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PHYTOCHEMICAL SCREENING FROM *FICUS RELIGIOSA* LEAVES AND DETERMINATION OF SEDATIVE-HYPNOTIC ACTIVITY IN MICE BY USING ETHYL ALCOHOL EXTRACT

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ABSTRACT

Ficus religiosa is a sizable broad-leaf evergreen woody plant have always been the important form of the medicinal drug in the south Asian country including Bangladesh, India Pakistan, Nepal, Bhutan, Sri Lanka in care of a variety of diseases and disorders such as insomnia, pain, inflammation, jaundice, and fever. *Ficus religiosa* has therapeutic properties due to the presence of the different complex chemical substance of different composition, which is found as secondary plant metabolites in one or many parts of these plants. The present aim evaluated the sedative and anxiolytic potentials of the ethanol extract of leaves of *Ficus religiosa* in various activity models in mice and update information on its phytochemistry and pharmacological activities. The sedative action of EEFR was investigated by using open field, hole cross, rotarod, and thiopental sodium- (TS-) induced sleeping time finding tests, where the overhead plus maze (EPM) and light-dark box (LDB) attention tests were employed to support the anxiolytic potentials in mice at the doses of 100, 150 and 200mg/kg. The issue illustrated that EEFR significantly inhibited the exploratory activity of the animals both in hole cross and in open field tests in a dose-dependent mode. It also reduced motor coordination and modified TS-mediated hypnosis in mice. On The other hand, EEFR showed anxiolytic potential by increasing the number and time of entries in the open arm of EPM, which is further strengthened by the increase in gross time spent in the light part of LDB. Therefore, this study suggests the sedative and anxiolytic properties of the leaves of *Ficus religiosa* and help the traditional utilization of this plant in the treatment of different psychiatric disorders including insomnia.

KEYWORDS

Ficus religiosa, Phytochemical, Sedative-Hypnotic and EEFR.

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INTRODUCTION

A neurological situation such that can be precipitated by insomnia are commonly activated with sedatives[1]. Insomnia and other sleep disorders are globally medical difficulty and most current physiological and psychological states defined by emotional, cognitive, and behavioral factor

affecting one-eighth of the universe population[2] and attempt to discover new remedies, particularly with herbs are stepped in the right direction. Presently there are no appropriate drugs for the treatment of chronic insomnia. People quickly develop tolerance to existing sleeping medications, leading them to take high doses and to mix medications. This can effect in unspeakable side effects and even worse insomnia when they attempt to decrease the medications[3]. Today, different types of sedative drugs (e.g., diazepam, which is selected as a reference standard in this study) come with sanative vital to succeed in sleeping disorder, which also could bring down anxiety[4,5]. However, in addition to their advantageous properties, these currently getable sedatives and anxiolytic therapies have serious adverse and side effects. Consequently, newer, more efficacious and best-tolerated care including alternative/complementary medicines would be a welcome addition in the therapeutic repertoire of insomnia and anxiety management. A *Ficus religiosa* (F. religiosa) tree has a major role in an indigenous structure of medicine like Ayurveda, Siddha, Unani and Homeopathy[6]. It is a popular bodhi tree and has got mythological, religious, and medicinal importance in Indian culture since times immemorial[7]. The plants have been used in traditional Indian medicine for various range of ailments. Traditionally the bark and leaves are used as an antibacterial, antiprotozoal, antiviral, astringent, antidiarrheal, in the treatment of gonorrhea, ulcers, and the leaves used for skin diseases. The leaves reported anti-venom activity and regulates the menstrual cycle[8,9]. In Bangladesh, it has been used in the treatment of different diseases such as cancer, inflammation, or infectious diseases[10]. In case of high fever, its tender branches are used as a toothbrush. Fruits are used as laxatives[11], latex is used as a tonic, and fruit powder is used to treat asthma[12,13]. The different parts of the tree are usually used to treat various human diseases such as diabetes, atherosclerosis, Alzheimer's, gastritis, cancer and AIDS[7]. The *F. religiosa* aqueous leaf extract has a rich source of bioactive functional compounds which includes, alkaloids, flavonoids, terpenoids, Saponins, Tannins and so forth[14]. Flavonoids are able to determining factor central nervous system activity (CNS) by binding to the benzodiazepine position on the GABAA receptor consequent in sedation, anxiolytic effects[15]. Various bioactive component have also been show from *Ficus religiosa* with antihyperglycemic[16], antioxidant, antinociceptive[17] anti-inflammatory[18] cytotoxicity and anthelmintic activities[19]. However, till now there is no scientific study revealing its activity (CNS) on the central nervous system. This influenced us to arrangement and conduct the present study is focused on *Ficus religiosa* leaves exert in sedative and anxiolytic activities on Central Nervous System (CNS) in various models in mice.

MATERIAL AND METHODS

Plant Material

The *Ficus religiosa* Leaves collected from the village Rambhadropur road side of Satkhira District, Bangladesh during August 2016 at the daytime. The plant identified by the experts of Bangladesh National Herbarium at Mirpur in Dhaka (Accession No. DACB 39952) and a voucher specimen deposited at the Pharmacy department, Stamford University Bangladesh. The date of the inquiry done by Bangladesh National Herbarium on 12th August 2016. The powdered dried leaves (250g) were macerated with 450mL of ethanol (100%; Merck, Bremen, Germany) with occasional stirring at room-temperature for three days. Then the filtrate was collected and totally dried using a rotary evaporator. After drying, 20.7g of dried extract (yield 17.82%) obtained from 120g of powder which was further used in the entire set of studies

Animals

Adult male Swiss albino mice (20-25g) collected from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). The standard environmental condition preserved for mice (temperature: 25 ± 2°C, humidity: 55-65% and 12 h light/dark cycle). Food and water of mice collected from ICDDRB. Mice adapted to the laboratory

environment over a period of seven days before performing the experiments. No food was given to the animals nightlong before the experimentation. Every experiment associated with new cohorts of mice. We measured the average body weight of mice for the experiments. All the experimental mice handling followed the Moral Principles and Guidelines for Scientific research on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences.

Drugs and Treatments

Mice were divided into five groups containing 5-7 animals each for control, standard, and test samples, for all experiment. Standard drug diazepam (1mg/kg; i.p.) (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), EEFR (50, 100, and 200mg/kg; p.o.), or vehicle (DMSO; 0.1mL/mouse; p.o.; Merck), was apply to the animals, instantly after taking the pre- treatment reading in hole cross and open field tests. For the rest of the models, diazepam was administered at 15 min and EEFR or medium at 30 min before the experiments. In sleeping time determination test, the sleep inducer thiopental sodium (20mg/kg) (Square) was administered 15 min post-treatment with diazepam and 30 min of vehicle or EEFR.

Acute Toxicity Test

Mice were separate into desired groups each containing 5-7 animals. EEFR was apply to the animals orally at the doses of 500, 1000, and 2000mg/kg. The mice were then allowed to take food and water ad libitum and observed for the next 72 h to check any allergic symptoms and mortality induced by EEFR[20].

Sedative Activity Analysis

Open Field Test

This test is a widely used model for the assessment of emotional activity of the animals, particularly the rodents. The method was accomplished as described by Gupta *et al*, (1971)[21]. The open field apparatus made of a wooden field of half square meter with a series of squares as an alternative painted in black and white. It had a wall of 50 height and was located in a dimly lit room. The animals were arranged in the middle of the open field to explore freely and the number of squares visited by them was counted for 3 minutes as pretreatment reading. Instantly after taking the reading the animals were treated with vehicle, extract, or diazepam and determined repeatedly at 30, 60, 90, and 120 min after the treatments.

Hole Cross Test

A cage having a size of 30 × 20 × 14 cm with a fixed partition in the center having a hole of 3 cm diameter was used in this research[22]. Mice were treated with either vehicle or drug or EEFR and allowed to cross the hole from one chamber to some other. The animals were then determined for 3 min and the number of passages was recorded before and at 30, 60, 90, and 120 min following the treatments.

Test for Motor Coordination (Rotarod Test)

The rotarod test was performed according to the operation described by Dunham and Miya (1957)[23]. This test is effective for the research of motor impairment due to pharmacological agents like muscle relaxants or CNS depressants. The apparatus consisted of a horizontal no slippery plastic rod, rotating at 20 rpm. The animals which can remain in the rotating rod for more than 180 sec were selected for this work. After desired treatments, each mouse was placed on the rod and the falling time of each mouse within 180 sec was recorded as an indication of muscle relaxation.

Thiopental Sodium-Induced Sleeping Time Determination

Thiopental sodium-induced sleeping time test was performed according to the previously described method acting[24]. Following desired sample or drug administration, the animals were observed for the latent period (time to lose their righting reflex, immediately after thiopental sodium injection) and the period of time of sleep (time between the failure and recovery of reflex) induced by thiopental sodium.

Anxiolytic activity test

Elevated plus-maze test

The plus maze apparatus consisting of two open arms (16 × 5 × 12 cm) and two closed arms (16 × 5

× 12 cm) with an open cover which was 50 cm elevated from the floor used to observe anxiolytic activity in animals[25]. All new cohort of the mouse placed on the elevated plus-maze apparatus 30 min after the administration of the dose. Each mouse situated in the middle of the elevated plus-maze with its head facing the open arms. The behavioral personality of the mouse observed for 3 min with a different kind of parameter (time spent in open arms, time spent in the closed arms, the number of the entry in the open arms, the number of the entry in the closed arms).

Light-dark box test

The light-dark test may be useful to predict anxiolytic-like or anxiogenic-like action in mice. Transitions have been reportable to be an index of activity-exploration because of habituation over time, and the period of time spent in each compartment to be a reflection of aversion[26]. This consist of the full automated box monitored by the observer. An open-topped rectangular box (46 × 27 × 30 cm high) separated into a small (18 × 27 cm) area and a big (27 × 27 cm) area with an opening door (7.5 × 7.5 cm) situated at the middle of the partition at the floor level. One room painted black and dark environment whereas the large room was painted white and brightly illuminated by a 60-W (400 lx) light source. The light placed in the center of the white room. The time spent in the illuminated and dark room, as well as the number of transitions in each space, the latency of the first crossing from one room to the other, recorded for 5 min. The environment during the test was the dark condition. The data for these four parameters directly collected by the observer. This test exploited the conflict between the animal's tendency to explore a new environment and its fear of brig.

Hole-board test

The apparatus was combined with a gray wooden box (40 cm × 40 cm × 25 cm) with sixteen equal holes 3 cm in diameter on the floor[27]. The center of each hole was 10 cm from the closest wall of the box. The level of the box was positioned 15 cm above the ground and separate into squares of (10 cm × 10 cm) with a water-resistant marker. An animal placed at the center of the hole-board and allowed to explore the apparatus for 5 min. The total number of heads-dips recorded. The head dip mark if both eyes disappeared into the hole.

Marble-burying test

Mice placed one by one in glass cages with the selected bedding material for 30 min (habituation period) and then located into waiting in cages[28]. Twenty-five glass marbles positioned evenly spaced 7 cm apart on a 4 cm layer of bedding material in the habituation cages. The mice inform in the same cage in which they habituated before the research project. After 30 min, the marble burying period concluded by removing the mouse, and the number of marbles that more than two-thirds covered with bedding material counted. Whether a marble buried was established by research and was confirmed by manual post-experimental assessment of an investigator. After each test, cages and bedding materials located by fresh ones and glass marbles clean with water, dried with a paper towel, and left to return to room temperature.

Statistical Analysis

The results are given as Mean ±SEM. The statistical investigation was performed using two-part analysis of variance (ANOVA) followed by Bonferroni's posthoc trial for hole cross and open field tests, where one-way ANOVA followed by Dunnett's post hoc test was employed for other scientific research performed in this study. Each statistical analysiswas performed using SPSS software system. In any case, the ED50 values were measured using GraphPad Prism and the figures were drawn using Sigma Plot software.

Methodology for phytochemical screening

Chemical substance tests were carried out on the extract and on the powdered specimens using standard process supported on the protocols of Edeoga *et al*, 2005[29], Harborne, 1973[30] and Sofowara, 1993[31], to determine the different component present.

Test for Alkaloids

Test solution (1ml) was taken over in a test tube and few drops of Mayer's reagent (Potassium mercuric iodine solution) were added into it and then cream

color precipitate was observed. To a couple of ml of filtrate, 1 or 2ml of Dragendorff's reagent was added by the side of the test[32] tube. An outstanding red precipitate indicates the test as positive.

Test for Tannins

To test solution added 10 ml distilled water(D.W), then filtered, in the filtrate 2ml FeCl3 (10%) was added blue-black or greenish precipitate formed, show the presence of tannins[33].

Test for Cardiac Glycosides

5ml of each extract was treated with 2ml of glacial acetic acid include one drop of ferric chloride solution. This was set with 1ml of concentrated sulphuric acid. A brownish ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid part, a greenish ring may form just step by step throughout a thin layer.

Test for Flavonoid

5ml of dilute ammonia solution was added to a part of the aqueous filtrate of each plant extract followed by addition of concentrated H2SO4. A yellow color observed in all extract indicated the presence of flavonoids. The yellow coloration disappeared on standing[29].

Test for Terpenoids

To the test solution, added 2 ml of chloroform and 1 ml H2SO4, reddish brown color at the interface, indicate the presence of terpenoids[34].

Test for Saponins

2gm of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken smartly for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken thoroughly, then observed for the formation of emulsion thereafter in plant sample (filtrate/powder)[29].

Test for Steroids

2ml of acetic anhydride was added to 0.5gm ethanolic extract of each sample with 2ml H2SO4. The color changed from violet to blue or green in some samples show the presence of steroids.

Detection of carbohydrates

Benedict's test-Test solution was mixed with some drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in a water bath, determined for the formation of red-brown precipitate to display a supportive result for the presence of carbohydrate[35].

RESULTS AND DISCUSSION

Phytochemical screening

Upon management standard procedures, preliminary phytochemical screening of EEFR was qualitatively test carried out for the detection of alkaloids, flavonoids, carbohydrates, glycosides, tannins, Saponins, steroids and so forth (Table No.1).

Where saponins are known to show amphetamine antagonism and sedative property and drop-off spontaneous motor activity in the experimental animal models[36]. It has also been reported that the presence of alkaloids, glycosides, and flavonoids in plant extract possess sedative and anxiolytic effect through the interaction with GABAA receptors[37,38]. Considering our results and previously published reports, it is possible that the abovementioned chemical components in the extract might contribute at least in part to the observed pharmacological activities. We may, therefore, believe that the ethanol extract of *Ficus religiosa* contains psychoactive principles that are sedative and anxiolytic in nature.

Reported to oral administration of EEFR at the doses of 100-750mg/kg did not show any visible sign of delay toxicity, activity changes, allergic manifestations (skin, rash, itching) or mortality rate during the 72-h observation time period. Therefore, it shows that the EEFR have low-level toxicity profile and the LD50 is 750mg/kg.

The hole cross and open field tests are the most popular research models used to analyze the exploratory activity of the animals. It is well accepted that different drugs like benzodiazepines suppress curiosity of the animals about a new environment resulting in a drop-off in their locomotor activity[22]. Likewise, our outcome

demonstrated that EEFR significantly ($p < 0.01$) decreased locomotion of the animals both in hole cross and in open field trial. The suppressive result was observed from 30min and continued up to 120min of EEFR administration (Figures (Figure No.1 and 2) accounting 81 and 92% of locomotor inhibition at the high time point of these tests, respectively. Besides, it is well accepted that the Central Nervous System (CNS) sedative drugs like benzodiazepines cause muscle weakness[27], reduced ambulatory action, and sedation which negatively affects the rotarod performance of the animals[39,40]. As depicted in Figure No.3, EEFR reduced the decreasing latency of the animals from rotarod, significantly ($p < 0.01$) with the doses of 100 and 200mg/kg. The highest motor coordination impairment and ED50 were calculated as 58% with 200mg/kg and 89.66mg/kg, respectively. In parallel, diazepam at 1mg/kg dose also produced a similar pattern of effects determined with EEFR in all experimentation. From these notices, it is possible that, like diazepam, EEFR may have the potential to act on CNS which was reflected by its locomotor inhibitory action as well as impaired motor coordination effects in the animals.

Further information of the central sedative action of EEFR is provided by TS-induced sleep raise the quality of the extract. Substantial scientific reports suggested that the CNS depressant barbiturates like TS bind to the barbiturate binding site of the GABAA receptor, which potentiates GABA-mediated hyperpolarization of the neurons[28]. In our study, the acute oral administration of EEFR significantly ($p < 0.01$) modulated the sleeping activity of the animals induced by TS. We found that EEFR reduced TS-induced onset of sleeping and enhanced the sleeping time period in a dose-dependent manner (Figures No.4(a) and 4(b)) with the ED50 value of 69.73mg/kg. Therefore, these results strengthened the sedative and muscle relaxation important of the extract observed in hole cross, open field, and rotarod tests.

The elevated plus maze is a widely used activity model in rodents and has been validated to look into the anxiolytic possible of different pharmacological agents. The open arm activities of the animals in EPM indicate a conflict between the animal's innate activity to keep itself in a sheltered area (e.g., closed arms) and motivation to explore in a novel environment, where the anxiolytic agents induce the exploratory activities of the rodents in the open arm[41,20]. Our results demonstrated that EEFR caused a marked increase in the number of entries as well as the time the animals spent in the open arms of EPM (Figures No.5(a) and 5(b)). However, the significant ($p < 0.01$) effect was observed with 100 and 200 mg/kg doses of LEGO and the ED50 values were calculated as 88.65 and 92.87 mg/kg for the number of entries and time spent, respectively. In addition, the effect of EEFR was also evaluated using LDB, a popular screening tool in the research of anxiolytic or anxiogenic agents[36]. The present study demonstrated that the oral administration of EEFR at 100 and 200 mg/kg doses could significantly ($p < 0.01$) rise the time the animals spent in the lighted area (ED50: 82.46mg/kg) without altering the total number of transitions in between the compartments. As expected, diazepam also exhibited similar patterns of effects of EEFR in these models (Figures No.6(a) and 6(b)). Considerable scientific reports demonstrated that, with anxiolytic drug treatment, animals enhanced their transitions in between the compartments of LDB. In contrast, there were no changes observed by other researchers when they administered standard anxiolytics to their animals. These discrepancies might be due to the genetic or strain variation of the animals used in their studies. Therefore, it has been concluded that simply the time spent in the lighted area, but not the total number of transitions, is the most useful and consistent parameter to evaluate an anxiolytic action. Therefore, our results and previously published reports suggest that EEFR may possess anxiolytic potentials along with its sedative properties (Figure No.7). Although this research has reached its goals, the audience of this paper might have the query regarding the effects of higher doses of LEGO on the above models. Therefore, it is

worthy to include a couple of more doses (higher than 200mg/kg) in future studies.

S.No	Phytochemical constituents	Tests	Inferences
1	Alkaloids	Mayer's test	+
		Dragendorff'stest	
2	Flavonoids	Lead acetate test	+
3	Carbohydrates	Molisch's test	+
		Fehling's test	
4	Steroids	Libermannburchard's test	+
5	Saponins	Frothing test	+
		Foam test	
6	Glycosides	Modified borntrager's test	+
7	Tannins	Gelatin test	+
8	Terpenoids	Chloroform test	+

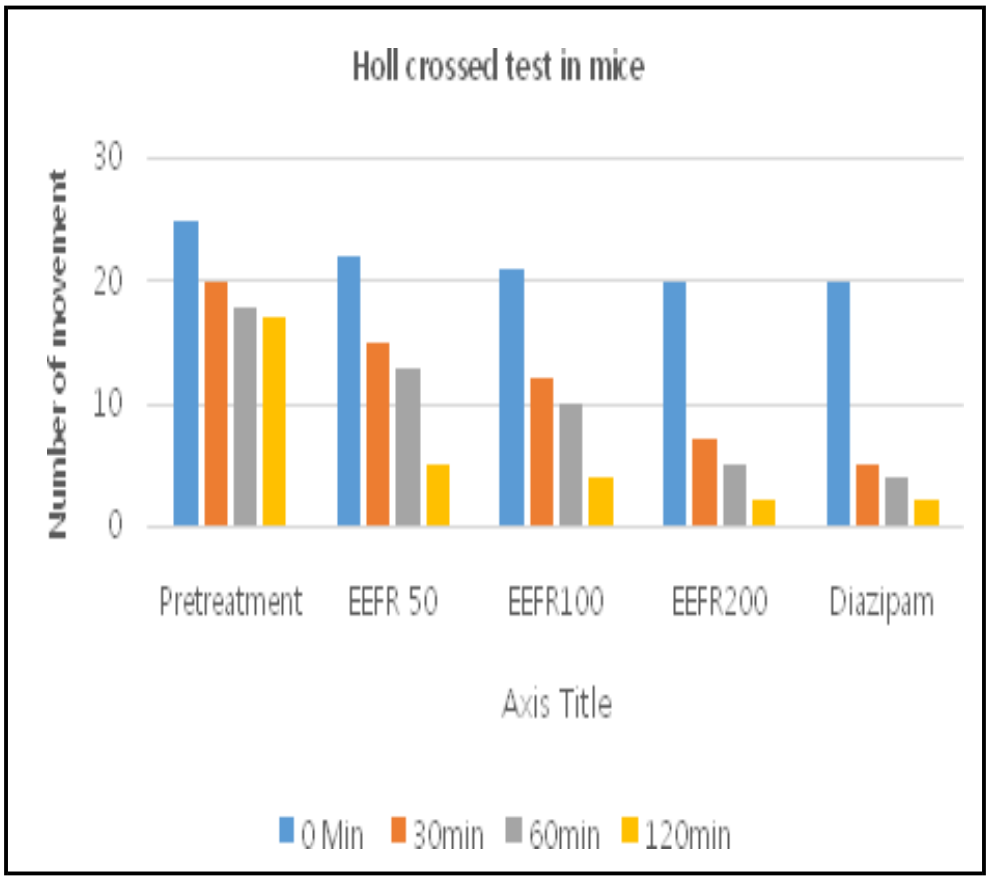


Figure No.1: Effect of EEFR on hole cross test in mice. Before and after treatments with diazepam, vehicle, or EEFR the number of holes crossed in the hole cross box was recorded at various time points, Data were presented as Mean ± SEM (n = 5-7). ¤p<0.01 compared to control

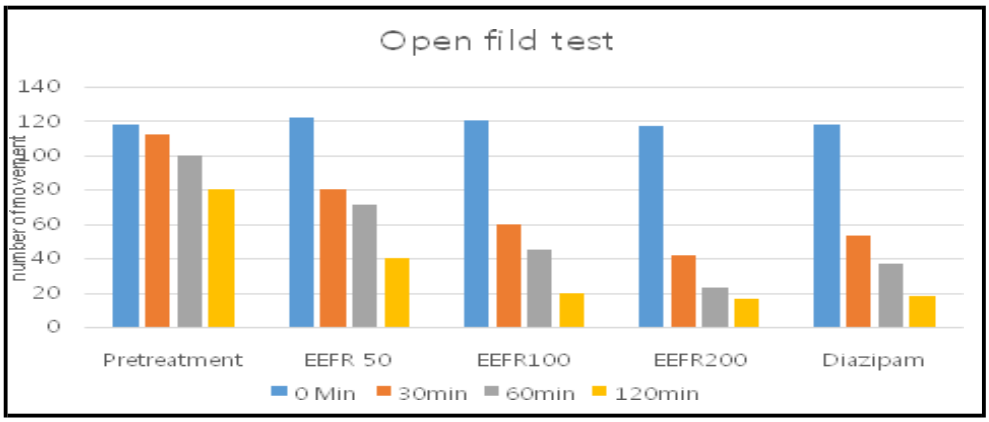


Figure No.2: Effect of EEFR on open field test in mice. Before and after treatments with diazepam, vehicle, or EEFR the number of squares crossed in the open field box was recorded at different time points. Data were presented as Mean ± SEM (n = 5-7), ▫p<0.01 compared to control

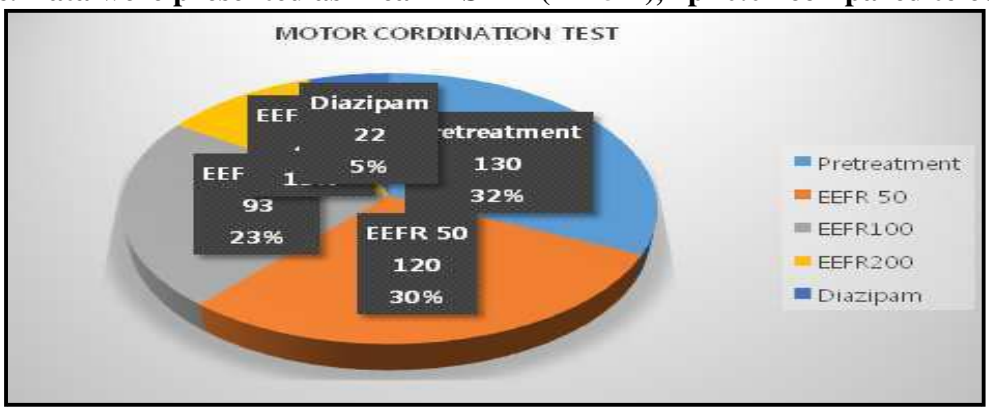
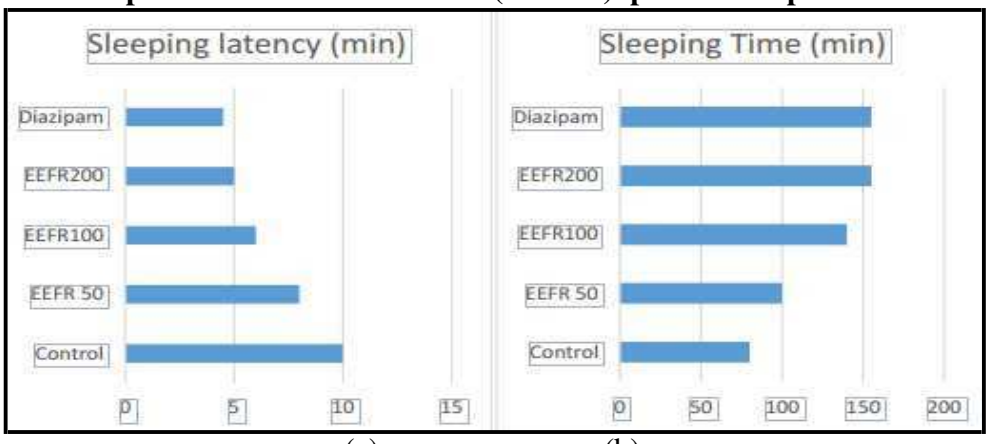
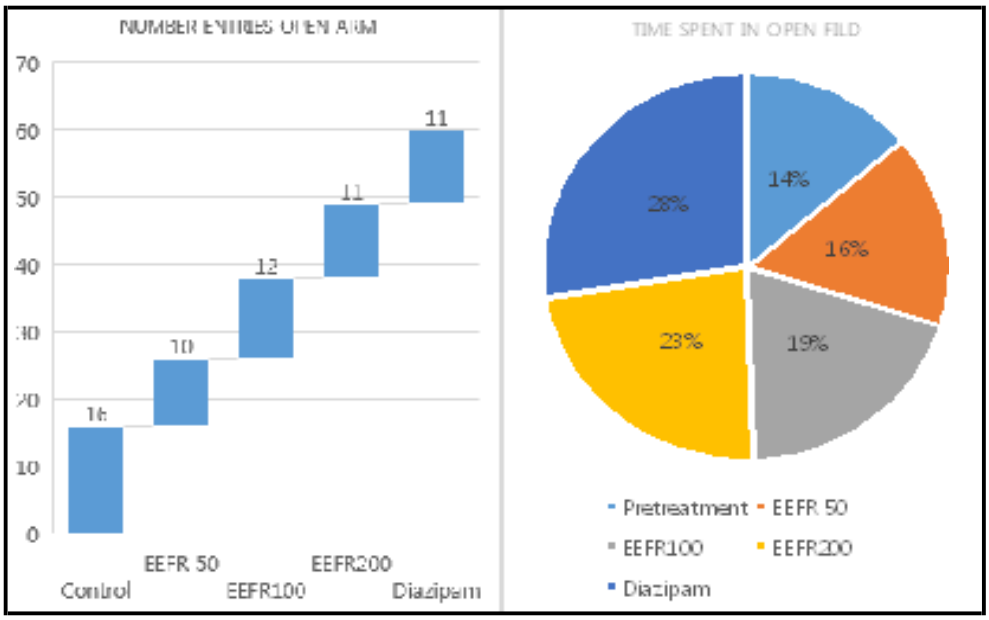


Figure No.3: Effect of EEFR on motor coordination of mice. Thirty min after the treatment with EEFR or vehicle and 15min after diazepam, rotarod performances by the animals were observed for 180sec. Data were presented as Mean ± SEM (n = 5–7). p<0.01 compared to control



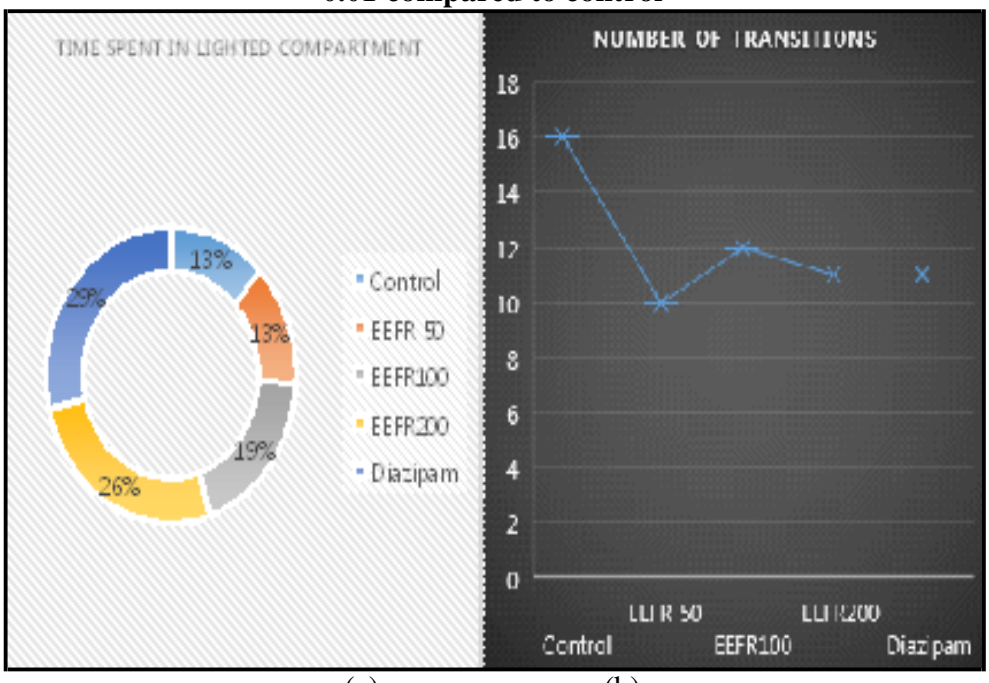
(a) (b)

Figure No.4: Effect of EEFR on TS-induced mental state in mice. Thirty min after the treatment with EEFR or vehicle and 15min after diazepam, TS was administered intraperitoneally, Then the latency to sleep (a) and total sleeping duration (b) induced by TS were observed, Data were given as Mean ± SEM (n = 5-7). ▫p< 0.01 compared to control



(a) (b)

Figure No.5: Effect of EEFR on elevated plus maze (EPM) test in mice. Thirty min after the treatment with EEGO or vehicle and 15min after diazepam, animals were observed for their number of entries (a) and total time spent (b) in the open arms of EPM. Data were presented as Mean ± SEM (n = 5-7), ▫p< 0.01 compared to control



(a) (b)

Figure No.6: Effect of EEFR on light-dark box (LDB) exploration test in mice. Thirty min after the treatment with EEFR or vehicle and 15min after diazepam, animals were observed in LDB and the time spent in the lighted part (a) and number of transitions between compartments (b) were recorded, Data were presented as Mean ± SEM (n = 5-7). ▫p< 0.01 compared to control

ABBREVIATIONS

BZN: Benzodiazepine
CNS: Central Nervous System
GABA: Gamma Amino Butyric Acid
ICDDR, B: International Center for Diarrheal Disease and Research, Bangladesh.
EEFR: Ethyl alcohol Extract of Leaves of *Ficus religiosa*
TS: Thiopental Sodium

CONCLUSION

Our preliminary pharmacological studies suggest that the ethanol extract of *Ficus religiosa* leaves possesses sedative properties, decrease locomotor action, and reason muscle relaxation in experimental animals, which permit the possible anxiolytic-like activity of the extract. Consequently, these outcomes provide the scientific establishment for the use of this plant in traditional medicine in the treatment of different ailments connected to CNS disorders. Nevertheless, advance pharmacological studies are necessary to clearly understand the sedative and anxiolytic actions of EEFR, where our finding could stand as a basis for further progress. In addition, whether these activities were generated by the actions of agonists or partial agonists present in EEFR, which could directly act on the receptor(s) responsible and/or interact with another molecular mechanism (s) active in the determined sedative-anxiolytic effects also needs to study. These bioactivity-guided phytopharmacological research will provide us the opportunity to find out pharmaceutical lead(s) with the best tolerability and low side effects in the new drug development.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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