

## Neuropharmacological Activities of Methanol Extract of *Albizia lebbbeck* (L.) Benth.

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### Abstract

*Albizia lebbbeck* (Linn.) Benth. is a deciduous tree which is planted as ornamental and avenue tree almost all over Bangladesh. Leaves of the plant are used in ethnomedicine for the treatment of convulsion and CNS related disorder. This study was aimed to evaluate the neuropharmacological effects of the methanol extract of *A. lebbbeck* leaves in Swiss albino mice. The locomotor effect of crude methanol extract of the plant was investigated by open field and hole cross tests while the anxiolytic activity was determined using elevated plus-maze (EPM) and light/dark box (LDB) tests. Furthermore, the sedative activity of the plant extract was assessed by sodium thiopental-induced sleeping time test. The results demonstrated that the methanol extract significantly ( $p < 0.001$ ) reduced locomotion of the animals in both hole cross and open field tests in dose-dependent manner at 200-400 mg/kg b.w. In both EPM and LDB tests, the plant extract produced significant anxiolytic effect ( $p < 0.05$ ) at the doses of 100-400 mg/kg b.w. In addition, it showed significant ( $p < 0.001$ ) dose-dependent decrease in the onset of sleep and an increase in duration of sleep in sodium thiopental-induced sleeping time test. Preliminary phytochemical analyses of the plant extract revealed the presence of alkaloid, flavonoid, glycoside, saponin, tannin and resin. In acute toxicity test, the leaf extract did not exhibit any adverse effect in mice during 7 days treatment. The results of the present studies suggest that the crude methanol extract of *A. lebbbeck* leaves possesses significant CNS depressant, anxiolytic and sedative properties and rationalize the traditional uses of the plant.

**Key words:** *Albizia lebbbeck*, ethnomedicine, CNS depressant, anxiolytic, sedative.

### Introduction

*Albizia lebbbeck* (L.) Benth. (Family: Mimosaceae) known locally as Shirish, is a fast-growing medium-sized to large deciduous tree (approximately 12-21 m in height) with compound leaves, grayish-brown bark, reddish mimosaceous flowers, flat oblong fruits and flat, round, cream-coloured seeds (Vozzo, 2002; Ghani, 2003). The genus *Albizia* comprises about 150 species, which are found mostly throughout India, Bangladesh, tropical and sub-tropical regions of Asia and Africa. The tree is widely cultivated as an ornamental and plantation tree in almost all districts in Bangladesh (Ghani, 2003; Faisal *et al.*, 2012).

It has been reported that the main bioactive constituents of *A. lebbbeck* are alkaloids, flavonoids, tannins, triterpenes, glycosides, proteins, saponins and various sterols like taraxerol, cycloartemol, lupeol, campesterol, and  $\beta$ -sitosterol. Recently, two tri-*o*-glycoside flavonols, kaempferol and quercetin, and a hexaglycosylated saponin, albizziahexoside, have been isolated from the leaves of *A. lebbbeck* (Yadav *et al.*, 2011; Faisal *et al.*, 2012). The leaves have been also found to contain non-protein amino acids and a large amount of keto acids including phosphoenol-pyruvate, glyoxylate, oxaloacetate and  $\alpha$ -oxoglutarate (Une *et al.*, 2001). The leaf extract has been reported to exhibit antibacterial (Chulet *et al.*,

2010), anticonvulsant (Kasture *et al.*, 2000), diuretic (Sivakumar *et al.*, 2013), immunomodulatory (Chaudhary *et al.*, 2012), anti-asthmatic and nootropic (Faisal *et al.*, 2012) activities.

*A. lebbeck* is extensively used in treatment of a wide range of diseases and disorders including asthma, arthritis, antiseptic, burns, boils, toothache, dysentery, allergic rhinitis, bronchitis, leprosy, leucoderma, ophthalmopathy, skin eruption, sprains, wounds, ulcers, paralysis and tuberculosis (Une *et al.*, 2001; Zia-Ul-Haq *et al.*, 2013). A decoction of leaves is also used for the central nervous system (CNS) depressant effect in folk medicine (Mathur, 2012). Therefore, considering the ethnomedicinal implications and previously reported bioactivities of *A. lebbeck* leaves, we have investigated neuropharmacological effects of methanol extract of the plant leaves. The plant extract was subjected for locomotor, anxiolytic and sedative activities in different animal models of CNS. Additionally, the acute toxicity of the crude plant extract was also estimated, and we here in, report the results of our preliminary findings.

## Materials and Methods

**Plant material and extraction:** The leaves of *A. lebbeck* were collected from Sonargoan, Narayanganj, Bangladesh in August 2013 and were identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A voucher sample (DACB: 38500) has been deposited in the herbarium for further reference. The collected leaves were washed, dried in a mechanical dryer at 40 °C and ground to coarse powder. The powdered leaf (108 g) was soaked in 500 ml of methanol. The mixture was occasionally stirred and kept at 25 ± 2 °C for 10 days. Then the mixture was filtered through a cotton plug followed by Whatman filter paper no 1 and the filtrate was completely dried using a rotary evaporator at 40°C and 50 r.p.m. under reduced pressure. Finally, 14.78 g extract (yield 13.69%) was obtained which was further used for the phytochemical and pharmacological studies.

**Preliminary phytochemical screening:** The crude methanol extract of *A. lebbeck* leaves was qualitatively analyzed to detect the presence of carbohydrates, glycosides, cardiac glycosides, saponins, flavonoids, tannins, steroids, coumarins, resins, and alkaloids, following the procedures as previously described by Ghani (2005).

**Experimental animals:** Swiss Albino mice of 8 - 10 weeks aged, weighing between 25 - 30 gm, were collected from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (icddr,b). They were given icddr,b formulated food and fresh water *ad libitum*. The animals were acclimatized to the laboratory environment for 14 days prior to the experiments and were maintained under standard environmental conditions (temperature: 25.0 ± 2.0°C; relative humidity: 55 - 60 % and 12 hrs light/12 hrs dark cycle). All the experimental animals were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) drafted described by the Swiss Academy of Medical Sciences and Swiss Academy of Sciences. All the protocols were approved by the Institutional Ethics Committee of Stamford University Bangladesh (SUB/IAEC/13.09).

**Drugs and treatments:** Standard drugs used in this study were diazepam (Square Pharmaceutical Ltd., Bangladesh) and sodium thiopental (Gonosshastho Pharmaceuticals Ltd., Bangladesh). The vehicle (0.9% saline water; 10 ml/ kg b.w., p.o.), methanol extract of *A. lebbeck* leaves (100, 200 and 400 mg/kg b.w., p.o.), diazepam (1 mg/kg b.w., i.p.) and sodium thiopental (40 mg/kg b.w., i.p.) were administered 30 min before the experiments started.

**Acute toxicity test:** The oral acute toxicity test of the plant extract was observed in four test groups (n = 5) at the dose of 500, 1000, 2000 and 3000 mg/kg b.w. After gavaging, the mice were kept in separate cages and allowed for free access of water *ad libitum* and food. Each animal was observed for mortality, allergic reactions, and any abnormal behaviors continuously for the first 4 h and next 7 days after treatment (Shang *et al.*, 2015).

### Locomotor activity

*Open field test:* This experiment was carried out on apparatus, measuring  $50 \times 50 \times 50 \text{ cm}^3$  in size. The floor of the open field was divided into series of squares and each square painted in black and white in an alternative format. Thirty minutes after the treatment with diazepam (1 mg/kg, i.p.) and experimental groups, mice were individually placed in the center area of the open field and then the number of squares traveled was counted for 3 min at 0, 30, 60, 90 and 120 min during the observation period (Gupta *et al.*, 1971). The percent inhibition was calculated as: % Inhibition =  $\{[\text{Mean squares travel (Control)} - \text{Mean squares travel (Treatment)}] / [\text{Mean squares travel (Control)}]\} \times 100$ .

*Hole cross test:* The hole cross test was performed according to the method described by Takagi *et al.*, (1971). The apparatus was  $30 \text{ cm} \times 20 \text{ cm} \times 14 \text{ cm}^3$  in size and provided with a fixed partition in the middle of the cage. A hole of 3 cm in diameter was made in the partition to move the mice freely from one chamber to other. Each animal was placed beside the wall of one chamber facing to the hole thirty minutes after the treatment with standard and experimental groups. Then the number of hole crossed was counted for 3 min at 0, 30, 60, 90 and 120 min during the observation period and the percent inhibition of number of hole crossed was calculated.

### Anxiolytic activity

*Elevated plus-maze test:* The apparatus consisted of two opposite open arms, two opposite enclosed arms and a central platform measuring of ( $50 \times 10 \text{ cm}$ ), ( $50 \times 10 \times 40 \text{ cm}$ ) and ( $5 \text{ cm} \times 5 \text{ cm}$ ), respectively. Each type of arms was arranged like plus sign and was elevated to a height of 50 cm from the ground level. Then, 30 min before the experiment, mice were individually placed in the center of the apparatus facing one of the open arms. The number of entries and time spent on the open and enclosed arms was observed during a period of 5 min (Pellow *et al.*, 1985). An arm entry was counted when all four paws were in the arm. The percentage

of open arm entries and the time spent in open arm were measured using the following formula:

% of open arms time spent =  $[\text{open arms time} / (\text{open arms time} + \text{closed arms time})] \times 100$

% of open arms entries =  $[\text{open arms entries} / (\text{open arms entries} + \text{closed arms entries})] \times 100$

*Light/dark box test:* The experiment was carried out as the procedure described by Hascoet and Bourin (1998). The light/dark box apparatus was consisted of a rectangular wooden box ( $46 \times 27 \times 30 \text{ cm}$ ) and was divided into the typical dimensions of one-third for the dark compartment and two-thirds for the light compartment. To move freely from one part of the chamber to other both sections were connected by an opening of  $7.5 \times 7.5 \text{ cm}$  at floor level. The light compartment was brightly illuminated with a light source of 400 lux which placed 35 cm above the box. 30 minutes after treatment with the vehicle, methanol extract or diazepam, each mouse was individually placed in the corner of the light compartment, facing away from the entry to the dark compartment. The mice were monitored for a period of 5 min and the following parameters were observed and quantified: (a) latency of the first crossing from one compartment to the other, (b) time spent into light and dark compartment, (c) the number of transition between the light and dark compartment.

### Sedative activity

*Sodium thiopental-induced sleeping time determination:* The experiment was conducted as previously described by Ferrini *et al.*, (1974). Mice were injected with sodium thiopental (40 mg/kg b.w.), 30 min after the vehicle, methanol extract of plant leaves or diazepam treatment to induce sleep. Immediately after administration of sodium thiopental, the animals were observed for the latent period (time between sodium thiopental injections to loss of righting reflex). The duration of sleep was recorded from the time of loss to the recovery of the righting reflex.

### Statistical analysis

The results were expressed as a mean  $\pm$  standard error of the mean (SEM). The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's *post hoc* test using SPSS 22.00 software.

### Results and Discussion

In the present study, we evaluated the neuropharmacological effects of the methanol extract of *A. lebbbeck* leaves in mice. Our results demonstrated that the oral administration of methanol extract of plant leaf elicit potent and dose-dependent CNS depressant, anxiolytic and sedative activities. Moreover, there were no death, allergic reaction or any behavioral changes observed at the doses of 500 – 3000 mg/kg b.w. in any animals during 7 days observation period, which can be suggested that the plant extract possesses a low toxicity profile.

Open field and hole cross tests are most widely used methods to investigate the locomotor activity of

the animals. Locomotor activity refers to an index of alertness and a decrease in that indicates a sedative effect (Thakur and Mengi, 2005). Our results demonstrated that the leaf extract significantly ( $p < 0.001$ ) reduced locomotion of the animals in both hole cross and open field tests. The number of squares traveled by the mice in open field test was suppressed significantly from its initial score by the treatment of the extract which was comparable to the reference drug diazepam. The dose (400 mg/kg b.w.) of the methanol extract exhibited maximum inhibition by 54.55 % at 120 min which was closer to the standard drug, diazepam (59.90 %) (Table 1). The extract at 200 and 400 mg/kg b.w. also produced significant ( $p < 0.001$ ) decrease of movement in hole cross experiment. The highest inhibition of the hole cross movement was demonstrated by the extract at 400 mg/kg b.w. by 76.74 % at 90 min where diazepam showed a maximum of the inhibition by 87.18 % at 120 min (Table 2).

**Table 1. Effect of methanol extract of leaf of *A. lebbbeck* during open field test in mice model.**

Treatment	Dose (mg/kg)	Number of square crossed (% inhibition)				
		0 min	30 min	60 min	90 min	120 min
Vehicle	-	84.80 $\pm$ 2.46	66.60 $\pm$ 3.28	49.80 $\pm$ 3.17	41.80 $\pm$ 3.89	38.40 $\pm$ 2.75
Diazepam	1	87.60 $\pm$ 2.54 (-3.30)	36.80 $\pm$ 2.11* (44.74)	24.40 $\pm$ 1.82* (51.00)	17.60 $\pm$ 0.92* (57.89)	15.40 $\pm$ 0.61* (59.90)
MEAL	100	85.20 $\pm$ 4.34 (-0.47)	49.8 $\pm$ 2.63 (25.23)	33.40 $\pm$ 2.24* (32.93)	28.20 $\pm$ 1.63 (32.54)	27.60 $\pm$ 0.73 (28.13)
MEAL	200	86.40 $\pm$ 4.04 (-1.89)	45.20 $\pm$ 1.78* (32.13)	29.40 $\pm$ 2.20* (40.96)	25.60 $\pm$ 1.66* (38.76)	24.40 $\pm$ 2.48* (36.46)
MEAL	400	86.80 $\pm$ 3.10 (-2.36)	39.40 $\pm$ 2.55* (40.84)	25.40 $\pm$ 1.85* (49.00)	19.00 $\pm$ 1.41* (54.55)	17.20 $\pm$ 1.07* (55.21)

Each value is presented as mean  $\pm$  SEM (n = 5). MEAL = methanol extract of *A. lebbbeck* leaves. \* $p < 0.001$ , compared with the control group (Dunnett's test).

The elevated plus maze (EPM) and light/dark box (LDB) test are commonly used behavioral models and have been validated to investigate both the anxiolytic and anxiogenic potential of different pharmacological agents. They both utilize the animals via naturally occurring anxiety-related

activities such as fear of novel environments including light, open space, height and fear of balancing on the narrow surface (Hascoet and Bourin, 1998; Dawson and Tricklebank, 1995). Results of the EPM test showed that the treatment of the plant methanol extract at 200 and 400 mg/kg b.w.

produced significant ( $p < 0.001$ ) increase in time spent on open arms and decrease in time spent in enclosed arms. Besides, the percent open arm entries of the extract treated mice were also significant ( $p < 0.001$ ) (Table 3). In the LDB test, the methanol extract treatment of mice on latency time, time spent

and transition in light area, were significant ( $p < 0.05$ ) compared to the control group as shown in table 4. The maximum percent inhibition was observed at the dose of 400 mg/kg in both tests (Tables 3 and 4).

**Table 2. Effect of methanol extract of leaf of *A. lebbbeck* during hole cross test in mice model.**

Treatment	Dose (mg/kg)	Number of hole crossed (% inhibition)				
		0 min	30 min	60 min	90 min	120 min
Vehicle	-	13.00 ± 0.49	11.20 ± 0.77	9.40 ± 0.83	8.60 ± 0.61	7.80 ± 0.59
Diazepam	1	12.60 ± 0.73 (3.08)	5.20 ± 0.33* (53.57)	2.60 ± 0.67* (72.34)	1.60 ± 0.22* (81.40)	1.00 ± 0.28* (87.18)
MEAL	100	13.40 ± 0.96 (-3.08)	9.40 ± 0.61 (16.07)	6.60 ± 0.46 (29.79)	5.00 ± 0.49* (41.86)	4.40 ± 0.61* (43.59)
MEAL	200	12.80 ± 0.66 (1.54)	7.80 ± 0.59 (30.36)	4.60 ± 0.61* (51.06)	3.60 ± 0.46* (58.14)	3.20 ± 0.44* (58.97)
MEAL	400	13.20 ± 0.77 (-1.54)	6.40 ± 0.46* (42.86)	3.40 ± 0.22* (63.83)	2.00 ± 0.28* (76.74)	2.20 ± 0.33* (71.79)

Each value is presented as mean ± SEM (n = 5). MEAL = methanol extract of *A. lebbbeck* leaves. \* $p < 0.001$ , compared with the control group (Dunnett's test).

**Table 3. Effect of methanol extract of leaf of *A. lebbbeck* during elevated plus-maze test in mice model.**

Treatment	Dose (mg/kg)	Enclosed arms time (sec)	Open arms time (sec)	% of open arm time (sec)	Enclosed arms entries	Open arms entries	% of open arm entries
Vehicle	-	271.55 ± 4.27	28.45 ± 4.27	9.48 ± 1.42	16.60 ± 1.22	4.80 ± 0.66	22.57 ± 3.10
Diazepam	1	146.34 ± 6.55*	153.66 ± 6.55*	51.22 ± 2.18	3.60 ± 0.46	8.20 ± 0.66*	69.77 ± 3.01
MEAL	100	211.05 ± 5.92*	88.95 ± 5.92*	29.65 ± 1.97	12.60 ± 1.97	5.60 ± 0.96	32.73 ± 7.32
MEAL	200	176.20 ± 6.70*	123.80 ± 6.70*	41.27 ± 2.23	7.60 ± 1.40	7.40 ± 0.54	51.77 ± 6.99
MEAL	400	160.98 ± 6.37*	139.02 ± 6.37*	46.34 ± 2.12	5.20 ± 0.87	8.00 ± 1.02*	60.79 ± 6.56

Each value is presented as mean ± SEM (n = 5). MEAL = methanol extract of *A. lebbbeck* leaves \* $p < 0.001$ , compared with the control group (Dunnett's test).

**Table 4. Effect of methanol extract of leaf of *A. lebbbeck* during light/dark box test in mice model.**

Treatment	Dose (mg/kg)	Latency time (s)	Time spent in light area (s)	% of light area time (s)	Transition in light area	% of light area entries	Total transition no.
Vehicle	-	11.18 ± 1.40	86.71 ± 4.62	28.90 ± 1.54	4.60 ± 0.61	25.97 ± 4.39	18.40 ± 0.96
Diazepam	1	32.08 ± 1.50*	177.26 ± 7.52*	59.09 ± 2.51	7.00 ± 0.63**	65.79 ± 6.53	10.80 ± 0.56*
MEAL	100	16.54 ± 1.22	117.87 ± 6.08**	39.29 ± 2.03	5.80 ± 0.52	39.13 ± 3.38	14.80 ± 0.33**
MEAL	200	22.60 ± 1.67*	133.83 ± 5.36*	44.30 ± 1.71	6.20 ± 0.52	50.71 ± 6.89	12.60 ± 0.67*
MEAL	400	29.36 ± 1.03*	157.17 ± 5.05*	52.39 ± 1.68	6.80 ± 0.33	67.58 ± 4.34	10.20 ± 0.59*

Each value is presented as mean ± SEM (n = 5). MEAL = methanol extract of *A. lebbbeck* leaves. \* $p < 0.001$ , \*\* $p < 0.05$ , compared with the control group (Dunnett's Test).

**Table 5. Effect of methanol extract of leaf of *A. lebbeck* during sodium thiopental-induced sleeping test in mice model.**

Treatment	Dose (mg/kg)	Onset of Sleep (sec)	Duration of Sleep (min)
Vehicle + ST	0.1mL/Mouse + 40	175.00 ± 2.51	32.61 ± 1.05
Diazepam + ST	1 + 40	94.84 ± 4.49*	93.30 ± 1.41*
MEAL + ST	100 + 40	154.67 ± 2.63	45.68 ± 1.64
MEAL + ST	200 + 40	123.66 ± 3.34*	67.65 ± 1.76*
MEAL + ST	400 + 40	91.99 ± 2.58*	79.07 ± 3.85*

Each value is presented as the mean ± SEM (n = 5), MEAL = methanol extract of *A. lebbeck* leaves, ST = sodium thiopental. \*p < 0.001, compared with the control group (Dunnett's test).

The sedative activity was evaluated by the sodium thiopental-induced sleeping test. Sedative-hypnotic agent, thiopental, which belongs to the barbiturates, at appropriate doses produced sedation or hypnosis by potentiating GABA mediated hyperpolarization of postsynaptic inhibition through allosteric modification of GABA<sub>A</sub> receptors. These results in CNS depressant effect by the means of decreasing sleep latency or prolonging the duration of sleep or both (Sieghart and Sperk, 2002). In our study, the *A. lebbeck* leaves extract treatment showed significant ( $p < 0.001$ ) dose-dependent decrease in the onset of sleep and an increase in duration of sleep (Table 5). So, it could be suggested that the leaves of the plant possess sleep-inducing property.

The phytochemical analyses of the crude methanol leaf extract of *A. lebbeck* revealed the presence of alkaloid, flavonoid, glycoside, cardiac glycoside, saponin, tannin, and resin. Moreover, *A. lebbeck* leaves have also been reported to have kaempferol, quercetin flavonoids and albizziahexoside saponin (Yadav *et al.*, 2011; Faisal *et al.*, 2012). It has been reported that quercetin and kaempferol possess anxiolytic and sedative activities (Aguirre-Hernández *et al.*, 2010; Jäger and Saaby, 2011). Saponins are also found to exhibit sedative activity (Wagner *et al.*, 1983). Considering our results and previously published reports, it can be suggested that the anxiolytic and sedative activities observed for the plant extract might be due to the presence of CNS active compounds.

In summary, the neuropharmacological study has shown that the methanol extracts of *A. lebbeck* leaves have central nervous system related effects. In hole cross and open field tests, suppression of locomotor activity was dose-dependent and significant. The plant extract also demonstrated a significant anxiolytic activity. In sodium thiopental-induced sleeping time test, the methanol extract of *A. lebbeck* showed significant dose-dependent effect in the sleep latency and duration of sleep. Based on the results of the present study, it could be concluded that the plant extracts possess remarkable CNS depressant, anxiolytic and sedative potential. Therefore, further studies regarding isolation and determination of the bioactivity of isolated compounds from the plants would be worthwhile for the development of CNS active agents.

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