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ANALGESIC AND NEUROPHARMACOLOGICAL INVESTIGATIONS ON *SESBANIA GRANDIFLORA*

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ABSTRACT

This study was designed to evaluate the analgesic and neurophramacological investigations of leaf extract of *Sesbania grandiflora* in Swiss albino mice following oral administration. *In-vivo* analgesic activity test was evaluated by acetic acid induced writhing method and tail immersion test. *In-vivo* neurophramacological investigations was determined by open field and hole cross test. *In-vivo* analgesic activity test shows that methanolic extract (250 & 500 mg/kg b.w.) performed good activity in mice comparing to the standard drug diclofenac Na. Methanolic extract of *Sesbania grandiflora* displayed a significant and dose dependent analgesic activity, the percent inhibition was 68.13%, 85.56% respectively in the test group (250 & 500 mg/kg b.w.).

Keywords: *Sesbania grandiflora*, analgesic activity, neurophramacological activity

INTRODUCTION

The plants which are useful for healing diseases are called medicinal plant. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." There are more than 500 medicinal plants growing in our country [1].

Recently World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care. It has been recorded that about 450 to 500 plants growing or available in Bangladesh have therapeutic values [1, 2]. In Bangladesh, people living in the remote hilly areas, such as, ethnic communities rely mostly on herbal medicines. Bangladesh, a country fertile deltaic land has a rich diversity of flora of medicinal plants scattered throughout the forests, crop fields, roadsides gardens and wastelands.

Sesbania grandiflora (syn. *Aeschynomene grandiflora, Agati grandiflora)* [3] also known as agati or hummingbird tree/scarlet wisteria, is a small tree in the genus *Sesbania*. *Sesbania grandiflora* is indigenous from Malaysia to North Australia; cultivated in many parts of India. It has a large number of traditional uses [4]. It grows where there is good soil and hot humid temperature. Die in snow, cold weather. It's a tropical plant. Leaves used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. Flower in headache, dimness of vision [3], Catarrh, Headache, cooling and improving appetite, bitter, astringent, acrid, antipyretic. Bark is used for cooling (ayurvedha and siddha medicinal terms), bitter tonic, anthelmintic, febrifuge, diarrhea, small pox, astringent [5]. Fruits in bitter & acrid, laxative, fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning. Root used in rheumatism, expectorant, painful swelling, Catarrh [6].

MATERIALS AND METHOD

Plant material: The plant leaves were collected during June, 2013, from Rangpur, Bangladesh. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 32531) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The leaves were sun dried for seven days. The dried plant was then ground in coarse powder using high capacity grinding machine.

Preparation of extract: Hot solvent extraction process was used for extraction of the plant material. Soxhlet extractor was used for the extraction procedure. Plant material was extracted by the solvent- methanol. After extraction, was kept at petri dishes and dried at room temperature. After drying, extracts were stored in petri dishes and kept in refrigerator for further use.

Preparation of animals: Adult Swiss albino mice (BALB/c) weighing between (12-30) gm of either sex were used for the studies. The animals were maintained under normal laboratory condition & kept in standard cages at room temperature of 30°C±2°C and 60% to 65% relative humidity and provided with standard diet & water *ad libitum.* The experimental protocols were approved by institutional Animal Ethical Committee to carry out and complete this study.

Analgesic activity test: Analgesic activity was evaluated by acetic acid writhing test and tail immersion test.

Acetic acid induced writhing: The acetic acid writhing test in mice as described by Koster *et al.*, 1959 [7] was employed with slight modification. Mice were divided into 4 groups of 5 mice each. The first group was given 10ml/kg of 1% Tween 80 i.p. and served as control. Group 2 was served as standard where Diclofenac Sodium has given to mice as dose of 50mg/kg of body weight. Group 3 and 4 received methanol extract of flower of *Sesbania grandiflora* 250mg/kg and 500 mg\kg of body weight. Thirty minutes later each mouse was injected intra-peritoneally with 0.7% acetic acid at a dose of 10 ml/kg body weight. Full writhing was not always completed by the animal, because sometimes the animals start to give writhing, but they do not finish it. This incomplete writhing was taken as a half writhing. Accordingly, two half writhing were considered as one full writhing. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min intra-peritoneally administration of Acetic acid and the mean abdominal writhing for each group was obtained.

The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (drugs)}}{\text{Mean no. of writhing (control)}}$$

Tail immersion test: Analgesic activity was also evaluated by tail immersion test according to Janssen *et al.*, 1963 [8]. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C – 55.5°C. The animal immersing the tail from hot water within 5 second was selected for the study. The selected mice (either sex) were then divided in to 4 groups of 5 mice each (total 20 mice). Control group received normal saline & standard drug was Diclofenac Na. After administration of the doses by gavages, the reaction time was measured at 0, 30, 60 and 90 minutes.

CNS depressant activity test:

Open field test: The Open Field Test (OFT) is clearly the most frequently used of all behavioural tests in pharmacology and neuroscience. Despite the simplicity of the apparatus, however, open field behaviour is complex. Consequently, it has been used to study a variety of behavioural traits, including general motor function, exploratory activity and anxiety-related behaviours. Open-field behavioral assays are commonly used to test both locomotor activity and emotionality in rodents. CNS depressant activity tests were evaluated by Gupta *et al*., 1971[9].

Hole cross test: The most consistent behavioral change is a hyperemotional response to novel environmental stimuli. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. Spontaneous movement of the animals through the hole from one chamber to the other was counted for 5 minuets in this test. The observations are made on 0, 30, 60 and 90 minutes after intraperitoneally injection of the test drugs. The experiment was carried out as described by Takagi *et al.*, 1971 [10]. Spontaneous movement of the animals through the hole from one chamber to the other was counted for 5 minutes in this test. The observations were made on 0, 30, 60 and 90 minutes after

intraperitoneally injection of the leaves extract of the *Sesbania grandiflora.* There were no effects of the test animals at 0 min. After 30 min observed that the mice began to sleep and therefore very little movement was observed. Even after 90 min of administration of the extract they were still sleeping.

For both the Open Field Test (OFT) and Hole Cross Test, eexperimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV, consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. To prepare solution of the plant extract at a doses of 250mg/kg and 500mg/kg body weight, 20 mg extract was measured and added with it 4 ml of distilled water and mixing with the help of vortex apparatus. From this solution 0.20ml was taken for 50mg/kg and 0.40ml for 100mg/kg dose.

Statistical analysis: Data was expressed as Mean ± SEM (Standard error of Mean).

RESULTS AND DISCUSSION

Analgesic activity test:

Acetic acid induced writhing: The results of acetic acid induced writhing test for methanolic extract of *Sesbania grandiflora* is presented in **Table 1.** Methanol extract inhibited writhes in a dose dependent manner. Methanol extract at 500 mg/kg showed highest inhibition (85.56%) which is even higher than the standard drug (78.09%). Literature revealed that the acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [11]. The constriction response of abdomen produced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. It has been associated with prostanoids in general, for example, increased levels of PGE2 and PGF2a in peritoneal fluids as well as lipoxygenase or cyclooxygenases products [12] and acid sensing ion channels [13].

Tail immersion test: The results of tail immersion method for methanolic extract of *Sesbania grandiflora* is shown in **Table 2.** Tail immersion method, the heat itself acts as a source of pain. The different concentrations of methanol extract of *Sesbania grandiflora* (group-I, 250 and group-II, 500 mg/kg) and diclofenac Na (50 mg/kg) were administered to mice and observed the basal reaction time in different time intervals. The basal reaction time increased with increasing the concentrations along with increasing the time. The basal reaction time was more for standard drug when compared to plant extract.

CNS depressant activity test: The Open Field Test results are presented in **table 3** and the results of Hole Cross Test are presented in **table 4**. The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS [14] and this decrease may be closely related to sedation resulting from depression of the central nervous system. The extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests (**table 3 and 4**). The locomotor activity lowering effect was evident at the 3rd observation (60 min) and continued up to 4th observation period (90 min). Moreover, the validation of anxiety was carried out by measuring external signs, through hole-cross tests. Open field test showed that the depressing action of the extracts was not good enough.

CONCLUSION

The use of this plant in traditional medicine prompted us to investigate *Sesbania grandiflora* for possible analgesic and Central Nervous System depressant activity. Literature review of *Sesbania grandiflora* indicated that phytochemical and pharmacological investigations have been done on this plant extract previously. In this study on the leaves of the *Sesbania grandiflora*, effective analgesic activity was found. The investigations were preliminary type and more sophisticated technology should be adopted. More precise methods should be adopted for pain sensation test. During CNS depressant experiment and analgesic activity test surrounding environment was maintained properly. Traditionally, *Sesbania grandiflora* is used for some other treatment purpose also. Hence, some other pharmacological effect may be found. For the reason, some other pharmacological investigations should be adopted.

Table 1: Results of acetic acid induced writhing test

Group	Doses (mg/kg)	No of Writhing	% of Inhibition
Control	10 ml/kg of 1% Tween 80	50.2 ± 1.53	0.00
Std (Diclofenac)	50	11 ± 1.87	78.09
Methanol Extract	250	16 ± 1.645	68.13
	500	7.2 ± 3.62	85.56

Values are expressed as Mean ±SEM (n=5)

Table 2: Results of tail immersion test

Test Group	Dose (mg/kg)	0 min	30 min	60 min	90 min
Control		2.200 ±0.750	3.20 ± 1.16	2.600 ± 0.49	3.400 ± 1.020
Positive Control	50	4.200 ±0.750	8.06 ± 0.54	8.120 ± 0.45	7.540 ± 0.810
Group-I	250	5.240 ±1.350	5.792 ±2.96	3.182± 0.55	2.418± 0.799
Group-II	500	6.974 ±0.860	5.39 ±3.05	3.650 ± 1.17	3.220 ± 2.740

Values are expressed as Mean ± SEM, (n=5)

Table 3: Effect of *Sesbania grandiflora* on Open Field Test

Group	Observation			
	0 min	**30 min**	**60 min**	**90 min**
Control	113 ±3.22	106.6±1.69	91.2 ±1.51	87.4 ±1.63
Group-1	92.4 ±2.46	0 ±0	0 ±0	0 ±0
Group-2	49.4± 1.36	0±0	0 ±0	0 ±0

Values are expressed as Mean ±SEM (n=5)

Table 4: Effect of *Sesbania grandiflora* on Hole Cross Test

Group	Observation			
	0 min	**30 min**	**60 min**	**90 min**
Control	18.40±0.93	11.80±0.66	11.40±0.75	7.80±0.86
Group-1	6.60±0.60	0.00±0.00	0.00±0.00	0.00±0.00
Group-2	2.40±0.24	0.00±0.00		0.00±0.00

Values are expressed as Mean ±SEM (n=5)

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