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Sedative and Cytotoxic Properties of the Leaf Extract of *Desmodium paniculatum.*

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ABSTRACT

The present study was done to investigate the sedative and cytotoxic properties of methanol extract of *Desmodium paniculatum* Leaves. Sedative activity was evaluated by using hole cross, open field and thiopental sodium-induced sleeping time methods in swiss albino mice at 400 mg/kg body weight dose orally and the extract was evaluated by in-vitro brine shrimp lethality bioassay for investigating cytotoxic activity. The extract decreased the locomotor activity of mice in hole cross, open field test and minimize the onset of sleep and maximized the duration of sleeping time significantly ($p<0.05$) when administered with thiopental sodium. Moreover the extract showed remarkable cytotoxic activity, LC_{50} value of the extract was 50.01µg/ml which was compared with the standard drug vincristine sulfate as a positive control. In conclusion, the methanol extract of *Desmodium paniculatum* contains significant sedative and cytotoxic activity.

Keywords: *Desmodium paniculatum,* Sedative, Cytoxic, Hole Cross, Open Field, Cytotoxic, Brine Shrimp Lethality

1. Introduction

Desmodium paniculatum is a native, perennial, wild flower that grows up to 3 feet tall. The genus *Desmodium*: originates from Greek meaning "long branch or chain," probably from the shape and attachment of the seedpods. The central stem is green with clover-like, oblong, multiple green leaflets proceeding singly up the stem. The showy purple flowers appear in late summer and grow arranged on a stem maturing from the bottom upwards. In early fall, the flowers produce leguminous seed pods approximately ⅛ inch long. *D. paniculatum* plants have a single crown. This wild flower is a pioneer species that prefers some disturbance from wildfires, selective logging, and others causes. The sticky seedpods cling to the fur of animals and the clothing of humans and are carried to new locations. The Houma Indians of Louisiana used an infusion of the roots in whiskey to treat weakness and cramps.

Desmodium exhibit a wide spectrum of pharmacological activities. *D. gangeticum* protects heart against myocardial ischemia reperfusion injury [1]. Analgesic, anti-inflammatory, and antipyretic activities were observed in *D. caudatum* [2] and *D. podocarpum* [3]. *D. adscendens* possesses anti-anaphylactic property [4], analgesic and hypothermic actions as well as inhibitory influence on the propagation of clonic-tonic PTZ (pentylenetetrazole) seizure [5]. Recently, Tsai *et al.* (2011) studied 10 *Desmodium* species from Taiwan and found that *D. sequax* is a potent antioxidant medicinal plant, and chlorogenic acid may be an important factor in the antioxidant activity of this plant [6]. On the other hand, chemical investigations of *Desmodium* species have revealed the presence of isoflavones, Cglucosyl flavonoids, coumarono-chromones, and pterocarpans [7, 8]. Triterpenoid saponins, tetrahydroiso-quinolones, phenylethylamines and indole-3-alkyl amines have been isolated from the leaves of *Desmodium adscendens* [9]. Several flavonoid glycosides, pterocarpanoids, lipids, glycolipids, and alkaloids were isolated and identified from *D. gangeticum* [10]. *D. canum* is known to contain isoflavan, isoflavanones [11], Antimicrobial, Cytotoxic and Antioxidant Activities of *Desmodium heterocarpon* were identified by Hasan AA *et al*. (2011) and *Desmodium puchellum* have anti-diarrheal activity which was assessed by Rahman MK *et al.* in 2013 [13].

However, no phytochemical studies of *Desmodium paniculatum* have been found in literature to date. The present work was an endeavor to screen the methanolic extract (ME) of *D. paniculatum* for probable sedative and cytotoxic activities.

2. Materials and Methods

2.1 Plant materials

Adequate amounts fresh leaves of *Desmodium paniculatum* (L.) for this study were collected from the hill tracts of Cox's Bazar area of Chittagong, Bangladesh and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, and University of Chittagong, Bangladesh. The leaves were dried at room temperature in the shade and away from direct sunlight for 5 days and in hot air oven for 2 days.

2.2 Extraction

The dried leaves were coarsely powdered and extracted by dissolving 7 days with methanol. The sediments were filtered and the filtrates were dried at 40 ºC in a water bath. The solvent was completely removed by filtering with Hartman filter paper and obtained dried crude extract which was used for experiment.

2.3 Drugs and chemicals

The following drugs and chemicals were used in this study: diazepam (Square Pharmaceutical Ltd., Bangladesh), thiopental sodium (Gonoshastho Pharmaceuticals Ltd., Bangladesh), methanol (Sigma Chemicals Co., USA), dimethyl sulfoxide (DMSO) and Vincristine sulfate (2mg/vial; Techno Drugs Limited Bangladesh).

2.4 Animals

White female albino mice (Swiss-webstar strain, 25-35 gm body weight) were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). The animals were provided with standard laboratory food and tap water and maintained on a natural day night cycle. All experiments were conducted under isolated and noiseless conditions. Test animals were divided into one group at dose of 400 mg/kg body weight. The animals were acclimatized to laboratory conditions for one week prior to experimentation.

2.5 Hole cross test

The method was carried out as described by Takagi *et al.* [14]. A steel partition was fixed in the middle of a cage 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 cage. The animals were divided into control, positive control, and test groups containing five mice each. The test groups received methanol extract of *D. paniculatum* at dose of 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The number of passages of a mouse 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 both doses of the test drug.

2.6 Open field test

In the open field test, the animals were divided into control, positive control, and test groups containing five mice each. The test groups received methanol extract of the leaves of *D. paniculatum* at dose of 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The floor of a half square meter open field [15, 16] was divided into a series of squares each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of both doses of the test drug.

2.7 Thiopental sodium induced sleeping time test

The animals were randomly divided into five groups consisting of five mice each. The test groups received methanol extract from the leaves of *D. paniculatum* at dose of 400 mg/kg body weight while the positive control was treated with diazepam (1 mg/kg) and control vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40 mg/kg) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex [17].

2.8 Cytotoxicity test (brine shrimp lethality bioassay method)

Methanol extract was subjected to cytotoxic study. 1.0 mg sample was taken and a stock solution of 1000 µg/ml was prepared with pure dimethyl sulfoxide (DMSO). A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as−400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml. Then the samples were subjected to brine shrimp lethality bioassay [18] for cytotoxic studies. In each test tube, containing different concentrations of test sample, 20 brine shrimp nauplii (*Artemia salina)* were added.

Table 1: CNS depressant activity of methanol extract of leaves of *D. paniculatum* (MEDP) on hole cross test in mice.

Group	Treatment	Dose, Route	Numbers of movements				
			0 min	30 min	60 min	90 min	120 min
Control	1% tween 80 in water	10 mg/kg, p.o	18.67±0.882	16.33±0.67	14.67± 1.202	15.67± 1.202	14.67± 1.202
Standard	Diazepam	1.00 mg/kg, i.p	17.33±1.202	6.00± 1.528*	4.33± 0.33*	3.67± 0.67*	1.33± 0.33*
Test	MEDP	400 mg/kg, p.o	17.33±2.028	9.00± 1.741*	5.33± 1.453*	4.00± 1.732*	3.00± 1.155*

All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Tukey HSD test.
*P<0.05, significant compared to control.

Two control groups were used in cytotoxicity study, to validate the test method and results obtained due to the activity of the test agent. In the study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml and serial dilutions were made using DMSO to get 40µg/ml, 20µg/ml,

10µg/ml, 5µg/ml, 2.5 µg/ml, 1.25µg/ml and 0.625µg/ml of concentration. 30 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as negative control groups. After 24 hours, the test tubes were observed and the numbers of survived nauplii in each test tube were counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

3. Results

3.1 Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min (Table 1). In the hole cross test, the extracts showed a decrease in locomotion in the test animals from the second observation period as evident by the reduction in number of hole crossed by the treated mice compared to the control group. The result was comparable to the reference drug diazepam and was statistically significant ($p< 0.05$).

3.2 Open field test

In the open field test, the number of squares traveled by the mice was suppressed significantly in the test group throughout the study period. The CNS depressant activity obtained for extract was more than that of standard drug and the result was statistically significant.

Table 2: CNS depressant activity of methanol extract of leaves of *D. paniculatum* on open field test in mice.

Group	Treatment	Dose, Route	Numbers of movements				
			0 min	30 min	60 min	90 min	120 min
Control	1% tween 80 in water	10 mg/kg, p.o	68.00±1.528	65.67±0.882	49.33±1.856	46.33±1.202	48.33±2.728
Standard	Diazepam	1.00 mg/kg, i.p	66.33±4.256	54.67±4.33	34.00±2.646*	19.67±0.882*	17.67±1.33*
Test	MEDP	400 mg/kg, p.o	68.00±1.879	44.33±6.642*	36.33±2.963*	20.00±3.056*	12.33±3.283*

All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Tukey HSD test. *P<0.05, significant compared to control.

3.3 Thiopental sodium induced sleeping time test

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 400 mg/kg showed significant ($p<0.05$) decrease in onset of action and increased the duration of sleep. The extract and standard drug diazepam showed nearly similar sedative activity regarding both onset of sleep and duration of sleep (Table 3).

Table 3 CNS depressant activity of methanolic extract of leaves of *L. repens* on thiopental sodium induced sleeping time test in mic

Group	Treatment	Dose, Route	Onset of sleep (min)	Duration of sleep (min)
Control	1% tween 80 in water	10 mg/kg, p.o	38.67± 2.028	46.33± 0.67
Standard	Diazepam	1.00 mg/kg, i.p	12.33± 2.404*	147.33±6.566*
Test	MEWT	300 mg/kg, p.o	16.00± 5.568*	143.67±18.55*

All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Tukey HSD test. *P<0.05, significant compared to control.

Table 4: Brine shrimp lethality bioassay of methanol extract of *D. paniculatum.*

Concentration, C, µg/ml	LogC	No. of nauplii taken	No. of nauplii Dead	% mortality	LC_{50}, µg/ml
6.25	0.79588	10	3	30	50.01
12.5	1.09691	10	3	30	
25	1.39794	10	4	40	
50	1.69897	10	4	40	
100	2	10	5	50	
200	2.30103	10	7	70	
400	2.60206	10	9	90	

3.4 Cytotoxicity test

In the present bioactivity study, the crude methanol extract showed positive results indicating that the test samples are biologically active. Plotting of log of concentration (log C) versus percent mortality (% mortality) for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined. The crude extract of *D. paniculatum* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was 50.01µg/ml (Table 4 and Figure 1). Moreover the LC_{50} value of the vincristine sulfate as positive control was 0.95µg/ml (Table 5 and Figure 2) and the DMSO as negative control have no mortality

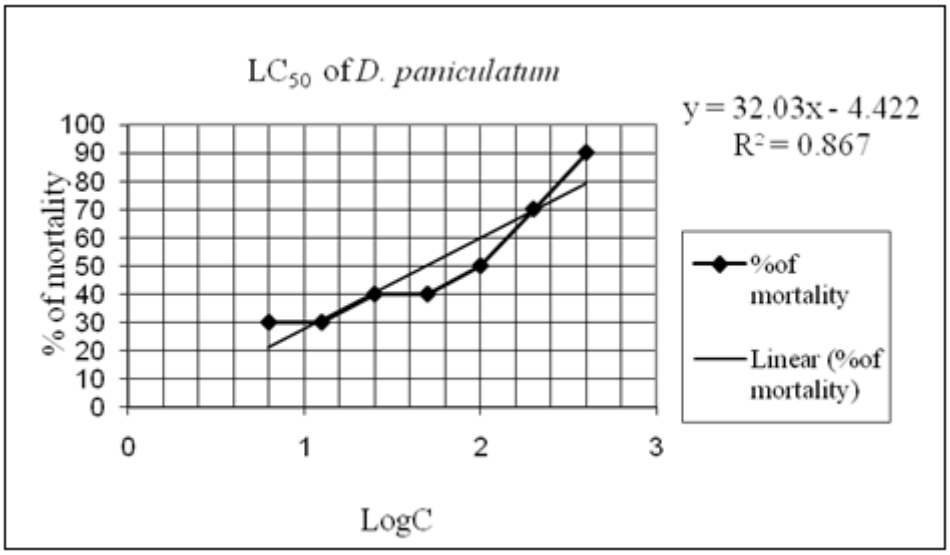

Fig 1: Determination of LC_{50} of methanol extract of D. paniculatum

Table 5: Brine shrimp lethality bioassay of vincristine sulfate as positive control.

Concentration, C, µg/ml	LogC	No. of nauplii taken	No. of naplii Dead	% mortality	LC_{50}, µg/ml
0.625	-0.20412	10	4	40	0.95
1.25	0.09691	10	5	50	
2.5	0.39794	10	7	70	
5	0.69897	10	8	80	
10	1	10	9	90	
20	1.30103	10	10	100	
40	1.60206	10	10	100	

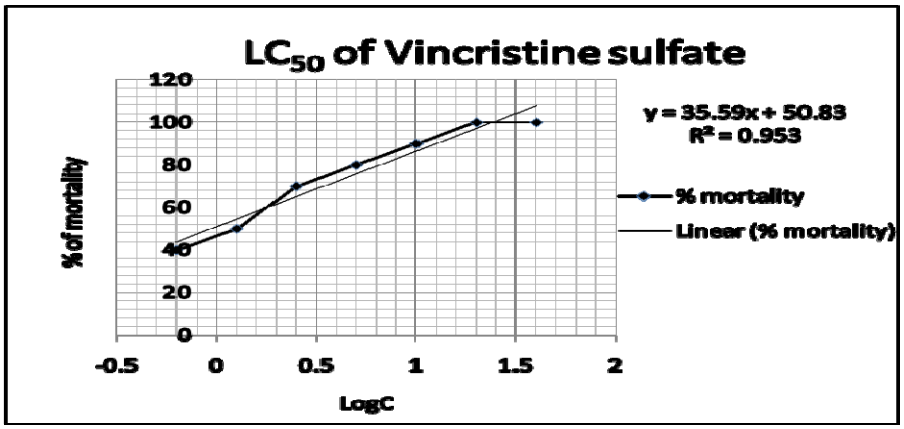

Fig 2: Determination of LC_{50} of vincristine sulfate as positive control

4. Discussion

The study has examined some neuropharmacological activities of methanol extract of *D. paniculatum*. The plant extract possessed central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross and open field test. The marked sedative effect of the extract was also found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. CNS depressant drugs mainly exert their action through $GABA_A$

receptor [20]. So, the extract of *D. paniculatum* may acts by hyperpolarization of the CNS via GABA receptor or benzodiazepine receptor located adjacent to the GABA receptor. Moreover the extract of *D. paniculatum* possessed significant cytotoxic activity.

5. Conflict of Interest

The Authors have declared that there no conflict of competing interest.

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