Biosensors Journal
ISSN: 2090-4967

Islam et al., Biosens J 2015, 4:2
DOI: 10.4172/2090-4967.1000132

Research Article Open Access

Analgesic and CNS Depressant Effect of the Crude Ethanolic Extract of the *Operculina turpethum*

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Abstract

The *Operculina turpethum* (Fam. Convolvulaceae) is a traditional medicinal plant for the treatment of anthelmintic, expectorant, antipyretic, anti-inflammatory and purgative. The work reached the acute toxicity of *Operculina turpethum* and its action on the ethanolic extract of the central nervous system (CNS) because on data in the literature have been found of pharmacological activity of this plant in the CNS. The *Operculina turpethum* was extracted with ethanol and investigated for its CNS activity of experimental model in mice. The extract produce a dose dependent reduction of the onset and duration of pentobarbitone induced hypnosis, reduction of locomotor and exploratory activities in the open field, hole cross at the dose level of 250 mg/kg and 500 mg/kg body weight. At the same dose labels, the extract dose dependently inhibited acetic acid induced writhing in mice. This study suggested that the extract possess CNS depressant activity.

Keywords: *Operculina turpethum*; Central nervous system; Analgesic activity

Introduction

Operculina turpethum is a perennial herb with milky juice and its root is incorporated in the Avipattikara churna (An Ayurvedic preparation used for the treatment of hyperacidity, gastric ulcer and related gastrointestinal disturbances) [1].

In Ayurveda, root of *Operculina turpethum* is used internally to treat fevers, anorexia, edema, anemia, ascites constipation, hepato-splenomegaly, hepatitis, intoxication, abdominal tumors, ulcers, wounds, worm infestation, pruritis and other skin disorders [2].

Root is also administered to treat obesity, hemorrhoids, cough, asthma, [3] dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia, scorpion sting, and snake bites. The paste of root powder of *Operculina turpethum* is used topically to treat vitiligo and other skin disorders, alopecia, cervical lymphadenitis, hemorrhoids, fistulas, ulcers, and chancres [4]. Oil extracted from the root bark of Trivrit is used in skin diseases of a scaly nature [5]. A processed ghee with *Operculina turpethum* or fresh juice of *Operculina turpethum* leaves is dropped into the eyes to treat diseases like corneal opacity or ulcer and conjunctivitis. Root powder of Trivrit mixed with ghee and honey is also used to treat hematemesis, tuberculosis and herpes.

Root bark, root stem and leaves of this herb have high medicinal value [6]. It is one of the plants mentioned in the literature having claims of activity against liver disorders [7]. It also has anthelmintic, expectorant, antipyretic, analgesic, anti-inflammatory and purgative properties. It contains a wide variety of phyto constituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oils [8].

The root bark of *Operculina turpethum* is rich in turpethum resin consisting of 10% 'turpethin' which is a glycoside analogue of Jalapine and Convolvulin and is insoluble in ether, benzene, carbon sulphide and essential oils. Under the action of alkaline bases, turpethin is transformed into turpethic acid, while it gets converted into turpetholic acid, Glucose and fructose in presence of hydrochloric acid. Trivit also contains Turpethinic acids- A, B, C, D, and E [9] some ether soluble resin, volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol and beta- sitosterol Turpethin is mainly responsible for purgative action of Trivit and is an excellent relatively safer substitute for jalap [10].

As a part of our on-going investigation on Bangladeshi plants for phytochemical, biological and pharmacological properties [11-13], we now report on the neuropharmacological potential of the ethanolic extract of the *Operculina turpethum* in mice.

Materials and Methods

Plant material

Operculina turpethum was collected from Gazipur, Dhaka, Bangladesh in December 2006 and identified by the experts of Bangladesh National Herbarium, Dhaka. The specimen sample was preserved in the phytochemical Laboratory (No. PL-24).

Preparation of ethanol extracts

The *Operculina turpethum* plants are carefully cut with the help of a scissor and separated from other parts. About 400 g of the *Operculina turpethum* was dried for 15 days without the direct contact of sunrays. The dried *Operculina turpethum* was extracted with 95% ethanol in a Soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 400°C using buchi rotary evaporator to yield a gummy reddish black colored extract (yield App.6.6%).

Animals

Male and female Swiss mice of either sex (20-25 g body weight)

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Received October 18, 2015; **Accepted** November 26, 2015; **Published** November 28, 2015

Citation: Islam MN, Nyeem MAB, Taher MA, Awal A (2015) Analgesic and CNS Depressant Effect of the Crude Ethanolic Extract of the *Operculina turpethum*. Biosens J 4: 132. doi:10.4172/2090-4967.1000132

bred in the animal house of the Department of Pharmacology, Bangladesh Agricultural University, were collected from the animal resources branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-60%, r.t 23 ± 2°C and 12 h light: dark cycle). The animals were fed with standard diet and water *ab libitum*. The animals were divided in groups of 6, with each group balance for sex and body weight. The institutional animal ethical committee approved the study protocol.

Acute toxicity test

Acute oral toxicity [14] study was performed as per OMCD-423 guidelines. Test animals (n=6) of either sex selected by random sampling technique were for the study. The animals were kept fasting for overnight providing only water, after which the ethanolic extracts were administrated orally at the dose label of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50,300, and 2000 mg/kg body weight

Pentobarbital induced sleeping time test

Pentobarbital sleeping time test carried out by the method of Williamson et al., [15]. The animals were randomly divided into four groups containing five mice each. The test group received *Operculina turpethum* at the dose 250 and 500 mg/kg body weight while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (50 mg/kg i.p) was administrated to each mouse to induce sleep. The animals were observed for latent period (time between pentobarbitone administrations to onset of sleep) and duration of sleep (time between the loss of writhing reflex to recovery of writhing reflex). The test drugs were administrated per oral 30 min before the administration of pentobarbital. The animals were observed for the onset and the duration of sleep, as evidenced by the observation of the loss of writhing reflex.

Open field test

This experiment was carried out as described by Gupta et al. [16]. The animals were divided into control and test groups containing six mice each. The test group received *Operculina turpethum* at the doses of 250 and 500 mg/kg body weight orally where as the control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter divided in to a series of square each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of square visited by the animals was counted for 3 min at 0,30,60,90,120, 180 and 240 min after oral administration of the test drugs.

Hole cross test

The method described by Takagi et al., [17] was adopted for this study. A steel portion was fixes in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the case. The number of a mouse through the hole from one chamber to other was continued for a period of 3 min at 0,30,60,90,120, 180 and 240 min after oral administration of the test drugs.

Antinociceptive activity study

Effect on nociception was studied using acetic acid induced writhing model in mice (Uddin et al.,). After an overnight fast, the animals were divided into control, positive control and test groups containing six mice in each group. The animals were fed with test substance at the doses of 250 and 500 mg/kg body weight, reference drug (Diclofenac sodium) and control vechile 45 min before intraperitonial administration of 0.7% acetic acid. After five minutes interval for proper absorption of acetic acid, the mice were observed for specific contraction of body referred as 'writhing', which is an indication of pain sensation in test animals. A comparison of writhing was made between positive control, control and test sample.

Discussion

Acute toxicity studies indicated that *Operculina turpethum* extract can be used safety and should no mortality up to the dose of 2000 mg/kg body weight. So the extract safe for long term administration.

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 ethanolic extract indicated depressant activity.

In the pentobarbitone induced hypnosis test, *Operculina turpethum* at the dose of 250 mg and 500 mg/kg significantly induced the sleep at an earlier stage and also prolonged the duration of sleeping time in test animals as compared to control (Table 1).

In the open field test, the extracts showed a noticeable decrease in locomotion in the test animals from the second observation period at both dose levels (250 mg and 500 mg/kg body weight). The depressant actions were slowly reduced with the time. The results were dose depended and statistically significant (Table 2).

In the hole cross test, the extract also showed a decrease in locomotion in the test animals from the second observation period at both dose levels (250 mg and 500 mg/kg body weight). The results were dose depended and statistically significant (Table 3).

In acetic acid induced writhing test, the extract significantly and dose dependently suppressed the frequency of acetic acid induced writhing in mice. At the dose 250 mg/kg body weight the extract of *Operculina turpethum* showed 36.3% writhing inhibition whereas at 500 mg/kg body weight produced 49.63% writhing inhibition, which is comparable to a standard drug and all the result are statistically significant ($p<0.001$) (Table 4). Diclofenac-Na, used as the positive control exhibited a writhing inhibition of 48.89% as compared to

Treatment	Dose (mg/kg)	Route of administration	Onset of sleep (min)	Duration of sleep (min)
Control (1% aq. tween 80)	10 ml/kg	p.o.	7. 4 ± 0.41	46.66 ± 1.92
Diazepam	1	i.p.	5.8 ± 0.38[c]	65.33 ± 1.41[a]
Operculina turpethum	250	p.o.	6.03 ± 0.27[c]	60.83 ± 1.57[a]
Operculina turpethum	500	p.o.	5.8 ± 0.47[c]	74.5 ± 1.62[a]
[a]$p<0.001$ [b]$p<0.01$ [c]$p<0.05$ vs. control, Student's *t*-test; values are mean ± S.E ($N=6$).				

Table 1: Effect of *Operculina turpethum* ethanolic extracts on pentobarbitone induced sleeping tine in mice.

Treatment	Dose (mg/kg, p.o.)	Number of Movements						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (1% aq. tween 80)	10 ml/kg	104.4 ± 3.51	92.83 ± 2.05	93.16 ± 2.91	91.33 ± 2.45	78.16 ± 2.53	70 ± 2.44	65.17 ± 2.58
Operculina turpethum	250	101 ± 3.39	76.5 ± 2.74[b]	58.33 ± 3.05[a]	44.83 ± 3.13[a]	41.16 ± 3.12[a]	30.16 ± 1.97[a]	27.83 ± 2.12[a]
Operculina turpethum	500	96.33 ± 4.13	70.66 ± 2.48[a]	42.16 ± 2.02[a]	30 ± 1.94[a]	27.83 ± 2.12[a]	26.75 ± 2.45[a]	23.5 ± 1.41[a]
[a]$p<0.001$ [b]$p<0.01$ [c]$p<0.05$ vs. control, Student's *t*-test; values are mean ± S.E (*N*=6).								

Table 2: Effect of *Operculina turpethum* ethanolic extracts on Open field test in mice.

Treatment	Dose (mg/kg, p.o.)	Number of Movements						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (1% aq. tween 80)	10 ml/kg	8.5 ± 0.65	8.33 ± 0.57	10.41 ± 0.89	9.08 ± 0.93	7.4 ± 0.61	6.83 ± 0.79	6.11 ± 0.65
Operculina turpethum	250	7.01 ± 0.62	5.16 ± 0.7[b]	5.33 ± 0.49[a]	3.55 ± 0.51[a]	3.23 ± 0.47[a]	3.63 ± 0.32[b]	2.86 ± 0.23[a]
Operculina turpethum	500	6.9 ± 0.63	4.96 ± 0.85[b]	4.1 ± 0.48[a]	4 ± 0.3[a]	2.21 ± 0.33[a]	2.16 ± 0.66[a]	1.26 ± 0.39[a]
[a]$p<0.001$ [b]$p<0.01$ [c]$p<0.05$ vs. control, Student's *t*-test; values are mean ± S.E (*N*=6).								

Table 3: Effect of *Operculina turpethum* ethanolic extracts on Hole Cross test in mice.

Treatment	Dose (mg/kg)	Route of administration	Writhing	% of writhing inhibition
Control (1%aq. tween 80)	10 ml/kg	p.o.	22.5 ± 0.88	100
Diclofenac -Na	25	i.p.	11.5 ± 0.92[a]	48.89
Operculina turpethum	250	p.o.	14.33 ± 1.57[a]	36.3
Operculina turpethum	500	p.o.	11.33 ± 1.54[a]	49.63
Administered 45 min before 0.7% acetic acid administration (10 ml/kg, i.p.) Counted foe 15 min, starting 5 min after acetic acid administration; [a]$p<0.001$ [b]$p<0.01$ [c]$p<0.05$ vs. control, Student's *t*-test; values are mean ± S.E (*N*=6).				

Table 4: Effect of *Operculina turpethum* ethanolic extract on acetic acid induced writhing in mice.

control and the result was statistically significant ($p<0.001$).

In vivo methods using intact animals are considered to be the best method for investigation the action of drugs on the CNS. The most impotent step in evaluating drug action on the CNS is to observe the behavior of the test animals. To obtain meaningful results regarding the effect of *Operculina turpethum* flowering tops ethanolic extracts on the CNS in mice, a number of methods namely pentobarbitone –induced hypnosis, open field, hole cross, hole-board and acetic acid induced writhing test were adopted. In the pentobarbitone induced hypnosis test, both extracts, at the doses of 250 mg and 500 mg/kg body weight dose dependently induced sleep at a rapid stage as compared to control, and increased the duration of sleep. Pentobarbitone is a barbiturate type of hypnotic agent, when give at appropriate dose, induces sedation or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors [18]. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolonged the duration of sleep of both. The results obtained in this test, indicate that these extracts might have depressant action on the CNS.

An important step in evaluating drug action on CNS is to observe its effects on locomotor activity of the animal. The extract significantly decreased the locomotor activity as shown by the result of the open field and hole cross test. The locomotor activity lowering effect was evident at the second observation (30 min) and continued up to seventh observation period (240 min) (Table 2). Thus decreased spontaneous motor activity and potentiation of pentobarbitone-induced sleep could be attributed to the CNS depressant activity of the extracts. Moreover, the validation of anxiety was carried out by measuring external signs, through hole-board and evasion tests. In the hole cross experiment, the depressing action of the extract was evident from the second observation period in the test animals at the doses of 250 mg and 500 mg/kg body weight. Maximum depressant effect was observed from third (60 min) to seventh (240 min) observation period. The results were also dose dependent and statically significant.

The ethanolic extract of the *Operculina turpethum*, at the doses of 250 mg and 500 mg/kg body, weight showed significant and dose dependent deceased in the acetic acid induced writhing in mice and the results followed a dose dependent response. Intraperitoneal administration of acetic acid causes algesia by liberating noxious endogenous substances including serotonin, histamine, prostaglandin and bradykinin that sensitize pain nerve endings [19,20]. Of the rostanoids, mainly prostacycline has been held responsible for the causation of pain followed acetic acid administration [21]. It has been suggested that acetic acid stimulates the vanilloid receptor and bradykinin B2 receptor in the pathway comprising sensory afferent C-fibers [22]. The reason behind the observed activity of the ethanolic extract of the *Operculina turpethum*, may be due to the effect of the extract in decreasing the synthesis and /or release of those endogenous substances or depressant effect of the extract on the nerve fibers involved in the pain transmission pathway [23].

Finally overall results obtained from this study showed CNS depressant activity of the *Operculina turpethum* on experimental animal models. Among the extracts the ethanolic extract of *Operculina turpethum* 500 mg/kg dose showed more prominent depressant activity than the 250 mg/kg dose. The mechanism of this depression is not early understood at this point, but it can be assumed that the drug may exert CNS depressant effect by interfering with the function of cortex

Further studies to determining underlying mechanism of action and to isolate the active principle(s) responsible for such activity are also needed.

Acknowledgement

Research work has been performed in Pharmacology Graduate Laboratory, Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202.

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