Original Research Article

Evaluation of sedative and anxiolytic activity of methanolic extract of *Lablab purpureus* (L.) sweet seeds in experimental mice model

ABSTRACT

Aims: Lablab purpureus (Fabaceae) a well-known Bangladeshi vegetable combines a great number of qualities with its adaptability and functional food properties. But still there is no evidance based report on neuropharmacological activities of Lablab purpureus seeds. For this reason, the present study was designed to analyze in vivo CNS effect of methanolic extract of Lablab purpureus seeds.

Place and Duration of Study: Department of Pharmacy, USTC between April 2017 and May 2018.

Methodology: In this study, the crude seeds extract of Lablab purpureus was evaluated for its sedative and anxiolytic effect using animal behavioral models, such as open field and hole cross tests for its sedative properties and an elevated plus maze (EPM) test for its anxiolytic potential.

Results: In open field and hole cross test, the seeds extract of Lablab purpureus showed a dose dependent suppression of motor activity and exploratory behaviour. In EPM test, animals treated with higher dose of methanolic extract showed significant ($p<0.01$) increased exploration to and time spent in EPM open arms.

Conclusion: This study provide in vivo evidence that the methanolic extract of Lablab purpureus has significant sedative and anxiolytic activity. Further studies on active constituent of the extract can provide approaches for therapeutic intervention.

Keywords: Neuropharmacology, open field, hole cross, elevated plus maze, Lablab purpureus

1. INTRODUCTION

Anxiety and depressive disorders are the most common psychiatric conditions now-a-days. As indicated by the World Health report approximately 450 million people are suffering from mental or behavioral disorders. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [1,2]. Over 20% of the adult populations suffer from these illnesses

at some time during their lives [3]. Previously, psychoactive synthetic drugs were recognized as most effective in the management of central nervous system (CNS)- related disorders. Due to presence of some adverse effects of these drugs searching for new pharmacotherapy from remedial plants for psychiatric illnesses has proceeded appreciably in the past decades [4]. Medicinal plants are the prominent source of secondary metabolites as well as active compounds. It plays a pivotal role for the discovery of new drug molecules. Additionally, medicinal plants contain different essential bioactive compounds such as alkaloids, phenols, flavonoids, terpenoids, tannins, saponins, steroids, polysaccharides, and so on which are the important part of modern and traditional medicines. Medicinal plants therapies may be effective alternatives in the treatment of depression. They possess least side effects compared to synthetic medicines [5-7]. Nutraceutical products have recently been at the forefront of research because of their enormous contribution as natural agents in the prevention of various health problems. *Lablab purpureus* (Fabaceae), a common vegetable plant combines a great number of qualities with its adaptability and functional food properties. *Lablab purpureus (L.)* is a woody climbing herb which can reach a length of 5 m. Leaves are pinnate and generally 3-foliolate. Leaflets are acute, entire, 6–12 cm by 5–9 cm. Flowers are white or purplish pink. Fruits are green pods, 6 cm long by 2 cm wide, flattened, contain 4–5 seeds and turn light brown when mature [8]. *L. purpureus* has been used in the Philippines and China and some other countries as a stimulant, to reduce fever, to reduce flatulence, to stimulate digestion, and as an antispasmodic [9]. So far there has been no scientific report in literature about the sedative and anxiolytic activity (in vivo) of methanolic extract of *Lablab purpureus* seeds. Therefore, in this research study, methanolic plant extract of *Lablab purpureus* seeds was evaluated to find its *in vivo* sedative and anxiolytic potential on some neuropharmacological experimental animal models.

2. MATERIAL AND METHODS

2.1 Plant material

The seeds of *L. purpureus(L)* sweet were collected from local market in 2017 and authenticated (Herbarium-MSAN 003) by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong. A voucher specimen has been deposited at the Department of Pharmacy, University of Science and Technology Chittagong (USTC).

2.2 Preparation of Plant Extract

After collection, seeds were properly weighed by electronic balance, dehulled manually and kernels and seed coats were separated. The kernels were then ground by using blender (Nowake, Japan), and powder was passed through a sieve with mesh number 80 (177 µm pore size) and stored in air tight plastic bags at 2-8ºC until used. About 500g of powdered material was macerated with methanol (1:10) at room temperature for a period of 7 days with occasional shaking and stirring. After that the plant extract was filtered with clean cotton filter followed by Whitman filter paper (No. 1). The solvent was evaporated by Rotary evaporator (Lab Tech EV311) at 40 ºC under reduced pressure. The L. purpureus methanolic extract (LPMEx) was then preserved in a refrigerator (2-8ºC) till further use.

2.3 Experimental animals

Swiss albino mice of either sex, weighing between 18-28g, were collected from Animal Research Branches of BCSIR, Chittagong. They were kept in clean and dry iron cages with 12 hours light and dark cycle at 25±3ºC and 45-55% relative humidity in the Animal house of Department of Pharmacy, USTC. The mice were fed with standard laboratory diet supplied

by BCSIR Laboratory and water ad libitum. Food was withdrawn 12 hours before and during experiment. As these animals are very sensitive to environmental changes, they were acclimatized with the laboratory condition 7 days before the experiment. The research was approved by the Institutional Ethics Committee. All experiments were conducted under isolated and sound attenuated room.

2.4 Sedative activity

2.4.1. Open field test

The method was adopted as described by Gupta et al. [10] with slight modification. Briefly, the animals were divided into control, positive control, and test groups. The test groups received *L. purpureus* methanolic extract (LPMEx) at a dose of 200 and 400mg/kg body weight p.o. whereas the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. The floor of an OFT of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a 40cm height wall. The number of squares traveled by the animales was counted for 3minutes, at 0, 30, 60, 90, and 120 minute during the study period after oral administration of both extract and standard.

2.4.2. Hole cross test

The method was carried out as described by Takagi et al., [11]. The animals were divided into control, positive control, and test groups with six animals in each group. The test groups received LPMEx at a dose of 200 and 400mg/kg body weight p.o. whereas the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. The number of passages of the animals through the hole from one chamber to the other was counted for 3min at 0, 30, 60, 90 and 120min after oral administration of the extract as well as diazepam and vehicle. The apparatus was thoroughly cleaned after each test. The apparatus was a cage of 30cm×20cm×14cm with a partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5cm diameter was made at a height of 7.5cm in the center of the cage.

2.5. Anxiolytic activity

2.5.1. Elevated plus maze test

The method was carried out according to Lister RG. [12] with minor modifications. The apparatus consists of two open arms(5 × 10 cm) and two close arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to form a plus –sign figure situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animals were divided into control, positive control, and test groups with 5 animals in each group. The test groups received LPMEx at a dose of 200 and 400mg/kg body weight p.o. whereas the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. Sixty minutes after administration of the test samples, each animal was individually placed in the center of the EPM and were allowed 5 min for free exploration. Next, the number of open and close arms entries, and time spent on open and close arms were manually registered. The whole test was carried out in a sound attenuated room. Entry into an arm was defined as the point when the animal placed all four paws onto the arm.

% of time spent in open arm = (Time spent in open arm)/(Time spent in open arm + Time spent in close arm)

% of entry in open arm = (No.of entry in open arm)/(No.of entry in open arm + No.of entry in close arm)

2.6. Statistical analysis

The data were expressed as mean ± Standard error mean (SEM). Statistical comparisons were performed using One way ANNOVA followed by Dunnett's multiple comparison test. The values were obtained were compared with the vehicle control group and were considered statistically significant when $p<0.05$.

3. RESULTS

3.1. Open field test

In the OFT, the number of square traveled by the mice suppressed significantly ($p<0.01$) in the third observational period (60 min.) at the dose of 400 mg/kg b.w. and showed dose-dependent reduction of movement from its initial value at 0 to 120 min (Figure 1). Diazepam was used as the standard drug in the experimental animals to evaluate the CNS depressant effect of the seeds extract.

3.2 Hole cross test

In the HCT, the number of hole crossed from one chamber to another by the experimental animals of the control group was similar throughout the observational period. In the animal treated with methanolic extract *L. purpureus* seeds exhibited a dose-dependent suppression in the locomotor activity and at higher dose (at dose of 400mg/kg b.w.) the locomotor activity was significantly ($p<0.01$) reduced from the third to fifth observational period, which was comparable to the reference diazepam ($p<0.001$) (Figure 2).

3.3 Elevated Plus Maze test

The EPM test is probably the most widely used model to evaluate the anxiolytic activity. In EPM test, the time and proportion of entrance into the open arms generally increases when a anxiolytic substance is given to the experimental animals. In this study, methanolic extract of *L. purpureus* seeds treated groups (200 and 400 mg/kg body weight) showed significant and dose-dependent increment of percentage of entries of mice in open arms , and the percentage of time spent in open arms of the EPM as shown in table 1. At a dose of 400 mg/kg body weight maximum anxiolytic activity was found as the maximum percentage of entries in open arms was displayed ($p<0.01$) which was comparable to the standard diazepam.

4. DISCUSSION

Anxiety and depression-related disorders are increasing day by day all over the world. Humanitarian emergencies and ongoing conflict add further to the need for upgrading of treatment. WHO estimates that, during emergencies, as many as 1 in 5 people are affected by anxiety and depression [13]. The CNS depression activity showed by methanolic extract

of L. purpureus seeds indicated the suppression of the locomotor activity. The present study demonstrated that the administration of methanolic extract of *L. purpureus* seeds at a dose of 400 mg/kg body weight showed strong sedative and anxiolytic properties. The study on locomotor activity showed significant ($p<0.05$) suppression in the movement of mice at higher dose (400 mg/kg b.w.) of methanolic extract as compared to the control group in open field and hole cross tests. Since locomotor activity is a measure of the level of excitability of CNS, [14] this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts [15]. In OPT and HCT, at a dose of 400mg/kg b.w. the sedative effect was evident at the 3rd observation (60 min) and continued up to the 5th observation period (120 min) (Figure 1 and Figure 2).

However, the anxiolytic effect was evaluated by the EPM test that has been recognized as a valuable model able to predict anxiolytic effects of drugs in rodents [15, 16]. The anxiolytic effect is observed when the experimental drug increases open arm entries [17]. At both doses of methanolic extract of L. purpureus seeds exhibited an increase in the percentage of entries into open arms and percentage of time spent into open arms. But at dose of 400 mg/kg showed a significant increase in percentage of open arm entries ($p<0.01$) and percentage of time spent into open arms ($p<0.05$). Compared to the reference standard drug diazepam test extract showed significant anxiolytic action at highest dose of 400 mg/kg b.w.

GABAA-benzodiazepine receptors are the most abundant inhibitory receptor [18] system in the CNS and binding of a benzodiazepine agonist to its recognizing site results in increased chloride ion flux [19] which in turn hyperpolarizes the postsynaptic membrane at a level below that at which spike generation is possible and for this reason some GABAA agonists are frequently used for their sedative effects. Some of the reported bioactive phytochemicals such as Lablabosides A, B and C, 3,7,11-Trimethylhentriacontane, (E)- 2-Octene, 7,11,17,21-Tetramethylhentriacontane, 6-Methyldotriacontane, Norbornene,, 4-methyl thiazole, 5,9,13-Trimethylnonacosane, Methyl Butyrate, 13,17-Dimethyl nonacosane,Santene, 5-Methyl hentriacontane, Luteolin, Cosmosiin, Luteolin-4/7-O-β-D glucopyronoside [20,21] present in the L. purpureus seeds, may act as GABAA agonists and this agonistic property could be attributed to the CNS depressant effect. These findings suggest that the seeds of *L. purpureus* could be useful as a therapeutic agent for anxiety-related disorders. Further investigations are needed to elucidate the bioactive constituents, responsible for sedative and anxiolytic activity of *L. purpureus* seeds.

4. CONCLUSION

From the above experiments, it could be concluded that the methanolic extract of L. purpureus seeds possesses a significant CNS effect and may fulfill the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders. However, further phytochemical and pharmacological studies would be necessary to evaluate the contribution of compounds for the activity showed as it still remains to be determined which components were exactly responsible for these effects.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" Reference No. USTC-EA-2017/10/025.

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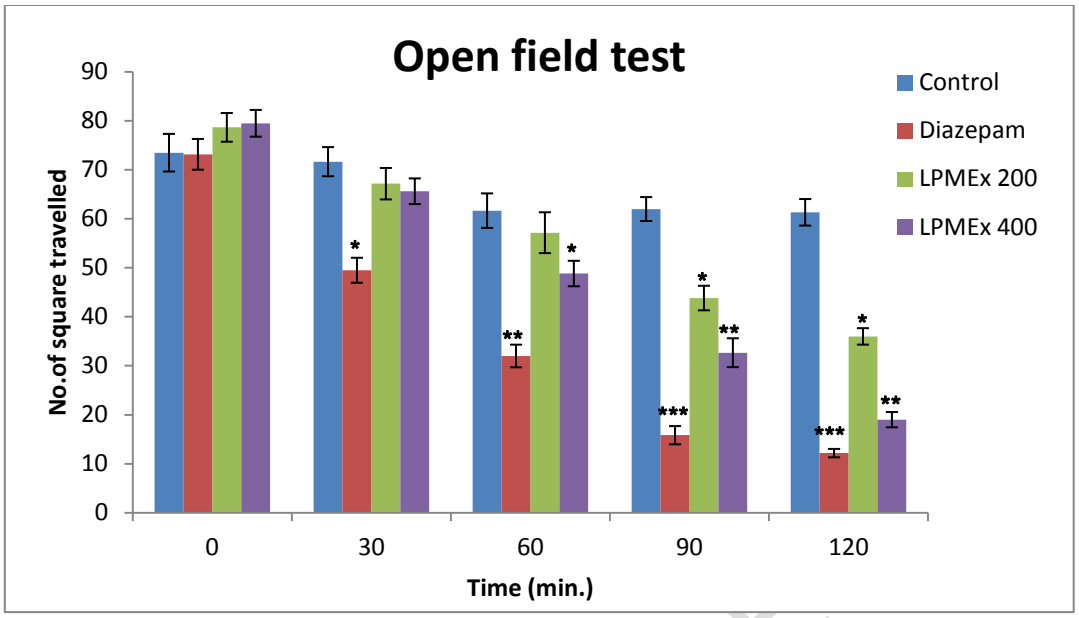

Figure 1: Effects of *L. purpureus* seeds extract on the open field test in mice. Values are mean±SEM., (n=6); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Dunnet test as compared to control (vehicle=10ml/kg)

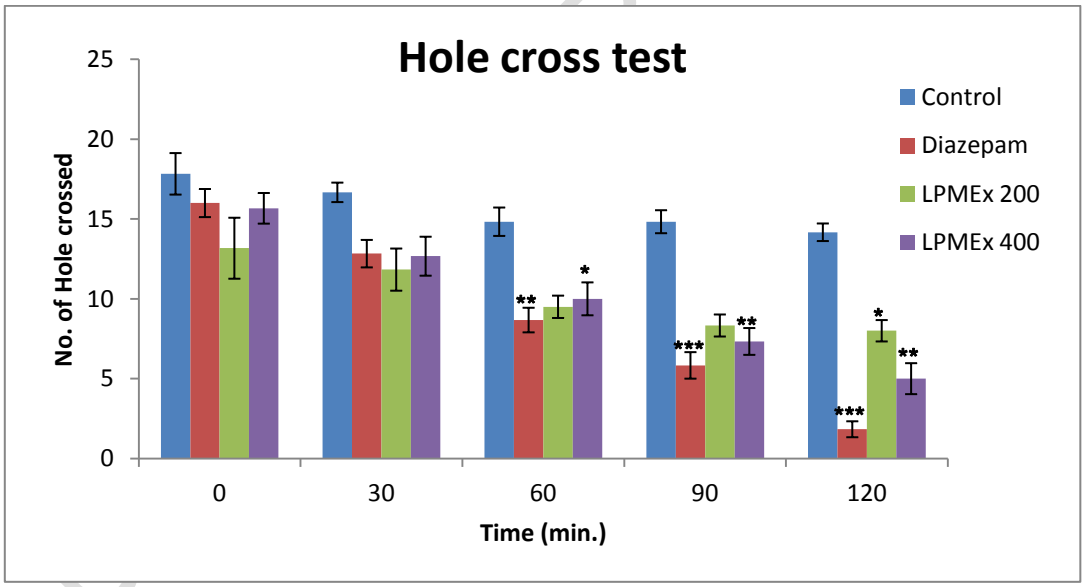

Figure 2: Effects of *L. purpureus* seeds extract on the Hole cross test in mice. Values are mean±SEM., (n=6); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Dunnet test as compared to control (vehicle=10ml/kg)

Table 1: Effect of methanolic extract of *L. purpureus* seeds on EPM test during 5 min test period.

Animal Groups	Dose	% of number of entry into open arm	% of Time (in seconds) spent in open arm
Control	10ml/kg	29.07±2.29	12.9±1.61
Diazepam	1mg/kg	73.37±1.72***	63.94±1.99**
LPMEx 200	200mg/kg	39.75±4.35*	23.90±1.67
LPMEx 400	400mg/kg	67.34±2.49**	51.03±2.86*

Values are mean±SEM., (n=5); * p<0.05, ** p<0.01, *** p<0.001, Dunnet test as compared to control (vehicle=10ml/kg

(A)

(B)

Image 1: Morphology of *Lablab purpureus* (A) Whole plant ; (B) Seeds

Image 2: Herbarium of *Lablab purpureus*