Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 2694-2699
Received: 23-03-2018
Accepted: 28-04-2018

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Acute toxicity and neuropharmacological activity of *Ficus hispida* leaves extract in mice

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Abstract
Generally *Ficus hispida* is used for treatment of neurological disorders, pain, inflammation, diabetes and fever in folk medicine. The present study was designed to evaluate the safety along with the neuropharmacological activity of the methanolic extract of *Ficus hispida* leaves by using OECD guidelines, pentobarbital induced sleeping test, elevated plus-maze test, open field test and hole cross test respectively. Mortality, behavioral changes or sign of any toxicity were not observed up to the dose as high as 4000mg/kg. Moreover, the extract of *Ficus hispida* leaves potentiated the pentobarbital induced sleeping time in mice at dose 200mg/kg and 400mg/kg. In elevated plus maze test, the extract at dose 200mg/kg and 400mg/kg showed a significant ($p<0.05$, vs. control) depressant and slight anti-depressant activity. Again, both lower and higher doses of extract (200mg/kg and 400mg/kg) of *Ficus hispida* leaves were decreased the open field score in open field test and also decreased the number of passage through the hole from one chamber to other in hole cross test at both doses. Therefore, results of above study indicate that *Ficus hispida* leaves can be a potential source of depressant as well as anti-depressant agent. For the conformation of their activities further investigation is required.

Keywords: *Ficus hispida*, acute toxicity, neuropharmacological study, elevated plus-maze test

1. Introduction
Ficus hispida is a medium but well distributed species of tropical fig tree or shrub that is coarsely hairy and dioeciously. *Ficus hispida* is a member of the Moraceae family. It is generally known as Dumoor in Bangladesh. Ficus hispida [Family: Moraceae; English Name: Hairy fig; Botanical name: *Ficus hispida*; Local name: Dumor, kack dumur] is a medicinal tree, which can attain a height up to 10 meters. It is commonly a popular plant which is widely distributed throughout subcontinent from Bangladesh to India and Malaysia and is also found in Australia [1]. The leaves are opposite, leaf blade ovate, oblong or obovatye-oblong. They measure 10-25cm x 5-10cm, thickly papery. Secondary veins are 6-9 on each side of the midvein. The petiole measure 1-4cm long with short thick hairs. The fig appears axillary on normal leafy shoots, measuring 1.2-3cm diameter with short scattered hairs. The male flowers are numerous near the apical pore; calyx lobes 3, thinly membranous; stamen single. The gall flowers are without calyx, style subapical, short and thick. The female flowers are also without calyx, the style is lateral and with hairs [2]. The plant generally contains ficushispimines A and B, ficushispidine, hispiloscine, β-amyrin acetate, N-triacontanyl acetate, Ficusin A, lupeol acetate, and 10-keto- tetracosyl arachidate [3, 4, 5] which are revealed from recent publication. Astringent, antidysenteric, antipsoriasis, antianemic, and antihemorrhagic properties of whole plant (bark, fruit, root, and leaves) has already been demonstrated and reported elaborately [6, 7]. The roots and leaves are comprehended for their antidiarrhoeal [8], antidiabetic [9], antibacterial [10], hepatoprotective [11], antioxidant [12], and cardioprotective [13] properties. The fruit is edible and acts as a coolant and tonic. A mixture of honey and its juice is a good antihemorrhagic [14].
Traditionally, *Ficus hispida* leaves is a regular folklore medicine in some regions of Bangladesh and used against neurological disorders such as epilepsy and depression, pain, diarrhea, diabetes fever, and inflammation. Therefore, the present study was designed to justify the neuropharmacological activity of *Ficus hispida* leaves, and evaluate the traditional usage scientifically.

2. Materials and Methods
2.1 Collection and identification of the plant
For conducted this study, the freshness leaves of *Ficus hispida* plant was collected from Jessore University of Science & Technology Campus, Jessore, Bangladesh, in September,

2017. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

2.2 Extraction

For methanol extraction, 250 gm of powdered leaves were taken. First, the leaves of *Ficus hispida* were thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature (25±2°C) for two weeks. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The ground leaves (250 gm) were soaked in sufficient amount of methanol for 14 days at room temperature with periodical shaking and stirring. The whole mixture was primarily filtered through cotton and then through Whatman No.1 filters. The solvent was evaporated with a rotary evaporator under reduced pressure at 40°C temperature to yield semisolid crude extract. The percentage yield of the extract was 3.73 % (w/w). The extract was then preserved in a refrigerator till further use.

2.3 Experimental animals

One hundred and fifteen Swiss albino mice of either sex, aged 4-5 weeks, weighing about 25-30 gm were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh to run the experiment of neuropharmacological activity. Before initiating the experiment, the animals were exposed to alternative 12:12 hours light and dark cycle at an ambient temperature of 26±2ºC. Proper supplies of foods and water ad *libitum* were ensured. All protocols for animal experiment were approved by the Institutional Animal Ethical Committee of Jessore University of Science & Technology, Jessore, Bangladesh. Mice were acclimatized for 7 days in the laboratory environment prior to the study, and maintained the constant environmental and adequate nutritional conditions throughout the period of the experiment.

2.4 Acute oral toxicity study

Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. According to Organization of Economic Cooperation and Development (OECD) guidelines, the acute toxicity study of *Ficus hispida* leaves was designed to estimate the half lethal dose (LD50) of the experimental samples [15]. Fifteen mice were divided into two groups: control group and test group (MFHL), with five animals per group. The experimental sample (MFHL) was administered orally at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After that the animals were observed every 1 h for next 5–6 h for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any sign of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [16].

2.5 Neuropharmacological Study

2.5.1 Pentobarbital-induced Hypnosis

The method of Williamson *et al*. [17] with slight modification was used for studying the pentobarbital-induced hypnosis test. The experimental animals were randomly divided into four groups consisting of five mice in each group. The groups were denoted from group-I to group- IV. Group I and II used as control and standard and group III and IV used as treatment groups. The experimental groups were administered with the methanolic extract of *Ficus hispida* leaves at dose of 200mg/kg and 400mg/kg body weight orally. Here, diazepam (1 mg/kg p.o.) was administered as positive control and negative control was treated with distilled water (10mL/kg, p.o.). Each mouse was placed in an observation box (a rectangular open box composed of hardboard floor (36 x 36 cm^2) with a surrounding wall 30 cm height. After 30 min from administration, pentobarbital (40 mg/kg, i.p.) was administered to each mouse to induce sleep. The total sleeping time were recorded for both controls as well as for treated groups. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

2.5.2 Elevated plus-maze test (EPM)

The elevated plus maze test was performed according to the method of Lister Elevated plus-maze test [18]. Twenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (MFHL at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. The apparatus was made of two opposing closed arms with a dimension of 50 cm x 10 cm x 30 cm (length x width x height) along with two opposing open arms 50 cm (length) x 10 cm (width) and it was placed at 70 cm high from the floor level. Each mouse was placed in the centre of elevated plus-maze apparatus. After respective treatment, the number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min and the every counting was continued for 3 min. Arm entry refers to the entry of all four paws into one arm.

2.5.3 Open field test

The method of Hawiset *et al*, [19] was applied for the open field test. Grouping of the mice and sample (MFHL at 200 and 400 mg/kg body weight, p.o.) administration were carried out as like as Elevated plus maze test. The evaluation of the CNS depression activity can be completed by this test. An apparatus that consists of a series of alternating white and black squares floor with a height of 40 cm was made for the open field test. The number of movement of the test animals i.e., total number of squares that every group of animals visited was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min.

2.5.4 Hole cross test

The hole cross test was conducted by slight modification of Takagi *et al* [20]. Twenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (MFHL at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. Here, 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 centre of the cage. After oral administration of the test drugs and the standard, the number of passages of each mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 120 and 180 min respectively. The apparatus was thoroughly cleaned after each trial.

2.6 Statistical analysis

The results of statistical analysis for animal experiment were

expressed as mean ± SEM (Standard Error of mean). Statistical analyses for neuropharmacological studies were performed by one-way ANOVA following Dunnet's test through the SPSS software (version 16; IBM Corporation, New York, USA). The obtained results were compared with the vehicle control group. The $p<0.05$ was considered to be statistically significant.

3. Results

3.1 Acute oral toxicity study

In acute oral toxicity study, no mortality was viewed up to the dose as high as 4000 mg/kg for MFHL or control group. Behavioral changes or sign of any toxicity were not observed up to the dose as high as 4000 mg/kg for MFHL (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently indicated that the test group does not show acute oral toxicity.

3.2 Neuropharmacological study

3.2.1 Pentobarbital-induced hypnosis

Statistical analysis of the data obtained in this test show that (table-1) both 200mg/kg and 400mg/kg dose of methanolic extract of leaves of *Ficus hispida* prolong the duration of the pentobarbitone-induced sleeping time. After completed this experiment, it was noted that the total sleeping time was about 59.56±1.84 and 73.51±2.07 min at dose of 200 and 400 mg/kg of body weight, respectively of the methanolic leaves extract of *Ficus hispida* whereas, in positive control group sleeping time was about 95.77±2.54 min.

3.2.2 Elevated Plus-maze Test (EPM)

The present result (showed in table-2 & 3) was noticeable that standard drug diazepam revealed depressive activity with time. In addition to, MFHL 200 mg/kg and 400 mg/kg exhibit both depressive and slight anti-depressive activities at 30 min and 60 min, 120 min and 180 min respectively. Besides, MFHL 400 mg/kg showed anxiolytic activity during all observations except fourth observation. There was a significant change both in number of opened arm entries and time spent in opened arm were observed after the single and repetitive administration of MFHL extract at dose 200mg/kg and 400mg/kg used in this study. Moreover, MFHL 200 mg/kg and MFHL 400 mg/kg both elicited depressive and/or anxiolytic activities and spent much more time in open arm at 30 min, 60 min and 120 min respectively but during fourth observation depressive activity was noticed.

3.2.3 Open Field Test

It was observed that the extract (200mg/kg and 400mg/kg) of *Ficus hispida* leaves was elicited anti-depressive activity in first observation period in the test animals and then till last period (180min) it elicited depressive activity. In this case, significant activities were noticed during all of the observations at MFHL 400 mg/kg ($p<0.05$, vs. control). Furthermore, slightly less result was obtained by MFHL 200 mg/kg than MFHL 400 mg/kg and extract shows both depressive and anti-depressive activities and all activities are dose dependent in manner. Results of open field test are showed in able-4.

3.2.4 Hole Cross Test

Results of hole cross test are showed in table-5. The observation was similar as like as the open field test. Both doses of extract were exhibited anti-depressive activity from first observation period in the test animals and then till last period (180min) it elicited depressive activity. Also here, significant activities were noticed during all of the observations at MFHL 400 mg/kg ($p<0.05$, vs. control). Furthermore, slightly less result was obtained by MFHL 200 mg/kg than MFHL 400 mg/kg and extract shows both depressive and anti-depressive activities and all activities are dose dependent in manner.

Table 1: Effects of methanolic extract of *Ficus hispida* leaves on Pentobarbital-induced hypnosis in mice.

Group	Dose	Time of onset of sleep (min)	Total sleeping time (min)
Control	10 mL/kg	15.36±1.13	35.60±0.93
Standard	1 mg/kg	4.73±0.54	95.77±2.54
MFHL	200 mg/kg	7.82±0.63	59.56±1.84
MFHL	400 mg/kg	5.73±0.49	73.51±2.07

Sleeping time and duration values are presented as mean ± SEM (standard error of mean). P<0.05, vs. control (Dunnett's t test).

Table 2: Effects of methanolic extract of *Ficus hispida* leaves on Elevated plus-maze apparatus after entrance into close arms.

No. of movement in closed arm						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	2.60±0.25	2.80±0.37	3.00±0.32	2.60±0.25	2.80±0.37
Standard	1 mg/kg	1.40±0.25	2.40±0.25	2.80±0.20	3.40±0.25	4.40±0.25
MFHL	200 mg/kg	1.20±0.37	1.80±0.20	2.00±0.32	2.40±0.25	1.60±0.25
MFHL	400 mg/kg	1.00±0.45	1.80±0.37	1.60±0.25	2.00±0.32	2.40±0.25

Numbers of movement in close arm are present as mean ± SEM (standard error of mean). P<0.05, vs control (Dennett's t test)

Table 3: Effects of methanolic extract of *Ficus hispida* leaves on Elevated plus-maze apparatus after entrance into open arms.

No. of movement in opened arm						
Group	Dose	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	3.00±0.32	3.40±0.25	3.20±0.37	2.80±0.37	2.40±0.25
Standard	1 mg/kg	4.80±0.37	4.20±0.37	3.80±0.37	2.80±0.37	2.40±0.25
MFHL	200 mg/kg	4.00±0.32	3.60±0.25	3.00±0.45	2.60±0.40	2.00±0.32
MFHL	400 mg/kg	5.20±0.37	4.40±0.25	3.60±0.25	3.00±0.32	3.20±0.37

Numbers of movement in open arm are present as mean ± SEM (standard error of mean). P<0.05, vs control (Dennett's t test)

Table 4: Effects of methanolic extract of *Ficus hispida* leaves on open field test in mice.

No. of movement in opened field						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	117.30±5.98	114.47±8.26	111.91±2.91	108.58±5.50	111.87±4.50
Standard	1 mg/kg	102.22±2.48	55.07±2.64	39.25±2.24	25.01±2.18	17.72±2.33
MFHL	200 mg/kg	110.01±4.71	77.91±2.83	64.59±3.11	45.70±3.24	40.99±3.12
MFHL	400 mg/kg	105.64±3.85	72.75±3.38	55.34±3.28	39.44±2.99	33.64±3.04

Numbers of movement in open field are present as mean ± SEM (standard error of mean). P<0.05, vs control (Dennett's t test)

Table 5: Effects of methanolic extract of *Ficus hispida* leaves on hole cross test in mice.

No. of movements						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	17.80±1.71	17.00±1.14	18.40±2.20	16.60±1.60	16.40±1.81
Standard	1 mg/kg	11.80±1.36	6.20±1.28	2.60±0.40	1.60±0.51	1.60±0.51
MFHL	200 mg/kg	14.60±1.33	10.00±1.41	9.20±1.46	8.60±1.21	6.60±1.08
MFHL	400 mg/kg	12.40±1.29	9.80±0.97	8.60±1.44	5.80±0.86	4.20±0.86

Numbers of movement are present as mean ± SEM (standard error of mean). P<0.05, vs control (Dennett's t test)

4. Discussion

For the investigation of therapeutic index of drugs and xenobiotics, the acute oral toxicity study is a vital factor [21]. As no mortality was observed up to the dose as high as 4000 mg/kg, LD50 of *Ficus hispida* leaves extract could not be obtained. For this, the extract was found to be safe with a broad therapeutic range and two comparatively high doses (200mg/kg and 400 mg/kg) of MFHL were used for in-vivo doses.

Neuropharmacological disorder such as- depression and anxiety both are important psychiatric imbalance that badly affects person's quality of life and social relations directly. The results of depression and anxiety in the community are very high and are associated with lot of morbidity. These are characterized by emotional symptoms such as loss of self-confidence, hopelessness, apathy, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like, sleep disturbances, psychomotor retardation, loss of libido, and loss of appetite. The major depression is considered when symptoms are very severe. In spite of, availability of several drugs in market, it is very important to address these problems and find effective remedies. Cause, all chemically synthetic drugs are associated with some limitations and there is an urgent need for alternative medications for these disorders. Medical therapies with medicinal herbs may be more effective alternatives in the treatment of depression and anxiety. And the research of their effects has progressed significantly since the past decade [22, 23].

This study examined some neuropharmacological effects of *Ficus hispida* leaves and established that it has antidepressant and anxiolytic activities. MFHL increased the pentobarbitone-induced sedative effect in mice and it is a dose dependent manner. Between two doses MFHL 400mg show more significant depressant activity than MFHL 200mg/kg. To generate their action, barbiturates naturally work on the cerebral cortex [24]. MFHL improved sleeping time that can be attributed to its action on the central sleeping mechanism. Moreover, MFHL decreased the locomotor activity which is a parameter of the level of excitability of the central nervous system. Decrease of locomotor activity is closely related to the depression of the central nervous system [25].

The elevated plus maze test is one of the most widely validated tests and is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma aminobutyric acid type A (GABAA)- benzodiazepine complex [26]. In case, normal mice will normally prefer to spend much of their allotted time in the closed arms. In this experiment, we observed that the administration of different doses (200 mg/kg and 400 mg/ kg body weight) of methanolic extract of *Ficus hispida* leaves induced a depressant -like effect in mice, as it increased open arm entries much more and the time spent in the open arms when compared to the control animals. Locomotor activity is considered as an index of alertness and a decrease in that indicates a sedative effect [27]. Both the doses (200 mg/kg and 400 mg/kg body weight) of the *Ficus hispida* leaves extract showed a gradually decrease in the locomotor score with a dose dependent manner, thus suggesting a profound sedative activity. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both [28].

In addition, the study on locomotor activity, as measured by hole cross and open field tests, showed that both doses (200mg/kg and 400mg/kg) of methanol extract from the leaves of *Ficus hispida* gradually decreased the frequency and the number of movements with time. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Many flavonoids and neuroactive steroids, and specially the β-amyrin acetate were found to be ligands for the GABAA receptors in the CNS; which led to the hypothesis that they act as benzodiazepine-like molecules [29]. This is supported by the present study on the behavioral effects in animal models of anxiety and sedation.

Probably, the mechanism of anxiolytic action of the methanol extract of *Ficus hispida* leaves could be due to the binding of any of the phytoconstituents to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor [30]. By the locomotor activity it is possible to measure the level of excitability of the CNS and sedation resulting from depression of the central nervous system [31].

5. Conclusion

From the results of existing study, it can be concluded that Methanolic extract of *Ficus hispida* leaves might possess remarkable neuropharmacological properties such as- depressive and/or anxiolytic and anti-depressive properties. Data obtained in this study showed that all activities were dose dependent and statistically significant. The presence of flavonoides, β-amyrin acetate, lupeol acetate, and phenolic compounds might be responsible for these activities. We hope

that, further detailed investigation is needed to confirm which neuropharmacological property become prominent and to find out the active components of the extracts for discovering the mechanism of actions in the improvement of neuropharmacological agents. Moreover, genotoxicity study of the extract may be a promising area for the researchers.

6. Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Acknowledgements

The authors would like to thank Department of Pharmacy, Jessore University of Science & Technology, Jessore, Bangladesh for providing facilities throughout the work.

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