OPEM

Oriental Pharmacy and Experimental Medicine 2002 **2(2)**, 101-105

Central nervous system depressant effect of hot water extract of *Ocimum sanctum* Linn. (Labiateae)

Mahiuddin Alamgir[1,*], Md. Shahabuddin Kabir Choudhuri[2], Shaila Jabbar[3], Sultana Rajia[2] and Mahmud Tareq Hassan Khan[3]

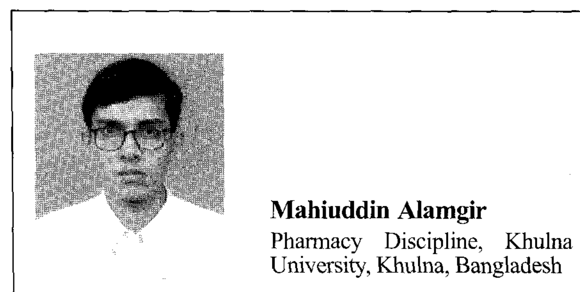
[1]*Pharmacy Discipline, Khulna University, Khulna, Bangladesh;* [2]*Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh;* [3]*Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh*

SUMMARY

A battery of neuropharmacological experiments showed the hot water extract of *Ocimum sanctum* Linn. (Labiateae) had a depressant effect on the central nervous system (CNS), but the aqueous extract showed no effect on it. The hot water extract reduced the spontaneous locomotor activity, exploratory head dipping, propulsive locomotion and exploratory ambulation as well as prolonged the pentobarbital induced sleeping time. The depressant effect starts from 60 minutes after the drug administration and continued to 180 minutes. The drug may exert central depressant effect by interfering with the function of the cortex.

Key words: *Ocimum sanctum* Linn.; Labiateae; Central nervous system; Depressant; Neuropharmacological

Ocimum sanctum Linn. (Family: Labiatae) is an erect, hairy annual herb found throughout the South Asian region. The leaves have ethnomedical reports of anthelmintic, antipyretic, antiashmatic, antidiabetic and various other properties (Kirtikar and Basu, 1984). A water extract of the leaves showed increase sleeping time induced by hexabarbitone in mice (Dhar *et al.*, 1968). A 50% alcohol extract showed no barbiturate potentiation (Bhargava and Singh, 1981), but the crude water extract potentiated hexobarbitone induced hypnosis in mice (Singh *et al.*, 1970). Ethanol extract of the leaves of *O. sanctum* prolonged the time of the lost reflex in mice due to pentobarbital, decreased the recovery time, the severity of electroshock, pentylenetetrazole-induced convulsions, and decreased apomorphine-induced fighting time, and ambulation in open field studies (Sakina *et al.*, 1990). *O. sanctum* leaves are rich of essential oils. The important constituents of the leaves are flavonoids (apigenin, luteolin, molludistin, vicenin and orientin); phenolic compounds (carvacol, chavibetol, estragole eugenol and ursolic acid) and terpenoids (cineole, linalool and caryophyllene) (Brooks, 1911; Dutt, 1940; Gonopati, 1952; Nair and Gunasegaran, 1982; Skaltsa *et al.*, 1987).



Mahiuddin Alamgir
Pharmacy Discipline, Khulna University, Khulna, Bangladesh

The present study was undertaken to evaluate the effect of the two commonly used extract of *O. sanctum* L on some neuropharmacological experimental models.

MATERIALS AND METHODS

Plant material

Ocimum sanctum Linn (Labiateae) leaves were collected from Jahangirnagar University campus, Savar,

*Correspondence: Dr Mahiuddin Alamgir, Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. E-mail: m19alamgir@yahoo.com

Dhaka and identified by the Bangladesh National Herbarium. A voucher specimen (MAB020) was deposited at the Ethnopharmacology laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. FTIR spectra of the extracts were recorded for fingerprinting.

Preparation of the extract

Two kinds of the extract were used in this study. The aqueous extract was prepared from fresh leaves cut into small pieces, homogenized in a mortar and pestle and then 100 gm leaves were blended with 100 ml of water in a kitchen blender. It was then filtered through a net filter. The hot water extract was prepared from 5 g dried (50°C) powdered leaves of *O. sanctum* L by adding 150 ml distilled water and thoroughly mixed to make a uniform suspension. It was then boiled till the volume was reduced to 40 ml and filtered. This filtrate was collection 1. The residue was again boiled with 150 ml of water till the volume was reduced to 40 ml and filtered. This filtrate was collection 2. The two filtrates were then added and reduced to 20 ml by gentle heating and the mixture was known as hot water extract.

Dose and route of administration

The drug was administered per oral 20 ml/kg body weight. For spontaneous motor activity test, the drug was given intraperitoneally 10ml/kg body weight. Control animals were administered with normal tap water.

Animals

Male and female mice (*Swiss-webstar* strain, 20-25 gm body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the experiments. The animals were provided with standard laboratory food and tap water *ad libitum* and maintained at natural day night cycle. The animals were divided in-groups of 6-10, with each group balanced for sex and body weight. All the experiments were conducted on every evening in an isolated and noiseless condition.

Spontaneous motor activity test

In this method (Svensson and Thieme, 1969) the animals were placed on an automatic activity cage (Model-7400, UGO Basile, Italy) with a recorder (Model-7401, UGO Basile, Italy) . The movements were recorded by the detector for 2 minutes and were considered as locomotion. The observation was conducted on 0, 30, 60, 120, 180 and 240 minutes after i.p. injection.

Hole board test

In this experiment (Nakama *et al.*, 1972) each animal was placed carefully in the center of the field and the number of holes passed, head dipping, and the number of fecal boluses excreted recorded for 3 minutes. The observation was made on 0, 30, 60, 90, 120, 180 and 240 minutes after oral administration of the test drugs.

Open field test

The number of squares traveled by the animal was recorded for 3 minutes in this experiment (Gupta *et al.*, 1971). The observations were made on 0, 30, 60, 90, 120, 180 and 240 minutes after oral administration of the test drugs.

Hole cross test

Spontaneous movement of the animals through the hole from one chamber to the other was counted for 3 minuets in this test (Takagi *et al.*, 1971). The observations were made on 0, 30, 60, 90, 120, 180 and 240 minutes after oral administration of the test drugs.

Hypnotic action of pentobarbital

Pentobarbital sleeping time test was carried out by the method of Williamson *et al.*, 1996. The test drugs were administered per oral 30 minutes before the administration of pentobarbital (i.p. 50 mg/kg body weight). The animals were observed for the onset and the duration of sleep, as evidenced by the observation of the loss of writhing reflex.

Statistical analysis

All analyses were done using the SPSS 7.5 for windows. Experimental values were expressed as Mean±SEM (Standard Error of Mean). Independent samples t-test was done for statistical comparison. Statistical significance was considered to be indicated by a p value of less than 0.05 in all cases.

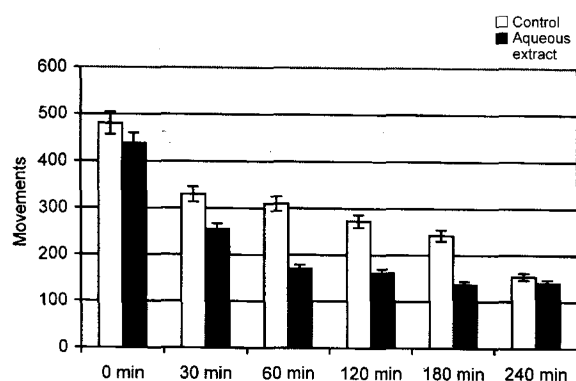


Fig. 1. Effect of *O. sanctum* L. on spontaneous motor activity.

RESULT AND DISCUSSION

Intact animals are considered to be the best method for investigating the action of drugs on CNS. A most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. Inhibitory effects on spontaneous motor activity of the hot water extract indicated depressant activity. The lowering began after 30 minutes of drug administration and continued up to 180 minutes (Fig. 1).

In order to provide the animals with a stronger stimulus for exploration the hole board test was performed (Table 1) evoking a pattern of behavior characterized by exploration (head dipping through the holes), locomotion (ambulation past the holes), and emotional defecation. The aqueous extract and hot water extract did not have any effect on ambulation and defecation, but the hot water extract reduced the exploratory head dipping behavior from 30 to 180 minutes ($P<0.001$) which indicates possible CNS depressant activity.

There is also significant decrease ($P<0.05$) in the open field scores from 60 to 180 minutes period of the hot water extract. The aqueous extract had no effect in this experiment. The hot water extract decreased ($P<0.05$) the propulsive locomotor activity in the hole cross test which further indicates the

Table 1. Effect of *Ocimum sanctum* L. on hole board test [Mean±SEM (*P* value)]

Group	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Ambulation							
Control (n=15)	20.33±2.62	24.67±2.38	22.67±3.25	23.93±2.77	20.80±2.80	17.60±1.98	14.93±1.69
Aqueous extract (n=7)	31.14±4.56 (0.040)[a]	25.85±2.46 (0.763)	26.71±4.65 (0.487)	27±2.97 (0.509)	22.14±2.89 (0.772)	20.71±3.38 (0.410)	26.86±2.67 (0.001)[b]
Hot water extract (n=8)	37.50±4.53 (0.002)[a]	24.37±5.01 (0.950)	20.87±3.97 (0.740)	21.25±2.61 (0.537)	24.37±4.44 (0.484)	15.13±2.68 (0.468)	27.25±2.74 (0.001)[b]
Head dipping							
Control (n=15)	21.27±3.18	27.4±3.89	22.73±2.94	25.47±2.67	20.87±2.79	20.87±2.53	17.27±2.69
Aqueous extract (n=7)	30.14±3.93 (0.115)	23±3.96 (0.496)	23.29±4.47 (0.918)	23±4.29 (0.619)	18.14±3.01 (0.560)	15.57±3.69 (0.250)	21.14±3.97 (0.427)
Hot water extract (n=8)	32.75±3.98 (0.040)[a]	18±1.99 (0.107)	17.37±2.59 (0.245)	16.13±1.22 (0.023)[a]	14.37±1.98 (0.129)	10.0±1.54 (0.001)[b]	16.63±1.87 (0.847)
Defecation							
Control (n=15)	5.0±0.49	3.47±0.29	2.53±0.32	2.33±0.33	3±0.36	2.93±0.43	2.33±0.29
Aqueous extract (n=7)	1.57±0.29 (0.000)[b]	2.57±0.43 (0.098)	1.85±0.46 (0.446)	2.28±0.64 (0.943)	1.29±0.28 (0.007)[a]	1.0±0.31 (0.009)[a]	0.71±0.42 (0.005)[a]
Hot water extract (n=8)	5.50±0.53 (0.532)	2.13±0.39 (0.013)[a]	1.88±0.52 (0.268)	2.37±0.60 (0.998)	1.13±0.29 (0.003)[a]	1.25±0.31 (0.015)[a]	1.87±0.72 (0.568)

n = number of mice
[a]$P<0.05$.
[b]$P<0.001$.

Table 2. Effect of *Ocimum sanctum* L. on open field and hole cross test [Mean±SEM (P value)]

Group	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Open field test							
Control (n=8)	117.13±6.3	85.13±8.68	61.25±7.67	50.63±3.86	44.63±3.52	39.63±5.43	35.13±4.42
Aqueous extract (n=8)	114.63±6.72 (0.790)	80.11±4.53 (0.609)	54.63±6.28 (0.515)	40.37±6.18 (0.181)	38.25±4.59 (0.289)	46.37±4.58 (0.358)	41.00±5.44 (0.416)
Hot water extract (n=8)	119.38±9.42 (0.846)	77.5±8.71 (0.545)	40.63±3.86 (0.047)[a]	37.37±4.54 (0.043)[a]	31.21±4.78 (0.038)[a]	25.63±3.03 (0.041)[a]	34.02±3.73 (0.849)
Hole cross test							
Control (n=8)	9.5±0.85	6.5±0.71	6.38±0.5	5.75±0.62	4.25±0.59	4.88±0.58	5.13±0.55
Aqueous extract (n=7)	9±0.71 (0.657)	6.63±0.75 (0.905)	5.88±0.61 (0.536)	5.75±0.45 (1.00)	4.75±0.59 (0.560)	5.25±0.65 (0.673)	6.10±0.48 (0.192)
Hot water extract (n=8)	9.85±1.41 (0.826)	5.14±1.60 (0.430)	3.86±1.22 (0.067)	3.57±0.87 (0.054)[a]	2.43±0.48 (0.035)[a]	5.28±1.57 (0.80)	4.14±1.16 (0.440)

n=number of mice.
[a]P<0.05.

Table 3. Effect of *Ocimum sanctum* L. on the hypnotic action of pentobarbital

Group	Onset of sleep (min) Mean±SEM (p value)	Duration of sleep (min) Mean±SEM (p value)
Control (n=8)	3.44±0.18	47.13±2.75
Aqueous extract (n=8)	3.72±0.39 (0.492)	58.70±8.03 (0.213)
Hot water extract (n=8)	3.63±0.46 (0.712)	74.38±10.78 (0.040)[a]

n=number of mice.
[a]P<0.05.

CNS depressant effect (Table 2).

The extracts showed no effect on the onset of the sleeping time and as with previous findings (Dhar *et al.*, 1968; Singh *et al.*, 1970). The hot water extract prolonged ($P<0.05$) the duration of the pentobarbital induced sleeping time (Table 3), which may be attributed to an action on the cerebral mechanism involved in the regulation of the sleep. Interestingly, this report of the hot water extract differed with the previous alcoholic extract report (Bhargava and Singh, 1981; Sakina *et al.*, 1990). Reduced locomotion and activity in the several neuropharmacological models justified the hot water extract had CNS depressant activity in animal models. The hot water extract may contain CNS depressant component(s), which could not be isolated by alcoholic extract.

CONCLUSION

The results obtained from the several neuropharmacological experiments showed CNS depressant activity of the hot water extract on experimental animal models. The depression started from 60 minutes and continued up to 180 minutes. Whereas the aqueous extract did not show any significant effect on the CNS. The mechanism of this depression is not clearly understood, the drug may exert central depressant effect by interfering with the functions of the cortex.

Further studies are required to identify the compound (s) responsible for this depression and its mechanism.

REFERENCES

Bhargava KP, Singh N. (1981) Anti-stress activity of *O. sanctum* Linn. Indian *J. Med. Res.* **73,** 443.

Brooks BT. (1911) Citrus oils in the Philippine Islands and the Essential oils of *Clausena anisumolens* BL and *Ocimum sanctum*. *Perf. Ess. Oil Record.* **2,** 264-265.

Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C. (1968) Screening of Indian plants for biological activity Part I. *Indian J. Exp. Biol.* **6,** 232.

Dutt S. (1940) New Indian essential oils. *Indian Soap Journal* **6,** 248-255.

Gonopoti R. (1952) Chemical composition of *Ocimum sanctum*. *Congr. Luso-espan. Farm.* **3,** 187-191.

Gupta BD, Dandiya PC, Gupta ML. (1971) A psychopharmacological analysis of behavior in rat. *Jpn. J. Pharmacol.* **21,** 293.

Kirtikar KR, Basu BD. (1984) *Indian Medicinal Plants*, Vol. 3, 2nd edn, pp. 1928, Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

Nair AGR, Gunasegaran R. (1982) Chemical investigation of certain South Indian plants. *Indian J. Chem.* **21B,** 979.

Nakama M, Ochiai T, Kowa Y. (1972) Effects of psychotropic drugs on emotional behavior on naïve rats in holded open field. *Jpn. J. Pharmacol.* **22,** 767.

Sakina MR, Dandiya PC, Hamdard ME, Hameed A. (1990) Preliminary psychopharmacological evaluation of *Ocimum sanctum* Leaf extract. *J. Ethnopharmacol.* **28,** 143.

Singh TJ, Das GP, Khan SY, Mishra KC. (1970) Preliminary pharmacological investigations of *Ocimum sanctum* Linn. *Indian J. Pharm.* **32,** 92.

Skaltsa M, Couladi M, Phillianos S, Singh M. (1987) Phytochemical study of the leaves of *Ocimum sanctum. Fitoterapia* **58,** 286.

Svensson TH, Thieme G. (1969) An investigation of a new instrument to measure motor activity of small animals. *Psychopharmacol.* **14,** 157.

Takagi K, Watanabe M, Saito H. (1971) Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethan. Its acylesters on the central nervous system. *Jpn. J. Pharmacol.* **21,** 797.

Williamson EM, Okpako DT, Evans FJ. (1996) Selection, Preparation and Pharmacological Evaluation of Plant Material. In: *Pharmacological Methods in Phytotherapy Research*, vol. 1, 1st edn, p.184, John Willey & Sons, England.