Pharmacologia 3 (9): 467-471, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.467.471

Evaluation of the Anxiolytic Effect of Methanol Leaf Extract of *Ficus hispida* Linn. in Corticosterone Induced Anxiety in Young Adult Mice

D. Sivaraman, P. Muralidharan and Habibur Rahman
Department of Pharmacology and Toxicology, C.L. Baid Metha College of Pharmacy, Jyothi Nagar, Thoraipakkam, Chennai 97, Tamil Nadu, India

Abstract: Objective: The aim of the present study is to evaluate the anxiolytic effect of a methanol extract of *Ficus hispida* Linn. Leaves in young adult mice. **Materials and Methods:** Anxiety in rodents was induced by administration of corticosterone (5 mg/kg/day), treated for 4 weeks developed an anxiety. Actophotometer, elevated plus maze, zero maze, hole board and rotarod paradigm were used to assess the anxiolytic activity of the methanolic leaf extract *Ficus hispida* Linn. (MEFH) at the dose of 200 and 400 mg kg^{-1}. p.o. and diazepam 1 mg kg^{-1}, i.p. were administered 30 min before the tests. **Result:** The results showed that the MEFH significantly increased the number of head poking and line crossing in the hole board test. In the elevated plus maze the MEFH at the dose of 400 mg kg^{-1} significantly increased the duration of exploration in open arm in similar way to that of diazepam. Further, in the zero maze the extract produced significant increase in time spent in open arm as compared to negative control. In the rotarod MEFH at the dose of 200 and 400 mg kg^{-1} significantly decreased the fall off time which shows the muscle relaxing property of the plant. The spontaneous locomotor activity count, measured using actophotometer, was significantly decreased in animal pretreated with MEFH. Indicating the remarkable sedative effect of the plant. **Conclusion:** The result of the present study suggests that leaves of *Ficus hispida* Linn. may possess an anxiolytic effect.

Key words: *Ficus hispida* Linn., anxiolytic effect, actophotometer, elevated plus maze, diazepam

INTRODUCTION

Anxiety is a feeling of apprehension and fear characterized by physical symptoms such as palpitations, sweating and feelings of stress. Anxiety disorders are serious medical illnesses that affect approximately 19 million American adults. These disorders fill people's lives with overwhelming anxiety and fear. Anxiety disorders are chronic, relentless and can grow progressively worse if not treated. With an increase in the number of individuals suffering from anxiety and stress-related disorders, there has been a rise in the use of antidepressant drugs and anxiety medications. Certain antidepressants and anxiety drugs are known to cause hallucinations and nightmares. Sleeping disorders like nightmares etc. Benzodiazepines that are prescribed for anxiety disorders and panic attacks can cause certain personality changes in individuals consuming them (Abid *et al*., 2006). Increased aggressiveness and violent behavior in some and depression accompanied by suicidal thoughts in others. Some anti-anxiety drugs result in weakness, loss of appetite, dry mouth, excessive sweating and nervousness while others may cause excessive increase in weight. Some anxiety medications can also lead to a disturbed sexual life as a result of impotence and sexual dysfunction. Blurred vision and skin rashes are some of the other common side effects of anti-anxiety drugs.

Researches done by FDA and other associations have claimed that herbs contain natural composition of various compounds that affects our internal system (brain, heart and mind). In conventional medicine, those formulations are synthesized by chemical associations and are given in different doses to the person. We should also keep in mind that around 30% of the ingredients that are added in conventional medicine are extracted from natural resources like plants and herbs (Winston, 2005). Healing anxiety with herbs has been a topic of hot debate in recent years as our ideas about wellness and natural living have begun to influence our lives.

Ficus hispida Linn. belongs to the family Moraceae is a widely distributed and most commonly found in the interior and coastal regions distributed throughout India, Srilanka, Myanmar and Southern region of the Republic

Corresponding Author: D. Sivaraman, Department of Pharmacology and Toxicology, C.L. Baid Metha College of Pharmacy, Jyothi Nagar, Thoraipakkam, Chennai 97, Tamil Nadu, India Tel: 04425522874

china (Kunwar and Bussmann, 2006). A mixture of honey and the juice of these fruit is a good antihemorrhagic (Peraza-Sanchez *et al*., 2002) but the barks and leaves are of particular interest from a medicinal point of view of as an antidiarrhoeal (Mandal and Kumar, 2002) Antidiabetic (Ghosh *et al*., 2004) and as cardio protective (Shanmugarajan *et al*., 2008) among others. The current study was undertaken to evaluate the anti-anxiety activity of methanolic leaf extract of FH by, till now no pharmacological evaluation has been done on FH especially in leaf for its anti-anxiety activity. This prompted us to pursue the activity and was examined for their efficacy.

MATERIALS AND METHODS

Plant material: The fresh *Ficus hispida* Linn. (FH) leaves were collected from (Perannakavur, Changlepet, Tamil Nadu, India) western Ghats of South India during June 2008. The plant was identified and authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Southern Circle, Coimbatore (BSI/SC/5/23/08-09/Tech-738). The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India.

Preparation of the methanolic extract of FH: The fresh leaves of FH were collected and washed with running water and shade dried at room temperature. One kilogram of the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. The grounded power was extracted with methanol in water bath at room temperature. The solvent was then removed by filtration and fresh solvent was added to the plant material. The extract process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give a dark green viscous mass. The extract was stored at 0-4°C. The percentage yield was 20.2% w/w.

Phytochemical screening: The freshly prepared methanol leaf extract of FH (MEFH) was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's and Dragendorff's reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's, Fehling's and Benedict's reagent; proteins and amino acids with Millon's, Biuret and xanthoprotein test. Saponins was tested using hemolysis method; Gum was tested using Molish's reagent and Ruthenium red; Coumarin by 10% sodium hydroxide and quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures (Trease and Evans, 1989).

These were identified by characteristic color changes using standard procedures.

The screening results were as follows: Alkaloids+ve; Carbohydrates+ve; Proteins and amino acids+ve; Saponins+ve, Steroids-ve; Sterols+ve; Phenols+ve; Flavonoids+ve; Gums and mucilage+ve; Glycosides+ve; Saponins+ve; Terpenes+ve and Tannins-ve, Where+ve and-ve indicates the presence and absence of compounds.

Animals: Young adult Swiss albino mice of either sex, weighing (18-20 g) were obtained from animal house of C.L. Baid Metha College of pharmacy, Chennai, Tamil Nadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy, The animals were maintained in colony cages at 25±2°C, relative humidity 50-55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd. Bangalore, India) and water *ad libitum*, animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conducted between 10-00 and 17.00 h and were in accordance with the ethical guidelines of the International association for Study of Pain (Zimmerman, 1983). All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by the Institutional Animal Ethical Committee. C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India.

Induction of anxiety: Anxiety in rodents was induced by administration of corticosterone (5 mg/kg/day) available *ad libitum* in the drinking water in opaque bottles to protect it from light, for 4 weeks developed an anxiety.

Acute toxicity studies: Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD) (Ecobichon, 1997).

Treatments: Animals were divided into four (I-V) groups. Group I was a control group and was given with CMC (Carboxy methyl cellulose) (1% w/v, p.o.). Group II was a negative control and was given corticosterone

(5 mg kg^{-1}). Group III was a positive control and was given standard drug, diazepam (1 mg kg^{-1}, i.p.). Group IV and V were treated as test groups and were given MEFH at doses of 200 and 400 mg kg^{-1} orally for 7 days. All the test solutions were administered 30 min prior to experiment.

Open field habituation: In order to control for possible effects on locomotor activity, animals were explored twice with a 24 h interval, to a 40× 40×60 cm open field whose brown linoleum floor was divided into 16 equal squares by white lines. After 1 h of administration of test drug, the animals were placed in the rear left square and left to explore it freely for 5 min during which time the number of lines crossing and head dipping were counted (Saitoh *et al*., 2006).

Test for locomotor activity: The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer. The movement of animal interrupts a beam of light falling on a photo cell, at which a count was recorded and displayed digitally. Each mouse was place individually in actophotometer for 10 min. The animal was divided in groups; each group consists of six animals. MEFH was administered at two doses (200 and 400 mg kg^{-1}, p.o.) and diazepam (1 mg kg^{-1}, i.p.), used as standard was given 30 min before the test. The negative control group was treated with corticosterone (5 mg kg^{-1}); mice were placed in actophotometer for recording the activity score (Yadav *et al*., 2008).

Muscle co-ordination test

Rotarod: The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 20 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller. Swiss albino mice underwent a pretest on the apparatus. Only those animals which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min were used for the test (Rakotonirina *et al*., 2001).

The two doses of MEFH (200 and 400 mg kg^{-1}) were administered orally, the standard group was treated with diazepam (1 mg kg^{-1}, i.p.) and negative control group received corticosterone (5 mg kg^{-1}).

Elevated plus maze: The apparatus consist of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to form a plus-sign figure. The apparatus was situated 40 cm 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. The animal was placed at the center of the maze, facing one of the closed arms. During 5 min test period the following measures are taken:

- (a) The number of entries into open arms and (b) Time spent in the open arms
- Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (Pellow *et al*., 1985)

Zero mazes: The Zero Maze consists of a circular platform (6.1-cm width with a 40-cm inner diameter) that is equally divided into four quadrants. Two quadrants on opposite sides of the platform are enclosed by walls (20.3 cm high); the other two quadrants are open and bordered by a lip (0.6 cm high). The maze is elevated 72.4 cm above the floor and there is an overhead camera and tracking system to monitor activity of the mouse. Place the mouse just inside a closed arm, with all four paws inside and its nose pointing inside the closed arm. Each mouse placed on the maze for 5 min and the time it spends in the open and closed sections is measured (Bakshi and Kalin, 2002).

Statistical analysis: All values are expressed as Mean±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

RESULTS

Effect of MEFH on open field habituation: Each mouse was placed individually in the hole board apparatus and the number of head pokes and line crossings were noted. MEFH at the dose of 200 and 400 mg kg^{-1} p.o. Increased the number of head pokes significantly ($p<0.05$) in dose dependent manner as shown in Table 1.

Table 1: Effect of MEFH on open field habituation

Groups	No. of head pokes	No. of line crossing
Control CMC (1% w/v, p.o.)	31.60±2.0	48.33±3.2
Negative control (corticosterone 5 mg kg^{-1}, p.o.)	13.00±0.5	31.60±2.64
Diazepam (1 mg kg^{-1}, i.p.)	1.60±0.91	8.30±2.3
MEFH (200 mg kg^{-1}, p.o.)	5.60±0.66*	21.00±1.14*
MEFH (400 mg kg^{-1}, p.o.)	5.75±0.15*	25.50±3.31*

All values are Mean±SEM, n = 6, *p<0.05 when compared with negative control

Table 2: Effect of MEFH on locomotor activity

Groups	Actophotometer score in 10 min
Control CMC (1% w/v, p.o.)	87±12.0
Negative control (corticosterone 5 mg kg^{-1}, p.o.)	337±17.0
Diazepam (1 mg kg^{-1}, i.p.)	40±8.13
MEFH (200 mg kg^{-1}, p.o.)	182±12.6*
MEFH (400 mg kg^{-1}, p.o.)	160±10.12*

All values are Mean±SEM, n = 6, *p<0.05 when compared with negative control

Table 3: Effect of MEFH on Muscle co-ordination test

Groups	Time spent on revolving rod in rotarod groups apparatus (sec)
Control CMC (1% w/v, p.o.)	42.00±3.6
Negative control (corticosterone 5 mg kg^{-1}, p.o.)	120.00±5.1
Diazepam (1 mg kg^{-1}, i.p.)	11.300±4.9
MEFH (200 mg kg^{-1}, p.o.)	76.75±11.0*
MEFH (400 mg kg^{-1}, p.o.)	46.33±12.5*

All values are Mean±SEM, n = 6, *p<0.05 when compared with negative control

Table 4: Effect of MEFH on elevated plus maze

Groups	No. of entries in time spent	Open arm (in sec)
Control CMC (1% w/v, p.o.)	14.50±1.33	259.8±3.81
Negative control (corticosterone 5 mg kg^{-1}, p.o.)	1.00±1.10	75.0±5.11
Diazepam (1 mg kg^{-1}, i.p.)	5.30±1.15	166.0±10.2
MEFH (200 mg kg^{-1}, p.o.)	2.30±0.50	115.0±9.52*
MEFH (400 mg kg^{-1}, p.o.)	3.30±0.90*	130.0±11.13*

All values are Mean±SEM, n = 6, *p<0.05 when compared with negative control

Effect of MEFH on locomotor activity: MEFH at the dose of 200 and 400 mg kg^{-1} p.o. and diazepam (1 mg kg^{-1} i.p.) decreased the locomotor activity significantly (p<0.05) when compared with the negative control animals. The diazepam treated group also revealed statistically significant decrease in locomotor activity (Table 2).

Effect of MEFH on muscle co-ordination test: MEFH at the dose 400 mg kg^{-1} significantly reduced the time spent by the animals on revolving rod when compared to negative control (p<0.05). The standard drug (diazepam) also showed significant effect when compared to negative control (p<0.05) Low dose of drug (200 mg kg^{-1}) shown less significant effect as shown in (Table 3).

Effect of MEFH on elevated plus maze: The present results demonstrated that diazepam at dose of 1 mg kg^{-1} significantly increased both number of the opened arm entries and time spent in the opened arm in elevated plus maze. 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 MEFH extract at dose of 200 and 400 mg kg^{-1} used in this study (Table 4).

Table 5: Effect of MEFH on zero maze

Groups	Total time spent in open arm (sec)	Total time spent in enclosed arm (sec)
Control CMC (1% w/v, p.o.)	70.50±1.33	259.8±3.81
Negative control (corticosterone 5 mg kg^{-1}, p.o.)	21.33±6.65	278.66±7.5
Diazepam (1 mg kg^{-1}, i.p.)	294.66±1.52	5.33±9.13
MEFH (200 mg kg^{-1}, p.o.)	210.80±8.33*	89.33±10.5*
MEFH (400 mg kg^{-1}, p.o.)	278.66±8.5*	21.33±3.12*

All values are Mean±SEM, n = 6, *p<0.05 when compared with negative control

Effect of MEFH on zero maze: The results obtained from the zero maze model, indicates that MEFH at 400 mg kg^{-1} showed significant (p<0.05) anti anxiety activity as compared to diazepam. The average time spent by the test group in open arms increased significantly when compare to the negative control group (Table 5).

DISCUSSION AND CONCLUSION

The study showed that MEFH (200 and 400 mg kg^{-1}, p.o.) possess sedative, antianxiety, muscle relaxant activity. The study on the spontaneous motor activity showed that MEFH decreased the frequency and the amplitude of movements. The reduction of the spontaneous motor activity could be attributed to the sedative effect of the extract. Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents (Nishikawa *et al*., 2004). The results showed that MEFH significantly increased the time spent on the open arms and also the number of entries into open arm. It was further observed that the result obtained from the elevated Zero maze test suggested that MEFH at both the dose level of 200 and 400 mg kg^{-1} significantly increased the latency and time spent by animal in open arm rather than closed arm.

A significant increase in the exploratory head dipping behavior was observed after treatment with 200 and 400 mg kg^{-1} of MEFH, thus reinforcing the hypothesis that it has anxiolytic activity. Since the decrease in falloff time determines the muscle relaxant property of the plant extract. MEFH at the dose of 200 and 400 mg kg^{-1} shown a significant decrease in the time spent by each animal on revolving rod.

Despite the wide spread traditional use of *F. hispida* for treating various disorders, there are no scientific report available so far demonstrating its anxiolytic activity. Results obtained from the preliminary phytochemical screening of the plant extract reveals that plant leaves containing flavonoids, Terpenes and saponins. The sedative, muscle relaxant and anxiolytic effects of extract could be due to the interaction of flavonoids (chemical constituent of the plant) with the GABA

(Gama aminobutyric acid)/BZD (benzodiazepine) receptor complex in brain (Nogueira and Vassilieff, 2000).

In conclusion the result obtained in the study suggested that the extract of the leaves of *Ficus hispida* Linn. posses anxiolytic and muscle relaxant activity, which is possibly mediated through the GABA-BZD mechanism. Thus *Ficus hispida* has potential clinical application in the management of anxiety and muscle tension disorders. Further investigation of the mechanism of action of the plant extract, as well as the active principle responsible for its biological action is necessary.

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Venkataraman (Director of C.L. Baid Metha Foundation for Pharmaceutical Education and Research, Chennai, Tamil Nadu, India) for his technical and secretarial assistances.

REFERENCES

Abid, M., H.J. Hrishikeshavan and M. Asad, 2006. Pharmacological evaluation of *Pachyrrhizus erosus* (L) seeds for central nervous system depressant activity. Indian J. Physiol. Pharmacol., 50: 143-151.

Bakshi, V.P. and N.H. Kalin, 2002. Animal Models and Endophenotypes of Anxiety and Stress Disorders. In: Neuropsychopharmacology-The Fifth Generation of Progress, Davis, K.L., D. Charney, J.T. Coyle and C. Nemeroff (Eds.). Lippincott Williams & Wilkins, USA., pp: 883-894.

Ecobichon, J.D., 1997. The Basis of Toxicity Testing. CRC Press, New York, pp: 43–49.

Ghosh, R., K. Sharatchandra, S. Rita and I.S. Thokchom, 2004. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian J. Pharmacol., 36: 222-225.

Kunwar, R.M. and R.W. Bussmann, 2006. Ficus (Fig) species in Nepal: A review of diversity and indigenous uses. J. Ecol. Appl., 11: 85-97.

Mandal, S.C. and C.K.A. Kumar, 2002. Studies on anti-diarrhoeal activity of *Ficus hispida*. leaf extract in rats. Fitoterapia, 73: 663-667.

Nishikawa, H., T. Hata, E. Itoh and Y. Funakami, 2004. A role for corticotropin-releasing factor in repeated cold stress-induced anxiety-like behavior during forced swimming and elevated plus-maze test in mice. Biol. Pharm. Bull., 27: 352-356.

Nogueira, E. and V.S. Vassilieff, 2000. Hypnotic, anticonvulsant and muscle relaxant effects of *Rubus brasiliensis*. Involvement of GABA(A)-system. J. Ethanopharmacol., 70: 275-280.

Pellow, S., P. Chopin, S.E. File and M. Briley, 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods, 14: 149-167.

Peraza-Sanchez, S.R., H.B. Chai, Y.G. Shin, T. Santisuk and V. Reutrakul *et al*., 2002. Constituents of the leaves and twigs of *Ficus hispida*. Plant Med., 68: 186-188.

Rakotonirina, S., E . Ngo-Bum, A. Rakotonirina and M. Bopelet, 2001. Sedative properties of the decoction of the rhizome of *Cyperus articulatus*. Fitoterapia, 72: 22-29.

Saitoh, A., M. Yamada, M. Yamada, S. Kobayashi, N. Hirose, K. Honda and J. Kamei, 2006. Rho associated kinase path way inhibition produces anxiety-related behaviors in mice. Psychopharmacology, 188: 10-21.

Shanmugarajan, T.S., M. Arunsundar, I. Somasundaram, E. Krishnakumar, D. Sivaraman and V. Ravichandiran, 2008. Cardioprotective effect of *Ficus hispida* Linn. on cyclophosphamide provoked oxidative myocardial injury in a rat model. Int. J. Pharmacol., 4: 78-87.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. In: Phenols and Phenolic Glycosides. Elsevier Science Ltd., Nottingham, UK, pp: 223-224, 246-249.

Winston, D., 2005. An introduction to herbal medicine. Am. Extra Pharmacopoeia, 2005: 1-8.

Yadav, A.V., L.A. Kawale and V.S. Nade, 2008. Effect of *Morus alba* L.(mulberry) leaves on anxiety in mice. Indian J. Pharmacol., 40: 32-36.

Zimmerman, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109-110.