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Analgesic and CNS depressant activity of the crude extract of *Sesbania grandiflora*

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ABSTRACT

Sesbania grandiflora, a plant of Fabaceae is full of various pharmacologically important components like, alkaloids, flavanoids, tannins, triterpenes, gums, mucilage, and anthraquinone glycosides. From different previous research documents, various functions of different parts like leaf, flower, seed and also other parts of the plant have been known. For this experiment the leaf extract is used. Basically, the objective of the research work was to bring out the pharmacological effects (CNS and analgesic activity) of the leaf extract of the plant. The investigations had brought out the significant effects of extract. Hence, CNS depressant and analgesic drug can be produced from the leaf of *Sesbania grandiflora* through a suitable formulation.

***Key Words*:** *Sesbania grandiflora*, bokphul, analgesic activity, CNS depressant activity.

INTRODUCTION

The exploration of medicinal properties of plants throughout the ages was accomplished principally through careful observation, trial and error, and accidental discovery, which are beneficial from nutritive and medicinal standpoints. Most of such indigenous knowledge was handed down, through the ages, by at first orally and later in written form as papyri, baked clay tablets, parchments, manuscripts, herbal, and finally printed herbals, pharmacopeias and other works (Ghani, 1998; Ghani, 2005). Like the other medicinal plants *Sesbania grandiflora* is full of different constituents which are used for different treatment purpose by the human beings.

Sesbania grandiflora, a fast growing tree belongs to the family, Fabaceae (Sunil *et al.*, 2006), is commonly known as sesbania and agathi. It is used as an important dietary nutritive source and often planted for its edible flowers and pods in Southeast Asian countries (Ramesh *et al.*, 2007). It is believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas of the world (Vijay *et al.*, 2009). It is found from the northern Luzon to Mindanao in settled areas in low and medium altitudes. It was certainly introduced to Phillipines. This tree occurs also in India to Mascarene Island, through Malaya to tropical Australia, and is planted to other tropical countries (Anonymous, 1980; Kirtikar and Basu, 1995).

The other scientific names of sesbania are *Robinia grandiflora* Linn, *Aeshynomene grandiflora* Linn, *Sesban grandiflora* Poir, *Agathi grandiflora* (L.) Desv. They are also known as agathi, agati sesbania, August flower, Australian corkwood tree, flamingo bill, sesban, swamp pea, tiger tongue, West Indian pea, white dragon tree etc. In bengali, they are called by different names like, agathi, agati, agusta, bokful, bak, bake and so on (Vijay *et al.*, 2009; Kar and Borthakur, 2008). It is a small growing; short-lived, white, soft-wooded tree sparsely branched. The tree is 5 to 12 meters in height. The leaves are 20 to 30 centimeters long. The flowers may be of white, red, or yellow color and 7 to 9 centimeters long (Figure 1). The chemical arrangements are different according to its different parts. Leucocyanidin and

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Figure 1: Flower of *Sesbania grandiflora*.

cyaniding are present in the seeds; olenolic acid and its methyl ester and kaemferol-3-rutinoside are present in the flower. The bark contains tannins and gum (Vijay *et al.,* 2009). In investigation, it is found that it contains alkaloids, flavanoids, tannins, triterpenes, gums, mucilage, and anthraquinone glycosides. Sesbania is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sore throat, and stonatitis. It is also used for the treatment of anemia, bronchitis, opthalmia, inflammation, leprosy, gout, and rheumatism. In addition, sesbania is mentioned as a potent antidote for tobacco and smoking-related diseases (Ghani, 1998).

Almost all parts of this plant can be used for different purposes. From this consideration, different types of experiments had been carried out by many investigators on this plant previously which brought out many fruitful results. Experimentations by different researchers indicate that, this plant has a protective effect against cigarette smoke-induced oxidative damage in rats (Ramesh *et al.,* 2008), against Erythromycin Estolate-Induced Hepatoxicity (Pari *et al.,* 2003), has cardioprotective effects against cigarette smoke-exposed rats (Ramesh *et al.,* 2008), has an anthelmintic property of various seed oils (Sunil *et al.,* 2006), it is effective as an Antidote to Tobacco (Ramesh and Begum, 2008), has antimicrobial activity (Krasaekoopt and Kongkarnchanatip, 2005), effect on digestion and of growing goat (Nhan, 1998), and so many other effects. This present study is carried out to study the analgesic activity and the neurological activity of the crude methanolic extract of *Sesbania grandiflora*.

MATERIALS AND METHODS

Plant material

Sesbania was collected from the local area of Rangpur. The time of collection was June, 08 at the daytime. Later, Bangladesh National Herbarium, Mirpur, Dhaka, identified the plant and gave an identification number (Voucher specimen: BOK-SK-05.12.26, Accession code: 32531). Date of the investigation by the herbarium was 05.12.2008. After collecting, the plant has been dried out by using an oven (L-C Oven) at 40°C for 7 days. Then the plant part was grinded by Blender machine (Nowake, Japan). After grinding, fine powder was obtained whose amount was about 1000gm/1kg. From this powder 100gm soaked in 300ml of 80% of methanol in a glass container for 7 days. The extract was separated from the leaf debris by filtration by filter paper (9 Whitman Filter Paper). The extract was concentrated by evaporation and dried to solid in an oven.

Chemicals and reagents

Methanol and acetic acid were obtained from Marc, Germany, Tween 80 and Diclofenac Sodium from Beximco Pharma Ltd. Distilled water, which had been used in this experiment was laboratory prepared.

Experimental animals

For the experiment, Swiss albino mice (wt. 18-20gm) were collected from ICDDR,B Animal House, Mohakhali, Dhaka, Bangladesh. They were about 120 in number. Enough amounts of food and water were given to them for their comfort.

Experimental protocol

Analgesic activity test

Acetic acid induced writhing test

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in

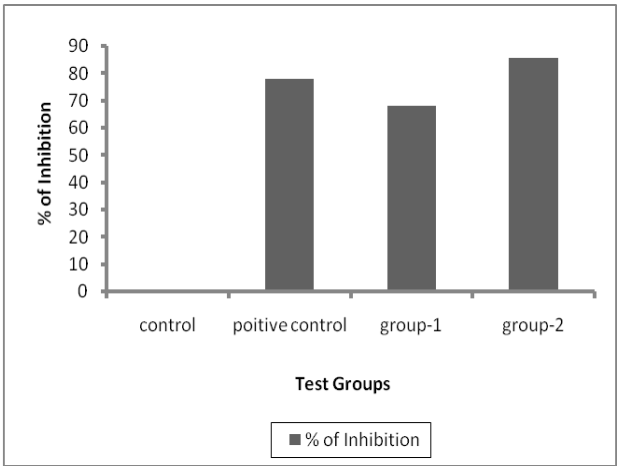

Figure 2: Bar Diagram showing the result of Writhing Test.

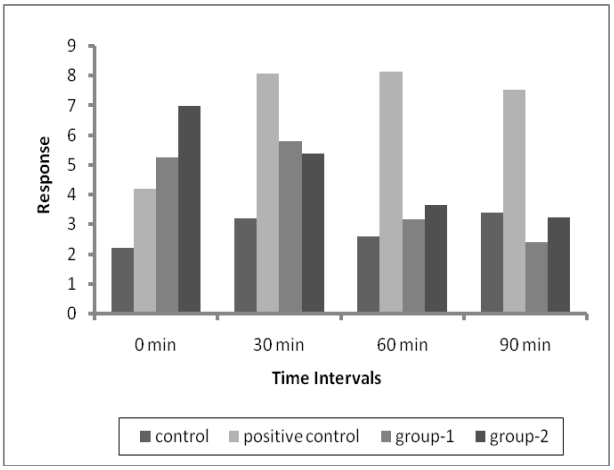

Figure 3: Bar Diagram showing the result of Tail Flick Test.

mice. The method described by (Howlader *et al.*, 2006). The animals were divided into four groups, each of which containing 5 mice. Group-1: Control group, group-2: Rats administered with Diclofenac Na, Group-3: Rats administered with *S. grandiflora* (250mg/kg), Group-4: Rats administered with *S. grandiflora* (500mg/kg).The control and the leaf extract were given orally by means of feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intraperitonally to each of the animals of a group. After an interval of fifteen minutes, this was given for absorption and no writhing was counted for 5 minutes. Then every mouse of all groups was observed carefully to count the number of writhing which made within 15 minutes.

Tail Flick Method

The tail flick test was used with modification described by Dambisya and Lee (1996). The extract was administered orally at two doses (250 and 500mg/kg body weight) using Diclofenac Na as standard. The post drug reaction time was measured at 0, 15, 30, 45, 60 and 90 minutes later. The tail of the mouse was immersed to a constant level (3cm) in a water bath maintained at 55°C. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 seconds was maintained to prevent thermal injury to the animals.

Neurological activity tests

Open Field Test

The Open Field Test (OFT) is clearly the most frequently used of all behavioral tests in

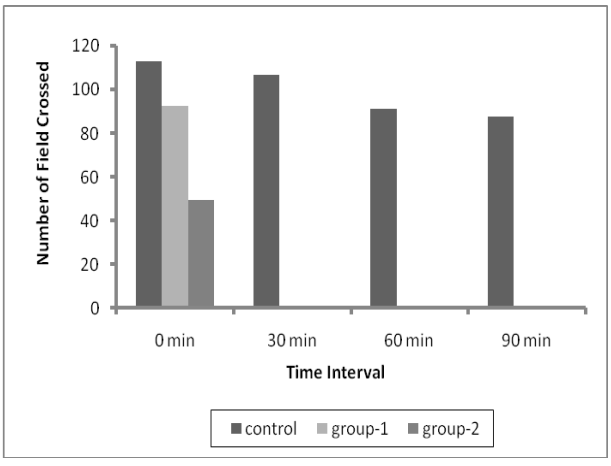

Figure 4: Bar Diagram showing the result of Open Field Test.

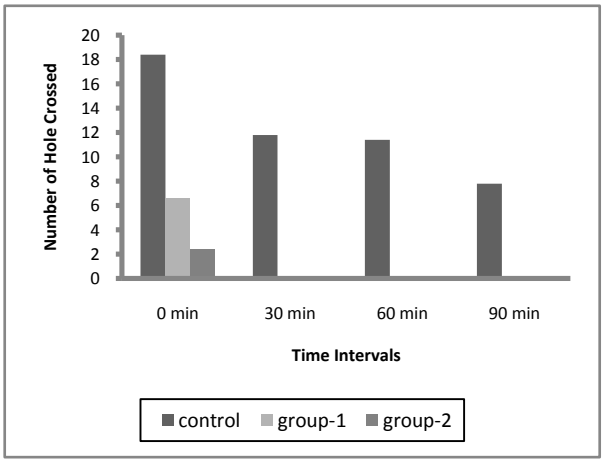

Figure 5: Bar Diagram showing the result of Hole Cross Test.

Table 1: Data of Analgesic Activity by Writhing Test of the leaves of S. grandiflora on mice.

Group	Avg. Body Wt. of Mice (gm)	Writhing Counting					Mean	% Writhing	SD	SE	SEM	Mean±SEM	% of Inhibition
Control	20	46	49	55	52	49	50.2	100	3.06	1.53	1.53	50.2±1.53	0.00
Positive Control		6	8	13	17	11	11	21.91	3.74	1.87	1.87	11±1.87	78.09
Group-1		13	18	12	21	16	16	31.87	3.29	1.64	1.64	16±1.64	68.13
Group-2		7	6	8	6	9	7.2	14.34	7.23	3.62	3.62	7.2±3.6	85.56

Control: Tween-80+water, Positive Control: Diclofenac Na (25mg/kg)

Group-1: Extract of the leaves of S. grandiflora (250mg/kg), Group-2: Extract of the leaves of S. grandiflora (500mg/kg)

Table 2: Data of analgesic activity by Tail Flick Test of the leaves of S. grandiflora on mice.

Test Group	Dose (mg/kg)	0min	30min	60min	90min
Control	-	2.200±0.750	3.20±1.16	2.600±0.49	3.400±1.020
Positive Control	25	4.200± 0.750	8.06±0.54	8.120±0.45	7.540±0.810
Group-1	250	5.240±1.350	5.792±2.96	3.182±0.55	2.418±0.799
Group-2	500	6.974±0.860	5.39±3.05	3.650±1.17	3.220±2.740

Results are presented as Mean ± SD

Control: Tween-80+water, Positive Control: Diclofenac Na (25mg/kg), Group-1: Extract (250mg/kg), Group-2: Extract (500mg/kg)

pharmacology and neuroscience. The method described by Gupta *et al.* (1971) was adopted for this test. The extract of *S. grandiflora* was administered to the mice. The numbers of field crossed by the mice were counted after every 30 minutes (From 0 minute to 90 minutes). At the 0 minute, there were no effects on the test animals. Within 30 minutes, it was observed that the mice began to sleep and therefore no movement was observed. Even after 90 minutes of administration of the extract they were still sleeping and there was no movement due to sleep.

Hole Cross Test

The most consistent behavioral change is a hyperemotional response to novel environmental. The experiment was carried out as described by Takagi *et al.* (1971). The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The leaf extracts were administered to the mice. Then their spontaneous movement of the animals through the hole from one chamber to another chamber of a wooden box was counted for 5 minutes in this test. The observations were made on 0, 30, 60, 90 minutes after intraperitoneally injection of the test drugs.

RESULTS AND DISCUSSION

Acetic acid induced writhing test

In this study, use of scientific methods to elucidate the anti-nociceptive properties of *S. grandiflora* has been attempted. The data obtained clearly indicated that the plant extract has anti-nociceptive activity by the highly significant responses. The tail-flick test and writhing test were used to elucidate central and peripheral anti-nociceptive effect, which was both central and peripheral. The mean number of abdominal constriction after I.P injection of acetic acid was 50.2 in vehicle treated control animals. Diclofenac Na treatment produced 78.09% inhibition of writhing response. A dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of methanolic extract of *S. grandiflora*. At the dose of 250mg/kg and 500mg/kg, inhibition of writhing response was observed 68.13% and 85.56% respectively (Figure 2). The experiment proved that the leaves extract of *S. grandiflora* has analgesic activity.

Tail Flick Method

In the experiment, we have seen that, the duration of heat tolerance is depending on the dose of our extract, and the onset of action is very rapid. From figure 3, it is observed that, in case of Diclofenac Na, the heat tolerance limit of the mouse was 4.20 seconds at 0min of drug administration, then the limit was almost 8 seconds at 30th min. It has gradually increased to 8.12 seconds at 60th min, but at 90th min, the tolerance limit decreased to 7.54 seconds. Besides, when we administered *S. grandiflora* at 250mg/kg dose, the tolerance limit was

Table 3: Data of neurological activity by Open Field Test of the leaves of *S. grandiflora* on mice.

Group	Routes of Administration	Number of Field Crossed			
		0 min	30 min	60 min	90 min
Control	Parenteral	11.3±3.22	106.6±1.69	91.2±1.51	87.4±1.63
Group-1	Parenteral	92.4±2.46	0±0	0±0	0±0
Group-2	Parenteral	49.6±1.36	0±0	0±0	0±0

Table 4: Data of neurological activity by Hole Cross Test of the leaves of S. grandiflora on mice.

Group	Route of Administration	Number of Hole Crossed			
		0 min	30 min	60 min	90 min
Control	Parenteral	18.40±0.93	11.80±0.66	11.40±0.75	7.80±0.86
Group-1	Parenteral	6.60±0.60	0.00±0.00	0.00±0.00	0.00±0.00
Group-2	Parenteral	2.40±0.24	0.00±0.00	0.00±0.00	0.00±0.00

5.24 at 0min, then, it increased to 5.792 at 30th min, but the limit decreased at 60th min to 3.182 seconds and finally to 2.41 seconds at 90th min. So here we can see that the onset of action of our sample is faster than Diclofenac Na. The experiment proved that the leaves extract of *S. grandiflora* has analgesic activity.

Open Field Test

After statistical analysis of many experimental data, it was observed that in open field test, the 250mg/kg extract of *S. grandiflora* shows significant CNS depressant effect and they felt asleep within 30 minutes and continued to sleep for about 2 hrs. Again, the 500mg/kg extract of *S. grandiflora* showed more significant CNS depressant effect and naturally they felt asleep (Figure 4). When we administered the extract at 250mg/kg, the mice felt asleep within 30mins, so, here, we cannot see any bar in the diagram for Group-1 and Group-2 after 30mins, which was indicating the number of the square they crossed. The experiment proved that the leaves extract of *S. grandiflora* has neurological activity.

Hole Cross Test

After statistical analysis of the experimental data, it was observed that both 250mg/kg and 500mg/kg extract of *S. grandiflora* gave rapid onset of action and had produced sleeping which may be attributed to an action on the cerebral mechanism involved in the regulation of the sleep. The experiment proved that the leaves extract of *S. grandiflora* has neurological activity. Just as the previous test, at 250 mg/kg the mice fell asleep within 30mins, so, in here also, we cannot see any bar in the diagram for Group-1 and Group-2 after 30 mins, which was indicating the number of hole crossing (Figure 5).

CONCLUSION

A number of experiments have been carried out on *Sesbania grandiflora* to assure its analgesic and CNS depressant effects on mice. The results definitely prove that the leaf extract of this plant has a good analgesic and CNS depressant activity which is different from the findings of the previous researchers. These findings could open a new window on the use of this plant in traditional medicine.

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