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Investigation of Neuropharmacological Effects of *Flemingia stricta* (Roxb.) Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MSB, MMR, MNA and MRI proposed and designed the study. Authors MSB, MNA and MJA conducted all laboratory experiments. Authors MSB, MMR, MNA and MRI analyzed and interpreted experimental results as well as participated in manuscript preparations. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Traditional preparation of the leaf of *Flemingia stricta* (Fabaceae) Roxb. , a medicinal plant of the Indian subcontinent, has been used for the treatment of different diseases as a herbal preparation. Our purpose was to analyze the neuropharmacological effects of different chemical extracts of *Flemingia stricta* Roxb. in mice.
Methods: In present study, the anxiogenic activity of crude extracts of *Flemingia stricta* leaves was determined using standard animal behavioral models, such as hole cross and open field; Sedative and anxiolytic potential were evaluated by performing thiopental sodium induced sleeping times tests and elevated plus maze test respectively.
Results: The crude extracts at the doses of 200 and 400mg/Kg exhibited a significant dose-dependent suppression of movement of mice in both open field and hole cross test. In the anxiolytic and sedative study, extracts displayed an increased percentage of entry of mice into open arm at both doses which are 200 and 400mg/Kg. Plant extracts produced a significant increase in sleeping

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duration and reduction of onset of sleep compared to control at the both doses of 200 and 400 mg/Kg.
Conclusion: This study clearly showed that the treated extracts have promising anxiolytic and sedative effect. Further studies on the prime constituent of this plant extract may provide lead compound in future.

Keywords: Flemingia stricta; sedative; elevated plus maze; anxiolytic; locomotor activity; neuropharmacology.

ABBREVIATIONS

F. stricta: *Flemingia stricta*; EPM: Elevated plus maze; GABA: Gamma-aminobutyric acid; CNS: Central nervous System, FSM: *F. stricta* Methanol, FSH: *F. stricta* N-hexane, FSC: *F. stricta* Chloroform, FSA: *F. stricta* Aqueous, FSE: *F. stricta* Ethanol.

1. BACKGROUND

From ancient times herbal drugs are called as green medicine for their safe and trustworthy health care paradigms. Furthermore, herbal drugs are cost effective than chemically manufactured drugs. Due to these benefits, public as well as industrial interests to the traditional herbal medicines are rising day by day [1]. People are leading a hectic life in this 21st century. Too much pressure in daily life increases anxiety and depression in people which results in psychiatric disorders around 20% of the adult population [2-4]. This is an alarming situation for our future generation. Therefore, there is an increasing demand for the discovery of anxiolytic, sedative drugs.

Anxiolytic and sedative drugs act on central nervous systems (CNS) which are extensively used as effective pharmacological agents to treat psychological disorders [5]. Benzodiazepines are among the most prescribed and effective anti-anxiety drugs used worldwide [6]. Barbiturates and ethanol are also frequently used. Both barbiturates and benzodiazepines demonstrate their effect by binding with gamma aminobutyric acid receptor ($GABA_A$ receptor) [7].

Day to day use of benzodiazepines and barbiturates might cause psychological and physiological dependence which leads to drug tolerance. As a result, repeated uses of this type of drugs can decrease the effectiveness. Safety is the main issue of barbiturates as depressant because only ten times of their pharmacological dose might be lethal [8-12]. According to the statistical data, alcohol addiction in American society for men and women is 5% to 10% and 3% to 5% respectively. Hence, a natural CNS depressant with minimum or no toxicity is therefore, essential [13].

Flemingia stricta (Fabaceae) Roxb. is distributed in Southeast Asia which includes Bangladesh, Bhutan, China, India, Indonesia, Laos, Myanmar, Philippines, Thailand and Vietnam [14,15]. It is an erect subshrub. It is found in hilly areas of Bangladesh and it has various traditional names given by local tribes [16]. Chakma healers traditionally use this plant for the treatment of polio. It has also several traditional benefits which consist of treat rheumatism followed by bone fracture, cough, asthma, goiter, urinary problems, snake bite, insect bite, leprosy, tumor and cancer, caries, hysteria, tuberculosis, insomnia and intestinal worms [17-19].

According to the literature review on this plant, it showed that the plant has been used as traditional medicine for the treatment of various diseases for many years. Furthermore, we also performed in-vitro antioxidant and phytochemical studies on this plant extract which paved our way to go for animal study [20]. Therefore, we undertook the study to assess the neuropharmacological potential of *F. stricta* leaves, by using animal models and studying the effect of the different chemical plant extracts on their exploratory behavior.

2. METHODS

2.1 Plant Collection and Identification

Whole plants of *F. stricta* were collected from Bhatiary, Chittagong region, Bangladesh. The plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

2.2 Preparation and Extraction of Leaf Extract

The collected leaves were thoroughly washed with distilled water and dried under the shade.

The dried sample was coarsely powdered (500 g) and extracted with methanol for 3 days to allow the total extraction process. After that the plant extract was filtered with sterilized cotton filter and the filtrate was gathered in a beaker. The plant extracts then kept in a water bath at 60 °C to evaporate the solvent from the solution. The container allowed to airtight for 72 h and filtrate thus obtained was concentrated by using a rotary evaporator. The extract was divided into two portions. One portion (2.5 g) was poured into glass vials to be tested as crude methanol extract, whereas the second portion (8 g) was dissolved in concentrated methanol and partitioned successively into four different extracts [21]. The fractions were then concentrated using a rotary evaporator.

2.3 Animal

Male Swiss albino mice, 3-4 weeks old, weighing between 20-25 g, were collected from the International Center for Diarrheal Disease and Research, Bangladesh. Animals were maintained under standard environmental conditions [temperature: (24±1)°C, relative humidity: 55%-65% and 12 h light/12 h dark cycle] and had free access to feed and water ad libitum. Prior to experimentation, animals were familiarized in laboratory conditions for one week.

2.4 Acute Toxicity Study

Mice were divided into control and test groups (n = 5). The test groups received the extract per orally at the doses of 400, 600, 800 and 1000 mg/Kg. Then the animals were kept in separate cages and were allowed to food and ad libitum. The animals were observed for possible behavioral changes, allergic reactions and mortality for the next 72 h [22].

2.5 Neuropharmacological Tests

The study was done to find out if extracts had any effect on the central nervous system. Effect on the exploratory behavior of mice was evaluated by hole cross test and open field test. Elevated plus maze test was conducted for determination of anxiolytic activity whereas thiopental sodium induced sleeping time test was for sedative activity.

2.6 Open Field Test

The method was adopted as described by Gupta et al. [22]. In the open field test, the animals were divided into control, positive control and test groups containing 5 mice each. The test groups received extract of *F. stricta* at the doses of 200 and 400 mg/Kg body weight orally whereas the control group received the vehicle (1% Tween 80 in water) and standard group received Diazepam at the dose of 1 mg/kg (i.p). The floor of half square meter open field was divided into a series of squares each alternatively colored black and white. The apparatus had a 40cm height walls. The number of squares traveled by the animals was counted for 5 min at 0, 30, 60, 90, 120 min after oral administration of both doses of the extract.

2.7 Hole Cross Test

The apparatus was a cage of 30 cm × 20 cm × 14 cm with a steel partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5 cm diameters was made at a height of 7.5 cm in the center of the cage. Animals were randomly divided into control, positive control and test groups containing 5 mice each. The test groups were treated with extract of *F. stricta* at the doses of 200 and 400 mg/Kg body weight orally whereas the positive control group with diazepam (1 mg/Kg) and control group with vehicle (1% Tween 80 in water). Number of passages of the animals through the hole from one chamber to the other was counted for 5 min at 0, 30, 60, 90 and 120 min after oral administration of the extract as well as diazepam and vehicle [23]. The apparatus was thoroughly cleaned after each trial.

2.8 Thiopental Sodium Induced Sleeping Time Test

For the experiment, the animals were randomly allocated to four groups, each with 5 mice. The test groups were given the leaf extract of *F. stricta* at doses of 200 and 400 mg/Kg body weight, while the positive control was treated with diazepam (1 mg/Kg) and control group with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40mg/Kg) was administered to each mouse to induce sleep. The animals were observed by placing them on separate chambers for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep i.e. time between the loss and recovery of righting reflex. The onset of sleep and total sleeping time was recorded for control, positive control and test groups [24].

2.9 Elevated Plus Maze Test

The method initially suggested by Handley and Mithani was employed with minor modifications

[25]. The apparatus consists of two open arms (5 × 10) cm and two closed arms (5 × 10 × l5) cm radiating from a platform (5 × 5) cm to form a plus-sign figure. The apparatus was situated 40cm 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. Sixty minutes after administration of the test drugs, each animal was individually placed in the center of the EPM and was allowed 5 min for free exploration. Next, the number of open and enclosed arm entries, and time spent on open arms was manually registered [26]. Entry into an arm was defined as the point when the animal placed all four paws onto the arm. The percentage of open arm entries (100 × open/total entries) and the percentage of time spent in the open arms (100 × open/(open + enclosed)) were calculated for each animal. Observations made from an adjacent corner produced significant (p< 0.05, p < 0.01) decreases of locomotion from its initial value during the period of the experiment. Maximum suppression of locomotor activity was displayed at the dose of 400 mg/Kg body weight, which was comparable to the reference drug diazepam.

2.10 Statistical Analysis

Statistical comparisons were performed using one-way ANOVA followed by post-hoc Dunnett's test with the SPSS program (SPSS 20.0, USA). The data were calculated as the mean ± standard error of mean (S.E.M.) and data of this graphs are deposited to the authors. The values obtained from plant extracts were compared with the control group and were considered statistically significant when P<0.05. Graphs were represented by Graph Pad Prism software.

3. RESULTS

3.1 Neuropharmacological Tests

3.1.1 Open field test

The open field test of *F. stricta* treated groups represented significant and dose dependent decrease of number of movements of mice from 0 minutes to 120 minutes. According to the study, 400 mg/Kg body weight treated groups showed more significant reduction of movements than 200 mg/Kg body weight. According to the chemical fractions, from 30 to 120 minutes, all the chemical extracts decreased the number of squares traveled by mice significantly at both 200 mg/Kg and 400 mg/Kg treated groups, but aqueous and n-hexane extract at 200 mg/Kg showed the lowest effect. (Fig. 1).

3.1.2 Hole cross test

Hole cross test of *F. stricta* treated groups showed a decrease of movement of mice. Data represented in Fig. 2 suggests that the number of

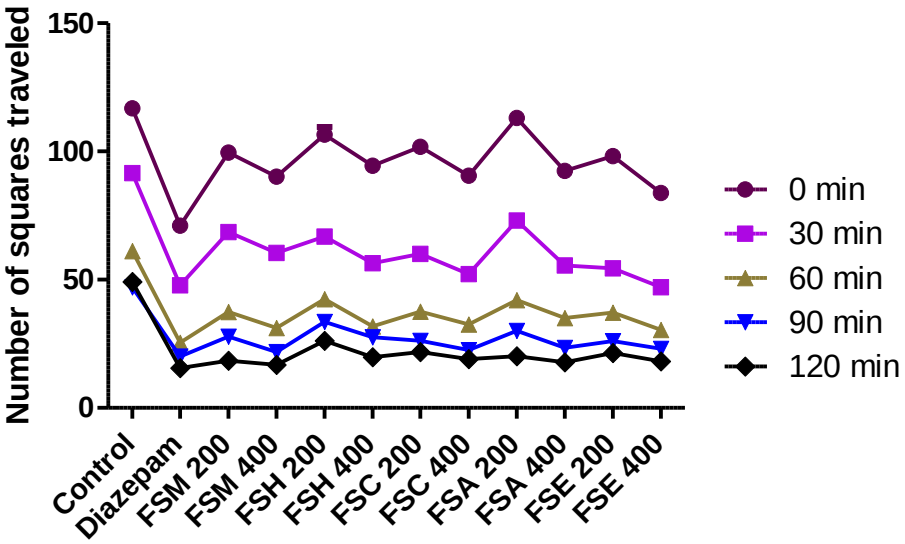

Fig. 1. Effect of extracts of *F. stricta* on exploratory behavior on mice (Open field test) (n=5); (P<0.05) Dunnett's test as compared to control

holes crossed from one chamber to another chamber by mice was decreased significantly for all chemical extracts of *F. stricta* leaves compared to control at doses of both 200 and 400 mg/Kg. Maximum suppression of locomotor activity was found at doses of 400 mg/Kg (Fig. 2). Among all the fractions, chloroform (FSC), methanol (FSM) and ethanol (FSE) extract showed comparatively better dose dependent reduction of movement of mice at both doses than other extracts after 60 minutes.

3.1.3 Thiopental sodium induced sleeping time test

In the thiopental induced hypnosis test, both 200 and 400 mg/Kg showed a significant reduction in the time of onset of sleep. Moreover, 200 and 400 mg/Kg treated groups also increase the duration of thiopental sodium induced sleeping time in mice in the case of methanolic (FSM) and ethanolic (FSE) plant extract. Though chloroform (FSC) and n-hexane (FSH) demonstrated promising effect (Fig. 3).

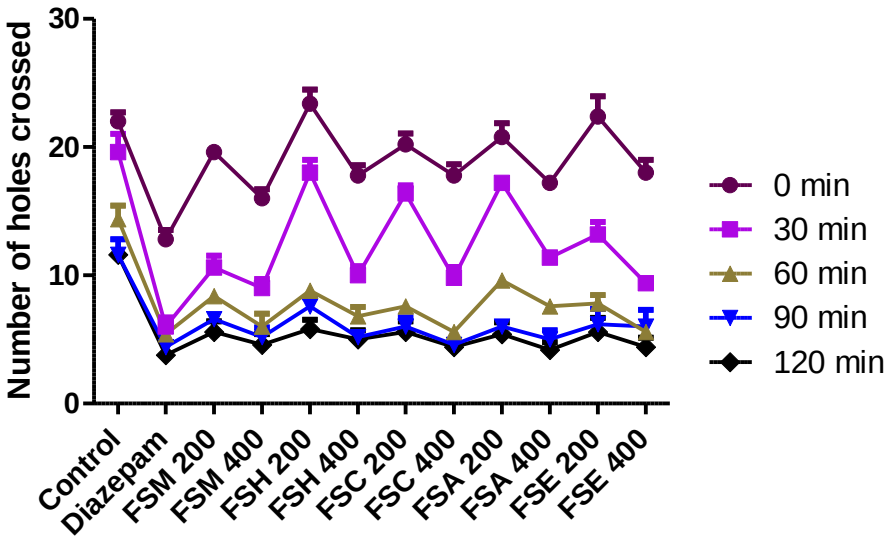

Fig. 2. Effect of extracts of *F. stricta* on exploratory behavior on mice. (Hole cross test) (n=5); (P<0.05) Dunnett's test as compared to control

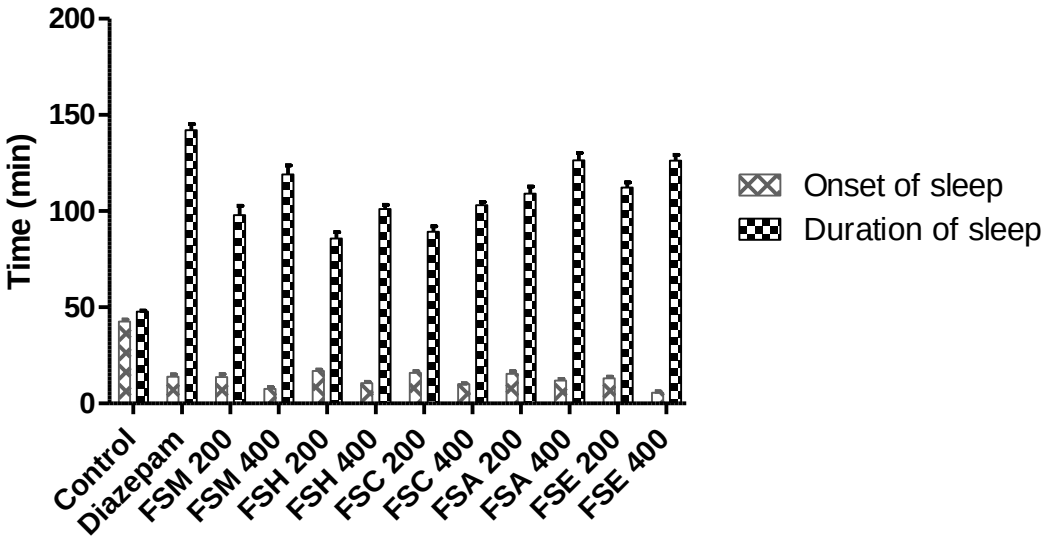

Fig. 3. Effect of extracts of *F. stricta* on thiopental sodium induced sleeping time (n=5); (P<0.05) Dunnett's test as compared to control

3.1.4 Elevated plus maze test

This test is used to detect the anxiolytic activity. The extracts of *F. stricta* at the dose of 200 mg/Kg and 400 mg/Kg significantly increased the percentage of entries of mice into the open arms, and the percentage of time spent in the open arms which are shown in Fig. 4. But, 400 mg/Kg showed more significant effect than 200 mg/Kg dose. Ethanol (FSE) extract showed comparatively more promising results than other extracts. But other extracts also showed promising effects (Fig. 4).

4. DISCUSSION

The result of open field and hole cross test demonstrated that this plant extracts reduced the frequency of movements of mice. Locomotor activity was dose dependently decreased from 30 minutes to 120 minutes which proved its sedative activity. Hence, it can be stated that both doses of chemical extracts of *F. stricta* leaves decreased the frequency and the amplitude of movements. The above result also showed that crude extracts of *F. stricta* plant had strong sedative and hypnotic action that mainly mediated in the CNS by the $GABA_A$ receptor complex. Thus, it can be said that the plant has promising sedative effects.

Thiopental sodium induced sleeping time test represented the depressant activity of central nervous system by decreasing the onset time of sleep as well as increasing the duration of sleep [27-29]. Chemical constituents of plant extracts might be the reason for its benzodiazepine like sedative activity. Previous literature review on this plant showed that this plant has several traditional uses. Furthermore, it also contains several chemical constituents such as tannins, glycosides, alkaloids, saponins, phytosterols, flavonoids. Elevated plus maze test is extensively used to determine the anxiolytic activity of drugs which is related to binding of drugs to GABAA receptor complex [30-32]. In this experiment, we observed that *F. stricta* increased the open arm entries along with time spent in open arms which precisely showed the anxiolytic effect of this plant extract in mice.

Generally, anxiolytic and hypnotic drugs such as benzodiazepines and their metabolites are highly lipid soluble which helps these drugs to reach the central nervous system readily. But this beneficial effect might turn into nightmare due to its high lipid solubility which can increase the risk of amnesia. Amnesia is a disease related with memory deficit which is caused by not only brain damage or disease but also by the use of several anxiolytic and hypnotic drugs [33-35].

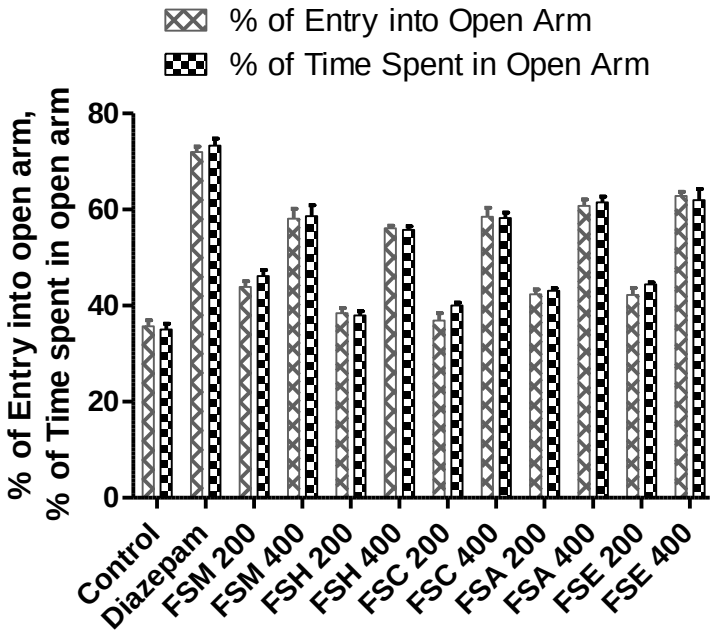

Fig. 4. Effect of extracts of *F. stricta* on EPM test during 5 min test session (n=5); (P<0.05) Dunnett's test as compared to control

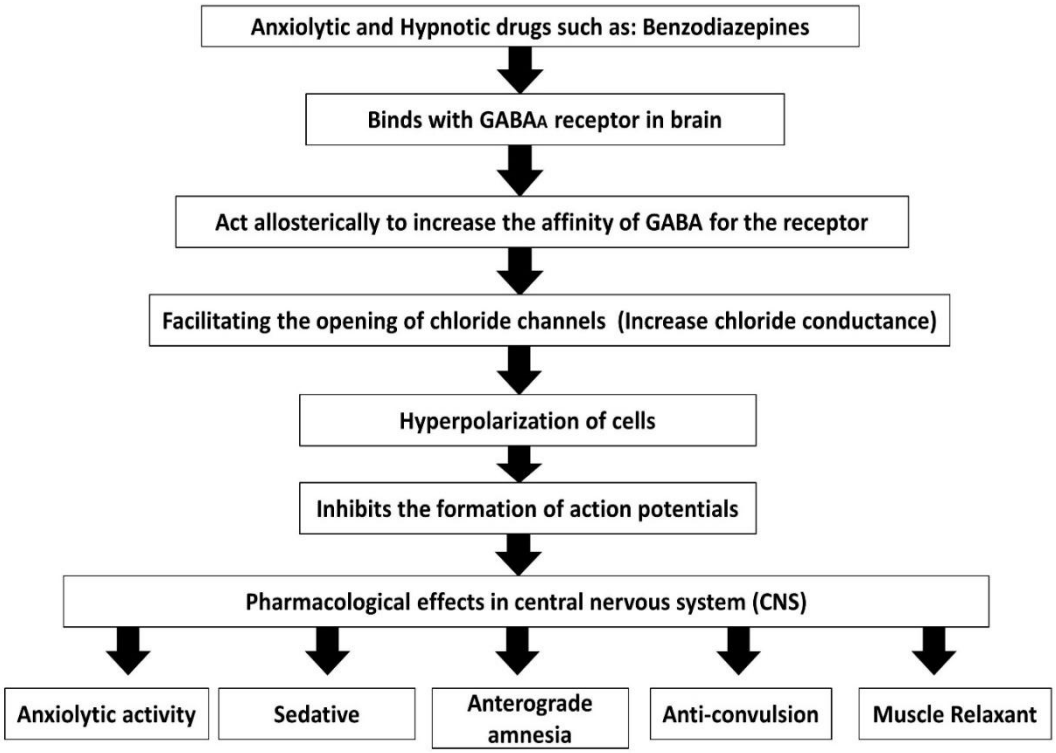

Fig. 5. Mechanism action of anxiolytic and hypnotic drugs

Therefore, optimization of lipid solubility is essential to decrease this type of side effect. Thus, excessive lipid soluble property of anxiolytic drugs might be harmful for consume. Hence, we need a compound which can make a great balance between lipophilicity and hydrophilicity means not excessive lipophilic or hydrophilic. Polarity of water is highest among five chemicals. Polarity of methanol and ethanol is less comparably to water. On the contrary, n-hexane and chloroform are more lipids soluble. According to our study, it could be said that, this plant might possess molecules which results in significant effects of *F. stricta* extracts made by different chemicals. Here, *F. stricta* extracts made by chemicals such as methanol, ethanol and chloroform extracts showed better effects than n-hexane and .aqueous extracts. However, further studies are needed to investigate the underlying mechanisms of this plant extract. There is a great need of the discovery of new anxiolytic drugs with fewer side effects. Hence, our experiment might provide a good drug candidate for future.

5. CONCLUSION

All of the results were dose-dependent and statistically significant. According to the results of this experiment, it can be conclude that the crude extracts of *F. stricta* possess significant neuro-pharmacological activity. Therefore, we can suggest that the extract may fulfill the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders. Nevertheless, further exploration is required to discover the exact mechanisms that are responsible for this anxiolytic activity of this plant extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The set of rules followed for animal experiment were approved by the institutional animal ethics committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh according to governmental guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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