Pharmacology Research Lab.[1], Department of Pharmacy, Faculty of Basic Medical and Pharmaceutical Sciences, University of Science and Technology Chittagong, Foy's Lake, Chittagong, Ethnopharmacology Lab.[2], Department of Pharmacy, Life Science Institute, Jahangirnagar University, Savar, Dhaka, Bangladesh

# The effects of aqueous extracts of *Desmodium gangeticum* DC. (Leguminosae) on the central nervous system

S. JABBAR[1], M. T. H. KHAN[1] and M. S. K. CHOUDHURI[2]

The aqueous extract of *Desmodium gangeticum* DC. (Leguminosae) showed no analgesic activity in the hot plate method, but it showed severe anti-writhing activity in the acetic acid-induced abdominal writhing assay. It exhibited moderate central nervous system depressant activity in the spontaneous motor activity, hole cross, and open field tests and hole board tests. The effects of this extract on locomotion were compared with some standard CNS drugs.

## 1. Introduction

In 1972 Ghosal et al. reported that, twelve alkaloids, of 4 broad structural types (carboxylated and decarboxylated tryptamine, β-carbolines, and β-phenyl ethyl amines) were isolated from *Desmodium gangeticum* DC. at different stages of development. Anticholinesterase, smooth muscle stimulant, central nervous system stimulant and central nervous system depressor responses to the alkaloid from the aerial portions of the legume were seen in test animals. The presence of catecholamine liberators (*tert*-β-phenyl ethylamines) and candicine in the roots was responsible for a nicotine-like effect on dog intestine in situ and on carotid blood pressure. The medicinal properties of this plant apparently reside in its alkaloids [1]. Addy et al. reported in 1984 that, both the aqueous and ethanolic extracts of *Desmodium adscendens*, when taken orally, reduce anaphylactic contractions, interfere with histamine-induced contractions, and reduce the amount of smooth muscle stimulating substances released from lung tissues of guinea-pigs [2].

In this study, we examined the effects of the aqueous extract, which was prepared according to the Ayurvedic method, on the central nervous system of laboratory animals.

## 2. Investigations, results and discussion

The aqueous extract of *Desmodium gangeticum* DC. (SLP) did not exhibit any analgesic effect (Table 1). The significant result at 0 min ($p < 0.001$) indicates a limitation with the animals utilized in the experiment. Even at 30 min the same problem is seen. This experiment should be repeated by an other experimenter to confirm this particular finding.

SLP (88.64%) exhibited a much more potent anti-writhing effect in comparison to the standard drug ASA (64.49%). The results (Table 2) were statistically highly significant. The writhing response produced in rats or mice by intraperitoneal injection of dilute acetic acid, phenylquinone, benzoquinone, or bradykinine is prevented by salicylates and similarly acting drugs, and this gives an indication of the possible therapeutic use. The test is not entirely specific; as several compounds without analgesic action in man also prevent the writhing response. Nevertheless, when taken in conjunction with other tests, including the ability to inhibit prostaglandin (PG) synthetase, especially that from nervous tissue, the anti-writhing test could provide further useful information [3].

Some standard drugs which also affect the central nervous system were studied in spontaneous motor activity tests simultaneously with the experimental aqueous extract of SLP. The standard drugs were amphetamine (1 mg/kg), which was taken as the CNS stimulator, and diazepam (12.5 μg/kg), which was taken as the CNS depressant. In this experiment the test results demonstrated that SLP has potent depressant activity on the central nervous system of laboratory mice. The data (Table 3) from the experiment at 30 min, 60 min, 120 min and 240 min were statistically highly significant.

The results of hole cross test (Table 4) demonstrated that SLP has a mild to moderate depressant activity on the central nervous system. The peak effect was observed 60 min after administration of the extract. The data from the experiment at 30 min, 60 min, 120 min and 240 min were statistically highly significant.

In the open field test, the results (Table 5) demonstrated that SLP has a mild to moderate depressant activity on the central nervous system. The peak effect was observed 120 min after administration of the extract. The data from the experiment revealed that with the exception of 30 min, the data at the times of 60 min, 120 min and 240 min were statistically highly significant.

**Table 1: Time taken to perceive pain after the administration of SLP in comparison with morphine**

| Groups | Dose (mg/kg) | Pain perception time (s) Mean ± S.E.M. (p value) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min | 240 min |
| Control | 100 | 16.42 ± 1.003 | 15.17 ± 1.103 | 17.0 ± 1.483 | 15.88 ± 1.268 | 13.66 ± 1.202 |
| SLP | 100 | 9.54 ± 0.666 (0.000)*** | 10.29 ± 0.993 (0.001)*** | 18.0 ± 4.068 (0.822) | 15.42 ± 1.998 (0.945) | 11.75 ± 0.793 (0.213) |
| Morphine sulfate (MPS) | 0.5 | 24.3 ± 4.177 (0.038)* | 494.30 ± 22.81 (0.000)*** | 411.17 ± 27.84 (0.000)*** | 398.3 ± 9.98 (0.000)*** | 385.17 ± 9.13 (0.000)*** |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

**Table 2: Effect of SLP in the acetic acid (AA)-induced writhing test**

| Groups | Doses (mg/kg) | Average number of writhings observed Mean ± S.E.M (p value) | % Protection |
|---|---|---|---|
| Control | 100 | 44.0 ± 2.449 | – |
| SLP | 100 | 5.0 ± 1.021 (0.000)*** | 88.64 |
| ASA | 5.0 | 15.63 ± 4.719 (0.001)*** | 64.49 |

*p < 0.05, **p < 0.01, ***p < 0.001

The results of the hole-board test on mice (Table 6) showed that SLP has a mild depressant effect on ambulation. The data from the experiment at 30 min and 120 min were statistically significant. Other data were not statistically significant. For head-dipping in the hole board test on mice SLP has a moderate decreasing effect on the number of head-dipps. The peak effect was observed 120 min after administration of the extract. The data from the experiment at 120 min are statistically highly significant. Other data are not statistically significant. For defecation (number of stool pellets) in the hole board test on

**Table 3: Effect of SLP on spontaneous motor activity**

| Groups | Doses (mg/kg) | Time of study (No. of Movements) Mean ± S.E.M. (p values) 95% CI val. | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min | 240 min |
| Control | 100 | 439.67 ± 49.29 | 415.5 ± 101.56 | 272.67 ± 40.96 | 227.17 ± 43.01 | 165.83 ± 19.93 |
| SLP | 100 | 334.4 ± 19.84 (0.1) −24.53, 235.07 | 96.4 ± 28.11 (0.02)* 58.62, 579.58 | 51.0 ± 21.38 (0.001)*** 110.28, 333.05 | 51.2 ± 24.09 (0.008)** 57.66, 294.27 | 34.4 ± 18.31 (0.001)*** 69.12, 193.75 |
| Amphetamine | 1.0 | 423.8 ± 27.96 (0.798) | 376.4 ± 68.18 (0.767) | 405.8 ± 46.44 (0.059) | 268.83 ± 14.62 (0.421) | 327.0 ± 54.39 (0.015)* |
| Diazepam | 12.5 μg/kg | 449.0 ± 24.16 (0.877) | 269.0 ± 34.12 (0.240) | 224.6 ± 29.75 (0.385) | 153.8 ± 10.02 (0.164) | 178.8 ± 63.18 (0.838) |

*p < 0.05, **p < 0.01, ***p < 0.001

**Table 4: Effect of SLP in the hole cross test**

| Groups | Dose (mg/kg) | Hole cross test (number hole crossed) Mean ± S.E.M. (p values) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min | 240 min |
| Control | 100 | 3.17 ± 0.55 | 3.61 ± 0.51 | 2.94 ± 0.44 | 3.17 ± 0.44 | 3.39 ± 0.37 |
| SLP | 100 | 2.83 ± 0.87 (0.760) | 0.17 ± 0.17 (0.001)*** | 0.0 ± 0.0 (0.001)*** | 0.0 ± 0.0 (0.001)*** | 0.0 ± 0.0 (0.000)*** |

*p < 0.05, **p < 0.01, ***p < 0.001

**Table 5: Effect of SLP in the open field test**

| Groups | Dose (mg/kg) | No. of movements Mean ± S.E.M. (p values) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min | 240 min |
| Control | 100 | 106.29 ± 4.14 | 49.71 ± 7.44 | 48.86 ± 5.72 | 47.14 ± 3.7 | 35.71 ± 5.76 |
| SLP | 100 | 146.33 ± 9.04 (0.001)*** | 28.33 ± 9.2 (0.095) | 14.33 ± 7.61 (0.004)** | 10.17 ± 1.92 (0.000)*** | 4.83 ± 2.87 (0.001)*** |

*p < 0.05, **p < 0.01, ***p < 0.001

**Table 6: Effect of SLP in the hole board test**

| Groups | Hole board tests Mean ± S.E.M. (p values) | | | | | |
|---|---|---|---|---|---|---|
| Control | Experiments | 0 min | 30 min | 60 min | 120 min | 240 min |
| | Ambulation | 9.0 ± 1.62 | 8.29 ± 1.09 | 4.57 ± 0.87 | 10.86 ± 3.10 | 3.43 ± 0.53 |
| | Head-dip. | 13.29 ± 2.72 | 5.57 ± 1.65 | 4.57 ± 1.59 | 4.71 ± 1.17 | 4.86 ± 5.27 |
| | Defaecation | 1.29 ± 0.42 | 1.41 ± 0.40 | 2.0 ± 0.93 | 1.57 ± 0.48 | 2.14 ± 0.74 |
| SLP | Ambulation | 16.33 ± 3.45 (0.068) | 2.5 ± 1.36 (0.006)** | 4.17 ± 1.78 (0.834) | 1.83 ± 0.543 (0.023)* | 2.33 ± 0.92 (0.306) |
| | Head-dip. | 12.67 ± 1.23 (0.849) | 1.5 ± 0.957 (0.066) | 2.17 ± 1.01 (0.246) | 0.83 ± 0.48 (0.01)** | 2.33 ± 0.96 (0.303) |
| | Defaecation | 0.67 ± 0.42 (0.324) | 0.0 ± 0.0 (0.025)* | 0.0 ± 0.0 (0.072) | 0.17 ± 0.17 (0.026)* | 0.0 ± 0.0 (0.022)* |

*p < 0.05, **p < 0.01, ***p < 0.001.

mice showed that SLP gives a drastic decrease in defecation. All through the experimental period there was no defecation, except at 120 min. The data from the experiment at 30 min, 120 min and 240 min are statistically significant. Other data are not statistically significant.

## 3. Experimental

### *3.1. Plant material*

Whole plants of *Desmodium gangeticum* DC, were collected and identified with the help of the herbarium of the Forest Research Institute, Bangladesh. The voucher specimen number was BFRI-7608. The collected whole plants were dried and then powdered by a grinding machine.

### *3.2. Preparation of the extract*

Kwath (aqueous extract) was prepared according to the procedure described in the Bangladesh National Ayurvedic Formulary [4]. 100 g of the powdered whole plant was heated in 1600 ml water at 80 °C and evaporated to 400 ml. Then the extract was filtered first with fine cloth and then with Whatman® filter paper. Yield of the extract was 4.52% from dry powdered plant. This extract (1 ml) was taken in a 10-ml beaker and dried at low temperature (20 °C) by a hot air dryer. Then the dried material was weighed in an electronic digital balance (Mettler, Switzerland). The weight in mg of dry material per ml of the aqueous extract and was adjusted to a concentration of 10 mg/ml.

### *3.3. Animals*

Male and female Swiss-Webster strain mice (20–25 g body weight) were used for the experiments. The animals were provided with food and tap water ad libitum. The animals were maintained at constant room temperature (22.0 ± 1.0 °C), humidity 55–65% and 12 h light/12 h dark cycle. Animals were divided in groups of 6, with each group balanced for sex and body weight. The control animals were given equal volume of physiological saline (0.9% NaCl solution).

### *3.4. Dose and route of administration*

The dose of the SLP was 100 mg/kg and route of administration was intraperitoneal (i.p.).

### *3.5. Analgesic effect evaluation*

#### *3.5.1. Hot plate method*

The analgesic study was conducted by the "Hot Plate" (Socrel-DS37, UGO Basile, Italy) method, described by Woolfe, et al. [5] and Wood [6]. The hot plate was maintained at a constant temperature of 55 ± 0.5 °C. Each mouse was placed on the hot surface and the time of response to thermal stimuli, indicated by the licking of hind and/or fore paws or by kicking of the legs or by trying to jumpout, was recorded. For comparison the same experiment was conducted with a standard analgesic drug, morphine sulphate (MPS).

#### *3.5.2. Acetic acid-induced abdominal writhing assay*

Muscular contraction was induced by a 0.6% solution of acetic acid (AA) (0.25 ml/animal). The plant extract or the vehicle (control) was administered intraperitoneally to mice, 30 min before acetic acid (AA) injection. After AA administration, the mice were individually placed in boxes. The number of muscular contractions were counted 15 min after injection for 5 min and data represent the average of the total number of writhes observed [7]. The percent protection was calculated as follows:

$$\%\text{Protection} = 100 - (\text{treated mean}/\text{control mean}) \times 100$$

For comparison the same experiment was conducted with standard analgesic acetylsalicylic acid [ASA].

### *3.6. Neuropharmacological study*

#### *3.6.1. Spontaneous motor activity tests*

Six mice, body weight 20–30 g, for each group were placed in an automatic activity cage (Model-7400) with recorder (Model-7401, UGO Basile, Italy), the floor of which comprised stainless steel grids. Through these steel grids current at 0.6 volts and 50 m.a. was passed. The positive and negative terminals were mounted consecutively. When the mice were positioned over these steel grids the terminals were connected so that movement of the mice could be detected. These movements were recorded by the detector (UGO Basile, Italy) and printed on thermal paper (UGO Basile, Italy).

#### *3.6.2. Hole cross tests*

The method of Takagi et al [8] was employed for this experiment. A steel partition was fixed in the middle of a cage 30 × 20 × 14 cm in size. A hole of diameter 3 cm was made at a height of 7.5 cm in the center of the plate. The number of passages of a mouse through the hole from one end of the cage to another was recorded for a period of 2 min at −60, +30, +60, +120 and +240 min. Similar recordings were made for the control animals [8].

#### *3.6.3. Open-field tests*

This experiment was carried out by the method of Gupta et al. [9]. The floor of an open field of half square meter was divided into a series of squares, each alternately colored black and white. The apparatus had a wall of 40 cm. The number of squares visited by the animals was recorded for a period of 2 min.

#### *3.6.4. Hole-board tests*

This experiment was carried by the method of Nakama et al. [10]. 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 centimeters square. The number of ambulations (expressed as the number of holes passed), head dipping and defecation was recorded for a period of 2 min.

### *3.7. Statistical analysis*

Data obtained from the experiments are expressed as mean and standard error of the mean (mean ± S.E.M.). Unpaired t-tests were performed by computer software SPSS (Statistical Package for Social Science) release 9.05 for Windows™, to test the level of significance. Probability (p) value of 0.05 or less ($p < 0.05$) was considered as significant.

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Mahmud Tareq Hassan Khan
56/E, Jamal Khan Road
Chittagong-4000
Bangladesh
mthkhan@usa.net