

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

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### SUMMARY

A battery of neuropharmacological experiments showed the hot water extract of *Ocimum sanctum* Linn. (Labiatae) 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.; Labiatae; 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, antiasthmatic, antidiabetic and various other properties (Kirtikar and Basu, 1984). A water extract of the leaves showed increase sleeping time induced by hexobarbitone 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

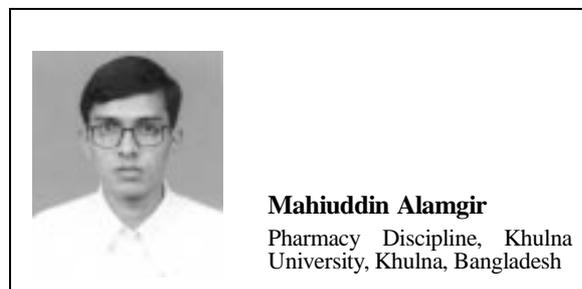
extract of the leaves of *O. sanctum* prolonged the time of the lost reflex in mice due to pentobarbital, decreased the recovery time, the severity of electroshock, pentylenetetrazole-induced convulsions, and decreased apomorphine-induced fighting time, and ambulation in open field studies (Sakina *et 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).

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 (Labiatae) leaves were collected from Jahangirnagar University campus, Savar,



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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**

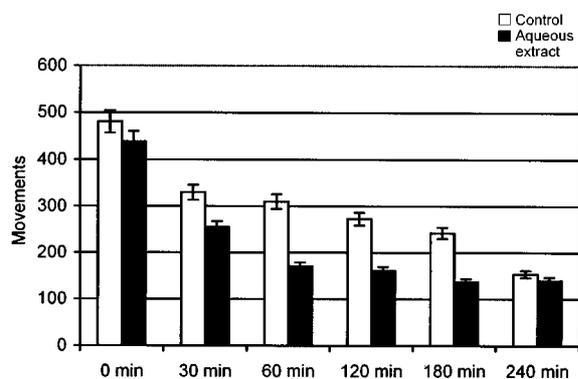
Spontaneous movement of the animals through the hole from one chamber to the other was counted for 3 minutes in this test (Takagi *et al.*, 1971). The observations were made on 0, 30, 60, 90, 120, 180 and 240 minutes after oral administration of the test drugs.

#### **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 $\pm$ SEM ( $P$  value)]

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

n = number of mice

<sup>a</sup> $P < 0.05$ .

<sup>b</sup> $P < 0.001$ .

**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
<b>Open field test</b>							
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) <sup>a</sup>	37.37±4.54 (0.043) <sup>a</sup>	31.21±4.78 (0.038) <sup>a</sup>	25.63±3.03 (0.041) <sup>a</sup>	34.02±3.73 (0.849)
<b>Hole cross test</b>							
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) <sup>a</sup>	2.43±0.48 (0.035) <sup>a</sup>	5.28±1.57 (0.80)	4.14±1.16 (0.440)

n=number of mice.

<sup>a</sup>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) <sup>a</sup>

n=number of mice.

<sup>a</sup>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.

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