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In vivo CNS Activity of Methanolic Extract of *Ficus racemosa* Fruits in Experimental Animals

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Abstract

The purpose of the study was to evaluate the CNS activity of the methanolic extract of *Ficus racemosa* fruits. The powdered fruits of *F. racemosa* were extracted with methanol to investigate the effect on central nervous system in Swiss albino mice. The CNS depressant activity was evaluated by observing the reduction of locomotor activity by open field and hole cross tests. The anti-depressant activity was tested by forced swimming and tail suspension methods. For CNS activity, two test doses of the extracts such as 200 and 400 mg/kg body weight were used. However, the extracts showed significant dose dependent CNS depressant activity when compared to the control in animal models. From our research, it can be concluded that the methanolic extract of *F. racemosa* fruits possesses significant CNS depressant activity. So, further studies are recommended for the isolation of compounds responsible for this activity.

Kew words: CNS depressant activity, *Ficus racemosa*, methanolic extract, locomotor activity.

Introduction

Ficus racemosa Linn (Family: Moraceae) has been widely used in traditional medicine for a widespread range of diseases. Its fruits, bark, leaves, roots, latex and seeds are medicinally used in different forms (Aiyegoro *et al*., 2009). The fruits arise from the main trunk, in large clusters, 2-5 cm in diameter, pyriform and the fruits become figs-like and are green when raw, turning orange and dull reddish. The fruits contain lupeol acetate, β-sitosterol, hentriacontane, gluanol acetate and tiglic acid ester of taraxasterol and glucose (Deep *et al*., 2013). The fruits are used as a remedy for the disease of kidney, spleen and dry cough. It is also used as a styptic, astringent and carminative as well as in the treatment of blood disorder, burning sensation, fatigue, urinary discharges, intestinal worms, leucorrhoea, miscarriage, menorrhagia, spermatorrhoea, cancer, scabies, haemoptysis and visceral obstructions (Vedavathy *et al*., 1995; Nadkarni *et al*., 1954; Sharma *et al*., 2008). The bark 0.5-1.8 cm thick, greyish green, inner surface light brown, fracture fibrous, taste mucilaginous without any characteristic odour, it has no aerial roots (Paarakh *et al*., 2009; Kirtikar *et al*.,1975). The bark contains tannin, wax, saponin, gluanol acetate, cerylbehanate, lupeol, lupeol acetate, α & β-amyrin, gluanol acetate, β-sitosterol, stigmasterol and a ketone. Gluanol acetate and β-sitosterol have also been isolatated from the heartwood (Husain *et al*., 1992). Bark is efficient in threatened abortion, leprosy, urological disorders, dysentery, piles, diabetes, hiccough (Paarakh *et al*., 2009; Chopra *et al*. 1986; Vedavathy *et al*., 1995). The leaves are dark green, 7.5-10 cm long, ovate, in large clusters from old nodes of main trunk. Leaves contain glycoside, gluanol acetate, β-amyrin and β-sitosterol (Deep *et al*., 2013). The leaves are used in the treatment of ulcer, diarrhoea and dysentery (Deep

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et al. 2013). As far our knowledge, no work has been found regarding the CNS activities of this plant. Present study has been undertaken to investigate the CNS activities of the crude extracts of *F. racemosa* in Swiss albino mice.

Materials and Methods

Plant material: The fresh fruits of *F. racemosa* Linn. were collected from Comilla, Bangladesh, 2017 and identified by an expert taxonomist. A voucher specimen No. DACB 45881 was given by the national herbarium, Mirpur, Dhaka, Bangladesh.

Preparation of the extract: About 650 gm of dried and powdered plant materials obtained from the plants were soaked in 3.5 liter of methanol in an amber glass container for about 14 days at room temperature with occasional shaking. After 14 days, the solution was filtered using cotton filter and Whitman's filter paper number 1. The filtrates were then concentrated to afford solid masses by using a rotary evaporator (Jeff *et al.*,1989; Haque *et al.*, 2014), which was used for the experimental purpose.

Animals: Swiss albino mice (20-30 g) of either sex were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions. The animals were fed with laboratory food and water *ad libitum*. The experiments were done in an isolated and noiseless room.

Drugs and chemicals: Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diazepam and nortriptyline were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Experimental design: The animals were randomly divided into four groups with each group consisting of four mice. The test groups received MFEFR (methanolic fruit extract of *F. racemosa* Linn) at the doses of 200 and 400 mg/kg body weight while positive control was treated with diazepam .

Open field test: The method described by Gupta *et al.* (Gupta *et al.*, 1971) was slightly modified and used for screening depressive action of the test drugs on CNS in mice. The animals were divided into control and test groups. The test groups received methanolic fruit-extracts at the doses of 200 and 400 mg/kg body weight orally, whereas the control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares where each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares visited by the animals was calculated for 3 min on 0, 30, 60, 90 and 120 min subsequent to oral administration of the normal saline (10 ml/kg), experimental crude extracts (200 mg/kg and 400 mg/kg b.w.) and diazepam as standard drug (1 mg/kg.).

Hole cross test: The method used was described by Takagi *et al*. (Takagi *et al*., 1971). The animals were divided into control, standard and test groups (n = 4 per group). The control group received vehicle (0.9% saline in water at the dose of 10 ml/ kg) whereas the test group received extract (at the doses of 200 and 400 mg/kg b.w.) and standard group received diazepam at the dose of 1mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90 and 120 min during the study period.

Forced swimming test (FST): The FST is the most widely used pharmacological model for assessing antidepressant activity. This method is based on the observation of animals exposed to a situation of forced swimming, in which they become passive and immobile after a period of vigorous activity (struggling), producing only the movements required to keep their heads above the water. The FST was carried out on mice according to the method of Porsolt *et al*. (Porsolt *et al*., 1977). Swimming sessions were conducted by placing the animals in individual Plexiglas's cylinders (40 cm height, 24 cm diameter) containing 20 cm of water. The animals were treated with the extracts (200 and 400 mg/kg/b.w. orally), nortriptyline (15 ml) or vehicle, 45 min before the test. All animals were forced to

swim for 6 min and the time spent in immobility during the last 5 min of a 6 min observation period was recorded as immobile when floating motionless or making only those movements necessary to keep the head above water. A decrease in the duration of immobility during the forced swimming test is taken as a measure of antidepressant activity. This is recorded manually by the competent observer.

Tail Suspension test: The total duration of immobility induced by tail suspension was measured according to the method described by Sterut *et al*. (Steru *et al*., 1985). Briefly, mice both acoustically and visually isolated were suspended 30 cm above the floor by adhesive tape and placed approximately 1-2 cm from the tip of the tail. Immobility time was manually recorded during a 5 min period (Machado *et al.*, 2017). Mice were considered immobile only when they hung passively or stayed completely motionless. Conventional antidepressants decrease the immobility time in this test. The animals were treated with the extracts (200 and 400 mg/kg b. w.), nortriptyline (15 ml) or vehicle, 45 min before the test.

Statistical analysis: The results were expressed as the mean ± SEM (standard error mean). ANOVA (analysis of variance) followed by Dunnett's 't' test was performed as a post hoc test to evaluate the statistical significance while taking vehicle treated animals as control, p value of < 0.05 was considered as statistically significant.

Results and Discussion

Open field test: In the open field test, it was found that, methanolic fruit extract of *F. racemosa* at the doses of 200 mg/kg b. w. and 400 mg/kg b. w. decreased number of hole crossed compared to the control group. Methanolic fruit extract exhibited a decrease in the movements of the test animals at all dose levels tested. The effect of the extract at the doses of 400 mg/kg b. w. significantly reduced the number to 76.25 ± 6.60, 68 ± 3.25, 53 ± 3.83 and 27 ± 2.16 after 30 min, 60 min, 90 min and 120 min respectively. The depressing effect was most intense during the 3rd (90 min) and 4th (120 min) observation periods. The results are shown in table 1.

Hole cross test: In the hole cross test, methanolic fruit extract of *F. racemosa* dose significantly decreased the number of hole crossed compared to the control group. Methanolic fruit extract of *F. racemosa* exhibited a decrease in the movements of the test animals at all dose levels tested. The depressing effect was moderately intense during the 3rd (90 min) and 4th (120 min) observation periods. The results are shown in figure 1.

Forced swimming test: In the forced swimming test, it has been found that, immobility time of extract at dose 200 mg/kg b.w. was similar to control. But at dose 400 mg/kg b. w. it was higher than control. After treatment with nortriptyline, immobility time was decreased. The results are shown in figure 2.

Table 1. Effect of methanolic fruit-extract of *F. racemosa* on open field test in mice.

Treatment	Dose (mg/kg)	Number of square crossed (minute)			
		After 30 min	60 min	90 min	120 min
Control	0.1ml/mice	136.6 ± 3.91	115.25 ± 5.52	86.2 ± 6.92	90.25 ± 7.43
Diazepam	1	88.75 ± 4.5*	54.25 ± 6.53*	19.5 ± 4.5*	6.5 ± 1.10*
MEFR 200	200	93 ± 5.25*	75.25 ± 6.13*	47.25 ± 8.81*	33.25 ± 3.99*
MEFR 400	400	76.25 ± 6.60*	68 ± 3.25*	53 ± 3.83*	27 ± 2.16*

Values are presented as the mean ± SD [SD=Standard Deviation]. N = 4, *p<0.05 compared with control (One way ANOVA followed by Dunnet's test). MEFR = Methanolic extract of *F. racemosa.*

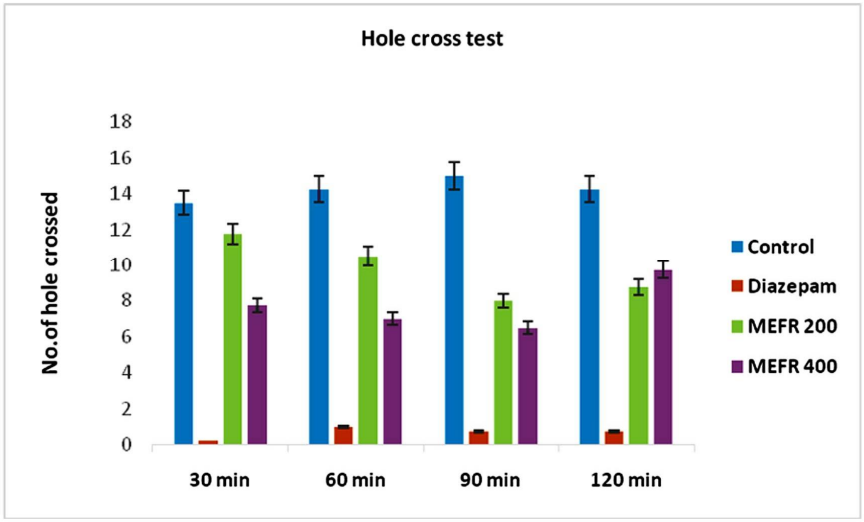

Figure 1. Number of hole crossed on hole cross test.

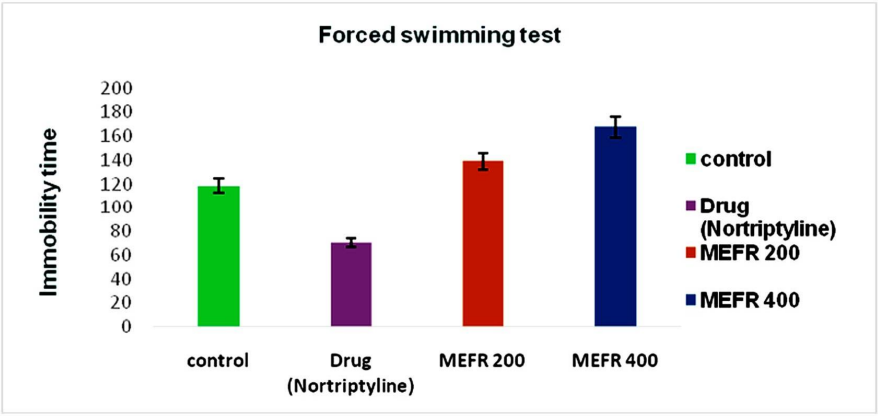

Figure 2. Immobility time on forced swimming test

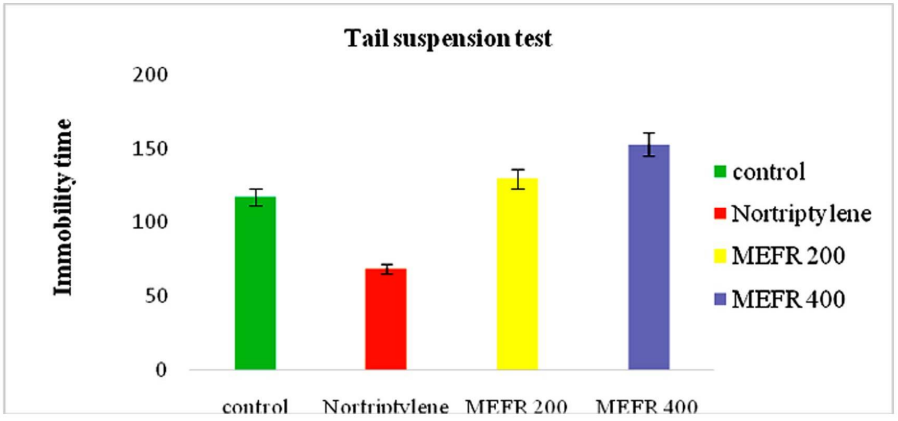

Figure 3. Immobility time on tail suspension test

Tail suspension test: In tail suspension test, it has been found that, immobility time of extract at dose 200 mg/kg b. w. was comparable to control. But at dose 400 mg/kg it was higher than control. After treatment with nortriptyline, the immobility time was decreased. The results are shown in figure 3.

In the present study, the effect of methanolic fruit extract of *F. racemosa* on CNS has been evaluated. The result indicated that the extract significantly decreased locomotor activity which indicates its CNS depressant activity. Locomotor activity refers to an increase in alertness and decrease in locomotor activity considered as sedative effect. The major inhibitory neurotransmitter in the central nervous system is gamma-amino-butyric acid (GABA). Different types of anxiolytic, muscle relaxant, sedative-hypnotic drugs show their effects through GABA. The extracts of *F. racemosa* may act by membrane hyperpolarization, potentiating GABA-ergic inhibition in the CNS which leads to either decrease in the firing rate of critical neurons in the brain or direct activation of GABA receptor by the extracts (Khatun *et al.,* 2011). Literature review of the plant reveals that *F. racemosa* contains terpenoids (Faiyaz *et al.,* 2010), flavonoids, cyanogenic glycosides and tannin. Different types of flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system, which indicate that they act as benzodiazepine-like molecules (Khatun *et al.,* 2011). The pharmacological investigation of the methanolic fruit extract of *F. racemosa* was similar to diazepam which led to assume that they might interact with benzodiazepine receptor located adjacent to the GABA receptor.

Conclusion

Results of the present study indicate that all tested doses (200 and 400 mg/kg b. w.) of fruit extracts of *F. racemosa* exhibited significant sedative effect. The effect is dose dependent, long lasting and statistically significant. However, further investigation is needed to isolate the pharmacologically active compounds responsible for this activity.

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Conflict of interest: Authors have no conflict of interest.

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