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Evaluation of neuropharmacological and analgesic activities of methanolic extract of *Diospyros blancoi* A. DC leaves in Swiss albino mice

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Abstract
This research work was carried out to evaluate the neuropharmacological and analgesic activities of methanolic extract of *Diospyros blancoi* leaves by experimenting Swiss albino mice. The neuropharmacological activities of methanol extract were conducted using hole cross, force swimming and tail suspension tests. On the other hand, analgesic activities of the extract were performed using acetic acid induced writhing and hot plate tests. The mice were treated orally at dose of 200 mg/kg and 400 mg/kg of leaves extract for neuropharmacological and analgesic tests. The methanolic leaves extract of *Diospyros blancoi* significantly (p<0.05) showed neuropharmacological and analgesic activity. This confirms the traditional uses and indicates further investigation to isolate the active phytochemical constituents and to understand the pharmacological mechanism of the chemical compounds present in the leave parts of this plant.

Keywords: Neuropharmacology, analgesic, *Diospyros blancoi,* forced swimming test

Introduction
Plants are the major source of medicine and most of the modern medicines are originated from the plants. Today, about 80% of the world's population still depends on the traditional medicine practices for the management of various diseases [1, 2]. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural product of origin [3]. *Diospyros blancoi* A. DC (Family: Ebenaceae) is a very common plant in Bangladesh. It grows well in areas with monsoon climate. In Bangladesh, *Diospyros blancoi* is widely known as 'Gab' (Bengali) [4]. Traditional uses of *Diospyros blancoi* include snakebites, heart problems, hypertension, spider bites, stomach aches, diabetes and eczema [5]. This plant showed antioxidant activity and anti-diarrheal property [6]. It has found that *Diospyros blancoi* possesses antimicrobial and cytotoxic properties [4]. The extracts of *Diospyros blancoi* exhibited anti-inflammatory activity [7]. This plant also showed moderate activity as an anti-cancer agent [8]. A study on *Diospyros blancoi* said that this plant can be a potent inhibitor of skin aging process [9]. The present investigations were carried out to identify the possible neuropharmacological activities of methanol extract of *Diospyros blancoi* leaves (MEDB) available in Bangladesh.

Materials and methods Collection of *Diospyros blancoi*
For the investigations, the leaves of *Diospyros blancoi* was collected from the village Churamankathi of Jashore district, Bangladesh.

Drying and grinding of the dried samples
The collected samples were dried for three days in the laboratory under electric fan. The dried samples were grounded to coarse powder with a blender and powdered samples were kept in close glass containers.

Preparation of methanol extract
About 300 gm of powdered leaves were soaked in 1200 ml of methanol for 7 days in beaker and mixture was stirred every 18 hours using a sterile glass rod. The filtrate obtained 3 times with the help of Whitman filter paper no 1 and the solvent was removed by heater with stirrer at 450 C temperatures. Then the collected extract was preserved in refrigerator for analysis.

Animals

The healthy Swiss albino mice were procured from the Jahangirnagar University, Bangladesh. The soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions in accordance with Helsinki Declaration in 1975 (revised in 2000). The study protocol involving animals was approved by the institutional ethical committee of Jashore University of Science and Technology.

Chemical and drug collection

The drugs (Imipramine HCl, Diazepam, Tramadol and Diclofenac sodium) used in the experiments were purchased from the local Pharmaceutical Company in Bangladesh. The DMSO was purchased from Hubei Xingfa Chemical Co., Limited, China.

Tests for Neuropharmacological activity

Hole cross test

The hole cross method was carried out with modification as described by Takagi *et al.* (1971) [10] and adopted by Apu *et al.* (2012) [11]. A wood partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into control, standard and two test groups containing five mice each. The test groups received the MEDB at doses level of 200 mg/kg and 400 mg/kg body weight orally whereas the control group received 10 ml/kg Deionized water (DW) + 1% DMSO and the standard group received Diazepam (1 mg/kg body weight) respectively. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test groups and the standard.

Forced swimming test

The forced swimming method was performed according to the method published by Porsolt *et al.* (1977) [12] and described by Kumar et al. (2014) [13]. All animals were housed in a controlled room (temperature, 25 ± 1ºC; humidity 45-50%; light-dark cycle, 12 h each) with free access to laboratory chow and tap water. The mice were divided randomly into control, standard and two experiment groups. Each group was contained 5 mice. The standard group was orally administered Imipramine HCl (25 mg/kg body weight), two test groups were orally administered MEDB (200 and 400 mg/kg body weight respectively) and the control group received orally 10 ml/kg Deionized water + 1% DMSO.

Tail suspension test

The tail suspension method was carried out as mentioned by Steru *et al.* (1985) [14] with modifications and experimented by Potdar *et al.* (2011) [15]. All animals were housed in a controlled room (temperature, 25±1 ºC; humidity, 45-50%; light-dark cycle, 12h each) with free access to laboratory chow and tap water. The mice were divided randomly into control, standard and two experimental groups. The standard group was orally administered Diazepam (1 mg/kg body weight), two test groups were orally administered MEDB (200 and 400 mg/kg body weight, respectively) and the control group received 10 ml/kg Deionized water + 1% DMSO. Two stands, each with a clamp located 22 cm from the floor, were placed at intervals of 23 cm. A mouse was hung 5cm from the end of its tail on a stand, and recorded with a video camera for 6 min. This test was performed between 1-3 p.m. The immobility time was evaluated by the observers

Acetic acid induced writhing test

This test was performed according to Koster *et al,* (1959) [16]. The experimental animals were randomly selected and divided into four groups where one control group received 10 ml/kg Deionized water + 1% DMSO, one standard group received 50 mg/kg Diclofenac Na and the two-test group received two different doses (200 and 400 mg/kg of MEDB). The test samples, control and Diclofenac-Na were given orally by means of a feeding needle. Then the writhing inducing chemical, acetic acid solution (0.7%, 10 ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of five minutes, which was given for absorption of acetic acid, number of writhing, was counted for 15 minutes.

Hot plate test

The hot plate test was performed according to the method of Turner *et al.* (1965) [17] and described by Rezaee-Asl *et al.* (2014) [18]. The mice were retained on a hot plate having a stable temperature of 55±1ºC. Each mouse was individually placed on the hot plate in order to find the animal's reaction to electrical heat-induced pain. The latency until mice showed first signs of discomfort were recorded and response was determined at 30, 45, 60, 75 and 90 min after the administration of 10 ml/kg Deionized water + 1% DMSO, MEDB at dose of 200 and 400 mg/kg per body weight and Tramadol (10 mg/kg) respectively.

Statistical Analysis

The results were statistically evaluated using SPSS software. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test. The results were presented as mean value ± SEM (n=5). The statistically significant results are marked with a star (*) sign in the table.

Result

Hole cross test

In the evaluation of neuropharmacological effect of *Diospyros blancoi*, we have started with hole cross test to record the spontaneous locomotor activity. The result was presented on Table 1 and figure 1.

Table 1: Effect of MEDB in hole cross test on mice

	(mg/kg)	0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg DW	0.80 ±0.58	0.80±0.37	1.40 ±0.75	1.40 ±0.51	2.00 ±0.71
+1% DMSO						
Standard (Diazepam)	1 mg/kg	0.30 ±0.07	3.60 ±1.32	4.60 ±1.03	4.40 ±1.50	3.00 ±1.00
Group-III	200 mg/kg	1.52±0.22	1.80 ±1.36	2.80 ±1.53	1.80 ±0.73	3.80±1.36
Group-IV	400 mg/kg	1.40 ±0.75	3.60 ±1.94	2.20 ±0.86	3.60 ±1.57	3.00±1.00

Values are presented as mean ± SEM (n = 5). P* < 0.05 compared with the control group (Dunnett's test)

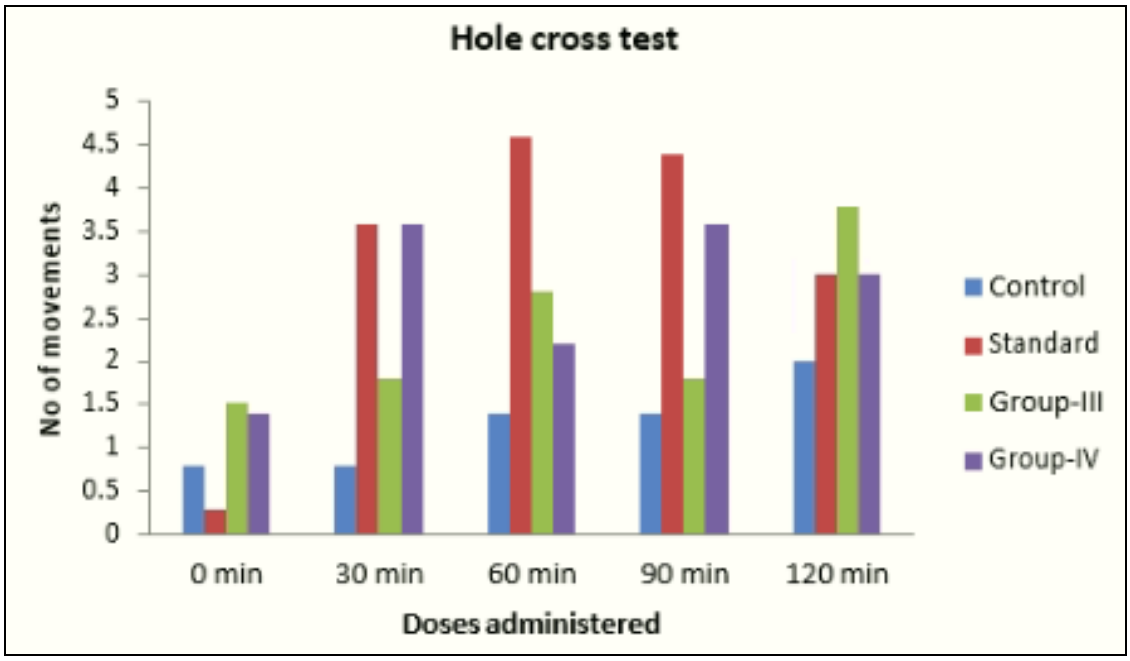

Fig 1: Graphical representation of effect of MEDB in hole cross test on mice. Control = 10 ml/kg Deionized water + 1% DMSO, standard = Diazepam, 1 mg/kg, Group- III = 200 mg/kg, Group-IV= 400 mg/kg

Forced swimming test

The MEDB (200 & 400 mg/kg) reduced the immobility time after one hour of administration. The test result of control, Imipramine HCl and methanolic leaves extract group are shown in the Table 2 and Figure 2.

Table 2: Effect of MEDB in forced swimming test on mice

Treatment	Dose (mg/kg)	Immobility Time
Control	10 ml/kgDW+1% DMSO	2.77±0.22
Standard (Imipramine HCl)	25 mg/kg	0.30±0.07*
Group-III	200 mg/kg	1.52±0.22*
Group-IV	400 mg/kg	1.15±0.46*

Values are presented as mean ± SEM (n = 5). P* < 0.05 compared with the control group (Dunnett's test)

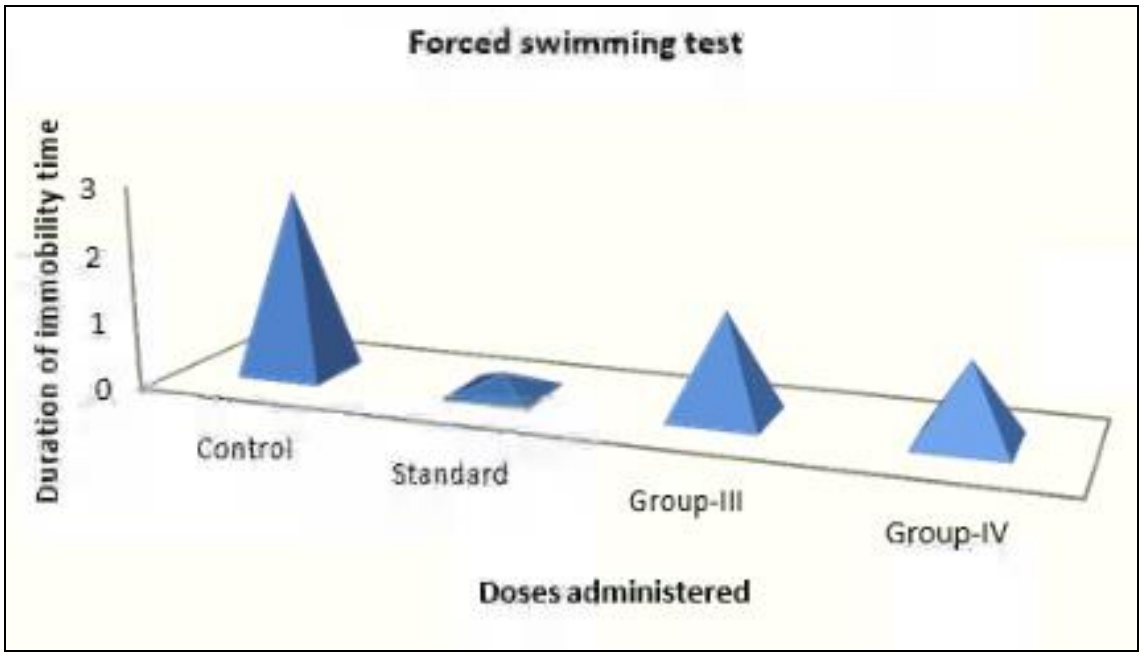

Fig 2: Graphical representation of effect of MEDB in forced swimming test on mice. Control = 10 ml/kg Deionized water +1% DMSO, standard = Imipramine HCl, 25 mg/kg, Group- III = 200 mg/kg, Group-IV= 400 mg/kg

Tail suspension test

The MEDB at doses level of 200 mg/kg and 400mg/kg body weight on tail suspension in mice were showed in positive result (Table 3 and Figure 3). This test using mice was treated with Diazepam (1 mg/kg). Administration of extracts of *Diospyros blancoi* at dosage of 200 and 400 mg/kg was markedly reduced the immobility time.

Table 3: Effect of MEDB in Tail suspension test on mice

Treatment	Dose	Immobility Time
Control	10 ml/kg DW+1% DMSO	3.20±0.06
Standard (Diazepam)	1 mg/kg	2.69±0.22
Group-III	200 mg/kg	1.62±0.22*
Group-IV	400 mg/kg	1.30±0.33*

Values are presented as mean ± SEM (n = 5). P* < 0.05 compared with the control group (Dunnett's test)

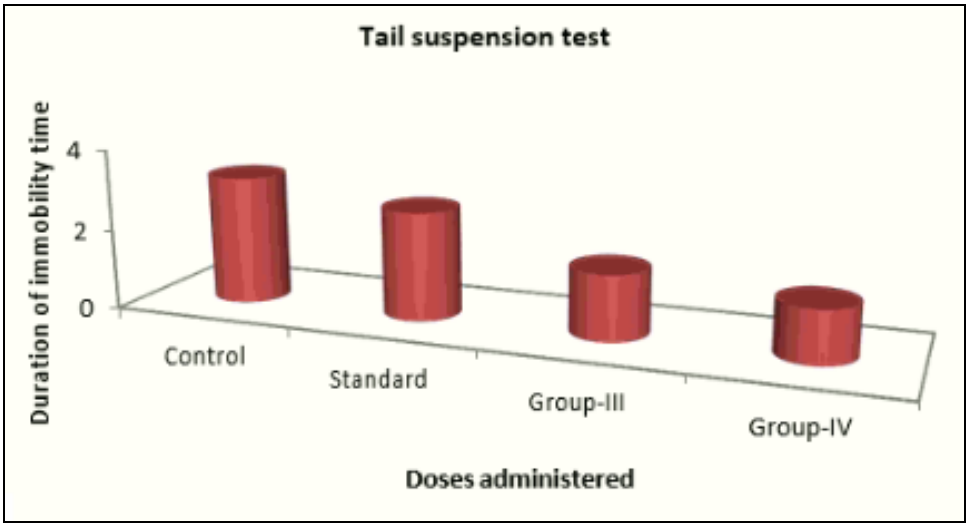

Fig 3: Graphical representation of effect of MEDB in Tail suspension test on mice. Control = Deionized water, 10 ml/kg +1% DMSO, standard = Diazepam, 1 mg/kg, Group- III = 200 mg/kg, Group-IV= 400 mg/kg

Acetic acid induced writhing test

The effect of MEDB on acetic acid induced writhing is presented in the Table 4 and Figure 4. The dose of extract at 200 mg/kg and 400 mg/kg body weight produced reduction in the number of writhing that produced by acetic acid in mice. The reference drug Diclofenac-Na 50 mg/kg also reduced the number of writhing.

Table 4: Effect of MEDB in Acetic acid induced writhing test on mice

Treatment	Dose	No of writhing	% of inhibition
Control	10 ml/kg DW+1% DMSO	91.40± 9.72	0.00
Standard (Diclofenac Na)	50 mg/kg	56.40± 12.04*	38.29
Group III	200 mg/kg	53.00± 11.20*	41.79
Group IV	400 mg/kg	48.00± 7.44*	47.26

Values are presented as mean ± SEM (n = 5). P* < 0.05 compared with the control group (Dunnett's test)

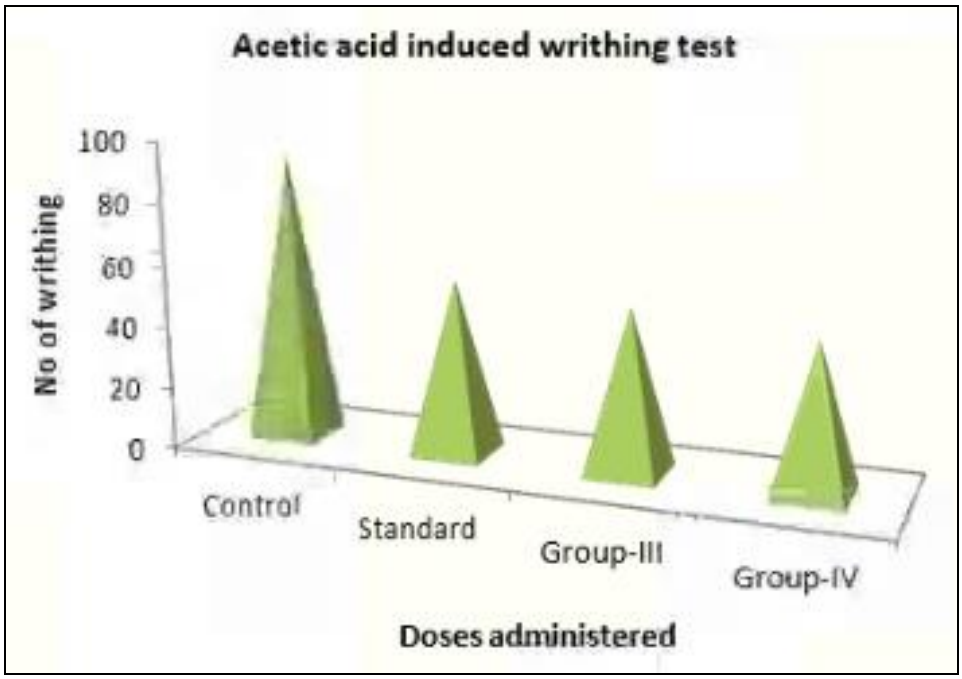

Fig 4: Graphical representation of effect of MEDB in Acetic acid induced writhing test on mice. Control = 10 ml/kg Deionized water+1% DMSO, standard = Diclofenac Na, 50 mg/kg, Group- III = 200 mg/kg, Group-IV= 400 mg/kg

Hot plate test

The results of analgesic effect of the MEDB using hot plate method are presented in Table 5 and Figure 5. The results showed the thermal stimulus in mice throughout the observation.

Table 5: Effect of MEDB in Hot plate test on mice

Latency Time				
Time	**Control (10 ml/kg DW+1% DMSO)**	**Standard (Tramadol)**	**Group-III (200 mg/kg)**	**Group-IV (400 mg/kg)**
30 min	0.63±0.11	2.67±0.51*	1.18±0.24	2.49±0.33*
45 min	0.63±0.08	1.09±0.27	0.60±0.08*	1.53±0.19
60 min	0.65±0.09	1.28±0.12*	0.92±0.15*	0.99±0.12
75 min	0.89±0.23	1.73±0.27	0.75±0.09	0.86±0.10
90 min	0.78±0.13	1.30±0.15	0.74±0.11	1.43±0.32

Values are presented as mean ± SEM (n = 5). P* < 0.05 compared with the control group (Dunnett's test)

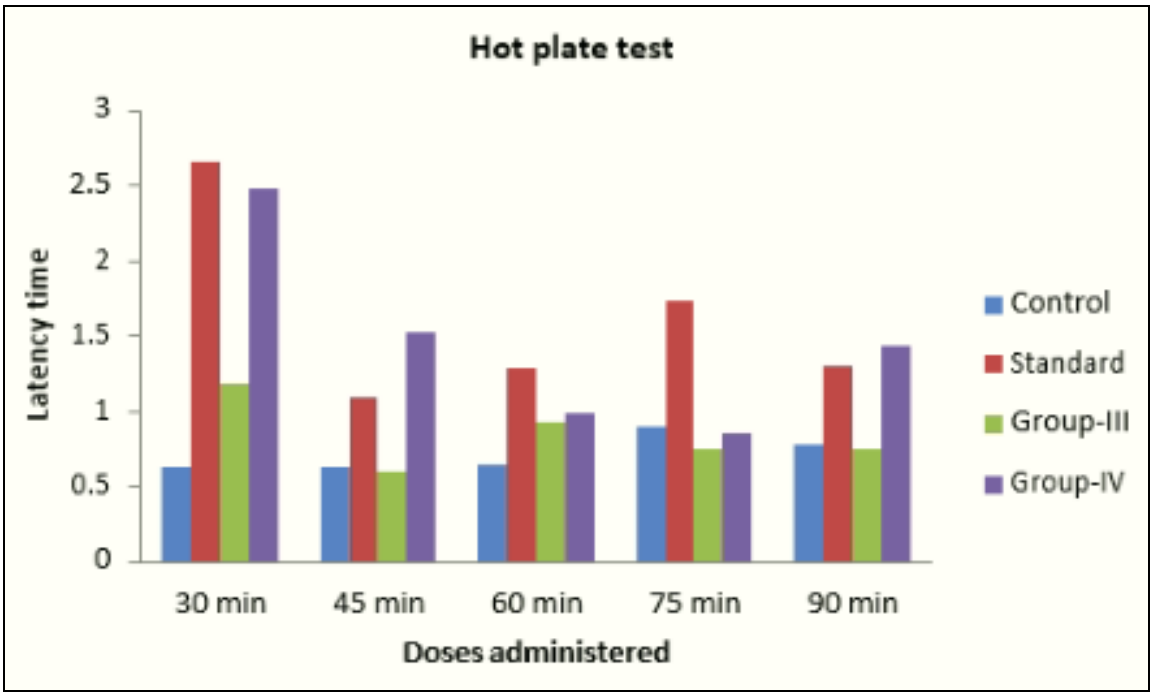

Fig 5: Graphical representation of effect of MEDB in Hot plate test on mice, Control = Deionized water, 10 ml/kg+1% DMSO, standard = Tramadol, 10 mg/kg, Group- III = 200 mg/kg, Group-IV= 400 mg/kg

Discussion

To prove the neuropharmacological effect of MEDB, hole cross, force swimming and tail suspension tests were performed. In hole cross test, *Diospyros blancoi* leaves extract decreasing the number of holes crossed compared to Diazepam was not time and dose dependent. During evaluation, *Diospyros blancoi* leaves extract reduced the immobility period during the forced swimming and tail suspension test in comparison with control and exhibited a dose dependent antidepressant activity significantly. The immobility time has been considered as depression, and hence any reduction in this parameter reflects antidepressant activity. Interestingly, our data indicates the higher doses (400 mg/kg) of plant extracts were more effective than smaller doses (200 mg/kg) both in forced swim test and tail suspension tests. In acetic acid induced writhing test, *Diospyros blancoi* leaves extract showed significant decrease in writhing movement. The hot plate assay was also employed to assess the effects of the extract in producing analgesic activity. There was no significant difference has been observed between the control and the test groups in the hot plate test. The findings of this study validate the presence of the antidepressant and analgesic effect of *Diospyros blancoi* leaves extract which is indicative of neuropharmacological activity of the leaf's parts of this medicinal plant.

Conclusion

The findings of our study find out statistically significant neuropharmacological and analgesic activity of *Diospyros blancoi* leaves extract. Hence, further extensive research is needed to isolate the active compound and to understand the underlying mechanism behind the neuropharmacological activity of the leave part of this plant.

Ethical approval

All procedures performed in this study involving animals were approved in accordance with the ethical standards of the Ethical Review Committee, Faculty of Biological Sciences and Technology, Jashore University of Science and Technology, Jashore, Bangladesh (Ref: ERC/FBS/JUST/2018 -11).

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