Neuropharmacological Profile of Methanolic Extract of *Leucas aspera* Leaves in Swiss Albino Mice

Rashed Reza[1], Md. Abdul Mannan[1*], Md. Sohrab Hosen[2] and Most. Nazma Parvin[1]

[1]Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

[2]Department of Pharmacy, University of Development Alternative, H#4/4-B, Block-A, Lalmatia, Dhaka-1205, Bangladesh

Abstract

Leucas aspera (*L. aspera*) is commonly known as "Darkolos or Dandokolos" in Bangladesh. The plant is used for the treatments of anti-pyretic, anti-rheumatic, anti-inflammatory, and anti-bacterial diseases. The purpose of this present study was to investigate the central nervous system (CNS) depressant activities of methanolic extract of *L. aspera* leaves (MELA) in mice models. The central nervous system (CNS) depressant activity of the MELA was evaluated using open field, whole cross, force swimming, tail suspension, and thiopental sodium-induced sleeping time tests. For CNS tests, diazepam (1 mg/kg, i.p.) was used as reference drug. In all mice models, MELA was administered orally at the doses of 250, 500, and 750 mg/kg, where as the control group expected deionized water (0.1 mL/mouse, p.o.). The present study indicated that MELA significantly decreased locomotor activity of open field and whole cross tests in mice. The extract significantly increased the duration of immobility time both force swimming and tail suspension tests. MELA significantly (*p< 0.05, vs. control) induced sleep at an earlier stage and lengthened the duration of sleeping time in thiopental sodium-induced sleeping time test. The findings of this study indicated a CNS depressant activity of *L. aspera* leaves extract. However, further studies are needed to evaluate the potential use of *L. aspera* for the treatment of CNS depressant diseases.

Keywords: *Leucas aspera;* Depressant; Extract; Diazepam; CNS

Introduction

Depression is a complex heterogeneous psychiatric condition that causes suffering to several millions of the world population [1]. It is characterized by emotional and physical manifestations such as energy, appetite, sleep, weight, and feelings of worthlessness, helplessness, hopelessness, guilt or indecision, loss of concentration, interest, agitation, mental and social withdrawal [2]. Although, market preparations are available which are used for the treatment of depression; but the efficacy of this drugs are restricted, and they are expensive. There is continuing research to develop highly efficacious, more tolerated and cost effective drugs. Plant derived medicines across a broad spectrum have been advanced as novel sources of psychiatric therapies [1], which have been reflected in the plenty of traditional therapeutic uses that have been researched and screened on behalf of their psychotherapeutic potential in animal models.

Leucas aspera (Family: Labiatae), is commonly known as "Darkolos or Dandokolos" in Bangladesh. The plant is found as weed in Asia, Africa, and Asia tropical countries. It is used for the treatments of analgesic, anti-pyretic, anti-rheumatic, anti-inflammatory, and anti-bacterial diseases. The paste of this plant is used topically to inflamed areas [3]. The entire plant is used as an insecticide, coughs, pain, and skin eruption conditions [4]. The anti-inflammatory activity of this plant has been reported in animal behavioral models [5, 6] through prostaglandin inhibition [7, 8]. The plant has wound healing activity that is used in cobra venom poisoning [9]. *L. aspera* leaves are useful in psoriasis, scabies, anti-bacterial and anti-fungal agents [10]. The root extract of *L. aspera* was studied for the central nervous system (CNS) depressant activity [11]. Preliminary phytochemical screening is reported that *L. aspera* is a source of triterpenoids, oleanolic acid, ursolic acid and 3-sitosterol [12, 13]. The plant contained sterols, alkaloids such as compound A, α-sitosterol, β-sitosterol, reducing sugars, and glucoside [14, 15]. Twenty-five compounds are identified from the leaf of *L. aspera*. Some of them are volatiles, u-farnesene, x-thujene, and menthol. Amyl and isoamyl propionate are major phytochemical constituents, which were found from the flower of *L. aspera* [16]. Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and ceryl alcohol are identified from the seeds of *L. aspera* [17, 18]. Some novel phenolic compounds, aliphatic ketols, and long-chain compounds are revealed from the shoot part of this plant [19-21]. Leucolactone (I) which has named by 3, 3, 16c-dihydroxyoleanan-28-1,3-olide, is isolated from the roots of *L. aspera* [22]. The anti-microbial, anti-malarial, larvicidal, and anti-sporiatic activities of *L. aspera* were well-established in research field [23-26]. It was also found to possess anti-plasmodial and pupicidal activities [27].

Based on the previous scientific reports, root of *L. aspera* showed the central nervous system (CNS) depressant properties [11]. For these reason, the purpose of this present study was to investigate the central nervous system (CNS) depressant activities of methanolic extract of *L. aspera* leaves (MELA) in mice models. However, further studies are needed to evaluate the potential use for the treatment of CNS depressant diseases.

Materials and Methods

Chemicals

Diazepam (Square Pharmaceuticals Ltd., Bangladesh), thiopental sodium (Sigma Chemicals Co., USA). Diazepam (1

mg/kg i.p.) was used in open field, hole cross, force swimming, tail suspension, and thiopental sodium-induced sleeping time tests as standard drug. For each experiment, the drugs were intraperitoneally (i.p.) administered 15 min before the experimental mice. For central nervous system (CNS) depressant mice models, MELA was orally administered 30 min previous to the experiments (Except open field and hole cross tests) at the doses of 250, 500, and 750 mg/kg, where the animals in control group received deionized water (0.1 mL/mouse, p.o.).

Collection of plant material

For this study, *Leucas aspera* leaves were collected from Padma Garden, Rajshahi, Bangladesh and were authenticated by Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The voucher number is "DACB: 38390". The number is deposited to the herbarium for further use.

Preparation of extraction

The leaves were dried out at room temperature in the away from direct sunlight for 5 days. The plant samples were ground into the fine powder-using blender and soaked by dissolving 7 days with methanol. Then, the extract was collected and the solvent was completely removed by rotary evaporator. 9.80 g extract (yield 3.92 % w/w) was obtained which was then used for all experimental studies.

Animals

Swiss albino mice (20-25 g) of both sexes were used for this study. The animals were purchased from Pharmacology Laboratory, Jahangirnagar University, Savar, Dhaka, Bangladesh. They were housed in polyvinyl cages with soft wood bedding materials and were kept under standard environmental surroundings of room temperature at 25 ± 2 ℃, 55-65% relative humidity with 12 h light/dark cycle. All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/17.02) of Stamford University Bangladesh approved all experimental rules.

Phytochemical analyses

The extract was subjected to qualitative screening for the detection of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids by established methods [28].

Acute toxicity test

Test mice were divided into six different groups containing five mice in each. The groups received *L. aspera* leaf extract orally at the doses of 100, 200, 500, 1,000, 2,000, and 4,000 mg/kg body weight where as the control group received deionized water. Immediately after dosing, the animals were observed continuously for the first 4 hrs for any behavioral changes. Thereafter, they were then kept under observation up to 14 days after drug administration to find out the mortality [29].

CNS depressant activity

Open field test

The method was described by Gupta et. al.[30] with few modifications. The apparatus was in light and sound control room. The mice were divided into five groups consisting of five mice in each (n=5).The control group received deionized water (0.1mL/mouse, p.o.) and the standard group received diazepam (1 mg/kg, i.p.). Three test groups received MELA at the doses of 250, 500, and 750 mg/kg body weight respectively (p.o.). The mice were individually placed in the open field box (100 cm × 100 cm × 40 cm h) which was divided into 16 square blocks. The square blocks visited by mice were recorded for 3 min on 0, 30, 60, 90, and 120 min intervals.

Hole cross test

The experiment was designed as described by Subhan et. al.[31] with minor modifications. The equipment was a fixed partitioning wall which having a dimension of 30 cm × 20 cm × 14 cm. In the middle of cage, 3 cm diameter hole was created at a height of 7.5 cm wall from the floor. Mice were separated into five groups containing five mice each (n=5).The control group received deionized water (0.1mL/mouse, p.o.) and the standard group received diazepam (1 mg/kg, i.p.). The three test groups received MELA at 250, 500, and 750 mg/kg doses body weight respectively (p.o.). Then, the number of passages from one chamber to other through the hole inside the cage was counted for a period of 3 min on 0, 30, 60, 90, and 120 min intervals.

Forced swimming test

The forced swimming test was conducted by using the method of Porsolt et. al.[32] with modifications. The experimental mice were placed in 45 cm glass cylinder of 20 cm diameter containing water at the temperature of 25 ± 1 ℃. Twenty-five mice were randomly separated into five groups in each five (n=5). The control group received deionized water (0.1 mL/mouse p.o.) and the standard received diazepam (1 mg/kg, i.p.). MELA was orally administered at doses of 250, 500, and 750 mg/kg body weight (p.o.) respectively. The immobility time was recorded for a period of 5 min in each mouse. They were considered in immobile when floated motionless in water, and producing small movements of forepaws to keep their head on top of water.

Tail suspension test

The test was adopted by the method of Steru et. al.[33] according to slight modifications. Mice were orally treated with deionized water (0.1 mL/mouse, p.o.); diazepam (1 mg/kg, i.p.) was used as standard drug and MELA at 250, 500, and 750 mg/kg doses body weight (p.o.) respectively. Each mouse was suspended on the table 50 cm above the floor with an adhesive tape placed 1 cm from the tip of the tail. Immobility time was calculated for the period of 6 min. Mice were considered immobile when they were totally static and hanged passively.

Thiopental sodium-induced sleeping time test

The experiment was evaluated through the method described by Turner with a minor change. The experimental animals were

erratically separated into five groups containing five mice in each. The control group received deionized water (0.1 mL/mouse, p.o.) and the standard group received diazepam (1 mg/kg, i.p.). The three test groups received MELA at 250, 500, and 750 mg/kg doses body weight respectively (p.o.). Thirty minutes later, thiopental sodium (40 mg/kg, i.p.) was administered to each mouse to make sleep. After that, mice were observed for the latent period (time between thiopental sodium administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of reflex) [34].

Statistical analysis

Data were presented as mean ± Standard Error of Mean (SEM). Statistical analysis was carried out using One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test through Statistical Package for the Social Sciences (SPSS) software (version 18.00).*p < 0.05, vs. control was considered to be statistically significant.

Results

Phytochemical screening

The present study showed that MELA contained alkaloids, flavonoids, terpenoids, tannins, steroids, saponins, and cardiac glycosides (Table 1).

Acute toxicity test

In this test, the animal decreased mobility without of convulsions or loss of writhing reflex. There was no mortality observed at the highest dose of 4,000 mg/kg in the test animals. Therefore, the LD50 of MELA is expected to be more than 4000 mg/kg

Table 1: Preliminary qualitative phytochemical screening of methanolic extract of L. aspera (MELA)

Plant constituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Carbohydrates	-
Reducing sugars	-
Proteins	-
Glucosides	-
Terpenoids	+
Steroids	+
+: Presence; -: Absence	

CNS depressant activity

Open field test

The test was carried out to determine the depressant action of the test drugs on CNS in test animals. MELA (250, 500, and 750 mg/kg; p.o.) significantly (*p< 0.05, vs. control) decreased the locomotor activity. The locomotor activity lowering effect of the extract was manifested at the 2nd observation (30 min) period and continued up to 5th observation period (120 min). Diazepam (1 mg/kg, i.p.) treated group also showed significant (*p< 0.05, vs. control) CNS depressant activity when compared with control (Figure 1 and Table 2).
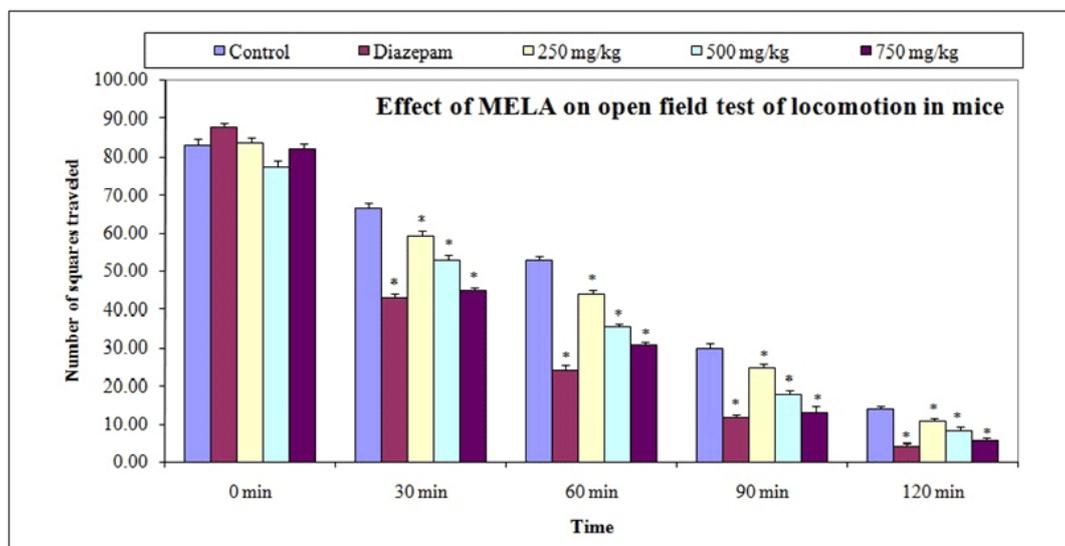

Figure1: Effect of MELA on open field test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).

Table 2: Effects of *L. aspera* extract and diazepam on the open field test

Treatment	Dose (mg/kg)	Number of square crossed				
		0 min	30 min	60 min	90 min	120 min
Control	0.1mL/mouse	83.00±1.54	66.40±1.20	52.80±1.15	29.80±1.06	13.80±0.73
Diazepam	1	87.80±1.11	43.00±0.94*	24.00±1.34*	11.80±0.66*	4.40±0.51*
MELA	250	83.60±1.36	59.20±1.24*	44.00±1.18*	24.80±0.80*	11.00±0.44*
MELA	500	77.20±1.68	52.80±1.59*	35.40±0.81*	17.60±1.20*	8.20±0.86*
MELA	750	82.00±1.18	45.00±0.83*	30.60±0.67*	13.00±1.58*	5.60±0.51*

Values are presented as mean ± SEM (n= 5). MELA= Methanolic extract of L. aspera.

*p< 0.05, vs. control (Dunnett's test)

Hole cross test

In the hole cross test, MELA showed a decrease in locomotion in the test mice. The number of hole crossed from one chamber to another by mice of the higher dose (750 mg/kg) is significantly (*p< 0.05, vs. control) decreased from 30 min to 90 min (Figure 2 and Table 3). On the other hand, the extract of *L. aspera* at 250, and 500 mg/kg doses showed significant decrease of movement from its initial value at 0 to 60 min. Diazepam (1 mg/kg, i.p.) showed significant depressant effect when compared to the control group (*p< 0.05, vs. control).
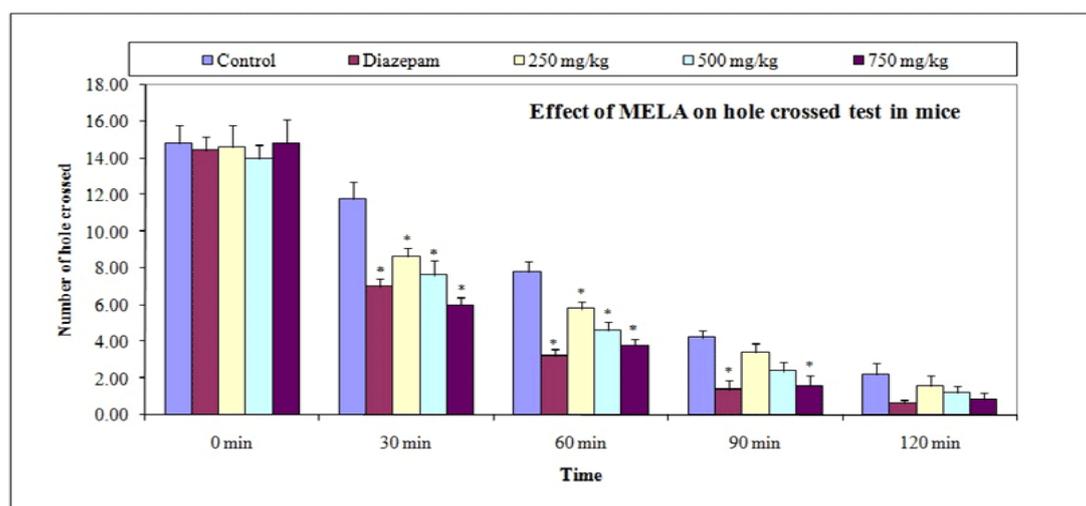

Figure 2: Effect of MELA on hole cross test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).

Table 3: Effects of L. aspera extract and diazepam on hole cross test.

Treatment	Dose (mg/kg)	Number of hole crossed				
		0 min	30 min	60 min	90 min	120 min
Control	0.1mL/mouse	14.80±0.97	11.80±0.91	7.80±0.58	4.20±0.37	2.20±0.66
Diazepam	1	14.40±0.74	7.00±0.44*	3.20±0.37*	1.40±0.51*	0.60±0.24
MELA	250	14.60±1.20	8.60±0.51*	5.80±0.37*	3.40±0.51	1.60±0.51
MELA	500	14.00±0.70	7.60±0.81*	4.60±0.51*	2.40±0.51	1.20±0.37
MELA	750	14.80±1.28	6.00±0.44*	3.80±0.37*	1.60±0.51*	

Values are presented as mean ± SEM (n= 5). MELA= Methanolic extract of L. aspera.

*p< 0.05, vs. control (Dunnett's test).

Force swimming test

In force swimming test, compared with the control group, MELA (250, 500, and 750 mg/kg; p.o.) significantly (*p< 0.05, vs. control) increased the duration of immobility time in mice. The standard drug, diazepam (1 mg/kg, i.p.) also significantly increased the duration of immobility time (Figure 3 and Table 4).
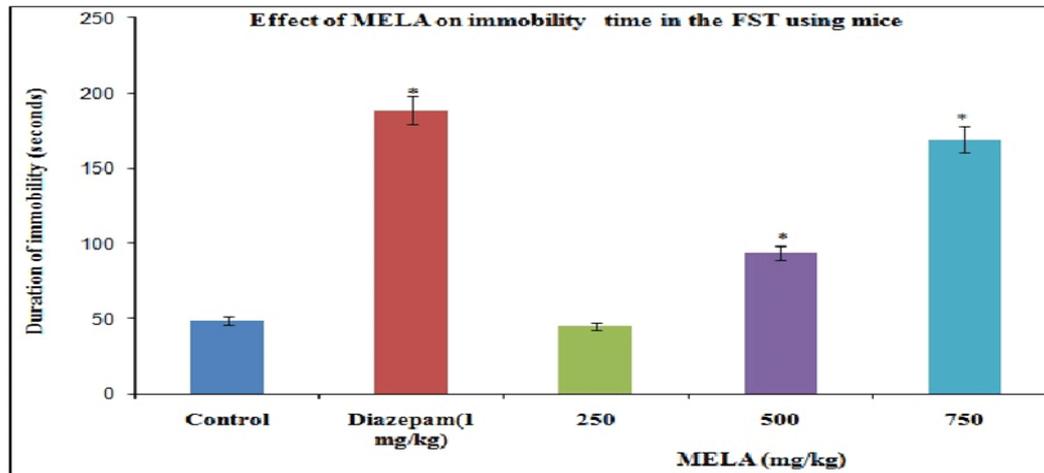

Figure 3: Effect of MELA on forced swimming test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).

Table 4: Effects of L. aspera and diazepam on forced swimming test		
Treatment	**Dose (mg/kg)**	**Immobility time (s)**
Control	0.1mL/mouse	48.60±1.36
Diazepam	1	188.80±1.28*
MELA	250	45.00±1.37
MELA	500	94.00±1.58*
MELA	750	169.00±2.47*

Values are presented as mean ± SEM (n= 5). MELA= Methanolic extract of L. aspera.

*p< 0.05, vs. control (Dunnett's test).

Tail suspension test

In tail suspension test, the duration of immobility time in mice with MELA (250, 500, and 750 mg/kg; p.o.) significantly (*p< 0.05, vs. control) increased when compared with the control group. Diazepam (1 mg/kg, i.p.) significantly increased the duration of immobility time (Figure 4 and Table 5).
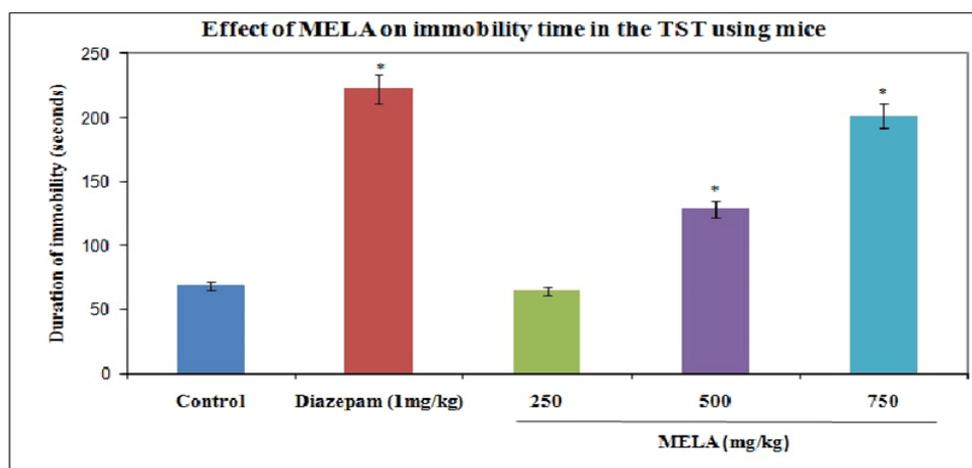

Figure 4: Effect of MELA on tail suspension test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).

Table 5: Effects of L. aspera and diazepam on tail suspension test

Treatment	Dose (mg/kg)	Immobility time(s)
Control	0.1mL/mouse	68.60±2.44
Diazepam	1	222.60±2.42*
MELA	250	64.60±3.26
MELA	500	128.40±2.65*
MELA	750	201.60±2.22*

Values are presented as mean ± SEM (n= 5). MELA= Methanolic extract of L. aspera.

*p< 0.05, vs. control (Dunnett's test).

Thiopental sodium induced sleeping time test

Thiopental sodium induced sleeping time test showed that MELA (250, 500, and 750 mg/kg; p.o.) significantly (*p< 0.05, vs. control) induced sleep at an earlier stage. It had a good effect on the onset of action (Figure 5 and Table 6). Mice when compared to control group enlarge the duration of sleeping time (Figure 6 and Table 6).
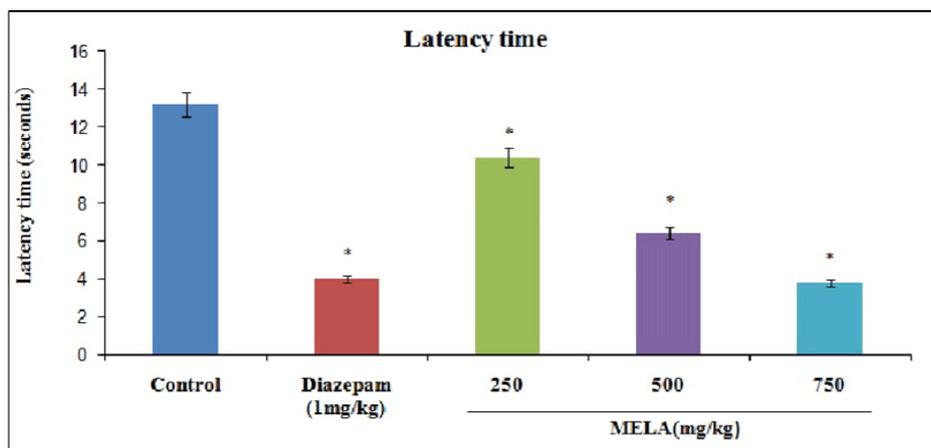

Figure 5: Effect of MELA on thiopental sodium-induced latency time test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).

Figure 6: Effect of MELA on thiopental sodium-induced sleeping time test in mice. Animals were treated with control (Deionized water, 0.1mL/mouse, p.o.), Diazepam was used as standard drug (1 mg/kg, i.p.) and MELA (250, 500 and 750 mg/kg, p.o.). *p< 0.05 compared with the control group (One-way ANOVA followed by Dunnett's post hoc test).
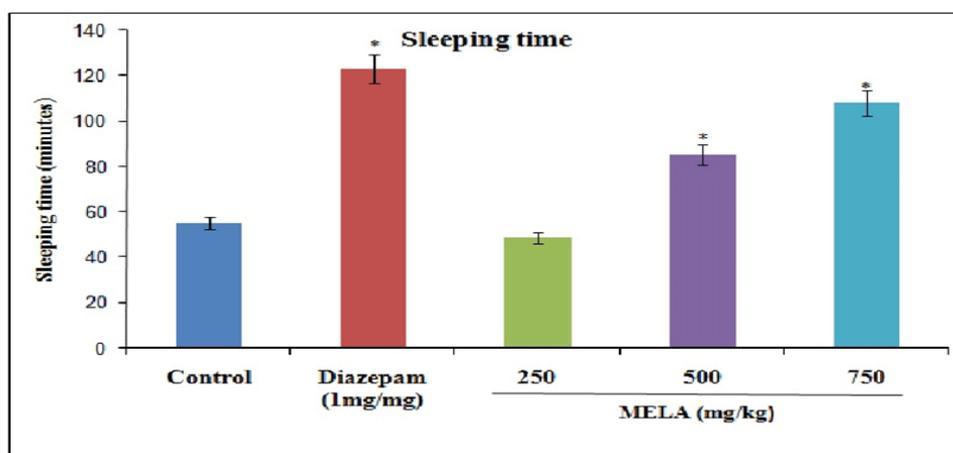
Table 6: Effects of *L. aspera* and diazepam on thiopental sodium-induced sleeping time test

Treatment	Dose (mg/kg)	Onset of action (s)	Duration of sleep (min)
Control	0.1mL/ mouse	13.20±0.58	55.00±1.41
Diazepam	1	4.00±0.44*	123.00±2.02*
MELA	250	10.40±0.51*	48.80±1.35
MELA	500	6.40±0.74*	85.20±1.39*
MELA	750	3.80±0.37*	108.00±2.09*

Values are presented as mean ± SEM (n= 5). MELA= Methanolic extract of *L. aspera*.*p< 0.05, vs. control (Dunnett's test)

Discussion

The recent study aims to evaluate the central nervous system (CNS) depressant activities of the methanolic extract of *L. aspera* leaves (MELA) in mice models namely open field, hole cross, force swimming, tail suspension, and thiopental sodium-induced sleeping time tests.

The locomotor activity is the most important action on CNS in mice. It refers to an increase in alertness and decrease in locomotor activity considered as sedative effect. In present study, locomotor activity measured by open field and hole cross tests, showed that the extract significantly decreased locomotor activity which indicates it has central nervous system (CNS) depressant activity. Diazepam, which was used to induce sleep in this study, believed to act at specific binding sites that are closely linked to γ-aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhancing GABA-ergic transmission. It has been reported that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system (CNS); that can act as benzodiazepine-like molecules [35]. However, the plant extract decreased the amplitude of movements which was marked at the 2nd observation (30 min) and sustained up to 5th observation period (120 min) (Figure 1, 2 and Table 2,3). In the hole cross test, maximum depressant effect was observed at 750 mg/kg. The present study showed that MELA contained alkaloids, flavonoids, terpenoids, tannins, steroids, saponins, and cardiac glycosides (Table 1). So, it is probable that flavonoids present in the extract may responsible for its CNS depressant activity.

The forced swimming and tail suspension tests which were claimed to reproduce a situation comparable to human depression [32]. The test models are based on the observation of mice when forced to swim or suspended in a restricted space from which there is no possibility of flee, eventually stop to struggle, surrendering themselves to the experimental conditions. This suggested that the helplessness or despair behavior reflected a state of lowered mood in mice and could serve as a valuable test for screening antidepressant drugs [36]. In this study, MELA significantly increased the immobility time of force swimming and tail suspension tests in mice (Figure 3, 4 and Table 4, 5).

Similar result happened on the standard drug diazepam. However, depression is a complex disorder resulting from changes in central noradrenergic, serotonergic and dopaminergic systems. Hence, it was thought to be worthwhile to estimate all the three neurotransmitters in the brain [37]. Based on these findings, it can be suggested that the extract has CNS depressant effect which supports the earlier report had been done by another method.

Thiopental, a barbiturate, produced a sedative-hypnotic effect at a specific dose because of its interaction with the gamma amino butyric acid (GABA)A receptors, which enhances GABAergic transmission. It potentiates the GABA activity, thereby allowing chloride to enter the neuron by prolonging the duration of the chloride-channel opening. Thiopental could block the excitatory glutamate receptors. These molecular activities lead to decreased neuronal activity, which supports the findings obtained for the reference drug diazepam, which is a CNS depressant drug that decreases the time of the onset of sleep or prolongs the length of sleep or both [38]. In this study, the extract showed significant CNS depressant effect in thiopental sodium-induced sleeping time test in mice. The standard drug diazepam was also shown the same effects (Figure 5, 6 and Table 6).

Conclusions

From the present study, it can be concluded that MELA possessed promising CNS depressant effects in the experimental mice models demonstrating its depressant action on the CNS, as manifested by these important neuropharmacological properties in mice. However, further studies are required to evaluate the potential use of *L. aspera* for the treatment of CNS depressant diseases.

Declarations

Ethics approval and consent to participate

This research work does not contain any individual person's data.

All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/17.02) of Stamford University Bangladesh approved all experimental rules.

Acknowledgements

The authors are grateful to Professor Dr. Bidyut Kanti Datta, Chairman, Department of Pharmacy, Stamford University Bangladesh for his permission to use the facilities of the Pharmacology and Phytochemistry Laboratory.

Authors' contributions

MAM and RR designed all the laboratory experiments, analyzed and interpreted results. RR, MAM, MSH and MNP conducted all experiments. MAM and RR did statistical analysis and drafted the manuscript. All authors read and approved the manuscript.

Competing interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Barrett JE. Animal behavior models in the analysis and understanding of anxiolytic drugs acting at serotonin receptors. In: Olivier B, Mos J, Slangen JL. Animal Models in Psychopharmacology. Switzerland (CH): Basel Birkhäuser. 1991;37–52.

2. Neal MJ. Medical Pharmacology at a Glance, Wiley-Blackwell. Singapore. 6th edition. 2009.

3. Ghani A. Medicinal plant of Bangladesh, Chemical constituents and uses, The Asiatic Society of Bangladesh, Dhaka, Bangladesh. 2003;277.

4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plant. Council of Scientific and Industrial Research (CSIR). 2002;153.

5. Goudgaon NM, Basavaraj NR, Vijayalaxmi A. Anti-inflammatory activity of different fractions of *Leucas aspera* Spreng. Indian J Pharmacol. 2003;35(6):397-398.

6. Srinivas K, Rao MEB, Rao SS. Anti-inflammatory activity of Heliotropium indicum Linn and *Leucas aspera* Spreng in albino rats. Indian J Pharmacol. 2000;32(1):37-38.

7. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Separation of Leucas aspera, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem Pharma Bull. 2003;51(5):595-598.

8. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Diterpenes from *Leucas aspera* inhibiting prostaglandin-induced contractions. J Nat Pro. 2006;69(7):988-994.

9. Mangathayaru K, Thirumuragan Patel DPS, Pratap DVV, David DJ, Karthikeyan J. Isolation and identification of nicotine from *Leucas aspera* (wild) link. Indian J Pharma Sci. 2006;68(1):88-90.

10. Rahman MS, Sadhu SK, Hasan CM. Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. Fitoterapia. 2007;78(7-8):552-555.

11. Rahman S, Ali MSY, Rashid A. Central nervous system depressant activity of *Leucas aspera* root. Orien Pharma Exper Med. 2006;6(3):174-178.

12. Kamat M, Singh TP. Preliminary chemical examination of some compounds in the different parts of the genus Leucas. Geobios. 1994;21:31–33.

13. Chaudhury NA, Ghosh D. Insecticidal plants: Chemical examination of Leucas aspera. J Indian Chem Socie. 1969;46:95.

14. Khaleque A, Huq ME, Huq MS, Mansoor MH. Chemical investigations on Leucas aspera. I. Isolation of compound-A, 3-sitosterol and et-sitosterol from the aerial parts. Sci Res.1970;7:125–127.

15. Chatterjee SK, Majumdar DN. Chemical investigation of Leucas aspera. J Ins Chem. 1969;41:98–101.

16. Kalachaveedu M, Ghosh A, Ranjan R, Vedam VK. Volatile constituents of *Leucas aspera* (WilId.). J Essen Oil Res. 2006;18:104–105.

17. Jam MP, Nath HB. Examination of the component fatty acids of the oil from the seeds of Leucas aspera. Lab Dev. 1968;6:34–36.

18. Badami RC, Patil KB. Minor seed oils. X: Physico-chemical characteristics and fatty acid composition of seven minor oils. J Oil Tech Asso India. 1975;7:82–84.

19. Misra TN, Singh RS, Pandey HS, Singh S. A novel phenolic compound from *Leucas aspera* Spreng. Indian J Chem. 1995;34:1108–1110.

20. Misra TN, Singh RS, Prasad C, Singh S. Two aliphatic ketols from Leucas aspera. Phytochem. 1992;32(1):199–201.

21. Misra TN, Singh RS, Pandey HS, Singh S. Long-chain compounds from Leucas aspera. Phytochem. 1992;31(5):1809–1810.

22. Pradhan B, Chakraborty D, Subba G. A triterpenoid lactone from Leucas aspera. Phytochem. 1990;29(5):1693–1695.

23. Mangathayaru K, Lakshmikant J, Shyam SN, Swapna R, Grace XF, Vasantha J. Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia. 2005;76(7-8)752-754.

24. Suganya G, Karthi S,Shivakumar MS. Larvicidal potential of silver nanoparticles synthesized from *Leucas aspera* leaf extracts against dengue vector Aedes aegypti. Parasitol Res. 2014;113(5):1673-1679. doi: 10.1007/s00436-013-3718-3

25. Singh SK, Chouhan HS, Sahu AN, Narayan G. Assessment of in vitro antipsriatic activity of selected Indian medicinal plants. Pharma Biol. 2015;53(9):1295-1301. doi: 10.3109/13880209.2014.97671

26. Kamaraj C, Kaushik NK, Rahuman AA, Mohanakrishnan D, Bagavan A, Elango G. Antimalarial activities of medicinal plants traditionally used in the villages of Dharmapuri regions of South India. J Ethnopharmacol. 2012;141(3):796-802. doi: 10.1016/j.jep.2012.03.003

27. Kovendan K, Murugan K, Vincent S, Barnard DR. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd.(Lamiaceae) and bacterial insecticide, Bacillus sphaericus, against malarial vector, Anopheles stephensi Liston, (Diptera: Culicidae). Parasitol Res. 2012;110(1):195-203. doi: 10.1007/s00436-011-2469-2

28. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. The Asiatic Society of Bangladesh, Dhaka, Bangladesh, 2nd edition. 2003;331-332.

29. Lorke D. A new approach to acute toxicity testing. Arch Toxicol.1983;54(4):275-287.

30. Gupta BD, Dandiya PC, Gupta MI. A psychopharmacological analysis of behaviour in rats. Japanese J Pharmacol. 1971;21(3):293–298.

31. Subhan N, Alam MA, Ahmed F, Shahid IJ, Nahar l, Sarker SD. Bioactivity of Excoecaria agallocha. Brazilian J Pharmacog. 2008;18(4):521-526.

32. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacod Ther. 1977; 229(2):327–336.

33. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol. 1985;85(3):367-370.

34. Turner RA. Anticonvulsant screening methods in pharmacology. New York and London: Academic Press. 1965;64–69.

35. Ozturk Y, Aydini S, Beis R, Baser KH, Berberoglu H. Effect of Hypericum pericum L. and Hypericum calycinum L. extracts on the central nervous system in mice. Phytomedicine. 1996;3(2):139–146.

36. Porsolt RD. Behavioral despair, Antidepressants: neurochemical, behavioral and clinical perspectives. In: Enna SJ, Malick JB, Richelson E editors. New York: Raven Press. 1981;121–139.

37. Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A. Pharmacological evaluation of Saraca indica Leaves for central nervous system depressant activity in mice. J Pharma Sci Res. 2010;2(6):338-343.

38. Nyeem MAB, Alam MA, Awal MA, Mostofa M, Uddin SJ, Islam N. CNS depressant effect of the crude ethanolic extract of the flowering tops of Rosa damascena. Iranian J Pharmacol Ther. 2006;5(2):171–174.