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Phytochemical Screening and Central Nervous System Activity of Methanolic Extract of *Cucumis sativus* Leaves.

F. Nasrin[1*], S. Ahmad[2], M.T. Hossen[1], Kamrunnahar[3]

[1]*Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh.*

[2] *Department of Electrical & Electronics Engineering, BUET, Dhaka-1000, Bangladesh.*

[3]*Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh.*

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ABSTRACT

Bioactive plant parts remain popular as therapeutics in all age. Their significance is intensifying day by day to provide safe, affordable and obtainable drugs for all classes of people. The present investigation was designed to evaluate the activity of *Cucumis sativus* leaves as analgesic and CNS depressant in model mice along with phytochemical screening. The analgesic activity of the extract was examined using acetic acid-induced writhing test (chemically induced pain) and tail immersion test (thermally induced pain) at the dose of 200 and 300 mg/kg body weight. CNS depressant activity was evaluated by pentobarbitone-induced hypnosis, neuropharmacological experiment by open field and hole cross tests at the doses of 200 and 400 mg/kg body weight. The results of the statistical analysis showed that the plant extract had significant ($P<0.001$) dose dependent CNS depressant and antinociceptive activities. The extract, at the dose of 300mg/kg showed better analgesic effects in tail immersion test, indicating its centrally acting analgesic property and exerted 63.64% inhibition of writhing response in the acetic acid-induced writhing model, which is very close to the reference drug Indomethacin (65.91%). The extract also produced 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 tests. This analgesic and CNS-depressant activity of the extract might be because of presence of different chemical compounds in it. Altogether, these results suggest that the leaves extract of *Cucumis sativus* possesses remarkable CNS depressant and analgesic properties.

Key Words: CNS-depressant, pentobarbitone, Antinociceptive, Centrally acting analgesic, Writhing, Locomotor activity.

INTRODUCTION

Central nervous system (CNS) is made up of the brain and spinal cord which acts as control for regulating physical and mental processes in developed animals. A large number of drugs e.g. sedative, stimulant and analgesic (narcotic) exert their action by inhibiting CNS. These drugs act directly on receptors in various parts of the brain while non –narcotic analgesics inhibit synthesis of pain stimuli (prostaglandins) that activate free nerve endings of peripheral nerves. The sedatives are used to calm anxiety, to induce sleep and manage tremor but analgesics are medications used to relieve pain without reducing the consciousness of the patient. Combination of the sedative with an analgesic often gives a synergistic effect in cases where pain and excitement co-exist. Pain management has become the focus of global scientific research because of its inference in virtually all human and animal diseases[1].The conventional pain management drugs are either possesses well known side and toxic effects or too expensive[2]. As a result, search of new analgesic drugs from traditionally used plant species as pain killers should still be seen as a fruitful explore strategy. The most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources and natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy.

***Corresponding author.**
Fatema Nasrin
Lecturer
Department of Pharmacy,
Southeast University
Banani, Dhaka-1213, Bangladesh.

Cucumis sativus Linn. (Family: Cucurbitaceae), local name shosa is one of such plant that have been part of the traditional healing practices among the indigenous people for centuries. It is widely distributed all over the world particularly in Asia, Africa and South America[3]. Traditionally, this plant is used for headaches; the seeds are cooling and diuretic, the fruit juice of this plant is used as a nutritive and as a demulcent in anti-acne lotions; Juice of leaves used as an emetic in acute indigestion in children. The fruits contain erepsin enzyme, Vitamin B_1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases, and so on. The seeds contain α- and ß-amyrin, sitosterols and cucurbitasides, proteins, fat, minerals and calcium[4] whereas, the leaves contain free cucurbitasides B and C and ferredoxin[5-6]. Identified phytoceuticals from its leaves are acylated flavone C-glycosides such as isovitexin 2”-O-(6’”-(E)-p-coumaroyl) glucoside, isovitexin 2”-O-(6’”-(E)-p-coumaroyl)glucoside-4’-O-glucoside, isovitexin 2”-O-(6’”-(E)-feruloyl) glucoside-4’-O-glucoside and isoscoparin 2”-O-(6’”-(E)-p-coumaroyl) glucoside[4]. Different pharmacological studies revealed the seed extract were effective on control

ling the loss of body weight in diabetic rats[3] and against tapeworms [7]. The fruit extract exhibited significant antidiabetic property[8] antiulcer property[9] and moisturizing property due to the presence of cucurbitacins[10]. It is also rich in flavonoids and tannins that exhibited antioxidant and analgesic property[11]. Cytotoxic and antifungal activities have been reported from leaves extracts[12]. The crude extract of *C. sativus* stems showed the antibacterial activity[13] cytotoxic and antifungal activities[12].Exhaustive literature survey revealed no report of the *C. sativus* leaves on CNS activity; so an effort is made to evaluate scientifically the CNS depressant and analgesic effects of methanolic extract of *Cucumis sativus* leaves in model mice.

EXPERIMENTAL

Identification of plant and preparation of the extract

The leaves of plant *Cucumis sativus* were collected from Gazipur in the month of March, 2012 and identified in the Bangladesh National Herbarium (Voucher Specimen No-34479), Dhaka, Bangladesh. The leaves were first washed with water to remove adhering dirt and then dried at 45°C for 36 hrs in an electric oven, then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powdered material (1kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The total filtrate was concentrated to dryness, *in vacuo* at 40^0C to render the methanol extract (390 g) of brownish red color.

Drugs and chemicals

The active drugs Indomethacin, Nalbufine, Pentobarbitone and Diazepam were the generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Formalin was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Animals

Swiss albino mice of either sex weighing about 23-27 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee[14].

Acute toxicity study

The median lethal dose (LD_{50}) of the extract in mice was estimated by the up and down method[15]. Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

Phytochemical analysis

The extract was subjected to qualitative chemical screening for the identification of bioactive constituents using standard procedures[16].

In vivo analgesic activity

Acetic acid-induced writhing test

The analgesic activity of the methanolic extract of *C. sativus* leaves was evaluated using acetic acid-induced writhing method in mice[17] as intraperitoneal administration of acetic acid in experimental animal causes pain. At first, twenty four animals were divided into four groups with six mice in each.

Group I: Treated with vehicle (1% Tween 80 in water, 10 ml kg^{-1} (p.o.)
Group II: Received Indomethacin (10mg/kg) body weight (p.o.)
Group III and Group IV: Treated with 200 and 300 mg kg^{-1} body weight (p.o.) of the extract, respectively.

The test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% v/v acetic acid but Indomethacin (reference drug) was administered orally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min. Full writhing was not always accomplished by the animal; this incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated groups was compared to that of a control group. Samples having analgesic activity will reduce number of writhes of treated mice. The percent inhibition (% analgesic activity) was calculated by

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

Where, A= Average number of writhing of control per group; B= Average number of writhing of test per group.

Tail immersion method

This study is a thermal method to assess central analgesia according to the method of Luiz[18]. Mice divided as above mentioned groups of six each, were held in position in a suitable restrainer with the tail extending out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. 0.2 ml of 0.9% NaCI solution was administered to control animals, extract in doses of 200 and 300 mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and centrally acting standard drugs (Nalbufin, 10 mg/kg) and then 30, 60 and 90 minutes after the administration. The criterion for analgesia was post-drug latency which was greater than two times the pre-drug average latency as reported by Janssen[19].

CNS Depressant Activity

Pentobarbitone induced sleeping test

This method is done by administering pentobarbitone, a hypnotic drug in experimental animal to evaluate CNS depressant activity (sleep) of the test samples. The animals were randomly divided into four groups consisting of six mice each. The test groups received the extract at the doses of 200 and 400 mg/kg 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 (40 mg/kg, i.p., Sigma Chemicals, USA) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex)[20].

Open Field Test

This experiment was carried out to evaluate the effect of extract on locomotor activity of mice [21]. The animals were divided as mentioned above. The test groups received the extracts at the doses of 200 and 400 mg/kg b. w. orally. The floor of an open field was divided into a series of squares each alternatively colored black and white. The apparatus had area of half square meter and a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min, on 0, 30, 60, 120 and 240 min during the study period.

Hole Cross Test

The method described by Takagi *et al.*,[22] was implemented for this study. A steel partition was fixed 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 partition. The number of passages of mice through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 120 and 240 min after the oral treatment with extract at the doses of 200 and 400 mg/kg.

Statistical analysis

All the values in the test are expressed as mean ± standard deviation (SD). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc Dunnett's tests with the Statistical Package for Social Sciences (SPSS 16.0, USA) program. Dissimilarity between the means of the various groups were measured significant at *P < 0.05, **P < 0.01 and ***P < 0.001.

RESULTS

Phytochemical screening

The methanolic extract of *C. sativus* leaves gave positive result for protein, carbohydrate, tannins, flavonid, saponin, steroid, alkaloid and glycosides (Table 1).

Acute toxicity

Oral administration of graded doses of the methanolic extract of *C. sativus* leaves (500 – 5000 mg/kg, body weight) did not cause any death in the different dose groups. The LD_{50} value for oral administration of the plant extract was found to be greater than 5000 mg/kg.

***In vivo* analgesic activity**

Acetic acid-induced writhing test

Table 2 showed the effects of the extract on acetic acid-induced writhing in mice. The oral administration of both the doses of 200 and 300 $mgkg^{-1}$ of the extract significantly (P<0.01and P<0.001, respectively) inhibited writhing in a dose dependent manner. The exerted inhibition of writhing at dose 300 $mgkg^{-1}$ was very much close to standard non-narcotic drug, indomethacin.

Table 2: Analgesic effect of methanolic extract of *Cucumis sativus* leaves in Acetic acid induced writhing test

Group	Dose (mg/kg b.w.)	No. of writhing	Percent of inhibition (%)
Control	-	11±.894	
Indomethacin	10	3.75±.524***	65.91
Extract	200	6.92±.585**	38.94
Extract	300	4.02±.585***	63.63

*All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P < 0.05, *** P <0.001, significant compared to control.*

Tail immersion test

The results of this study showed that oral administration of the extract significantly amplified the response time of the animals to the thermal stimuli. The extract at 300mg/kg dose significantly increased the reaction time (P<0.001)) from 300 to 90 min after administration and produced very close activity than nalbufin at 4th observation period (Table 3).But dose 100mg/kg increased the reaction time (P<0.001) after 60 min of observation.

Table 3: Analgesic effect of methanolic extract of *C. sativus* leaves. in Tail Immersion test

Treatment	Tail flict time (min) 0	30	60	90
Control (Vehicle)	1.198±0.0204	1.236±0.05465	1.21±0.052	1.225±.048
Control (Nalbufin) 10mg/kg	1.23±0.022*	2.47±0.10***	3.22±0.035***	5.56±0.37***
Extract 200mg/kg	1.20±0.024	1.23±0.11	2.65±0.28***	3.25±0.068***
Extract300mg/kg	1.21±0.012	2.27±0.05***	3.33±0.13***	5.04±0.18***

*All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P < 0.05, *** P <0.001, significant compared to control.*

Table 1: Phytochemical screening of methanolic extract of *Cucumis sativus* Leaves.

Test for	Protein	Carbohydrate	Tannin	Resin	Flavonoid	Saponin	Alkaloid	Steroid	Phenol	glycoside
Inference	++	++	++	-	+	+	+	+	-	++

+: Indicates the presence and -: Indicates the absence of the phytoconstituents.

CNS Depressant Activity

Pentobarbitone induced sleeping

In the pentobarbitone induced hypnosis test, both the doses of the *C. sativus* extract significantly (P<0.001) provoked the sleep at an earlier stage and also lengthened the duration of sleeping time in test animals as compared to control (Table 4).

Table 4: Effect of methanolic extract of *C. sativus* leaves on Pentobarbitone induced sleeping test.

Treatment	On set of sleep(min)	Duration of sleep(min)
Control (Vehicle)	12±1.26	26.83±1.83
Control(Diazepam,1mg/kg)	5.5±1.52***	66.±2.76***
Extract 200mg/kg	9.37±0.765**	37.33±1.86***
Extract400mg/kg	7.13±3.82***	52.67±1.97***

*All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. **P < 0.01, *** P <0.01, significant compared to control.*

Open field test

The extract was evaluated by open field test to determine the decreasing capability of CNS-locomotor activity in animal model. The extract significantly decreased the locomotor activity and this effect of the extract was evident from the initial observation (0 min) period and continued up to 5th observation period (120 min). The results were also dose dependent and statistically significant (Table 5).

Hole cross test

In this test, the extracts showed a decrease in locomotion in the test animals. The number of crossing hole from one chamber to another by mice of the control group was remain almost steady to slight decrease from 0 minutes to 120 minutes (Table 6). But the extract at 200 mg/kg & 400 mg/kg dose showed significant (P<0.001) gradual decrease of movement from 0 to 120 minutes. The extract showed dose dependent activity and maximum depressive effect was observed at fifth (120 min) observation period. Depression produced by dose 400 mg/kg was found more potent than that of standard drug.

DISCUSSION

Present study was conducted to elucidate CNS activity of the methanolic extract of *C. sativus* leaves. The relatively high oral median lethal dose (LD_{50}) in mice suggests that the extract is relatively non toxic when taken orally [23].

Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia as intraperitoneal administration of acetic acid in mice releases prostaglandins and sympathomimetic system mediators like PGE_2 and PGF_{2a} [24] as well as lipoxygenase products[25]. Inhibitory effect on the writhing response of the extract might be due to the presence of analgesic principles which either acts on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances (peripheral mechanism of pain inhibition) [26] or the inhibition at the central level of the transmission of painful message[27].On the other hand, tail immersion tests are most sensitive to centrally acting analgesics indicating narcotic involvement[28] and are highly correlated with human pain relief[29]. In this model, the extract in both the doses increased the pain threshold significantly during the observation period which indicated its CNS depressive effect. Thus, the extracts inhibited both mechanisms of pain, suggesting that the plant extract may act as a centrally acting analgesic. This analgesic activity might be due to the presence of alkaloid, glycoside and steroids in the extract[30].

CNS depressing agents are gaining importance in treating mental disorders like anxiety, dizziness and restlessness. The methanolic extract of *C. sativus* exhibited its CNS depressive activity in the entire conducted test. Pentobarbitone, a barbiturate type of hypnotic agent, when given at appropriate dose, induces sedation or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors[31]. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. The results ob-

Table 5: CNS depressant activity of methanolic extract of *C. sativus* leaves on Open Field test in mice.

Treatment	No. of movement				
	0 min	30 min	60 min	90 min	120 min
Control(Vehicle)	146.67±2.34	116±2.9	127±2.07	107±1.72	100.5±1.52
Control(Diazepam)1mg/kg	124±2.61***	86.33±2.66***	56.5±2.35***	25.83±2.48***	8.67±1.51***
Extract 200mg/kg	128.5±2.07**	116.5±1.52***	83±2.58***	57±1.41***	25.7±1.83***
Extract400mg/kg	127.6±2.34***	90.5±1.37***	42±2.09***	24.67±2.07***	14.17±1.93***

*All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. **P < 0.01, *** P <0.01, significant compared to control.*

Table 6: CNS depressant effect of methanolic extract of *C. sativus* leaves on Hole cross test in mice

Treatment	No. of movement				
	0 min	30 min	60 min	90 min	120 min
Control(Vehicle)	32.67±1.37	29±1.09	27.33±1.03	23.33±1.86	21.17±1.60
Control (Diazepam)1mg/kg	28.67±1.03***	18.83±1.33***	12.171±1.52***	11.5±1.52***	5.33±1.03***
Extract 200mg/kg	30.83±1.47	23.5±1.05***	15.67±0.816***	13.17±1.47***	7.67±1.03***
Extract400mg/kg	30±1.26**	12.17±2.56***	4.33±2.42***	2.83±1.72***	1.83±1.17***

*All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. **P < 0.01, *** P <0.01, significant compared to control*

tained in this test, indicated that the extract might have depressant action on the CNS. However, this test could provide false results, because some substances which interference with the biotransformation of pentobarbital, as Cytochrome P450 complex, could induce apparently the identical effects of the depressor CNS drugs [32]. So we carried out locomotion test to confirm the CNS activity of the extract with same doses. Increased locomotor activity (by excitatory neurotransmitter) considered as alertness and decrease in locomotor activity (due to inhibitory neurotransmitter) indicated sedative effect[33]. Our extracts had decreased locomotor activity indicating its CNS depressant activity. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS and different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through $GABA_A$. Therefore it is possible that extract may acts either by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extract[34]. Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders[35] and many flavonoids and neuroactive steroids were ligand capable for the $GABA_A$ receptors in the central nervous system; which led to the assume that they can act as benzodiazepine like molecules[33].Our phytochemical screening also showed the presence of alkaloids, flavonoids, tannin and steroids in the extract. So might be this phytoconstituents are responsible for its CNS depressant activity.

CONCLUSION

Based on the results of the present study, we conclude that the methanolic extract of *Cucumis sativus* leaves possesses remarkable analgesic and mild CNS depressant activity in dose dependent manner. However, further studies are indispensable to examine underlying mechanisms of such CNS effects and to isolate the active compounds responsible for these pharmacological activities.

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