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Evaluation of neurobehavioral activities of ethanolic extract of *Psidium guajava* Linn leaves in mice model

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Abstract

Background: The complementary and alternative medicines have particular importance in treating various comorbid conditions including anxiety and depression which prevalence will be raised to the second highest risk of morbidity, triggering a noteworthy socioeconomic burden. Ethanolic extract of leaves of *Psidium guajava* Linn (EEPG) was investigated to evaluate the anxiolytic and anti-depressant activity into two different doses (200 mg/kg and 400 mg/kg of body weight) on Swiss Albino male mice utilizing experimental paradigms of anxiety and depression. The extract was also subjected to phytochemical screening.

Results: Phytochemicals screening showed the presence of numerous types of active constituents in extract, for example, flavonoids, alkaloids, terpenoids, and steroids. The experimental results revealed that in case of anxiolytic activity tests, a statistical significant ($p < 0.05$ vs group I) effect is observed in EPMT model, hole cross model, light and dark model in both doses, whereas in hole-board model, marble burying model tests, a statistical considerable effect is observed only at the dose of 400 mg/kg although at the dose of 200 mg/kg, anxiolytic effect is also expressed and in case of anti-depressant activity test, the statistical significant effect is observed only at the dose of 400 mg/kg. All the results are comparable with the effect of standard drugs used.

Conclusions: Taken together, the present research work evidences the anxiolytic and anti-depressant effects of EEPG, but further investigation needed to find out the underlying mechanism of action and to isolate and purify the specific components that are responsible for aforementioned activities.

Keywords: *Psidium guajava* Linn, Anxiolytic, Anti-depressant, Phytochemical screening, Neuropharmacological activity

Background

The complexity of daily lifestyle in modern society leads to numerous degrees of two important seriously comorbid mental disorders—anxiety and depression. Anxiety and depression may occur coincidently, complying with the requirements for both disorders. The interpretation regarding comorbidity of these ailments is predominantly given by a shared genetic vulnerability to both diseases, or by one disease being an epiphenomenon of the other [1]. Though differentiation between anxiety and depression is badly effortful, identification and treatment of both disorders are paramount important since association of significant morbidity and mortality with them is inevitable. Most of the clinically existing anxiolytics and anti-depressants are not effective for all patients and overwhelmed by adverse effects, slow onset of actions, and poor patient compliance [2]. That being the situation, for the welfare of the mankind, it is inevitably required to search such types of novel anxiolytic and anti-depressant agents which will overweigh the demerits of presently existing agents. There are quite a lot of plant species

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showing their pharmacological effectiveness in the variety of animal models of psychiatric disorders and are being used as complementary and alternative medicines for the management of these ailments [3]. These medicinally valued plants are important elements of traditional medicine in virtually all nations. Despite having fewer adverse effects of phytomedicines, common naturally origin products have been symbolized as the reliance of people because of its marvelous safety and security in comparison with synthetically origin products resulting in reducing the dependency of people [4]. Over the years, there are several types of pharmacological models employing in the estimation of medicinal plants for neuropharmacological activities headed for the identification of botanicals and drugs with beneficial effects in the treatment of diverse central nervous system (CNS) ailments. The selection of the test methods not only measures efficiency, but in some instances also contributes to an indication of the mechanism(s) of the test ingredient. *Psidium guajava* Linn belonging to the family Myrtaceae, (commonly known as poor man's apple) is originated in the tropical South America and grows wild in Bangladesh, India, Thailand, Brazil, West Indies, and also in several other countries [5]. The tree is mainly known for its fruit namely guava, but different parts of the plant are used in traditional medical practice in several countries since the ancient history of herbal medicine [6]. The plant possesses several pharmacological activities such as anti-stress, anti-diabetic, anti-microbial, depressant, antioxidant, anti-diarrheal, anti-inflammatory, antinociceptive, and anti-cancer activities [7–9].

Though the plant has medicinal values regarding neuropharmacological activities along with its traditional use in neurological disorders in India and West Indies [10–12], there are little knowledge and scientific data on these activities of the plant in Bangladesh. Therefore, we designed our study to evaluate the effect of ethanolic extract of *Psidium guajava* Linn leaves on two significant comorbid psychiatric conditions-anxiety and depression by using universally accepted animal models.

Methods

Plant sample collection and extract preparation

Fresh leaves of *Psidium guajava* Linn were collected from Jashore District of Bangladesh. For the preparation of extract, the collected leaves were washed by clean and fresh water and were dried in the shade for 10–12 days before grinding into a coarse powder using grinder. Then, the powder was stored in an air tight glass container and kept in a cool, dark, and dry place until analysis started. After that, the leaves powder (approximately 250 gm) was kept in a clean glass container previously filled with 700 ml absolute ethanol (99.99%, Mark, Germany). Also, the opening of the container was sealed with foil paper and kept at room temperature for 7 days. The solution of the glass container was shaken and stirred irregularly. After 7 days, the mixture was filtered through a cotton plug followed by Whatman No. 1 filter paper. The filtrate was concentrated by rotary evaporator (RE 200 Sterling, UK) at control temperature (45 °C). The obtained extract was stored at − 20 °C for additional studies.

Drugs and chemicals

Diazepam and imipramine were received as a gift sample from Square Pharmaceuticals Ltd., Bangladesh and used as standard drug in this study. Tween 80 (Mark, Germany), absolute ethanol, and normal saline were purchased from local chemical market. All of these reagents were analytical grade. Phenobarbitone sodium was bought from local market.

Preliminary phytochemical investigation

The preserved fresh extract of the leaves of *Psidium guajava* Linn was screened for the presence of various phytochemical constituents [13].

Experimental animal

Swiss albino male mice weighed about 20–22 g were purchased from the animals' house of Pharmacology Lab, Dhaka-1342, Bangladesh, and were kept in large spacious hygienic polypropylene animal cases under standard environmental conditions (temperature ranging from 22 to 25 °C, humidity 60–70%) and 12-h natural light and dark cycle. Prior to commencing the experiments, all the mice were acclimatized to a new environmental condition for a week. During the experimental period, the mice were kept in a well-ventilated animal house at room temperature and were supplied standard pellets (laboratory) fed and fresh drinking water.

Experimental design

The mice were randomly selected and divided into four groups having five mice in each group denoted as experimental group. Therefore, total twenty mice were used for each test.

Group I: control group—received the vehicle (1% Tween-80 solution prepared by using normal saline)

Group II: positive control group—received 1 mg/kg BW diazepam for anxiolytic activity tests and 30 mg/kg BW imipramine for anti-depressant activity tests

Group III: *Psidium guajava* Linn low strength group—received 200 mg ethanolic extract/kg BW

Group IV: *Psidium guajava* Linn high strength group—received 400 mg ethanolic extract/kg BW

The extract was suspended in the vehicle. The solution of the extract was administered orally with the help of oral gavage to test groups (group III and IV) and only

vehicle to group I. The diazepam (1 mg/kg) and imipramine (30 mg/kg) were administered by intraperitoneally to group II.

For all of the experiments, the EEPG (200 and 400 mg/kg) and vehicle were administered 60 min prior to experiment, but in case of hole cross test, it was given just before starting the experiment. The same time interval of dose was applied for both the standard drugs, diazepam and imipramine, except hole cross test where only diazepam was given just before starting the experiment. After completion of this experiment, all mice were sacrificed by injecting a dose of 150–200 mg/kg phenobarbitone sodium intraperitoneally followed by surgical resection of heart.

Evaluation of anxiolytic activity
Elevated plus maze test (EPMT)
The method suggested by Adeyemi et al. was employed with slight modification of Lister RG, 1987 [14]. The apparatus is made up of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a central platform (5 × 5 cm). The apparatus was positioned 40 cm on high of the floor. Each mouse was independently placed in the center of the EPMT and were permitted 5 min for fair exploration. During the experiment, the number entries into open and enclosed arms as well as the spending time on open arms were manually recorded. Entry into an arm was defined as the print when the animal placed all four paws onto the arm. Additionally, the anxiety index was estimated according to Cohen et al. [15] as follow:

$$\text{Anxiety Index} = 1 - [[(\text{Open arm time}/\text{Test duration}]$$
$$+ [\text{Open arms entries}/\text{Total number of entries}])/2]$$

Light-dark model transition test [16]
The light-dark apparatus having two compartment chamber (40 × 60 × 20 cm/h) was comprised of a brightly illuminated region (40 × 40 cm) with a 24 watt white light source located 17 cm above the box and a dark region (40 × 20 cm) divided by a partition containing a 7 cm diameter round hole. The individual mouse was kept in the lightened part of the chamber. After that, total number of passages from one area (illuminated) to another (dark) and total spending time in the bright illuminated part were recorded for the 5 min.

Hole-board test [17]
The hole-board apparatus was constructed by using gray colored wood box (40 × 40 × 40 cm) having 16 evenly spaced holes of 3 cm diameter in the floor. The floor of the box was placed 12 cm above the ground. The mice were kept singly in the middle of the hole-board, and the number of head dips in 5 min investigation was counted and recorded manually. Disappearing both the eyes of mouse into the hole was scored as a complete head dip.

Marble-burying behavior test [18]
In this method, the mice were singly positioned in translucent polycarbonate cage (22 × 32 × 13.5 cm) having a 5-cm film of saw dust and 24 glass marbles of 1.5 cm in diameter. These marbles were uniformly dispersed on the saw dust. The mice were placed in the cage for a period of 30 min, and the number of marbles at least two-third buried in the saw dust was counted.

Hole cross test [19]
The experiment was carried out as defined by Takagi et al. A steel partition having a hole of 3 cm in diameter and 7.5 cm in height at the middle was installed in the middle of a cage of 30 × 20 × 14 cm. After oral administration of both strengths of the extract, the number of crosses of a mouse through the hole from one compartment to another was recorded for a period of 3 min at different time intervals beginning from 0, 30, 60, 90, to 120 min.

Evaluation of anti-depressant activity
Forced swim test
The forced swim test (FST) was performed suggested by Porsolt et al. [20].The apparatus was constructed by a transparent plexiglass cylinder (45 cm in height and 20 cm in diameter) filled to a 17-cm depth with water (25 ± 1 °C). Then, the behavioral evaluation was estimated during the last 4 min of 6 min test period.

Tail suspension test [21]
The tail suspension test (TST) was directed as primarily defined by Steru et al. with minor modification of Berrocoso et al. The mice were singly suspended 50 cm above the stage of table with a gum tape pasted 1 cm apart from the tail tip. Immobility duration was recorded for the final 5 min during of 6 min test period. Mice were taken into consideration as immobile only when they suspended inactively and were utterly motionless.

Statistical analysis
The data obtained from the experiment were analyzed by Statistical Product and Service Solution (SPSS) windows version 16.0 using one-way analysis of variance (ANOVA) followed by Dennett's post hoc analysis. All the results recorded from the experiment were expressed as mean ± standard error of mean (SEM) and level of significance was set at $p < 0.05$.

Results

Phytochemical Screening

Phytochemical analysis of the extract revealed the presence of alkaloids, steroids, flavonoids, glycosides, tannins, terpenoids, carbohydrates, and proteins but absence of saponins and anthocyanins (Table 1).

Effect of *Psidium guajava* Linn leaves extract on elevated plus maze test

As shown in Table 2, a statistically significant effect on increase in the time spent in open arms was observed for both doses ($p < 0.05$ for 200 mg/kg dose and $p < 0.001$ for 400 mg/kg dose) compared with group I (183.20 ± 2.60 s), whereas the effect was more at the dose of 400 mg/kg (219.80 ± 1.02 s) than at the dose of 200 mg/kg (194.40 ± 3.40 s). In the same way, the result from group II (233.80 ± 2.20 s) gave statistical significant effect ($p < 0.001$). On the other hand, the extract at low strength dose produced an increase in the number of entries (6.60 ± 1.03) into the open arms which was statistically non-significant relative to group I (3.80 ± 0.66). However, comparing to group I, a statistically significant ($p < 0.01$) increase in the number of entries into the open arms at high strength dose (8.00 ± 0.71) was observed, but not more than that of group II ($p < 0.001$; 8.60 ± 0.93).

Moreover, a statistically considerable lower anxiety index (Fig. 1) was observed in case of the group treated with plant extract at the dose of 400 mg/kg (0.53 ± 0.01; $p < 0.05$) and the group treated with standard drug diazepam (0.51 ± 0.014, $p < 0.01$), whereas the group treated with plant extract at the dose of 200 mg/kg (0.58 ± 0.02) showed a statistically non-significant lowering effect comparing with group I (0.59 ± 0.02).

Effect of *Psidium guajava* Linn leaves extract on light and dark model transition test

The high strength dose of extract (400 mg/kg) showed a statistically significant increment in both time spending (190.20 ± 6.78; $p < 0.001$) and the number of entries (20.60 ± 2.09; $p < 0.05$) into bright illuminated part of the light-dark apparatus compared to Group I (TSLB 128.00 ± 3.64; NELB 13.80 ± 1.77). However, considering low strength dose of extract (200 mg/kg), a statistically significant ($p < 0.01$) rise in time spending (155.800 ± 5.78) into bright illuminated area was observed, but in case of the number of entries (15.20 ± 1.46), a statistically non-significant increase was found relative to group I. The group II also contributed a statistically significant increase in both time spending (213.40 ± 6.38; $p < 0.001$) and the number of entries (22.40 ± 2.23; $p < 0.01$) into bright illuminated part when compared with group I. The respective data of this experimental paradigm was presented in following Fig. 2.

Effect of *Psidium guajava* Linn leaves extract on hole-board model

The data provided in Fig. 3 indicated that a statistically significant ($p < 0.05$) effect in increasing the number of head dips into the hole (51.00 ± 2.51) was found at 400 mg/kg extract, but the total count of head dips was non significantly increased (40.20 ± 2.27) for the 200 mg/kg extract in comparison with group I. Following the same pattern, highest number of head dips (54.80 ± 3.34) among the four groups was found for group II which was statistically significant ($p < 0.001$) in comparison with group I.

Effect of *Psidium guajava* Linn leaves extract on marble-burying behavior model

The group II receiving 1 mg/kg dose of diazepam showed statistically considerable ($p < 0.001$) decrease in the number of marble buried (8.00 ± 0.95) in the saw dust compared with group I (20.40 ± 2.06). In case of plant extract, the higher dose (13.00 ± 1.58) provided a statistically significant ($p < 0.01$) decrease in the number of marble buried, whereas the lower dose (16.60 ± 1.29) gave a statistically non-significant decrease relative to group I. The respective data was presented in Fig. 4

Effect of *Psidium guajava* Linn leaves extract on hole cross model

As shown in the Fig. 5, immediately (0 min) after given both of doses of plant extract, at dose 200 mg/kg (8.40 ± 0.98), 400 mg/kg (8.20 ± 1.39) and also for standard

Table 1 Phytochemical screening of ethanolic extract of *Psidium guajava* Linn leaves

Sl. no.	Phytoconstituents	Test method	Result
01	Carbohydrates	1. Molisch's test	+ve
		2. Benedict's test	+ve
		3. Fehling's test	+ve
02	Proteins	1. Ninhydrine test	+ve
		2. Biuret test	−ve
03	Steroids	1. Liberman-Burchard test	+ve
04	Terpenoids	1. Salkowski test	+ve
05	Flavonoids	1. Lead acetate test	+ve
		2. Alkaline reagent test	+ve
06	Alkaloids	1. Wagner's test	+ve
		2. Hager's test	−ve
07	Glycosides	1. Keller-Kiliani test	+ve
08	Anthocyanins	1. NaOH test	−ve
09	Saponins	1. Froth test	−ve
10	Tannins	1. Lead acetate test	+ve
		2. Ferric chloride test	+ve

+ve = presence, −ve = absence

Table 2 Effect of leaves extract on time spent and the number of entries into open arms in elevated plus maze test

Group	Dose	N	Time spending in open arms	No. of entries in open arms
Group I	10 ml/kg	5	183.20 ± 2.60	3.80 ± 0.66
Group II	1 mg/kg	5	233.80 ± 2.20***	8.60 ± 0.93***
Group III	200 mg/kg	5	194.40 ± 3.40*	6.60 ± 1.03
Group IV	400 mg/kg	5	219.80 ± 1.02***	8.00 ± 0.71**

Group I = control group, *Group II* = positive control group, *Group III* = *Psidium guajava* Linn low strength group, *Group IV* = *Psidium guajava* Linn high strength group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs group I. The data are presented as mean ± SE

drug diazepam (8.80 ± 0.86), a statistically non-significant decrease in the number of hole crossing was observed relative to group I (9.00 ± 1.48). After 30 min of given doses, the group II (5.60 ± 0.68) and group treated with 400 mg/kg (6.00 ± 0.71) of extract showed a statistically significant ($p < 0.01$ for both groups) decline in the number of hole crossing, but in case of 200 mg/kg (7.60 ± 0.75), a statistically non-significant decline audited in comparison with group I (9.40 ± 1.03). After 60 min of given doses, it was seen that group II (4.80 ± 0.37, $p < 0.01$), group III (6.20 ± 0.97, $p < 0.05$), and group IV (5.20 ± 0.80, $p < 0.01$) showed a statistically significant decline in the number of hole crossing, compared with group I (9.20 ± 0.86). The group II (3.60 ± 1.52 after 90 min; 3.00 ± 0.71 after 120 min, $p < 0.001$ for both intervals), group III (5.00 ± 1.58, $p < 0.001$ after 90 min; 4.80 ± 0.58, $p < 0.01$ after 120 min), and group IV (4.20 ± 1.48 after 90 min; 3.40 ± 0.93 after 120 min, $p < 0.001$ for both intervals) provided statistically significant effect on decrease in the number of hole crossing relative to group I (9.40 ± 2.79 after 90 min; 9.20 ± 0.58 after 120 min), whereas group IV showed more effect than group III at both intervals.

Effect of *Psidium guajava* Linn leaves extract on forced swimming test

A statistically significant ($p < 0.01$) decrease of immobility time in the forced swimming test was found at dose of 400 mg/kg (42.20 ± 4.65 s), but not at dose of 200 mg/kg (57.20 ± 5.28 s) when compared with group I (63.20±6.00 s). The imipramine (30 mg/kg) treated group II (32.60 ± 3.17 s) also resulted statistically considerable ($p < 0.001$) effect on decreasing immobility time. All the respective data of the test were provided in Fig. 6.
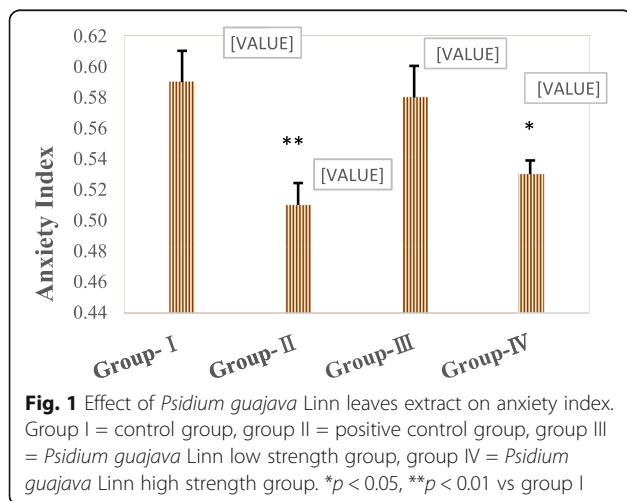
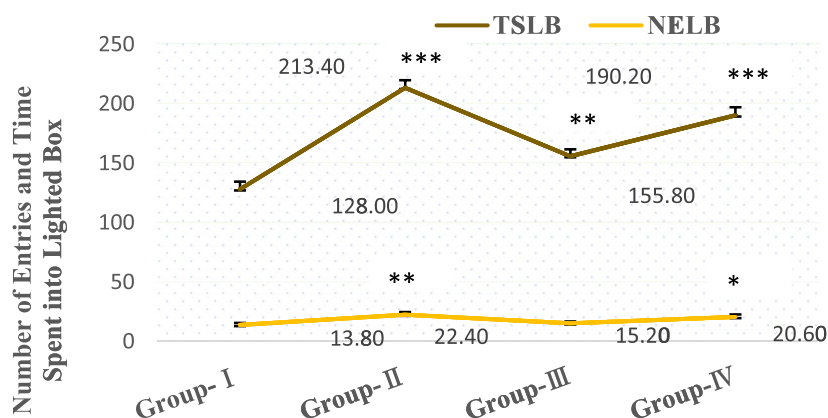### Effect of *Psidium guajava* Linn leaves extract on tail suspension test

The standard drug imipramine (30 mg/kg) treated group II (51.20 ± 6.11) gave a statistically signify cant ($p < 0.001$) effect on decline of the duration of immobility in TST in comparison with group I (98.40 ± 8.45). On the other hand, the plant extract of dose 400 mg/kg (68.80 ± 8.11 s) produced a statistically significant ($p < 0.05$) effect on decreasing immobility time but at dose 200 mg/kg, a statistically non-significant decrease (85.20 ± 8.91) in immobility time was observed relative to group I. Respective data was given in Fig. 7.

Discussion

There is a significant dimension in the development of novel anxiolytic and anti-depressant constituents that possess fewer side effects with fast onset of action and a comprehensive safety margin. In the current work, we investigated the neurobehavioral effects of oral treatment using well-validated animal models with the ethanolic extract of *Psidium guajava* Linn leaves at two different distinct doses as 200 mg/kg and 400 mg/kg of BW.

The phytochemical screening evaluates the presence of carbohydrates, proteins, steroids, terpenoids, flavonoids, alkaloids, glycosides, and tannins in *Psidium guajava* Linn leaves extract (Table 1). According to previous studies [22, 23], the presence of many biologically active phytochemicals such as flavonoids, triterpenes, alkaloids, steroids, tannins, and glycosides in different plant extracts may be accountable for their corresponding pharmacological activities. It is stated by several

Fig. 1 Effect of *Psidium guajava* Linn leaves extract on anxiety index. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. *$p < 0.05$, **$p < 0.01$ vs group I
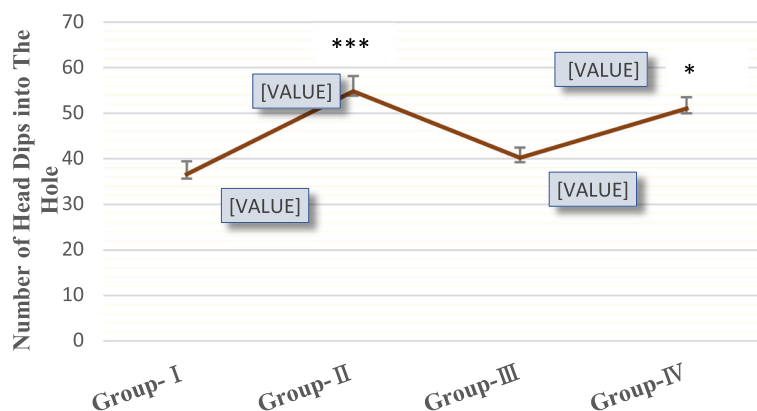
Fig. 2 Effect of *Psidium guajava* Linn leaves extract on time spent and number of entries into bright illuminated part in light and dark model transition test. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs group I. TSLB = time spent into lighted box and NELB = number of entries into lighted box

experimental investigations on different plant extracts that flavonoids, alkaloids, and terpenoids have been testified to be accountable for anxiolytic and sedative effects [24]. According to the study performed by Arcos-Martínez [25] and Graham [26] demonstrated that existing alkaloids might have the role to show the anxiolytic-like effects of the plant extract which was probably mediated through gamma-aminobutyric acid (GABA) receptor [27].

To assess anxiolytic properties of the extract, we use the elevated plus maze model, a most frequently employed test for screening the effect of anxiolytics on behavioral parameters of animals, and it is based on the fact that exposure of animals to an elevated plus maze evokes conflict of approach-avoidance stronger than revealed by the exposure to an enclosed arm [28–30]. In

EPMT, normal mice will habitually prefer to spend much of their assigned time in the closed arms. This preference seems to reveal an aversion in the direction of open arms that is generated by the fears of open zone. Anxiolytic effect is indicated by reduction in aversion headed for open arms by the way of increase in time spent and number of entries into the open arms [30], whereas, anxiogenics lessen the value of these parameters [31]. In our study, it is seen that *Psidium guajava* Linn leaves extract (200 mg/kg and 400 mg/kg of BW) increases the time spent in the open arms, and number of entries into open arms (Table 2). These results are similar to the effects observed after administration of the reference anxiolytic drug diazepam. Therefore, according to the result, it is evident that the extract shows dose dependent anxiolytic effect as higher dose shows better
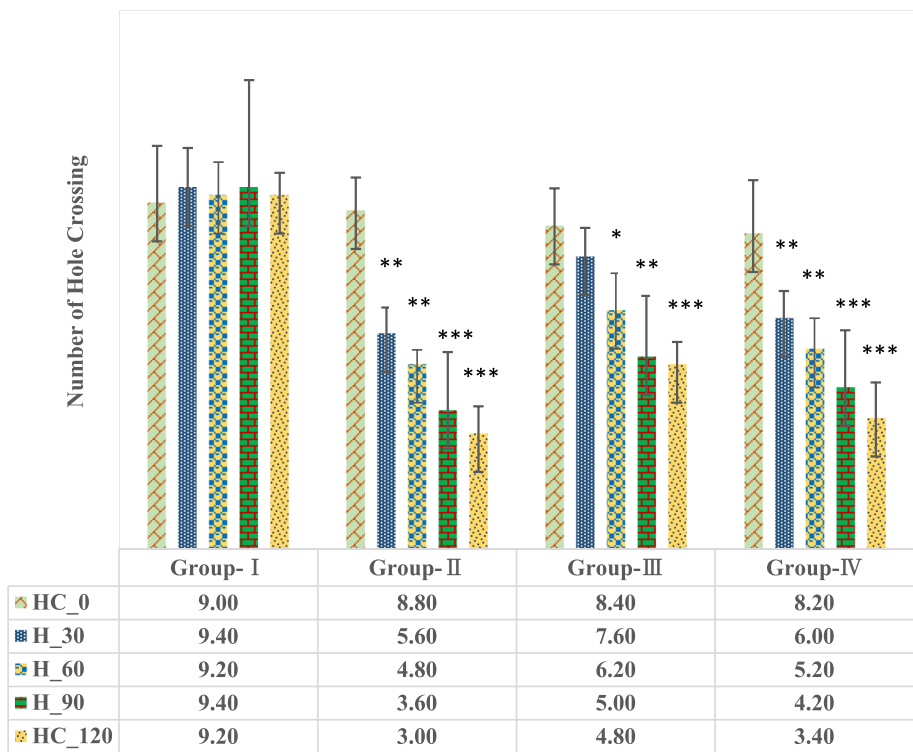

Fig. 3 Effect of *Psidium guajava* Linn leaves extract on the number of head dips into the holes in hole-board model. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. *$p < 0.05$, ***$p < 0.001$ vs group I
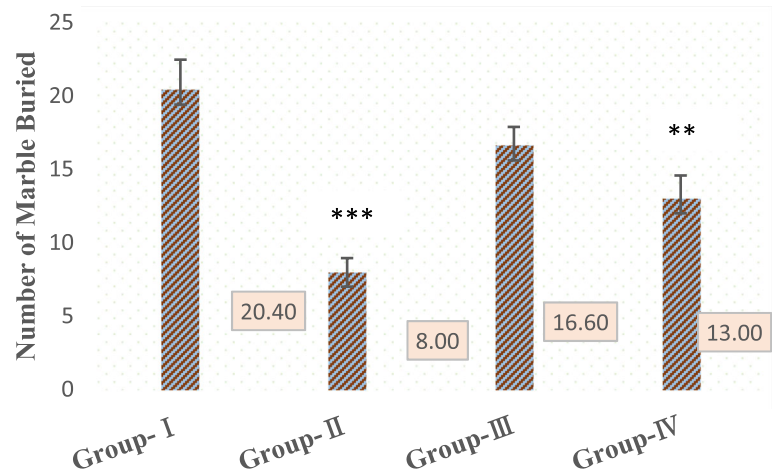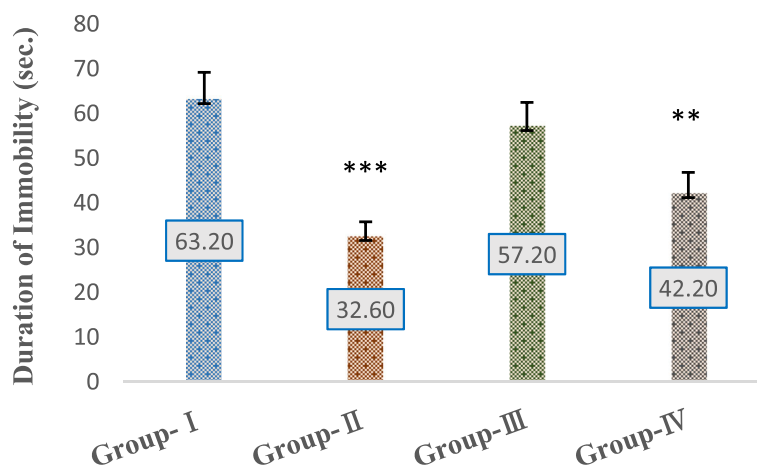
Fig. 4 Effect of *Psidium guajava* Linn leaves extract on the number of marble buried in the saw dust in Marble-Burying Behavior model. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. **$p < 0.01$, ***$p < 0.001$ vs group I

effect than lower, and these findings are supported by the previously published studies conducted with different plant extracts [32, 33]. Also, these treatments reduce the anxiety index (Fig. 1), a parameter that unifies the results of the number of entries to the open arms and the time spent in these spaces correspondingly to diazepam, and the findings are also supported by the previously conducted studies [34]. The anxiolytic effect of the

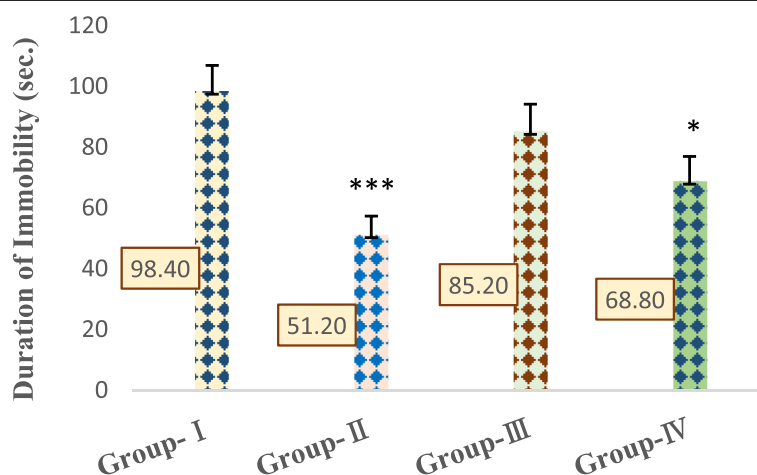|	Group- I	Group- II	Group- III	Group- IV
HC_0	9.00	8.80	8.40	8.20
H_30	9.40	5.60	7.60	6.00
H_60	9.20	4.80	6.20	5.20
H_90	9.40	3.60	5.00	4.20
HC_120	9.20	3.00	4.80	3.40

Fig. 5 Effect of *Psidium guajava* Linn leaves extract on number of hole crossing in hole cross model. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs group I. HC_0 = number of hole crossing at 0 min after treatment; HC_30 = number of hole crossing after 30 min of treatment; HC_60 = number of hole crossing after 60 min of treatment; HC_90 = number of hole crossing after 90 min of treatment; HC_120 = number of hole crossing after 120 min of treatment

Fig. 6 Effect of *Psidium guajava* Linn leaves extract on the duration of immobility in forced swimming test. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. **$p < 0.01$, ***$p < 0.001$ vs group I

extract is also prefaced through an experimental model principally designed as a potency predicting model of clinically available anxiolytic agents called light/dark test. It is taken into consideration that time spent by the mice on the illuminated part is mostly fruitful and consistent parameter of anxiety. The basic principle of the light/dark test is that the inherent aversion of rodents to intensely illuminated zone and the natural exploratory behavior of rodents responding to mild stressors [35]. In that point of view, the experiment may have efficiency for the prediction of anxiolytic or anxiogenic-like effect of plant extract in mice. In the light/dark test, anxiety is engendered by the conflict between the tendency to explore and the preliminary tendency to avoid the unfamiliar [36], and can be evaluated by taking into

consideration the number of transition into and the time spent in the light chamber, wherein increasing pattern of these parameters is regarded to reveal the anxiolytic-like features. The outcome obtained from our study indicates that the extract gives a statistically significant rise in time spending into bright illuminated part of the light-dark apparatus while comparing with group I, and the effect is comparable with the effect observed in group II (Fig. 2). For detecting anxiolytic effects, another advantageous experiment due mainly to its methodological simplicity and remarkable behavioral responses of the rodents when unveiled to an unacquainted environment is the hole board model, which is used to analyze head dipping behavior. This type of behavior is sensorial to the alterations in the emotional state of the animal, and

Fig. 7 Effect of *Psidium guajava* Linn leaves extract on the duration of immobility in tail suspension test. Group I = control group, group II = positive control group, group III = *Psidium guajava* Linn low strength group, group IV = *Psidium guajava* Linn high strength group. *$p < 0.05$, ***$p < 0.001$ vs group I

indicates that the expression of an anxiolytic-like state may be mirrored by the enhanced behavior of head dipping [37, 38]. In our study, both strengths of the extract and also the reference drug increase the number of head dips into the hole, in comparison with group I (Fig. 3). So, these findings may indicate the presence of the anxiolytic effect of the extract [39]. It has mostly been suggested that for the evaluation of drugs regarding anti-obsessive-compulsive disorder, using marble-burying behavior model would be beneficial and this is due to that no alteration related to the intensity of marble-burying behavior arisen during repeated testing, called as compulsive behavior [40]. *Psidium guajava* Linn extract of dose 200 mg/kg BW shows statistically non-significant decrease in the number of marble buried, but extract at 400 mg/kg BW dose shows a statistically significant decrease in the number of marble buried compared with group I. Group II taking diazepam produces similar pattern of results (Fig. 4).

In hole cross test, any agent having anxiolytic property will reduce the number of locomotion, assumed as of deficiency in interest on new environment [41]. Locomotor activity works as an indicator of mental wakefulness or alertness and thus, reduction of which signifies the tranquility and sedation elucidating as decreased CNS excitability [42].

In our present study (Fig. 5), the number of hole crossing by experimental mice of group I from one chamber to another group is slightly fluctuated from 0 to 120 min.

The group treated with the plant extract of 200 mg/kg shows statistically considerable decreasing effect at 60 min, 90 min, and 120 min interval of administration, but more decreasing effect on hole crossing is found at 120 min interval than other two intervals, and at interval 0 min and 30 min, a statistically non-significant effect is observed. On the other hand, a statistically considerable decreasing effect on hole crossing is found at all intervals except at 0 min in case of group IV, and the effect is more than that of in group III. In case of group II, the same statistically significant effect but more decrease is found as shown in group IV in all time intervals (Fig. 5). This locomotor activity suppressing capability as well as the sedative effect of EEPG demonstrates that the extract possesses CNS depressant activity. Drugs having sedative and anxiolytic agents, such as benzodiazepines, act to increase GABA mediated synaptic inhibition by facilitating the action of GABA upon the $GABA_A$ receptor. From our experimental findings, it is evident that the extract mainly at higher strength provides effect almost similar to that of the reference drug diazepam, a well-known benzodiazepine. Therefore, it could be supposed that substances of our extract able to mitigate the anxiety of rodents exposed to these paradigms could exert their

effect through the mechanism of action similar to that of the benzodiazepines [43], although there are numerous anxiolytic compounds with different action mechanisms through glutamatergic, noradrenergic, and serotoninergic receptors [44]. A molecular docking study may be conducted to elucidate the binding pattern of phytochemicals against $GABA_A$ receptor.

In our study, we use two most extensively preferable animal models for anti-depressant screening, the FST and TST, which are behavioral despair tests expedient for piercing the pathological mechanism of depression and for the evaluation of anti-depressant drugs [45]. These tests are quite sensitive and comparatively specific to all principal classes of anti-depressants including tricyclics, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs) [20]. These are based on the inspection of an immobile behavior developed by the rodents, after preliminary escape-oriented movements, when kept in an inescapable stressful condition. In this study, the higher dose (400 mg/kg BW) of extract shows statistically significant reduction in the immobility time of mice in both FST (Fig. 6) and TST (Fig. 7), whereas, a statistically non-significant reduction of the immobility time of mice is shown by the lower dose of extract (200 mg/kg BW) in both tests in comparison with group I. Similar effect is observed after administration of the anti-depressant drug imipramine. The compounds are said to have anti-depressant effect, those are involved in decreasing immobility time in FST [46]. Therefore, the compounds with higher concentration (400 mg/kg BW dose) present in *Psidium guajava* Linn extract might have anti-depressant effect.

It has been recognized that the shortening of immobility time in the forced swimming and the tail suspension tests relies predominantly on the augmentation of central 5-HT and catecholamine neurotransmission [47]. The majority of the conventional anti-depressants directly affects serotonin turnover in the brain [48], by exerting their inhibitory effect on serotonin reuptake and also by their efficacious interaction with 5-HT_{1A} and 5-HT_2 receptors [49]. It is demonstrated by a number of studies that the mechanism of action of different groups of anti-depressant drugs, including tricyclics, selective serotonin reuptake inhibitors, and (MAOIs), is involved with 5-HT_{1A} receptors [50]. In parallel with the serotonergic system, noradrenergic system is evidently involved depression and noradrenaline reuptake inhibitors or (MAOIs) exert their effect by enhancing the synaptic concentration of noradrenaline by interacting with α_1 and α_2-adrenoceptors, and also with β-adrenoceptors [51]. The standard anti-depressant drug (imipramine) used in our study averts reuptake of noradrenaline and serotonin resulting in their increased availability in the synapse and therefore, an increase in adrenergic and

serotonergic neurotransmission [52]. A relationship between dopamine neurotransmission and depression is demonstrated by several preclinical and clinical studies, and it is also claimed that the intensity of depression is inversely correlated dopamine metabolite levels in CNS [53, 54]. The activation of dopamine D_1 and D_2 receptors is involved with the mechanism of action of different anti-depressant drugs such as SSRIs, imipramine (a tricyclic anti-depressant) and bupropion (dopamine reuptake inhibitor) in the FST [55, 56], where dopamine D_2 receptors are related to the anti-immobility action of anti-depressants in mice reported by preclinical data [56].

The components present in the extract of *Psidium guajava* Linn leaves may show anti-depressant effect through above mechanisms. Also, many studies have demonstrated that protopine alkaloid possesses effects similar to anti-depressants in case of FST in mice by inhibiting transporters of serotonin and noradrenaline [57] and shows affinity for the receptor of $GABA_A$ [1].

Concerning the medical treatment of psychiatric disorders, the results obtained in this work has become important because not only anxiolytic effects were observed, but also anti-depressant activity was found. Developing biochemical and pharmacological studies are necessary for the purpose of establishing whether the observed effects in our study are produced by one chemical substance by itself as a result of distinct activation of nervous structures, or the several secondary metabolites of the plant are accountable for those biological activities.

Conclusions

The higher prevalence of anxiety and depression in the community at the present era ultimately leads to lot of morbidity. That being so, along with addressing these problems, it is too much important to discover efficacious remedies. Since some remarkable limitations are reported for the available drugs of these disorders, seeking alternative medications is compulsory for the welfare of human being. Our results demonstrate that the administration of the ethanolic extract of the leaves of *Psidium guajava* Linn in different doses applied in mice induces sedative anxiolytic and anti-depressant effects in dose dependent manner. However, further studies are necessary to confirm and extend these results.

Abbreviations
EEPG: Ethanolic extract of leaves of *Psidium guajava* Linn; EPMT: Elevated plus maze test; GABA: Gamma- aminobutyric acid; SSRIs: Selective serotonin reuptake inhibitors; MAOIs: Monoamine oxidase inhibitors; 5-HT: 5-hydroxy tryptamine; CNS: Central nervous system; BW: Body weight; SPSS: Statistical Product and Service Solution; ANOVA: Analysis of variance; SEM: Standard error of mean; FST: Forced swimming test; TST: Tail suspension test

Plant authentication approval
The plant was authenticated to be of the correct species by Dr. Mohammad Sayedur Rahman, Senior Scientific Officer, Bangladesh National Herbarium with a voucher specimen (Acc. Number: DACB-47697), which was prepared and deposited in the Bangladesh National Herbarium for future reference.

Authors' contributions
The idea of performing this research and designing of methodology came from SB and DM assisted by FI. All authors contributed equally to perform the analysis and to collect experimental data under the supervision of FI. Data analysis was conducted by DM, SS, AKT, MAS, and PJ. The draft manuscript was prepared by SB with the assistance of DM, MAS, AKT, and SS critically reviewed the manuscript. It was firmly assured that all authors went through the manuscript after reviewing and approved the manuscript as a final version before submission.

Availability of data and materials
The datasets used and/or analyzed during the study are available from the corresponding author on request.

Ethics approval and consent to participate
The experimental animals were treated in accordance with the Canadian Council on Animal Care (CCAC). The ethical review committee of Jashore University of Science and Technology, Bangladesh, also approved the handling of animals and the experimental design [Ref: ERC/FBST/JUST/2019-25(A)].

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests to conduct the study.

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