Sedative and Anxiolytic Activities of *Geodorum densiflorum* Roots in Swiss Albino Mice

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Abstract: Fresh root of *Geodorum densiflorum* (Lam.) (Orchidaceae) has applications in regularizing menstrual cycle and as topical aids in insect bites and wounds. The tuber extracts of some plants belonging to *Geodorum* have folkloric reputation in the management of transient anxiety. The current study was undertaken to investigate the sedative/ anxiolytic effects of *G. densiflorum* root extracts using rodent behavioral models, such as open field, hole cross, thiopental sodium-induced hypnosis and elevated plus maze test. Present data shows that the organic extracts of *G. densiflorum* root increase the thiopental sodium induced sleeping time and reduce the time for the onset of sleep. A statistically significant reduction in motor activity and exploratory behavior were observed in the open field and hole cross tests. The results of the current studies provide scientific evidence for its uses in traditional medicines as sedative and anxiolytic agents.

Keywords: Neuropharmacology, locomotor activity, thiopental sodium, transient anxiety.

1. INTRODUCTION

Anxiety and depressive disorders are considered as the commonest psychiatric disease burden striking between 10-30% of the general population [1-3]. Currently available medicines used in the treatment of such neurological problems have potential adverse effects and compatibility issues which provide us continuous impetus to explore alternative medicines and medicinal plants that have ethnopharmacological reputation [4]. The plant, *G. densiflorum*, locally known as Shankhyamul or Shankhyamoni, is an endangered terrestrial ground gem orchid that is distributed in the humid tropical forests of the east and west Ghats of south India and also in Bangladesh [5,6]. Although traditional uses vary among the local practitioners, fresh root paste of the orchid is administered orally on an empty stomach to regularize menstrual cycle in women and applied externally for insect bite and wounds [7]. The tuber extract of some plants belonging to *Geodorum* have folkloric reputation in the management of transient anxiety. A comprehensive literature survey shows that no chemical or biological studies on *G. densiflorum* root has so far been conducted to provide ample data in favor of the reported traditional uses. In our bioassay-guided approach for isolating compounds from the root parts of *G. densiflorum*, we have evaluated various activities in rodent models and herein, report the results of our preliminary investigations.

2. MATERIALS AND METHODS

2.1. Plant Materials

The roots of *G. densiflorum* were collected from a local market of Savar, Dhaka, Bangladesh in May 2009. The plant was cultivated from these roots for identification purpose at the Department of Pharmacy, Jahangirnagar University, Dhaka. The plant was identified by Dr Mahbuba Khanam, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh, where a voucher specimen representing the collection has been deposited (Accession Number- DACB 34377). Standard drugs used in the current study, diazepam and thiopental sodium were obtained from Square Pharmaceuticals Ltd. and Gonoshasthaya Pharmaceuticals Ltd., Bangladesh. All the solvents and reagents were purchased from Sigma-Aldrich and were of highest available purity and quality.

2.2. Extraction

The air-dried roots (178.0 g) were coarsely powdered with a mechanical grinder and extracted with methanol (500 mL) in a Soxhlet apparatus. The crude extract was concentrated *in vacuo* using a rotary evaporator (4.1 g) and was successively partitioned in

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petroleum ether (100 mL) and chloroform (100 mL) using modified Kupchan's partitioning scheme [8]. These partitionates along with the residual methanol extract were evaporated to dryness under reduced temperature and pressure and were used for further investigation.

2.3. Animals

Swiss Albino mice (weighing 20-25 g) of either sex, obtained from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Dhaka, Bangladesh (ICDDR, B), were used for investigating the neuro-pharmacological effects. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0 ± 2.0°C and 12 h light:12 h dark cycle) and fed with a standard diet and water *ad libitum*. The guidelines of the Animal Experimentation Ethics Committee, ICDDR, B were followed in all animal experiments. For each experiment (described below), the animals were randomly divided into positive, negative controls and test groups (n = 5 per group). The test groups received three different partitionates of *G. densiflorum* roots at 400 mg/kg body weight (b.w.) p.o. whereas the negative and positive controls had vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) and a standard drug, diazepam (1 mg/kg i.p.), respectively.

2.4. Thiopental Sodium Induced Sleeping Time

The thiopental sodium-induced hypnosis test was conducted as described previously [9,10]. Briefly, 30 min after administration of the test and control drugs, thiopental sodium (40 mg/kg b.w.) was administered to each mouse for inducing sleep. The animals were observed for the latent period (time between the thiopental administration and the onset of sleep) and the duration of sleep (time between the loss and recovery of the righting reflex).

2.5. Open Field Test

The experiment was carried out by following previously published protocol [11]. Here, the animals were placed on the floor of a steel cage (100 cm×100 cm×40 cm h) divided into a series of squares that were alternatively colored black and white. The number of squares traveled by the mice was counted for experimental and control groups for 3 min at 0, 30, 60, 90 and 120 min during the study period.

2.6. Hole Cross Test

This test was performed as described [12]. A steel partition was fixed in the middle of a cage (30 × 20 × 14 cm h). A hole of 3 cm in diameter was made in the steel partition at a height of 7.5 cm above the floor of the cage. The mice were placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs and controls.

2.7. Elevated Plus-Maze (EPM) Test

The plus-shaped maze consisted of two open (5 × 10 cm) and two enclosed arms (5 × 10 × l5 cm) with an open roof and a 5 × 5 cm platform arranged such that the two arms of each type were opposite to each other. The apparatus was elevated 40 cm above the floor. The open arm edges were 0.5 cm in height to keep the mice from falling and the closed-arm edges were 15 cm in height. The maze floor and walls were constructed from dark opaque wood. Sixty minutes after administration of the test drugs, each mouse was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, were recorded [13]. Entry into an arm was defined as the point when the animal placed all four paws onto the arm. The

Table 1: Effects of Different Fractions of *G. densiflorum* on Thiopental Sodium-Induced Hypnosis in Mice

Group	Onset of sleep (min)	Duration of sleeping (min)
Negative control (vehicle)	40.20 ± 1.65	47.00 ± 0.949
Positive control (diazepam)	14.80 ± 0.86*	149.80 ± 3.44*
G. densiflorum (methanol extract)	12.40 ± 0.51*	121.40 ± 15.46*
G. densiflorum (chloroform soluble)	15.00 ± 0.55*	114.80 ± 5.89*
G. densiflorum (pet ether soluble)	10.60 ± 0.51*	127.20 ± 9.03*

All values are expressed as mean ± S.D. (n=5); One-way Analysis of Variance (ANOVA) followed by Dunnet's test. *P <0.05, significant compared to control.

procedure was conducted in a sound attenuated room and observations were made from an adjacent corner.

3. RESULTS

All partitionates of *G. densiflorum* were evaluated for their possible sedative effects in a barbiturate-induced hypnosis model. The extracts at 400 mg/kg b.w. demonstrated prolonged durations of sodium thiopental-induced sleeping and shorter onsets (Table **1**). The depression intensities in the treatment groups were comparable to the standard drug diazepam with pet ether soluble fraction showing the highest activity (Table **1**). Consistent with results of barbiturate-induced hypnosis, the open field tests revealed that the organic fractions of *G. densiflorum* roots (400 mg/kg b.w.) had a statistically significant decrease in the locomotor activity in an order of chloroform- > pet ether- > methanol-solubles (Figure **1**). In addition, the extracts also demonstrated a decrease ($P < 0.05$) in locomotion of mice as evident by the reduced number of holes crossed from one chamber to the other (Figure **2**). The maximum suppression of locomotor activity that was comparable to the reference drug diazepam was observed with the methanol-fraction. The elevated plus-maze test showed no significant effect by the chloroform-soluble and in contrast, demonstrated an apparent anxiogenic response by the pet-ether/ methanol-soluble with a reduction in the percentage of entries and the time spent by the mice at the open arms compared to the controls (Table **2**).

4. DISCUSSION

In the current study, neuropharmacological effects of *G. densiflorum* roots were evaluated using a number of non-invasive locomotor activity tests including barbiturate-induced hypnosis and open field, hole cross, elevated plus-maze tests. Our preliminary results show that the organic soluble fractions of *G. densiflorum* roots have produced variable degrees of alterations in general behavioral pattern with a reduction in spontaneous mobility and stimulation of thiopental sodium-induced sleeping time in mice.

In vertebrates, central nervous system (CNS) depressants exert their effects through a number of biochemical mechanisms, including excitation of gamma-aminobutyric acid (GABA) or opioid activity, and inhibition of glutamatergic or catecholaminergic activity. Both thiopental sodium (barbiturate) and diazepam (benzodiazepine), used in the current study, produce sedative-hypnotic effects due to their interaction with $GABA_A$ receptors (also known as ionotropic receptors) that potentiates GABA activity [14,15]. In addition, thiopental sodium can block the excitatory AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor, a subtype of glutamate receptors. All of these molecular actions lead to decreased neuronal activity. The enhancement of barbital hypnosis (characterized by a reduced time for the onset and/or prolonged duration of sleep) can be a useful index of CNS depressant activity [16]. In this study, the reduction in time for the onset of sleep and

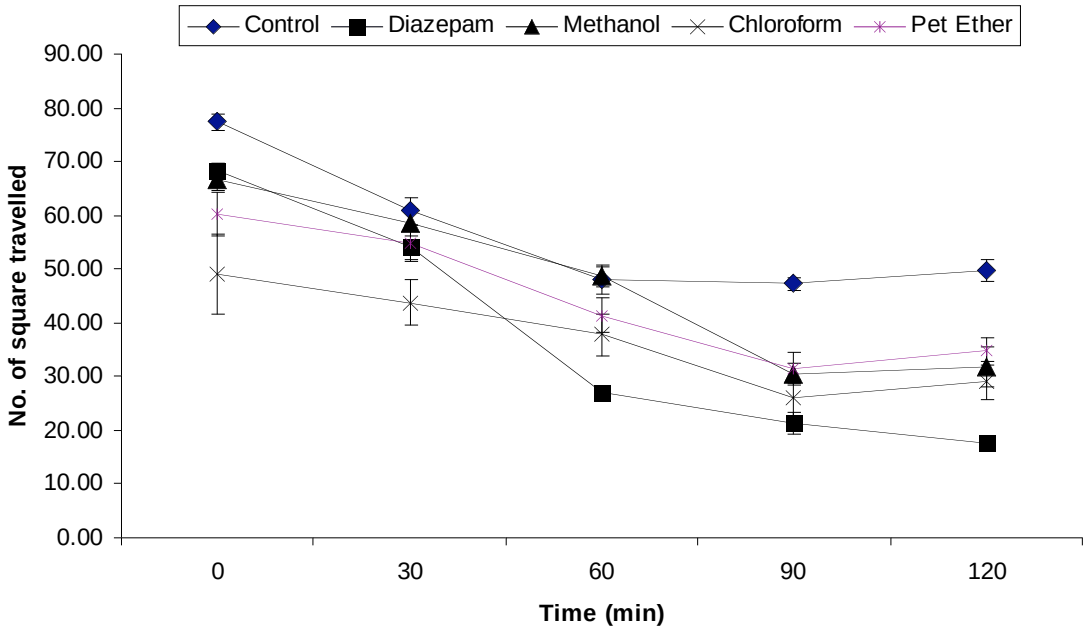

Figure 1: Effects of the different fractions of the root parts of *G. densiflorum* on open field test in mice. Values are mean ± SEM (n = 5); p < 0.05, Dunnet test as compared to control.

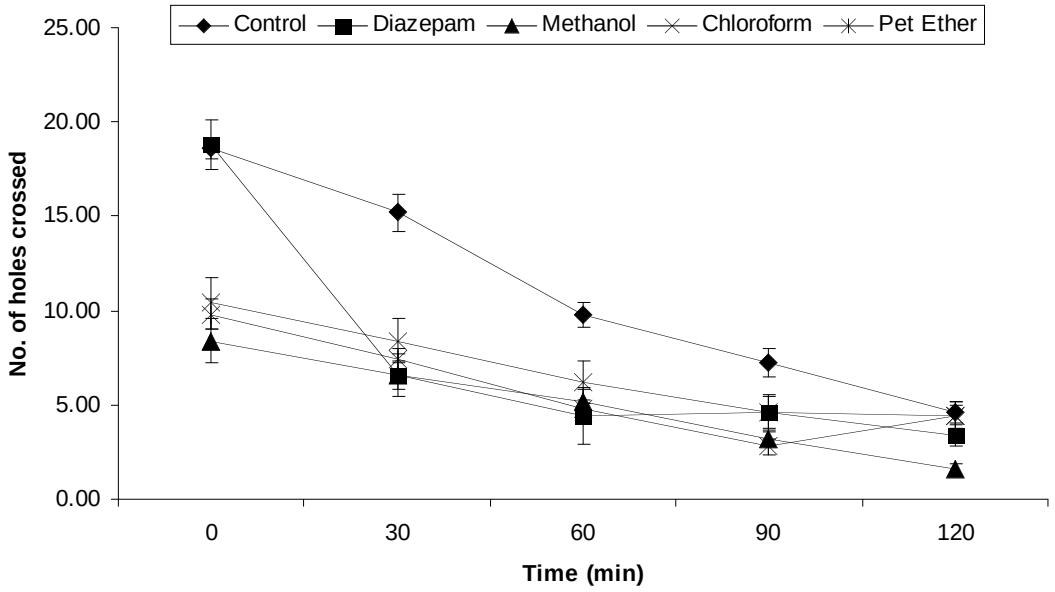

Figure 2: Effects of the different partitionates of the root parts of *G. densiflorum* on hole cross test in mice. Values are mean ± SEM (n = 5); $p < 0.05$, Dunnet test as compared to control.

Table 2: Effects of Different Fractions of the Roots of *G. densiflorum* Extract on the Elevated Plus-Maze (EPM Study) During the 5-Min Test Session

Groups	% entry into open arms	% time spent into open arms
Negative control (vehicle)	55.88 ± 1.90	51.93 ± 7.37
Positive control (diazepam)	76.28 ± 1.65*	79.39 ± 5.18*
G. densiflorum (methanol extract)	30.11 ± 3.14*	30.69 ± 3.30
G. densiflorum (chloroform solubles)	43.24 ± 3.16*	39.41 ± 1.49
G. densiflorum (pet ether solubles)	27.33 ± 6.09*	25.12 ± 5.38*

All values are expressed as mean ± S.D. (n=5); One-way Analysis of Variance (ANOVA) followed by Dunnet's test. *P <0.05, significant compared to control.

increase in the duration of total sleeping time caused by the partitionates of *G. densiflorum* roots were comparable to that observed with the standard drug diazepam (Table **1**).

To investigate further, the level of anxiety/sedation after administering the title plant extracts was evaluated by measuring external signs, through the open field and hole cross tests [11,12]. The exploration capacity of mice may be considered as an index of anxiety although it is difficult to dissect it from motor activity. In other words, locomotor activity can be considered as an index of alertness and a decrease of locomotion in test animals is generally indicative of sedative action [17]. All the organic fractions of *G. densiflorum* showed a remarkable loss of locomotor activity from the 3rd observation period (60 min) to 5th observation period (120 min) at 400-mg/kg. The depressant actions were slowly compensated with time and the outcomes were statistically significant (Figures **1** and **2**).

When tested for the anxiolytic activity in an elevated plus maze test, all the fractions except chloroform-soluble, showed a reduction in the percentage of entries and the time spent by the mice at the open arms compared to the controls. According to the hypothesis by Pellow *et al*. [18], this would reflect an increased aversion to the open arms due to increased fear or anxiety. In contrast an anxiolytic effect, as observed with the positive control (diazepam), is envisaged when the test drug increases open arm entries without altering the total number of arm entries [19]. Diazepam has been used as a standard anxiolytic and frequently applied in behavioral pharmacology as a reference compound [20].

The elevated plus-maze is considered as a measure of anxiety in rats [13]. This test revealed that it was sensitive to the effects of non-benzodiazepine anxiolytics as well as several other putative anxiogenic compounds. However, the test failed to detect activity of such clinically effective anxiolytics as pipequaline

(PK 8165), buspirone or tofisopam [13]. In fact, PK 8165 was found anxiogenic in their test and proconvulsant in a separate study [21]. The anxiolytic properties associated with these non-classical anxiolytics in the clinic differs quantitatively from that observed with classical anxiolytics. Indeed, PK 8165 was later found as a non-selective $GABA_A$ receptor partial agonist [22], whereas buspirone (under the trade name, Buspar) is a serotonin 5-$HT1_A$ receptor partial agonist [23], primarily used to treat generalized anxiety disorder (GAD); tofisopam (a benzodiazepine derivative) is more recently claimed to be a phosphodiesterase (PDE_{10A}) inhibitor [24] that is used in the treatment of schizophrenia (marketed under brand names Emandaxin and Grandaxin). Therefore, the reduced locomotor activity of *G. densiflorum* extracts observed in the open field and hole cross tests coupled with an apparent anxiogenic response from elevated plus-maze test is suggestive of the potential anxiolytic principles in the extracts operating *via* alternative novel mechanism(s) which remains to be elusive at the moment.

CONCLUSION

Our combined results suggest that the organic soluble of *G. densiflorum* roots contain biologically active compounds with good sedative/anxiolytic activity. This plant could be a potential candidate for the treatment of anxiety and related neuropsychiatric disorders. However, the lack of our current knowledge on chemical constituents of this plant limits us to attribute its sedative/anxiolytic properties to one or several active principles. Therefore, in order to investigate the mechanism of neuropharmacological action and to identify the active compound(s) responsible for these bioactivities in the animal models, further studies need to be carried out.

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