CNS ACTIVITY OF THE METHANOL EXTRACT OBTAINED FROM THE ROOTS OF *OCIMUM SANCTUM* LINN.

Jayanti Mukherjee*, Uttam Bhaumik, Pulok K. Mukherjee, B.P. Saha

Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700 032, India.

* Corresponding Author's contact id: jayanti_26@rediffmail.com

Summary

The aim of the present work was to investigate several neuropharmacological effects of the methanol extract of *Ocimum sanctum* roots (Family Labiatae) in Wister albino rats and Swiss albino mice. The dried powdered root was extracted with methanol and the solvent was removed under reduced pressure to obtain a dried extract material. With that extract the general behavior, exploratory behavior, muscle relaxant activity and phenobarbitone sodium-induced sleeping time were studied. The results revealed that the crude extract at 50-150 mg/kg per oral (p.o) caused a significant reduction in spontaneous activity (general behavior profile), remarkable decrease in exploratory pattern (Y-maze and head dip tests), and a reduction in muscle relaxant activity (rotarod, 30^0 inclined screen and traction tests) and also significantly potentiated phenobarbitone sodium-induced sleeping time. Hence it can be concluded that the methanolic extract of the roots of *Ocimum sanctum* (in the doses examined) possess most of the pharmacological characteristics of the psychoactive group of drugs like minor tranquilizers.

Keywords: *Ocimum sanctum* roots; Methanol extract; CNS activity; Experimental animals.

Introduction

The drugs which act upon the Central Nervous System (CNS) were among the first to be discovered by primitive human beings and are still the most widely used group of pharmacological agents. In addition to their use in therapy CNS acting drugs are often used without prescription to increase one's sense of well being.

Besides the synthetic drugs acting on the CNS, many plant materials so far screened have been found to possess immense activity on the CNS. For example, Indian valerian (*Valeriana wallichii*) contains 2% Valepotriates [1] and is four times more potent than European valerian (*Valeriana officinalis*) which contains 0.5% Valepotriates [2]**.** These valepotriates have been used as sedatives and tranquilizers.

From the literature survey it has been found that the leaves of *Ocimum sanctum* have been used extensively in various mental illnesses in India. To substantiate this claim the present study was undertaken to evaluate various psychopharmacological effects of methanol extract of *Ocimum sanctum* roots (MEOS). The effects of the root extract on general behavioral pattern, potentiation of phenobarbitone sleep, exploratory behavior and muscle relaxant activity were studied on different animal models in rats and mice.

Materials and Methods

Plant Material

The roots of *Ocimum sanctum* were collected from Kolkata, West Bengal and were identified by Botanical Survey of India, Shibpur, Howrah, and a voucher specimen bearing the number CNH/I-I(37)/99-Tech.II/625. The roots were collected, dried under shade and pulverized by a mechanical grinder. The powder was passed through sieve no. 40 and stored in a closed vessel.

Preparation of the extract

Coarsely powdered dried roots (1kg) were successively extracted in cold condition with 95% methanol as solvent for 72 hour at room temperature. The whole extract was collected in a 5 liters conical flask, filtered and the solvent was evaporated to dryness under reduced pressure in a Eyela Rotary Evaporator (Japan) at 40-45^{o} C. The percentage yield (w/w) of the prepared extract was 12.745% with respect to the dry powder.

The preliminary photochemical group test of root extract was done by the standard methods [3]. The photochemical tests of the whole extract were done by qualitative analysis and confirmed by thin layer chromatography (TLC) [4].

Animals used

Swiss albino mice (20-25 g) and Wister albino rats (150-180 g) of either sex were used. The animals were housed in groups of 10 per cage (standard metal cages) prior to pharmacological studies with free access to standard diet and water ad libitum for at least 2 weeks on a 12/12 hour light/dark cycle (from 08:00 to 20:00 hour). All animals were fasted overnight before conducting the experiment. The ambient temperature was 22 ± 1^0 C, except phenobarbitone sodium induced sleeping time experiments, which were carried out at 30 ± 1^0 C. Plant extracts and standard drugs were suspended in propylene glycol immediately prior to use and given orally 1 hour before the experiments in a dose of 5 ml/kg body weight in mice (0.1 ml/20 g) and rats (0.75 ml/150 g). Control animals received the same dose of vehicle under the same conditions. Behavioral observations took place between 08:00 and 15:00 hour and each animal was used only once. Injections were normally made intraperitoneally unless otherwise mentioned.

Drugs

The drugs used for this experiment were chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Lupin Laboratories Ltd., India), phenobarbitone sodium (Rhone-Poulenc India Ltd., India), pethidine (Ranbaxy Laboratories Ltd., India) and propylene glycol (SRL Laboratories, India).

LD_{50} in mice

An acute toxicity study was done by determining LD_{50} calculated from the lethal dose within 3 days after p.o. administration, of different doses of the crude extract of *Ocimum sanctum* roots by Litchfield and Wilcoxon method [5].

General behavioral profiles

Evaluation of general behavioral profile was performed by the method of Dixit and Verma et al [6]. Fifty adult albino male mice were divided into 5 groups (n=10). The first three groups were injected with methanolic extract of *Ocimum sanctum* root (MEOS) at doses of 50, 100 and 150 mg/kg intraperitoneally.

The fourth group received chlorpromazine (CPZ, 5 mg/kg) and the fifth group was treated with propylene glycol (5 ml/kg) as vehicle control. The animals were under observation for their behavioral changes if any, at 30 min intervals in the first hour and at hourly intervals for the next 4 hour for the following parameters [7,8].

Awareness, alertness and spontaneous activity

The awareness and alertness was recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls [9]. The normal behavior at resting position was scored as 0, little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness as (++++). The spontaneous activity of the mice was recorded by placing the animal in a bell jar. It usually showed a moderate degree of inquisitive behavior. Less or moderate activity was scored as ++, strong activity as +++, slight or little activity as + and for the sleeping animals the score was -. Excessive or very strong inquisitive activity like constant walking or running was scored ++++. A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table [7,9].

Touch response

The touch response was recorded by touching the mice with a pencil or forceps at various parts of the body (i.e. on the side of the neck, abdomen and groin).

Pain response:

The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

Sound response:

Albino mice normally utter no sound, so that vocalization may indicate a noxious stimulus.

Analgesic activity

Analgesic activity was studied by (i) tail immersion and (ii) tail flick tests.

Tail immersion test

Swiss albino mice of either sex were divided into 5 groups of 10 animals each. Propylene glycol (5 ml/kg), MEOS at the doses of 50, 100 and 150 mg/Kg and pethidine (5 mg/kg) were administered intraperitonialy.

The tail (up to 5 cm) was then dipped into a pot of water maintained at 55 ± 0.5^0 C. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 min of administration of the test drugs [10].

Tail flick test

Wister strain of albino rats of either sex weighing between 150 and 180 g were selected and divided into 5 groups of 10 animals each. The tail of the rat was placed on the nichrome wire of an analgesiometer (Techno, Lucknow, India) and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The MEOS in doses of 50, 100 and 150 mg/kg and pethidine (5 mg/kg) were injected intraperitonialy. Propylene glycol at **(**5 ml/kg) was served as control. Analgesic activity was measured after 30 min of administration of test and standard drugs [10].

Effect of sodium phenobarbitone-induced sleeping time:

Mice were divided into 4 groups of 10 each. Animals received 40 mg/kg (i.p.) phenobarbitone sodium 30 minutes after injection of MEOS at dose of 50, 100 and 150 mg/Kg and control vehicle propylene glycol (5 ml/kg). The sleeping time was recorded, and measured as the time interval between the loss and regaining of the righting reflex [11,12].

Exploratory behavior:

This was performed by (i) Y-maze and (ii) Head dip tests

Y-maze test:

This was performed in group of 10 albino rats at 30, 60, 90, and 120 min after injection of either propylene glycol (5 ml/kg) , MEOS (50, 100 and 150 mg/Kg), or diazepam (10 mg/Kg), respectively. The rats were placed individually in a symmetrical Y-shaped runway (33 cm x 38 cm x 13 cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4 feet (an entry) were counted [12,13].

Head dip test

Five groups of female albino mice (n=10) were placed on top of a wooden box with 16 evenly spaced holes, 30 min after injection of the MEOS (50, 100 and 150 mg/Kg), vehicle propylene glycol (5 ml/kg) and diazepam (10 mg/Kg) respectively. The number of times that each animal dipped its head into the holes was counted for a period of 3 min [14].

Muscle relaxant activity

The effect of extract on muscle relaxant activity was studied by the (i) Traction test and (ii) Rotarod test (iii) 30^0 inclined screen test.

Traction test

The screening of animals was done by placing the forepaws of the male mice in a small twisted wire rigidly supported above a bench top. Normally the mice grasp the wire with forepaws, and place at least one hind foot on the wire within 5 sec when allowed to hang free. The test was conducted in five groups of animals (n=10) which were previously screened, 30 min after the injection of either MEOS (50,100 and 150 mg/kg), diazepam (10 mg/Kg) or propylene glycol **(**5ml/kg) as vehicle control. Inability to put up at least one hind foot considered failure in the traction test [15].

Rotarod Test

Fresh mice were placed on a horizontal wooden rod (32 mm. diameter) rotating at a speed of 5 rpm. The mice capable of remaining on the rod for 3 minutes or more, in three successive trials were selected for the study. The selected animals were divided into 5 groups (n=10). Groups 1, 2 and 3 were injected intraperitoneally with MEOS (50, 100, 150 mg/kg). Groups 4 and 5 were received either propylene glycol (5 ml/kg) or diazepam (10 mg/kg). Each group of the animals was then placed on the rod at an interval of 30, 60, 90,120 and 150 minutes. The animals failed more than once to remain on the rotating rod for 3 minutes were considered as positive result [16].

30^oInclined screen test

In this test the groups of 10 male mice, in 5 groups, were injected intraperitoneally with either propylene glycol **(**5 ml/kg) or MEOS (50, 100, 150 mg/Kg) or diazepam (10 mg/kg) respectively. The animals were left on a 30^0 inclined screen at least for 4 hours to observe a paralysant effect sufficient to cause the mouse slide off the screen [17].

Statistical Analysis

The data were expressed as the mean ± S.E. Significance was evaluated by the Student's t- test in all the experiments, and Chi-square tests for muscle relaxant activity [18]. A value less than 0.05 were considered significant.

Results

Toxicity study

The root extract was found to be non-toxic up to doses of 3.0 g/kg and did not cause any death of the animals tested.

Effects on general behavioral profiles

The results obtained from different experiments are presented in Table1. The MEOS affected spontaneous activity, sound and touch responses at doses above 50 mg/kg and produced moderate/slight depression related to awareness and alertness. However, the standard drug chlorpromazine hydrochloride (5 mg/kg) caused a significant depression of these responses compared with the methanolic extract of the plant.

Table:1 Effect of the MEOS on general behavioral profiles in mice and rats

Behavior Type	MEOS (mg/kg)			Chlorpromazine (5 mg/kg)	Propylene glycol (5 ml/kg)
	50	100	150		
Spontaneous activity	+	++	+++	++++	-
Alertness	+	+++	+++	+++	-
Awareness	+	+++	++	+++	-
Sound response	+	++	++++	++++	-
Touch response	++	+++	+++	++++	-
Pain response	+	+++	++++	++++	-

MEOS, methanol extract of *Ocimum sanctum* root; (-), no effect; (+), Slight depression; (++), moderate depression; (+++) , strong depression; (++++), very strong depression; n=10

Analgesic activity

The result of the analgesic activity of MEOS by tail immersion and tail flick methods is presented in Table2. In both the tests the reaction time was significantly increased in treated animals compared with the standard drug pethidine.

Table:2 Analgesic effect of MEOS on tail flick and tail immersion tests in mice and rats

Treatment	Dose	Tail flick test (reaction time, s)	Tail immersion test (reaction time, s)
Propylene glycol	5 ml/ kg	2.15 ± 0.14	2.30 ± 0.16
Pethidine	5 ml/ kg	4.25 ± 0.18	4.45 ± 0.12
MEOS	50 ml/ kg	2.50 ± 0.16	2.40 ± 0.02
	100 ml/ kg	3.02 ± 0.07	3.05 ± 0.02
	150 ml/ kg	3.85 ± 0.21	3.83 ± 0.15

Values are mean ± S.E., n =10. All the data are significant at P<0.001 *vs*. control, Student's t-test.

Exploratory behavior potentials

In Y-maze test, the animals treated with MEOS in doses of 50mg/kg and above showed a marked decrease in exploratory behavior compared with controls (Table 3). In head dip test, there was a significant reduction in head dip responses occurred in mice treated with MEOS at doses of 50 mg/kg and above as compared with the control (Table 4).

Table: 3 Effect of *Ocimum sanctum* root extract on exploratory behavior (Y-maze test) in rats

Experiment	Dose	Number of Entries after treatment (mins.)			
		30	60	90	120
Control	0.1ml/kg	10.33±0.556	10.66±0.667	10.33±0.556	10.33±0.556
Diazepam	10 mg/kg	3.83±0.477*	4±0.258*	4.67±0.667*	5.16±0.526*
MEOS	50 mg/kg	8.5±0.763[a]	7.33±0.667*	8.33±0.421[b]	8.45±0.421[c]
MEOS	100 mg/kg	6.0±0.365*	6.83±0.653*	7±0.577*	6.33±0.614*
MEOS	150 mg/kg	5.33±0.667*	5.5±0.223*	5.33±0.780*	5.16±0.401*

MEOS, methanol extract of *Ocimum sanctum* root; Values are the number of entries in 3 min (mean ±S.E.; n = 10)

Table: 4 Effect of MEOS on exploratory behavior (head dip test) in mice

Experiment	Dose (body weight)	Head dip test
Propylene glycol	5ml /kg	29.16±1.402
Diazepam	10 mg/kg	9.67±0.434*
MEOS	50 mg/kg	23.0±0.9660**
MEOS	100 mg/kg	17.5±0.885**
MEOS	150 mg/kg	13.16±0.991**

MEOS, methanol extract of *Ocimum sanctum* root. Values are the number of head dips in 3 mins. (mean ±S.E.; n=10)
*P<0.01 compared with control
**P<0.001 compared with control

Effect on phenobarbitone sodium-induced sleeping time

The MEOS significantly potentiated the phenobarbitone sodium-induced sleeping time at dose of 50mg/kg and above with respect to the control drug diazepam. (Table 5)

Table:5 Effect of MEOS on phenobarbitone sodium induced sleeping time

Experiment	Dose	Sleeping time (mins.)
Control	0.1ml saline/10gm	45±1.62
MEOS	50 mg/kg	53.4±1.49*
MEOS	100 mg/kg	64.5±1.147*
MEOS	150 mg/kg	84.6±2.082*

MEOS, methanol extract of *Ocimum sanctum* root. Values are (mean ±S.E.; n=10)

*P<0.001 compared with control

Effect on muscle relaxant activity

In the traction test, the mice treated with MEOS showed a significant failure in traction at all doses tested (Table No.6). The result obtained from the rotarod test, showed that MEOS at 50mg/kg and above significantly reduced the motor co-ordination of the tested animals; while in 30^{o} inclined screen test, the extract showed a significant loss of coordination and muscle tone of all the tested animals.

Table: 6: Percentage effect of MEOS on Muscle relaxant activity in mice

Treatment and dose	% of animals showing negative test		
	Traction Test	30^{o} Inclined Test	Rotarod Test
Propylene glycol	0	0	0
Diazepam(10mg/kg)	100	100	100
MEOS (50mg/kg)	68.3[a]	50[a]	70[a]
MEOS (100mg/kg)	79.9[a]	68[a]	70[a]
MEOS (150mg/kg)	88.8[a]	79[a]	80[a]

MEOS, methanol extract of *Ocimum sanctum* roots. Values are the percentage of animals showing a negative result; n=10. a= $p< 0.5$ compared with control Chi-square test

The results of the preliminary photochemical group test of *Ocimum sanctum* root extract have been presented in Table7.The photochemical tests with the MEOS indicated the presence of tannin, flavonoid, alkaloid, sterol and reducing sugar.

Table:7 Preliminary phytoconstituents in MEOS

Serial No.	Phytoconstituents	Root extract of O.sanctum
1	Alkaloids	+
2	Anthraquinones	-
3	Steroids	+
4	Amino acids	-
5	Flavonoids	+
6	Gums	+
7	Reducing sugars	-
8	Tannins	+
9	Saponins	-

(-), Absence; (+), presence.

Discussion

The results indicated that the (MEOS) influences general behavioral profiles, as evidenced in the spontaneous activity, sound, touch and pain responses. The MEOS possesses significant analgesic activity compared with the standard drug pethidine in a dose-dependent manner. This activity may be due to its action on central nervous system like pethidine.The extract significantly potentiated the phenobarbitone sleeping time, possibly through a CNS depressant action [19] or a tranquilizing action [12]. The possible CNS activity of the methanol extract was further investigated by other common psychopharmacological tests like the rotarod test, 30^{o} inclined screen test, and traction test. The reduction in exploratory behavior in animals treated with the methanol extract of the roots of Ocimum sanctum is similar with the action of other CNS depressant agents. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with the crude extract.

Ursolic acid, a triterpene, has been reported to obtain from the leaves of Ocimum *sanctum* [20]. Contemporary scientific research revealed that the ursolic acid isolated from herbal sources have inflammatory, hepatoprotective, antimicrobial and antiviral activity [22]. Ursolic acid isolated from several medicinal plants is found to inhibit human leukocyte elastase [23], 5-lipooxygenase and cyclooxygenase [24], concavalin A-induced histamine release [25], and enzymes of arachidonate cascade [26]. The β-sitosterol, on the other hand, reported to have anticancerous, antiulcer, antidiabetic, anti-inflammatory and antipyretic activities and is helpful in controlling rheumatoid arthritis [27]. Isolation of ursolic acid from the roots of this plant is still under investigation.

The present investigation for the first time, confirms that there is a moderate to strong degree of antipsychotic activity in the MEOS. In conclusion, the vernacular medicinal use of *Ocimum sanctum* roots for mental tension and disturbance as well as to induce sleep is validated by our findings.

Acknowledgements

The authors are grateful to the Late Dr. Mrs. Manjushree Pal, Ex-reader, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India for her constant encouragement, help and suggestions. The author also acknowledges to University Grant Commission (UGC) for providing financial support to carry out this research project.

References

1. R. Kaur, M. Sood, S. Chander, R. Mahajan, V. Kumar and D.R. Sharma. Plant Cell, Tissue and Organ Culture 1999; 59: 33,227-229.
2. Becker H et al. Isolation and characterization of cell lives of *Valeriana wallichii* resistant to trifluoroleucine. Z Plazenphysiol 1983; 112:43-51.
3. Trease GE, Evans WC. Pharmacognosy, 12th ed. ELBS Publication, Baillier Tindall, East Bourne. 1983; 344: 539.
4. Pollock JRA, Stevens R. Dictionary of Organic Compounds, Vol. 4. Oxford University Press, New York, 1965; 2030.
5. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. Journal of Pharmacology and Experimental Therapeutics 1949; 96: 99-133.
6. Dixit VK, Varma KC. Effects of essential oil of leaves of *Blumea lacera* DC on central nervous system. Indian Journal of Pharmacology 1976; 18: 7-11.
7. Mukherjee PK, Saha K, Balasubramanium R, Pal M, Saha BP. Studies of psychopharmacological effects of *Nelumbo nucifera* Gaertn Rhizome extract. Journal of Ethnopharmacology 1996; 54: 63-67.
8. Murugesan T, Ghosh L, Das J, Pal M, Saha BP. CNS activity of *Jussiaea suffruticosa* Linn. Extract in rats and mice. Pharmacy and Pharmacology Communication 1999; 5: 663-666.
9. Turner RA. Screening Methods in Pharmacology. Academic Press, New York. 1965; 26-35.
10. Ghosh MN. Fundamental of Experimental Pharmacology, 2nd ed. Scientific Book Agency, Calcutta. 1984; 153.
11. Dandiya PC, Collumbine H. Studies on *Acorus calamus* (III): some pharmacological action of the volatile oil. Journal of Pharmacology and Experimental Therapeutics 1959; 125: 353-359.
12. Mandal SC, Dhara AK, Maiti BC. Studies of psychopharmacological activity of *Andrographis paniculata* extract. Phytotherapy Research 2001; 15: 253-256.
13. Rushton R, Steinberg H, Tinson C. Modification of the effects of an amphetamine barbiturate mixture by the past experience of rats (Y- shaped runway). Nature 1961; 192: 533-535.
14. Dorr M, Steinberg H, Tomkiewicz M, Joyce D, Porsolt RD, Summerfield A. Persistence of dose related behaviour in mice. Nature 1971; 121–123 ()
15. Rudzik AD, Henter JB, Tag AH, Straw RN, Friis W. Triazolobezodiazepine, a new class of Central Nervous System depressant compound. The Benzodiazepines Reven Press. New York. 1973; 286-298.
16. Dunham NW, Miya TS. A note on simple apparatus for detecting neurological deficit in rats and mice. Journal of American Pharmacology 1957; 46: 208-209.
17. Randall P. Studies of the neurological effects using simple experimental model. Journal of Pharmacology and Experimental Therapeutics 1960; 135: 543-545.
18. Woodson RF. Statistical Method for the Analysis of Bio-Medical Data, Probability and Mathematical Statistics. Wiley, Chichester. 1987; 313-316.
19. Fastier, F.N., Spenden, R.N., Hendrieka, W., 1957. Prolongation of chloral hydrate sleeping time by 5-HT and by certain other drugs. British Journal of Pharmacology 12, 251-259.

20. De et. al. Plant with hepatoprotective activity- A review. Indian Drugs 1993; 30(8): 355-363.
21. Kim KW. Anticancer activities of plant triterpenoids, ursolic acid and oleanoid acid. Journal of Korean Association and Cancer Prevention 1972; 38-44.
22. Liu J. Pharmacology of oleanolic acid and ursolic acid. Journal of Ethnopharmacology. 1995,49, 57-68.
23. Safayhi H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes. Planta Medica 1997, 63,1-7.
24. Najid A, Simon A, Cook J, Chable-Rabinovitch H, Chulia AJ, Rjgaud M. Characterization of ursolic acid as a lipoxygenase and cyclooxygenase inhibitor using macrophages, platelets and differentiated HL60 leukemic cells. FEBS Lett. 1992; 299: 213-217.
25. Tsuruga T, Chun YT, Ebizuka Y, Sankawa U. Biologicallyactive constituents of Melaleuca leucadendron: inhibitorsof induced histamine release from rat mast cells. Chem. Pharm. Bull. 1991: 39: 3276-3278.
26. Diaz AM., Abad MJ, Fernandez L, Recuero C, Villaescusa L, Silvan AM, Bermejo P. In vitro anti-inflammatory activity of iridoids and triterpenoid compounds isolated from *Phillyrea latifolia* L. Biological & Pharmaceutical Bulletin 2000; 23: 1307-1313.
27. Pegel KH, The importance of sitosterol and sitosterolin in human and animal nutrition. Plant Medica 1980: 39:157-163.