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Assessment of Neuropharmacological and Analgesic Potentials of *Geodorum densiflorum* (Lam.) Schltr Root Extracts in Experimental Animals

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Abstract

The aim of the present study was to investigate the neuropharmacological and analgesic properties of n-hexane and dichloromethane soluble extract of root of terrestrial orchid, *Geodorum densiflorum* (Lam.) Schltr (Family: Orchidaceae) using *in vivo* models. Its sedative activity was assessed by hole cross and open field tests and anxiolytic property was evaluated by hole board test. Peripheral analgesic activity was also investigated by conducting acetic acid induced writhing test. Both extracts at doses of 200mg/kg and 400mg/kg, p.o. showed statistically significant ($p<0.001$ and $p<0.01$) suppression of locomotor and exploratory behaviors of mice compared to that of diazepam in the hole cross test. In the open field test, all test samples except 400mg/kg dose of dichloromethane soluble root extract showed same results that were observed in the hole cross test. A significant ($p<0.001$) dose dependent anxiolytic activity was also observed in both extracts as confirmed by the hole board test. At higher dose (400mg/kg, p.o.), both extracts exhibited significantly ($p<0.001$) moderate analgesic property compared to that of diclofenac sodium. The results of this study disclosed a way of getting new therapeutic agent(s) from this plant against sedative, anxiolytic and related central nervous system disorders.

KEY WORDS: GEODORUM DENSIFLORUM, SEDATIVE, ANXIOLYTIC, ANALGESIC

Introduction

Orchids, one of the largest plant families available almost every habitat apart from glaciers, are economically important in horticulture as ornamental plants but medicinal uses of these have not been extensively studied and are almost neglected [1, 2]. But recently it has been reported that alkaloids, flavonoids, phenanthrenes, dibenzyl derivatives and terpenoids are present in orchids and these molecules possess diuretic, anti-inflammatory, anti-rheumatic, anticarcinogenic, hypoglycemic, anticonvulsive, antimicrobial, neuroprotective and antiviral properties [2]. As part of exploring natural sources of potential therapeutic agents, our endeavor was to study on an endangered terrestrial orchid named *Geodorum densiflorum* (Lam.) Schltr of Orchidaceae family. It is widely distributed throughout tropical Asia and Australasia [3]. It is locally known as 'Shankhamul' in Bangladesh which has long been used as a medicine to regularize menstrual cycle [2, 4].

Preliminary phytochemical screening of root of *G. densiflorum* revealed the presence of alkaloids, carbohydrates, phenols, glycosides, steroids and flavonoids. It also showed that the root part of this plant have potential antioxidant and antibacterial activities [4]. From the literature review, it is proved that the plants under Orchidaceae family are important source of biologically active compounds. However, no major investigative reports on their pharmacological properties have still been reported on the root part of this plant. That's why, the study was designed to explore the neuropharmacological (especially sedative and anxiolytic potential) and anti-nociceptive effects of *G. densiflorum* by using different experimental mice models.

Materials and Methods

2.1 Collection of plant materials

Fresh roots of the plant *G. densiflorum* was collected from the area of Savar, Dhaka district on October, 2011 and taxonomically identified by Bangladesh National Herbarium, Dhaka, Bangladesh. A sample (Accession Number DACB 34377) of this plant has been deposited in the Bangladesh National Herbarium as future reference.

2.2 Preparation of extracts

Collected roots of the plant were dried after cutting and slicing in the sun light for about two weeks. The dried roots were ground to coarse powder with a mechanical grinder and were stored in an airtight container. Powdered plant material (500 gm) was divided equally to soak in *n*-hexane and dichloromethane following occasional shaking and stirring for 7 days. The extracts were filtered through cotton and Whatman's filter paper No1. The filtrates were then concentrated using rotary evaporator (IKA, Germany) at 50°C and 50 rpm under reduced pressure. Residues obtained were kept in a beaker end air dried until complete evaporation of solvents. Finally, the dry extracts of *n*-hexane and dichloromethane were kept in a refrigerator (4°C) for further use.

2.3 Experimental models

Swiss Albino mice of either sex, 3-4 weeks of age, weighing between 20-25gm, were collected from the animal research branch of International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed in a group of six in polypropylene cages having a dimension of 28×22×13cm and maintained under standard environmental conditions: 24.0±1.0°C temperature, 55-60% relative humidity and 12hrs light/12hrs dark cycles with free access to food and water *ad libitum*. The experimental protocols were approved by the Animal Experimentation Ethics Committee (AEEC) of East West University, Dhaka, Bangladesh.

The animals were divided into negative control, positive control and test groups with five mice in each group. The mice of test groups received test samples at doses of 200mg/kg and 400mg/kg body weight. Mice of the positive control group of acetic acid-induced writhing test received diclofenac sodium (Square pharmaceuticals Ltd., Bangladesh)

at the dose of 10mg/kg body weight and for neuropharmacological tests, diazepam (Square pharmaceuticals Ltd., Bangladesh) was given to mice of positive control group at the dose of 1mg/kg body weight. The control group was treated with 1% Tween-80 in normal saline (Beximco Infusions Ltd., Bangladesh) at the dose of 10ml/kg body weight. All experiments took place in quiet laboratory environment at the same period of the day to reduce the influence of diurnal variation.

2.4 Hole cross test

The method was carried out as described by Takagi et al. (1971) [5] to evaluate sedative property of the extracts by measuring locomotor activity. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14cm. A hole of 3cm diameter was made at a height of 7.5cm in the centre of the cage. The numbers of passages of mice through the hole from one chamber to other were counted for a period of 3min at 0, 30, 60, 90 and 120 min after oral administration of the test samples and the standard drug.

2.5 Open field test

The method described by Gupta et al. (1971) [6] was adopted to evaluate the sedative property of the extracts. The apparatus was constructed of white plywood and measured 72cm × 72cm with a 40 cm wall. The floor of an open field of 0.5m^2 was divided into a series of squares each alternatively colored black and white. The frequency and duration of different line crossing by the animals were counted for 3min at 0, 30, 60, 90, and 120 min after oral administration of test samples and the standard.

2.6 Hole board test

The apparatus used in the hole-board test consisted of a wooden chamber measuring 40cm by 40cm with 16 evenly spaced holes (diameter 3cm) on the floor [7] and elevated to a height of 25cm from the ground so that the mice could peep through the holes. Thirty minutes after oral administration of the extract and the standard, each mouse was placed on the center of the board and the number of times the mice dipped their heads into the holes at the level of their eyes during a five-minute trial period was counted using a tally counter.

2.7 Acetic acid induced writhing test

The analgesic activity of plant extracts was studied using acetic acid-induced writhing model in mice [8, 9]. Test samples and the standard drug were administered orally 30min before intraperitoneal administration of 0.7% acetic acid (Merck, Germany). Due to intraperitoneal administration of acetic acid, pain sensation was generated to the experimental animals and they squirmed (i.e. abdominal stretching and contraction) their bodies at a regular interval termed as "writhing". After an interval of 5min of intraperitoneal administration, the mice were observed for writhing for the next 15minutes which were recorded. Analgesic agents reduce the pain sensation which could be observed from reduced number of writhing compared to control group. As the negative control group contains no known compounds with analgesic properties (only saline and Tween 80 present), this group's response (writhing) was considered as maximum and the percentage of writhing inhibition was minimum. For other groups percentage of writhing and percentage of writhing inhibition were calculated based on the following formulas:

a) Writhing (%) = ((Wc - Wt)/Wc) x 100%

b) Writhing inhibition (%) = Writhing of control group (%) - Writhing of test group (%)

Where, Wc = Mean writhing of the control group and Wt = Mean writhing of the test group.

2.8 Statistical analyses

Experimental data were analyzed applying one

way analysis of variance (ANOVA) and repeated measures ANOVA by using the statistical package of social sciences (SPSS) version 15.00 for windows. A post hoc comparison was made using Dunnett *t*-test (2 sided). Values were expressed as mean ± standard error mean (SEM). A *p* value of less than 0.05 was taken to be statistically significant and $p<0.001$ was taken to be highly significant.

Results

3.1 Hole-cross test

In this experiment, *n*-hexane extract at 400mg/kg body weight and dichoromethane extract at both doses of 200mg/kg and 400mg/kg body weight decreased locomotor activity from the first observation period in comparison to that in the negative control group whereas *n*-hexane extract at 200mg/kg body weight and diazepam exhibited this activity from second and third observation period respectively. Locomotor activities of mice of three experimental groups [*n*-hexane at dose of 400mg/kg body weight and both doses (200mg/kg and 400mg/kg body weight) of dichloromethane] sharply declined in the second observation period and then steadily dropped off up to the fifth observation period. However, the 200mg/kg body weight dose of *n*-hexane extract and the positive control group showed reduction in the locomotor activity in the third observation period. Data also revealed that test samples of plant extracts, more specifically the dichloromethane extract at 400mg/kg body weight showed earlier decrease of locomotor activity in mice than that of the positive control group.

See Table 1.

3.2 Open field test

The *n*-hexane treated group showed a noticeable decrease of locomotor (Table 2) and exploration (Table 3) activities from the second observation period (30min) to onwards at both dose levels (200mg/kg and 400mg/kg body weight) in comparison to that of the positive control. In addition, a significant reduction of locomotor and exploration activities were also observed in mice treating with 200mg/kg body weight dose of dichloromethane extract. But, 400mg/kg body weight dose of the dichloromethane extract increased the locomotor and exploration activities in mice compared to the control group.

See Table 2.

See Table 3.

3.3 Hole board test

Both *n*-hexane and dichloromethane extracts of *G. densiflorum* showed significant ($p<0.001$) and dose dependent increase in the number of head dipping in mice (Table 4) and the results were comparable to that of diazepam.

Groups	Dose and route	Number of head dipping (Mean ± SEM)
Control	10ml/kg, P.O.	64.6±1.78
Positive control	1.0mg/kg, P.O.	35.4±1.40*
n-hexane extract	200mg/kg, P.O.	47.6±1.89*
	400mg/kg, P.O.	70.2±2.22
Dichloromethane extract	200mg/kg, P.O.	19.6±1.44*
	400mg/kg, P.O.	51.2±1.50*

P.O. = per oral; Positive control = Diazepam; n=5; *$p<0.001$ against negative control group by Dunnett's *t* test

Table 4: Effects of root extracts on the number of head dipping in the hole board test

3.4 Acetic acid induced writhing test

Both the *n*-hexane and the dichloromethane extracts showed highly significant ($p<0.001$) inhibition of writhing induced by the injection of 0.7% acetic acid in a dose dependent manner. The *n*-hexane extract exhibited 25.96% and 75.96% of inhibition of writhing whereas 22.12% and 72.12% inhibition of writhing were observed when treated with the dichloromethane extract at doses of 200mg/kg and 400mg/kg body weight, respectively. At the dose of 400mg/kg body weight, both the *n*-hexane and the dichloromethane extracts showed almost same inhibition of writhing that were com-

parable to that of diclofenac sodium (86.54%).

See Table 5.

Discussion

The animal model is considered as one of the most widely validated tests of assaying sedative and anxiolytic substances [10]. Therefore, the hole cross test and the open field test were carried out to assess the sedative activity while, the hole board test was employed to investigate any potential anxiolytic activity of the root extracts of *G. densiflorum.*

The locomotor activity is considered as an index of alertness, and a decrease in this activity indicates a sedative effect [11]. In the hole cross test, all the test samples decreased the movement of the mice across the hole with time that was comparable to the values of the standard group. It was also observed from this experiment that 400mg/kg dose of dichloromethane soluble root extract showed rapid reduction of locomotor activity in mice. But in the open field test, the same dose demonstrated higher number of line crossing and centre square entry compared to the negative control group. The possible reason of this contradiction is that both excitation and inhibition in the central nervous system are regulated by GABA, inhibitory neurotransmitter, through the interaction with two different types of receptor [12]. $GABA_A$ receptors (ligand-gated ion channels) convey GABA's effect on fast synaptic transmission [13] through opening the chloride ion channel. This allows chloride ions (Cl^-) to enter the cell thereby slowing down the neuronal activity through hyperpolarization of the cell membrane potential [12]. On the other hand, $GABA_B$ receptors mediating GABA's action modulates the action of certain guanine nucleotide binding proteins (G proteins) [14] which alter the signal transduction and gene expression, or open ion channels that are dependent on the G-protein subunit activities [15]. Moreover, there is extensive heterogeneity in the structure of the $GABA_A$ receptor members of the ligand-gated superfamily [12]. Therefore, both excitatory and inhibitory activities can be obtained from the same dose of the test sample. Other test samples except 400mg/kg dose of dichloromethane soluble root extract exhibited decrease in locomotor and exploratory behaviors of mice compared to the negative control group in open the field test. So, it can be claimed that the extracts were able to induce a depressant effect.

The head-dipping behaviour in the hole board model is sensitive to the emotional state of animals and suggests that the expression of the anxiolytic state in animals may be reflected by an increase in head-dipping behavior [16]. All the test samples except 200mg/kg dose of dichloromethane soluble root extract showed significant increase in the number of head dipping which is comparable to that of the standard group. The result indicated that the *n*-hexane extract at both low & high doses and the dichloromethane extract at high dose exhibited an anxiolytic activity.

Phytochemical screening of the root of this plant [4] revealed the presence of high amount of flavonoids. Recent study on flavonoids demonstrated that these have anxiolytic, sedative and anticonvulsant activities. Although their actions in the central nervous system occur through a variety of interactions with different receptors and signaling pathways, it is believed that some of these effects are mediated by inotropic GABA, in particular $GABA_A$ receptors [17]. Thus the observed sedative and anxiolytic effects of test samples might be the result of interaction of flavonoids with the GABA receptor complex in the central nervous system.

Acetic acid induced writhing in mice is a model of visceral pain which is highly sensitive and useful for screening the activities of peripherally acting analgesic drugs [18]. This model is not only simple and reliable but also amenable to rapid evaluation of peripheral analgesic action. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that agitate pain at nerve endings. Acetic acid is also known to increase PGE_1 and PGE_2 peripherally [19]. The *n*-hexane and the Dichloromethane soluble root

extract of plant showed dose dependent antinociception against chemically induced pain. At the higher dose (400mg/kg), both test samples possibly block the release of endogenous substances at the nerve endings like NSAIDs. Inhibition of writhing also indicated that synthesis and/or action of prostaglandin was also prohibited by the test samples. One or more of the bioactive compound(s) in steroid, alkaloid, glycoside, flavonoid, tannin and carbohydrate groups [4] might be responsible for pain relieving activity.

Conclusion

Based on the results of the present study, it can be proposed that the root part of *G. densiflorum* has strong sedative and anxiolytic effects. It also showed moderate antinociceptive activity. However, further studies are required to understand the underlying mechanism of the observed activities and to isolate and characterize active phytochemical ingredient(s) responsible for these pharmacological activities.

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References

1. Dash P.K., Sahoo S., Bal S. Ethnobotanical studies on orchids of Niyamgiri Hill Ranges, Orissa, India. Ethnobotanical Leaflats 2008; 12: 70-78.
2. Yonzone R., Kamran A., Bhuje R.B. Orchids in Ethnobotany. Proceeding of 18th annual conference of IAAT and international Seminar on multidisciplinary approaches in angiosperm systematic, Nadia, India, 2012; p. 661-669.
3. Yukawa T., Kawaguchi D., Mukai A., Komaki Y. Discovery of Geodorum densiflorum (Orchidaceae) on the Ogasawara (Bonin) Islands: A case of ongoing colonisation subsequent to long-distance dispersal. Bull Natl Mus Nat Sci 2012; Ser.B, 38(3): 131–137.
4. Habib M.R., Rana M.S., Hasan M.R., et al. Antioxidant, cytotoxicity and antibacterial potential of different extract of Geodorum densiflorum (Lam.) Schltr. root. J Herb Med Toxicol 2011; 5 (1): 63-70.
5. Takagi K, Watanabe M, Saito H. Studies on the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethane and its acyl esters on the central nervous system. Jpn J Pharmacol 1971; 21: 797-810.
6. Gupta BD, Dandiya PC and Gupta ML. A psychopharmacological analysis of behavior in rats. Jpn J Pharmacol 1971; 21: 293-298.
7. Perez GRM, Perez IJA, Garcia D, Sossa MH. Neuropharmacological activity of Solanum nigrum fruit. J Ethnopharmacol 1998; 62: 43-48.
8. Ahmed, F., Selim M.S.T, Das A.K, Choudhuri M.S.K. Anti-inflammatory and antinociceptive activities of Lippia nodiflora Linn. Pharmazie 2004; 59: 329-333.
9. Hasan SMR, Jamila M, Majumder MM, et al. Analgesic and antioxidant activity of the hydromethanol extract of Mikania scandens (L.) Willd. leaves. Am J Pharmacol Toxicol. 2009; 4(1): 1-7.
10. Yogesh C.Y., Avijeet J., Lokesh D. A review: neuropharmacological screening techniques for pharmaceuticals. Int J Pharm Pharm Sci 2010; Vol 2(2): 10-14.
11. Thakur V.D, Mengi S.A. Neuropharmacological profile of Eclipta alba L. Hassk. J. Ethnopharmacol 2005; 102: 23–31.
12. John F.T., James C., John K. Mechanism of action of anxiolytics. In: Kenneth LD., Dennis C., Joseph T.C., Charles N, eds. Neuropsychopharmacology: the fifth generation of progress, 5th ed. Lippincott Williams & Wilkins, 2002; 993-1006.
13. Sieghart W. Structure and pharmacology of ã-aminobutyric acid: a receptor subtypes. Pharmacol Rev, 1995; 47: 181–234.
14. Kaupmann K, Nuggel K, Heid J, et al. Expression cloning of GABAB receptors uncovers similarity to metabotropic glutamate receptors. Nature, 1997; 386: 239–246.
15. Wess J. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. FASEB J 1997; 11: 346–354.
16. Takeda H, Tsuji M, Matsuyima T. Changes in the head-dipping behaviour in the hole-board test reflects the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol. 1998; 350(1): 21-29.
17. Jane R.H., Mary C., Graham A.R.J. Flavonoid modulation of GABAA receptors. Br. J. Pharmacol. 2011; 163(2): 234–245.
18. Hasan SMR, Hossain MM, Akter R. Analgesic activity of the different fractions of the aerial parts of Commelina benghalensis Linn. Int. J. of Pharmacol. 2010; 6(1): 63-67.
19. Kumar V, Singh PN, Bhattacharya SK. Anti- inflammatory and analgesic activity of Indian Hypericum perforatum L. Indian J Exp Biol. 2001;19:339–43.

Groups	Dose and route	Movement (number) as Mean ± SEM				
		0 min	30 min	60 min	90 min	120 min
Control	10ml/kg, P.O.	12.4±0.68	11.6±0.81	11.0±0.45	9.6±0.51	9.2±0.37
Positive control	1.0mg/kg, P.O.	12.6±0.93	11.0±0.77	5.6±0.24*	4.4±0.24*	2.2±0.37*
n-hexane extract	200mg/kg, P.O.	10.4±0.68	8.0±0.95**	5.2±0.66*	4.4±0.51*	3.4±0.24*
	400mg/kg, P.O.	9.2±0.37**	4.0±0.71*	4.2±0.58*	3.8±0.58*	3.4±0.24*
Dichloromethane extract	200mg/kg, P.O.	9.2±0.37**	5.0±0.71*	5.8±0.66*	3.6±0.60*	4.0±0.45*
	400mg/kg, P.O.	7.2±0.58*	2.0±0.40*	3.6±0.68*	2.0±0.55*	3.0±0.45*

P.O. = per oral; Positive control=Diazepam; n=5; *p<0.001, **p<0.01 against negative control group by Dunnett's t test

Table 1: Effects of root extracts on the movement of mice in the hole cross test

Groups	Dose and route	Line crossing (number) as Mean ± SEM				
		0 min	30 min	60 min	90 min	120 min
Control	10ml/kg, P.O.	189.2 ±4.15	81.4±1.57	76.8±2.35	52.0±3.21	45.0±3.03
Positive control	1.0mg/kg, P.O.	148.6±3.06*	185.8±3.31*	78.4±2.36	50.6±0.63	54.6±1.63**
n-hexane extract	200mg/kg, P.O.	105±2.02*	57.6±1.91*	57.8±2.58*	11.6±0.81*	19.4±1.03*
	400mg/kg, P.O.	140±2.62*	74.2±2.31	74.8±3.58	9.4±0.68*	8.8±0.73*
Dichloromethane extract	200mg/kg, P.O.	94.4±2.60*	2.8±0.37*	18.4±1.69*	19.2±1.91*	12.2±1.39*
	400mg/kg, P.O.	152.2±2.87*	147.6±3.54*	89.8±3.18**	54.2±3.28	74.6±2.38*

P.O. = per oral; Positive control=Diazepam; n=5; *p<0.001, **p<0.01 against negative control group by Dunnett's t test

Table 2: Effects of root extracts on the number of line crossing in the open field test

Groups	Dose and route	Centre Square Entry (number) as Mean ± SEM				
		0 min	30 min	60 min	90 min	120 min
Control	10ml/kg, P.O.	5.0±0.71	3.2±0.97	0.8±0.2	0.2±0.20	0.2±0.20
Positive control	1.0mg/kg, P.O.	5.6±0.93	4.4±0.75	1.8±0.58	0.4±0.24	0.6±0.40
n-hexane Extract	200mg/kg, P.O.	1.0±0.32*	0.0±0.00**	0.4±0.25	0.0±0.00	0.0±0.00
	400mg/kg, P.O.	3.2±0.58	2.4±0.68	1.0±0.32	0.0±0.00	0.2±0.20
Dichloromethane extract	200mg/kg, P.O.	1.2±0.20*	0.0±0.00**	0.0±0.00	0.4±0.25	0.4±0.25
	400mg/kg, P.O.	2.0±0.55**	2.8±0.58	2.0±0.55	1.4±0.40**	2.0±0.63**

P.O. = per oral; Positive control=Diazepam; n=5; *p<0.001, *p<0.01 against negative control group by Dunnett's t test

Table 3: Effects of root extracts on the number of centre square entry in the open field test

Groups	Dose and route	Writhing (Mean ± SEM)	% Writhing	% Inhibition of writhing
Control	10ml/kg, P.O.	20.8±0.73	100	0.000
Positive control	1.0mg/kg, P.O.	2.8±0.58*	13.46	86.54
n-hexane extract	200mg/kg, P.O.	15.4±0.51*	74.04	25.96
	400mg/kg, P.O.	5±0.45*	24.04	75.96
Dichloromethane extract	200mg/kg, P.O.	16.2±0.66*	77.88	22.12
	400mg/kg, P.O.	5.8±0.86*	27.88	72.12

P.O. = per oral; Positive control=Diazepam; n=5; *p<0.001 against negative control by Dunnett's t test

Table 5: Analgesic activity of extracts of *G. densiflorum* root