Lam. (Family-Moringaceae) is a widespread shrub in India. In the traditional medicinal system, almost all parts of this plant have been used in the treatment of various ailments. Fried leaves of this plant are useful in cold and fever (Biswas and Ghosh, 1950). The leaves are nutritive and cooling, and are useful in removing all kinds of excessive pain (Kirtikar and Basu, 1935). The aqueous extract of the leaves has been found to possess antifertility activity (Shukla *et al.*, 1981; Prakash, 1988). The juice of the leaves is used in cases of headache and it is also applied in the eyes in fainting fits due to nervous debility (Nadkarni *et al.*, 1954). Moreover, the local people of the Gangetic plain of West Bangal, India used this leaf juice as a cooling agent and refrigerant and also in anxiety. To substantiate these claims the present study was undertaken to evaluate various psychopharmacological actions of the leaf extract on different experimental models in animals.

MATERIALS AND METHODS

Plant material. Leaves of *Moringa oleifera* Lam. were collected from Bankura district of West Bangal, India and were identified by the Botanical Survey of India, Shibpur, Howrah. A voucher specimen herbarium (M-23) is kept in our laboratory for future reference.

Preparation of leaf-extract. Dried powdered leaves were defatted with petroleum ether (60°–80°C). The marc was then extracted with methanol. The solvent was evaporated *in vacuo* until constant weight was reached (yield was 8% w/w with respect to dry starting materials). Prior to use the extract (MOE) was dissolved in physiological saline solution.

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Experimental animals. Swiss albino mice (18–22 g) and Charles Forster rats (120–180 g) were used for experiments. The animals were housed in standard metal cages and provided with food and water *ad libitum*.

Effects on pentobarbitone sleeping time. This experiment was performed following the method of Dandiya and Cullumbine (1959). Mice were divided into groups of ten each. The leaf extract (MOE) at a dose of 100, 200 or 300 mg/kg, i.p. and normal saline (0.2 mL/20 g i.p.) were injected into six separate groups, 30 min after this each animal was injected with sodium pentobarbitone (40 mg/kg i.p.). The sleeping time was noted by recording the interval between the loss and regaining of the righting reflex.

Effects on exploratory behaviour pattern. This was performed by (a) head dip test, (b) Y-maze test.

The head dip test consisted of placing female mice 1 h after injection with MOE (100, 200 or 300 mg/kg i.p.), control vehicle and diazepam (10 mg/kg, i.p.), singly on a wooden board with 16 evenly spaced holes and counting the number of times the head was dipped into the holes during 3 min trials (Dorr *et al.*, 1971).

For the Y-maze test, female rats were placed singly in a symmetrical Y-shaped runway (33 × 38 × 13 cm) for 5 min and the number of times that the rat entered the arm of the maze with all four feet (an entry) was counted (Rushton *et al.*, 1961). Experiments were conducted in groups of six rats, 30 min after administration of MOE (100, 200 or 300 mg/kg, i.p.), control vehicle and diazepam (10 mg/kg, i.p.).

Effect on body temperature. Temperatures were recorded rectally with a thermometer (Apex India, DCT-1002) in groups of male mice (10 in each) at predetermined intervals before and after administration of test material (MOE) or control vehicle for 4 h.

Effects on muscle relaxant activity. This was studied by (a) rotarod test, (b) traction test and (c) chimney test.

For the rotarod test, untreated mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 5 rpm. Animals remaining on the rod for 3 min or more

CCC 0951–418X/96/050402–04

Accepted (revised) 10 August 1995

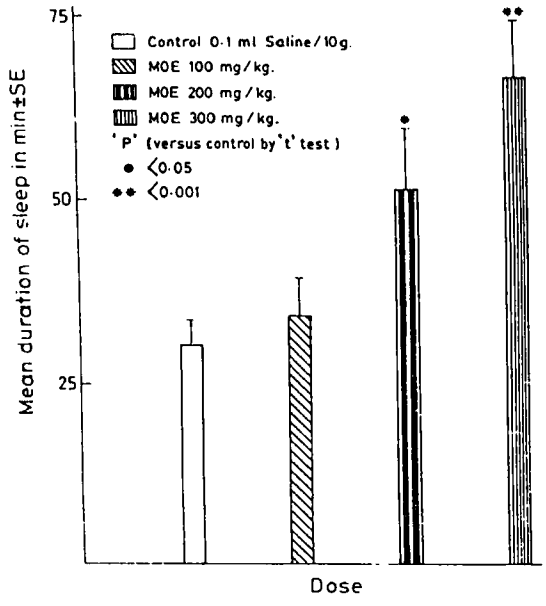

Figure 1. Potentiation of pentobarbital sleep with *M. oleifera* extract (MOE). Mean duration of sleep in min ± SEM from 10 animals in each group.

in two successive trials were selected for the experiment and were placed in groups of 10 animals each. Each group was then injected with control vehicle (saline) or MOE (100, 200 or 300 mg/kg i.p.) and placed on the rod at intervals of 30, 60, 90, 120 and 150 min. If the animals failed more than once to remain on the rotating rod for 3 min then the tests were considered to be positive (Dunham and Miya, 1957).

For the traction test, forepaws of a mouse were placed on a small twisted wire rigidly supported above a bench top. A normal mouse when allowed to hang free, grasped the wire with the forepaws and placed at least one hind feet on the wire within 5 s. Inability to put up at least one hind foot constituted failure to traction. This test was conducted in groups of 10 previously screened animals, 1 h after injection of MOE (100, 200 or 300 mg/kg, i.p.) or control vehicle (saline) (Rudzik *et al.*, 1973).

For the chimney test, a mouse was introduced into the base of a pyrex tube (30 cm long and 28 mm diameter) marked at 20 cm from the base. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb the tube backwards. Only those mice which reached the mark within 30 s were selected for further testing and placed in groups of 10 animals each. 1 h after injection of MOE (100, 200 or 300 mg/kg, i.p.) or control vehicle (saline) animals were tested (Turner, 1965).

Effects on residual curiosity. This was performed by an evasion test in mice. Groups of mice (10 in each) were kept in a rectangular box having an inclined plane and the mice

Table 1. Effect of MOE on exploratory behaviour (head dip test) (values are mean±SEM from 10 animals in each group)

Treatment	Dose	Mean number of entries in 5 min±SEM	*p* value (vs control)
Saline control	0.2 mL/20 g	13.6±1.71	—
MOE	100 mg/kg	8.2±1.54	<0.05
	200 mg/kg	6.1±1.17	<0.01
	300 mg/kg	4.6±1.45	<0.001
Diazepam	10 mg/kg	3.0±1.05	<0.001

'p' by Student's *t*-test.

Table 2. Effect of MOE on exploratory behaviour (Y-maze test) (values are mean ± SEM from 10 animals in each group)

Treatment	Dose	Mean number of head dips in 3 min ±SEM	*p* value (vs saline control)
Saline control	0.2 mL/20 g	33.65±3.41	—
MOE	100 mg/kg	22.25±3.11	<0.02
	200 mg/kg	17.50±2.21	<0.001
	300 mg/kg	11.20±3.25	<0.001
Diazepam	10 mg/kg	6.60±1.60	<0.001

'p' by Student's *t*-test.

remaining in the box after 5 min was noted. Those mice which escaped within 5 min of placing them in the box were selected for further testing. 1 h after administration of MOE (100, 200 or 300 mg/kg, i.p.) and control vehicle (saline) the animals were placed in the box. The number of mice remaining in the box after 5 min in each group was noted (Turner, 1965).

Statistical analysis.. In all the cases data were statistically analysed by Student's *t*-test excepting the studies of the effects on muscle relaxant activity which was performed by chi-square test (Woodson, 1987). Results are expressed as mean ± standard error of mean (SEM). Values of less than 0.05 imply significance.

RESULTS

Pentobarbitone sleeping time

Prior administration of MOE significantly potentiated pentobarbitone induced sleeping time in mice at doses of 200 and 300 mg/kg, i.p. (Fig. 1).

Exploratory behaviour pattern

On head dip test in mice treated with different doses of MOE, a significant reduction in head dip responses was observed compared with the control (Table 1). On the Y-maze test there was a marked decrease in exploratory behaviour of the rats treated with MOE in doses of 100 mg/kg and above, compared with the control (Table 2).

Body temperature

The administration of MOE produced a signficant decrease in normal body temperature in mice in doses of 100 mg/kg and above (Fig. 2).

Muscle relaxant activity

In the rotarod test, MOE in doses of 100 mg/kg and above produced significant motor incoordination of the animals. Significant loss of coordination and tone of muscles were also found to occur with different doses of MOE, as evident from the chimeny test. MOE in doses of 100, 200 and 300 mg/kg produced a failure in traction of 50%, 70% and 50% of animals, respectively in the traction test (Table 3).

Residual curiosity (evasion test)

In the evasion test MOE at doses of 100 mg/kg and above caused a significant inhibition of residual curiosity in mice compared with the control group (Table 4).

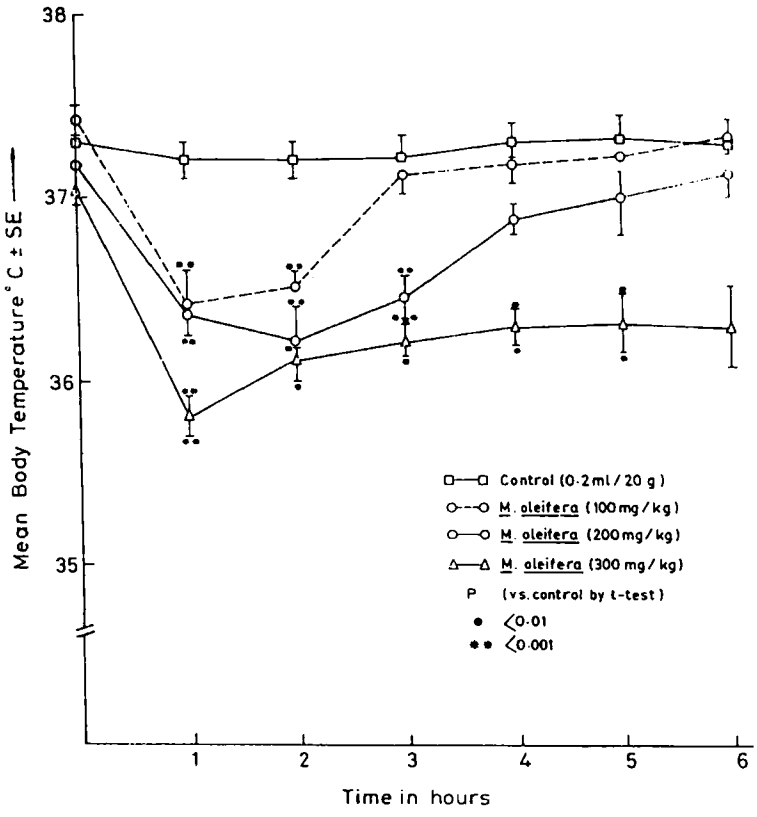

Figure 2. Effect of *M. oleifera* extract (MOE) on body temperature in mice. Mean body temperature (°C) ± SEM from 10 animals in each group.

DISCUSSION

It is evident from the results that the extract (MOE) has central nervous system (CNS) depressant effects as evidenced by its hypnotic potentiating effect in rats in a dose dependent fashion. The extract produced hypothermia in rats which was maximum with dose of 300 mg/kg, after 1 h of its administration. Reserpine and chlorpromazine have been shown to potentiate barbiturate hypnosis by virtue of their hypothermic action (Lessian and Parkes, 1957). So the extract may work by the same mechanism.

Accordingly, the effect of MOE was further investigated on certain other characteristic actions of CNS depressants, e.g. exploratory behaviour pattern and muscle relaxant activity. MOE produced a significant decrease in exploratory behaviour pattern as evidenced from the head-dip and Y-maze test. MOE produced significant results at a dose of 200 mg/kg and above on head dip responses and it caused a significant decrease in Y-maze entry behaviour compared with the saline control. Reduction in exploratory behaviour on treatment with MOE is in conformity with the actions occurring with other CNS depressant drugs (Dorr *et al.*, 1971).

In the tests concerning muslce relaxant activity, i.e. rotarod, traction and chimney tests, MOE was found to produce significant motor incoordination and muscle relaxant activity. In the test concerning residual curiosity, MOE at doses of 200 mg/kg and above caused significant inhibition of residual curiosity in mice as observed in the evasion test. Thus from the current investigation it is obvious that the extract of *M. oleifera* has a potent CNS-depressant action. However, it is difficult at the moment to indicate the precise nature and category of such CNS-depressant action and requires further experimentation.

Table 3. Effect on muscle relaxant activity, (A) rotarod test, (B) chimney test, (C) traction test

Treatment	Dose	Number of mice	Animals showing negative test (%) A	B	C
Saline control	2 mL/20 g	10	0	0	0
MOE	100 mg/kg	10	50[a]	60[a]	50[a]
	200 mg/kg	10	80[b]	70[b]	70[b]
	300 mg/kg	10	70[b]	50[a]	50[a]
Diazepam	10 mg/kg	10	100[b]	100[b]	100[b]

p-value (vs saline control, by chi-square test) [a]<0.05, [b]<0.001.

Table 4. Effects of MOE on residual curiosity in mice (evasion test)

Treatment	Dose	Number of mice	Number of mice remaining in box after 1 h	1.5 h
Saline	0.2 mL/20 g	10	0	0
MOE	100 mg/kg	10	5	6[a]
	200 mg/kg	10	6[a]	8[a]
	300 mg/kg	10	6[a]	9[a]
Diazepam	10 mg/kg	10	10[a]	8[a]

[a]*p* value (versus control, by chi-square test) [a]<0.001.

Acknowledgements

The authors are thankful to the CSIR Authority, New Delhi for providing financial support for the work, and the authorities of Jadavpur University for providing all the facilities. Thanks are also due to Dr D.C.S. Ranju of Botanical Survey of India, Howrah for taxonomic identification of the plant.

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