

**Original Article**

**EVALUATION OF ANTI-ANXIETY ACTIVITY OF *FICUS THONNINGII* ROOT IN MICE**

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**ABSTRACT**

**Objective:** To evaluate Anxiolytic activity of ethanolic extracts of *Ficus Thonningii* in male and female mice by light-dark model, open field test, Elevated plus maze models.

**Methods:** Healthy, adult mice of either sex weighing 25-40 g, maintained under standard laboratory conditions, at temperature  $25 \pm 2$  °C. The behavioral effects of an acute or sub-acute (14 d course) will be orally administered. *Ficus Thonningii* (200 and 400 mg/kg) ethanolic extract will be evaluated in male and female mice in various animal models. The animals were housed in plastic cages in groups of ten per cage, at room temperature, about  $21 \pm 1$  °C, and with free access to water and food. They were kept on an artificial 12 h/12 h d/night cycle.

**Results:** In the present study, we have undertaken the anxiolytic activity of ethanolic and aqueous extracts of *Ficus thonningii* in male and female mice, at the dose (200 and 400 mg/kg) mg/kg with oral administration respectively.

**Conclusion:** In conclusion, *Ficusthonningii* plant extract showed significant anxiolytic activity by using Elevated plus maze, open field test, light/dark exploration parameters. Probably due to GABA facilitatory action of phytoconstituents such as flavonoids, tannic acid, marmesinin, phenols, saponin etc.

**Keywords:** *Ficusthonningii*, Anxiolytic activity, Open field test (OFT), Light dark model (LDM), animals

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**INTRODUCTION**

A drug or other intervention that reduces anxiety is called an anxiolytic. Anxiolytic agents have the opposite meaning, causing anxiety; anxiolytic agents can refer to all of these different types of psychoactive drugs or interventions available. Research shows many recreational drugs are anxiolytic, but some substances, including alcohol, initiate the process with an anxiolytic effect initially. Anxiolytic agents have been used to treat anxiety disorder and associated physical and psychological conditions. Light therapy and other interventions have had anxiolytic outcomes [1].

Anxiety is a class of resonant emotional stimulation episodes that include feelings of worry or fear; in contrast to patients who have panic, patients with concern usually have an imprecise realization of the cause of danger. Research shows anxiety is the most frequent behavioral issue, and research related to the contributors of kids' anxiety shows that their physical development, social, family, and affective contributors are significant predictors of the child's anxiety level [2]. Anxiety is a frequently occurring psychological illness that comes with mood changes, irrational, violent behavior, low quality of life, bodily dysfunction, cognitive impairment. The most frequent phobias are agoraphobia and simple phobia, which have the most frequent prevalence. The lifetime prevalence of obsessive-compulsive disorder (OCD) and panic disorder (PD) is 2% while the prevalence of generalized anxiety disorder (GAD) is 3%–30% and social phobia (SP) is 2%–16%. Mood states of anxiety are shown to be governed by monoamines (dopamine, nor adrenaline, and serotonin), neuropeptides (galanin, neuropeptide Y, arginine vasopressin, tachycardia, and substance P), neurosteroids, and cytokines [3]. The two medications most frequently used for the treatment of anxiety are SSRIs and benzodiazepines. Synthetic medications used for the treatment of anxiety also have several adverse side effects. Natural substances have been shown to have the same ability to treat illness while possessing fewer side effects than synthetic drugs [4]. Since prehistoric times, mankind has relied on plants as a source of food, shelter and medicine. From the beginning of time, humans have utilized plants for food, shelter, and medicine. Before allopathic medicine arose, illnesses were treated

with remedies from plants. Plants are still commonly used in both ethnomedicine and ethnoveterinary practice, beyond their commercial use in manufacturing, cosmetics, and pharmaceutical purposes. Also, the development of traditional medicines has benefited as well. *Ficusthonningii* is one of the species of *Ficus*. It is native to Africa. Latest evolutionary research shows that *Ficusthonningii* may be the classification for many different species. One tree in the Moraceae plant family is *Ficusthonningii*. It is a fruiting tree that has been utilized for disease treatment for a long time. *Ficusthonningii* prefers light, deep, and well-drained soils and is found in upland woodlands in tropical and subtropical Africa [5-7]. It is a historically important plant species that has both medicinal and nutritional uses. The usually yellow fruits, 10-20 mm in diameter, change to pink when they are ripe. The well-known ornamental tree *Ficusthonningii* is also employed as an added-value species within agroforestry systems. Its bark is used to fabricate bark cloth, and leaves are used for fodder. Like many woody trees, *Ficusthonningii* is used extensively by ethnomedical practitioners to treat a wide range of maladies. Whereas every plant part has medicinal value, the latex-rich leaves are preferred as latex has historically been associated with efficacy. For mice treated with Alprazolam (Standard), open arms entries and total time in open arms were all significantly ( $p < 0.001$ ) greater than the baseline levels. For all *Ficusthonningii* root dosages (200 and 400 mg/kg), total time spent and entry into the open arms increased significantly ( $P < 0.05$ ) in a dose-dependent fashion [8]. The number of squares crossed and rearings were significantly ( $p < 0.05$ ,  $0.01$ ) increased by the root of *Ficusthonningii*. *Ficusthonningii* root significantly ( $p < 0.05$ ) increased the number of social interactions if we consider the social interaction test. In comparing to the vehicle treated control group (for the alprazolam and the extract (*Ficusthonningii* root 200 and 400 mg/kg p. o.)) there was a significant increase in rearing and squares crossed in the 5 min intervals. The best anti-anxiety action was seen primarily at the dose of 400 mg/kg. The mice in the various levels of treatment groups visited fewer squares over the two-hour time span in the open field test. Relative to control, it was shown that mice visited less squares overtime while they were exposed to the ethanolic extract of *Ficusthonningii* root

and ethanolic soluble fraction, suggesting the possibility of anxiolytic actions [9]. Elevated Plus Maze (EPM), Light Dark Model (LDM), and Open Field Test (OFT) are used to measure anxiolytic activity. Total locomotor time and the amount of time spent in open arms were both enhanced in EPM after treatment with ethanolic and aqueous extracts. Treatment with these extracts increased the amount of time spent in the light compartment in the light-dark experiment. The amount of time spent in the central compartment and motility both increased in the open field test treatment with extract. Root extract has demonstrated notable anxiolytic efficacy at 200 mg/kg and 400 mg/kg [10].

## MATERIALS AND METHODS

### Procurement of the extract

*Ficusthonningii* root ethanolic extracts were collected from the dealer of ShreedhaPhyto extracts Jaipur – 302019. The same group also provided a certification of the Plant's identify and quality.

### Experimental animals

24 adult either sex mice weighing between 25-30g were obtained from the animal house of Pharmacology Department. ST. Soldier

institute of pharmacy, Jalandhar-Amritsar Bypass NH-I, Behind NIT Jalandhar, Punjab India – 144011. Healthy, adult mice of either sex weighing (25-40 g), maintained under standard laboratory conditions; at temperature  $25 \pm 2^\circ\text{C}$  and a 12 h light-12 h dark period will be employed for the experimentation. Food and water will be provided *ad libitum*.

### Acute oral toxicity study

Acute toxicity study for the ethanolic extract of "*FICUS THONNINGII*" will be done according to the OECD guidelines No: 423 and low, medium and high dose will be selected for treatment [11].

### Methods

The overnight fasted mice will be divided into 04 groups, each group consisting of 06 animals. The *EERFT* will be given in various doses (0, 200, 400 and 600 mg/kg) by oral route with a gavage. After administration of the extract, the animal will be observed continuously for the first 2 h and at 24 h to detect changes in behavioural responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep, and coma and also will be monitored up to 14 d for the toxic symptoms and mortality.

Table 1: Treatment schedule

Groups (where N=4)	Treatment (For 14 d)
Group I	Naive animal, received standard pellet diet and tap water <i>ad libitum</i> daily.
Group II	Standard group received 0.25 mg/kg Alprazolam orally daily.
Group III	Test group-I received 200 mg/kg Ethanolic extract of <i>Ficus Thonningii</i> root orally daily
Group IV	Test group –II received 400 mg/kg Ethanolic extract of <i>Ficus Thonningii</i> root orally daily

### Total No. of animals required

No. of the animal in each group (n) = 06

No. of groups (N) = 04

Total no. of animals required = 24

### Parameters for antianxiety in mice

The behavioral effects of an acute or sub acute (14 h course) will be orally administered. *Ficus Thonningii* (200 and 400 mg/kg) ethanolic extract will be evaluated in male and female mice in various animal models. The animals were housed in plastic cages in groups of ten per cage, at room temperature about  $21 \pm 1^\circ\text{C}$ , and with free access to water and food. They were kept on an artificial 12 h/12 h day/night cycle.

### Body weight analysis

During the same time of study, weekly body weight analysis will be recorded.

### Statistical analysis

All the results will be expressed as standard error of mean (S. E. M.). Data will be analyzed using one-way ANOVA (Graph pad prism version 5.00 software) followed by suitable post test Dunnett's t-test.  $p < 0.05$  will be considered as statistically significant.

## RESULTS

All the parameters were performed with suitable time interval to prevent unwanted stress in animals. *Ficusthonningii* includes a variety of physiologically active chemicals with physiological effects that may account for its healing potential in a variety of diseases.

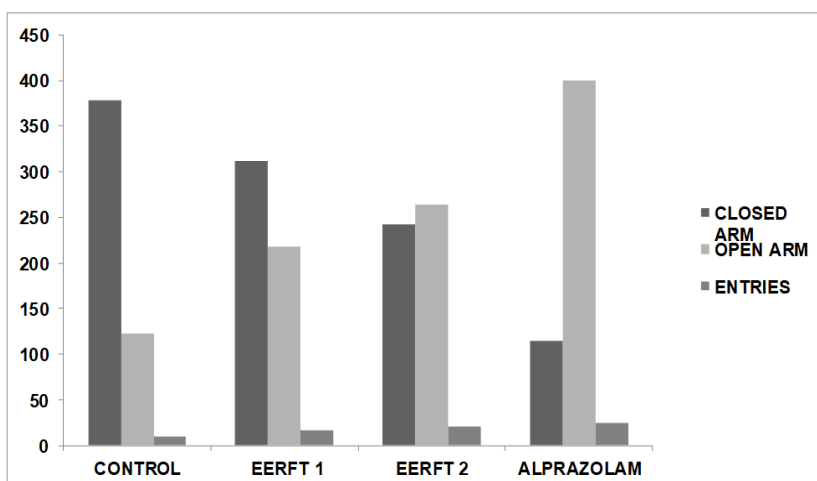


Fig. 1: Graphical representation of effects of different doses of *Ficus thonningii* root ethanolic extract on anxiolytic effect as compared to standard (Alprazolam)

Table 2: Phytochemical constituents of crude leaf/roots extracts of *Ficusthonningii*

Phytoconstituent	Aqueous extract	Ethanolic extract
Anthraquinones	+	+
Flavonoids	+	++
Alkaloids	-	+
Terpenoids	-	++
steroids	-	++
Saponins	++	+

(-) = absent, (+) = low, (++) = high, (+++) = very high

#### Elevated plus-maze test

The vehicle-treated mice (Normal control) had spent more time in closed arm and showed less entries in open arm compared to closed arm of the maze during 10 min.

**Group 1:** In this group animals were treated with normal saline solution in which mice spent  $122.8 \pm 2.34$  sec in open arm and  $378.6 \pm 1.53$  sec in closed arm and no. of crossings were  $10 \pm 2.01$ .

**Group 2:** In this group animals were treated with low dose of Ethanolic extract (200 mg/kg) solution in which mice spent  $218.1 \pm 1.99$  sec in open arm and  $312.1 \pm 1.22$  sec in closed arm and no. of crossings were  $17 \pm 1.59$

**Group 3:** In this group animals were treated with high dose of Ethanolic extract (400 mg/kg) solution in which mice spent  $264.6 \pm 2.31$  sec in open arm and  $242.1 \pm 0.56$  sec in closed arm and no. of crossings were  $20 \pm 0.22$ .

**Group 4:** In this group animals were treated with Alprazolam (0.25 mg/kg) solution in which mice spent  $400.6 \pm 0.22$  sec in open arm and  $114.6 \pm 0.21$  sec in closed arm and no. of crossings were  $24 \pm 0.21$ .

Animals treated with Alprazolam (Standard) showed significant ( $p < 0.001$ ) increase in the percentage of open arms entries as well as time spent in open arm. All the doses of *Ficusthonningi* root (200 and 400 mg/kg) showed a significant increase in the time spent and number of entries into open arms ( $P < 0.05$ ) in dose dependent manner.

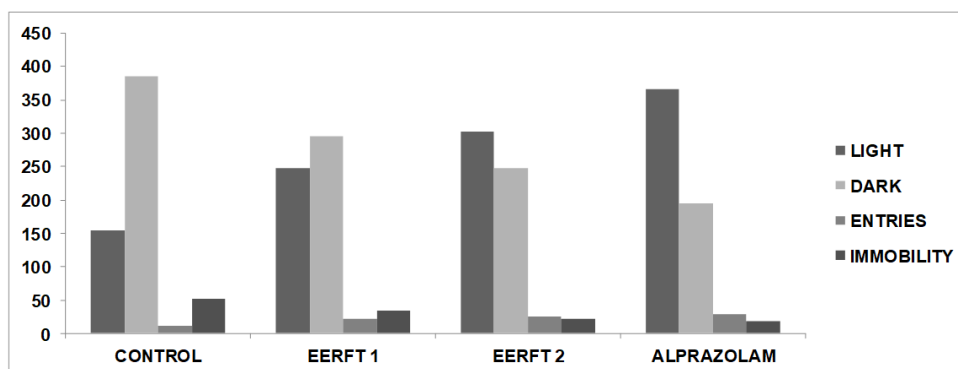


Fig. 2: Graphical representation of effects of different doses of *Ficusthonningi* root ethanolic extract on anxiety-like effect as compared to standard (Alprazolam)

#### Light dark model

The vehicle-treated mice (Normal control) had spent more time in dark, less number of entries and showed less mobility compared to test and standard drug treated group animals during 10 min.

**Group 1:** In this group animals were treated with Normal saline solution in which mice spent  $154.8 \pm 1.02$  sec in light chamber and  $384.6 \pm 0.24$  sec in dark chamber and no. of crossings were  $12 \pm 0.12$ . Immobility period was  $52 \pm 1.65$  sec.

**Group 2:** In this group animals were treated with low dose of Ethanolic extract (200 mg/kg) solution in which mice spent  $248.3 \pm 1.06$  sec in light chamber and  $294.5 \pm 0.55$  sec in dark chamber and no. of crossings were  $22 \pm 1.64$ . Immobility period was  $34.1 \pm 0.29$  sec.

**Group 3:** In this group animals were treated with high dose of Ethanolic extract (400 mg/kg) solution in which mice spent  $302.6 \pm 0.26$  sec in light chamber and  $248.1 \pm 0.21$  sec in dark chamber and no. of crossings were  $25 \pm 1.01$ . Immobility period was  $22.3 \pm 0.11$  sec.

**Group 4:** In this group animals were treated with Alprazolam (0.25 mg/kg) solution in which mice spent  $366.3 \pm 2.56$  sec in light chamber and  $194 \pm 0.25$  sec in dark chamber and no. of crossings were  $28 \pm 0.21$ . Immobility period was  $18.5 \pm 0.16$  sec.

In the light/dark exploration test, *Ficusthonningi* root significantly ( $p < 0.05$ , 0.01) increased the latency of entry into the dark box, time spent in the light box, and number of rearing and assisted rearing.

Treatment with Alprazolam significantly increased the time spent ( $P < 0.001$ ) in light box as well as the number of crossings between the light and dark boxes, but duration of immobility was significantly reduced. *Ficusthonningi* root treated mice also exhibited dose dependent significant increase in the time spent in light box and the number of crossings between light and dark boxes. The duration of immobility was also significantly reduced as compared to the vehicle treated group.

#### Open field test

The vehicle-treated mice (Normal control) had spent more time in center area, less number of grooming and showed less crossing compared to test and standard drug treated group animals during 10 min.

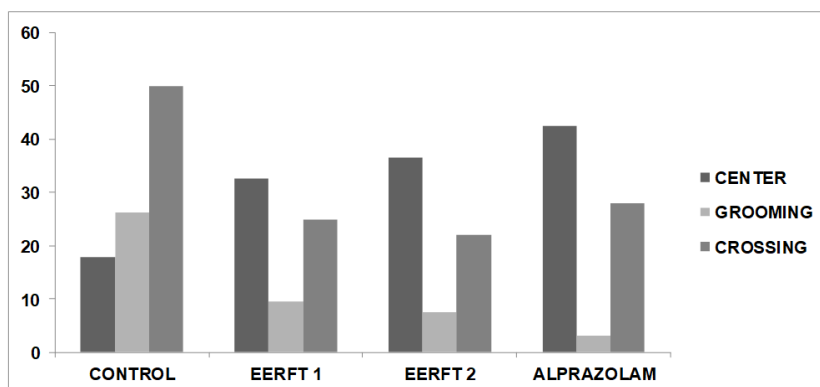
**Group 1:** In this group animal was treated with normal saline solution in which mice spent  $17.8 \pm 1.53$  sec time at centre,  $26.3 \pm 2.34$  sec. in grooming and  $50 \pm 2.01$  line of crossings.

**Group 2:** In this group animal was treated with low dose of Ethanolic extract (200 mg/kg) solution in which mice spent  $32.5 \pm 1.22$  sec time at centre,  $9.6 \pm 1.99$  sec. in grooming and  $25 \pm 1.59$  line of crossings.

**Group 3:** In this group animal was treated with high dose of Ethanolic extract (400 mg/kg) solution in which mice spent  $36.5 \pm 0.56$  sec time at centre,  $7.6 \pm 2.31$  sec. in grooming and  $22 \pm 0.22$  line of crossings.

**Group 4:** In this group animal was treated with Alprazolam (0.25 mg/kg) solution in which mice spent  $42.6 \pm 0.21$  sec time at centre,  $3.2 \pm 0.22$  sec. in grooming and  $10 \pm 0.21$  line of crossings. *Ficusthonningiroot* significantly ( $p < 0.05$ , 0.01) increased the number of sectional crossings and rearing. Considering the social interaction test, *Ficusthonningiroot* significantly ( $p < 0.05$ ) increased the number of interactions. Peak anti-anxiety effects were mostly observed at the dose of 400 mg/kg. Alprazolam and extract (*Ficusthonningiroot* 200 and 400 mg/kg p. o.) treated mice showed

significant increase in the number of rearing and number of squares crossed during 5-min interval as compared to vehicle treated control group. During Open field test, as time passed on, the number of squares visited by the mice of the different test groups decreased over time for an interval of 2h. It was seen that the ethanolic extract of *Ficusthonningiroot* and its ethanolic soluble fraction showed the lower number of squares visited by the mice over time, compared to the control, indicating plant extract might have potential of anxiolytic effect.



**Fig. 3:** Graphical representation of effects of different doses of *Ficusthonningiroot* ethanolic extract on anxiolytic effect as compared to standard (Alprazolam)

## CONCLUSION

The goal of the current study was to assess the anxiolytic impact of ethanolic extracts of the root portion of *Ficusthonningii* in mice using an elevated plus maze, an open field test, and a light-dark paradigm. The results were compared with those of a standard medication, alprazolam. Significant anxiolytic efficacy has been demonstrated by *Ficusthonningii* ethanolic extract in all LDM, OFT, and EPM models. *Ficusthonningii* extract given at two doses (200 mg/kg and 400 mg/kg) in a light-dark paradigm shown a reduction in the amount of time spent in the dark box and a notable increase in the amount of time spent in the light box, as well as the number of crossings between the light and dark boxes. *Ficusthonningii* extract given at two doses (200 mg/kg and 400 mg/kg) demonstrated a markedly higher number of rearings, fewer crossings, and a shorter grooming time in an open field test. As demonstrated by the common medication Alprazolam, *Ficusthonningii* exhibited anxiolytic effects. The outcomes were similar in all light-dark, open field, and elevated plus maze models. *Ficusthonningii*'s ethanolic extract has the ability to lengthen its duration of action. As a result, we may deduce that it may also have anxiolytic effects. It can also enhance the amount of time spent in the open arm, entry in the open arm (in the elevated plus model), and light field (in the light dark field).

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Nil

## AUTHORS CONTRIBUTIONS

All authors have contributed equally

## CONFLICT OF INTERESTS

Declared none

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