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PHARMACOLOGICAL EVALUATION OF ANXIOLYTIC ACTIVITY OF *FERONIA LIMONIA* (WOOD APPLE) LEAVES EXTRACT IN EXPERIMENTAL ANIMAL

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ABSTRACT

Anxiety disorder is one of the most frequent mental illnesses that people suffer from. It is characterized by motor stress, sympathetic overactive and nervousness and surveillance syndromes. Aside from the few chemical therapies that are accessible, such as benzodiazepine and serotonin modulators, not much treatment options are at hand that safely and effectively alleviate anxiety. The anxiolytic activity of ethanolic extract of *Feronia limonia* leaves is evaluated with two validated modes,elevated plus maze and light and dark exploration test. A total of 30 animals (*n* = 30) were used. They were divided into five groups of six animals each for both models. The effects of the test crude drug *Feronia limonia* at three different doses 2.5, 4.5, and 6.5 mg/kg were compared with the standard anxiolytic diazepam at 1 mg/kg dose and control group using 1% gum acacia at 10 ml/kg dose 1 h after administration of the drug. Anti-anxiety was evaluated by using elevated plus maze (EPM) and light and dark model methods. In experimental mice, the ethanolic extract has anxiolytic properties. So it is supports the use of *Feronia limonia* as anxiolytic agents. Further research should be conducted to determine the active ingredient responsible for the anti-anxiety activity.

KEYWORDS: Anxiety, anxiolytic, Ethanolic extract, Elevated plus maze, light and dark model, *Feronia limonia.*

1. INTRODUCTION

Anxiety is psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral component, associated with significant disability, uncomfortable emotional state, negative feelings about the future, or distress that triggers a sense of defense that serves as a warning so that the individual can prepare to face a possibly dangerous situation.[1] Anxiety disorders are psychiatric disorders affecting nearly 25% of the adult population at some purpose in their life. Anxiety disorders affect 30.5% of women and 19.2% of men, respectively. Anxiety disorders are extremely common among young people. Children aged seven to eleven years according a 15.4% prevalence rate of anxiety disorders. A survey has also stated that less than 14% of people with such psychiatric disorders receive treatment. Anxiety can aggravate many physical and mental ailments and also impede recovery from any other problems. Anxiety has become a significant are of psychopharmacological research during this decade, because it affects almost one-eighth of the globe's populations.[1-4]

Plants having therapeutic capabilities have been known for thousands of years and have been utilized as traditional medicine to heal diseases by the people. Traditional medicines are used all over the world due to the various adverse effects of modern science treatments and their exorbitant cost. Throughout history and prehistory, biologically produced medications have played an essential role in the way people lived.[3]

Feronia limonia L. (wood apple) is a member of the family, Rutaceae and is a religious tree planted in temples and gardens. It is a vital indigenous plant of India known for its medicinal and processing properties which is a moderate sized deciduous tree grown throughout India. It is a large tree growing to 9 meters (30 ft) tall, with rough, spiny bark. The leaves are pinnate, with 5-7 leaflets, each leaflet 25–35 mm long and 10–20 mm wide, with a citrus-scent when crushed. The fruit is a berry with a diameter of 5 to 9 cm that can be both sweet and sour. It has a very strong rind which can be difficult to break open, and contains sticky brown pulp and small white seeds. The fruits are woody, rough and used as a substitute for bael in diarrheoa and dysentery. The bark and leaves of the plant are used for vitiated conditions of vata and pita while the fruits are used for tumours, asthma, wounds, cardiac debility and hepatitis.4 And the leaves were reported to possess hepatoprotective effect**.** Flavanoids, glycosides, saponins, and tannins are found in the fruit. Limonia fruits have also yielded several coumarins and tyramine derivatives. Leaves contain stigmasterol, psoralen, bergapten, orientin, vitedin, saponarin, tannins and an essential oil3. While the fruit shells contain antifungal compounds,

namely- psoralene, xanthotoxin, 2, 6-dimethoxybenzoquenone and osthenol.[7-14]

2. MATERIALS AND METHODS

Experimental Animal

Healthy Swiss *albino* mice weighing 25-30g of either sex, aging 3-4 months was included in the study. Pregnant and diseased animals were not used in the experiments. Animals were provided free access to water and commercial food and were maintained under standard laboratory conditions with a natural light and dark cycle, under room temperature. The experiments were conducted in Department of Pharmacology, SVCP, Indore, Madhya Pradesh. The experiment was conducted 30 min after administrating the drug. A total of 30 animals (n = 30) were used. They were divided into 5 groups of six animals each (Table 1).[5]

Inclusion criteria

- Healthy Swiss albino mice of either sexes with normal behavioural activity
- Weight: 25-30g
- Age: 3-4 months

Exclusion criteria

- Unhealthy animal
- Obese animal
- Pregnant animal
- Animals previously used in other experiments

Table 1: The animals were divided into five groups with six animals in each group for both models.

Groups (n=6)	Treatment
I	Control - 1% Gum Acacia- 10 ml/kg
II	Diazepam - 1 mg/kg
III	FL - 2.5 mg/kg
IV	FL - 4.5 mg/kg
V	FL - 6.5 mg/kg

n: Number of animals in each group, FL: *Feronia limonia*

Plant Material

The Fresh leaves of *Feronia limonia* were collected from Local area, Indore (M.P.) and the leaves were authenticated by the botanist. The leaves were cleaned under tape water and leaves were cut into pieces and kept for shade drying. The dried leaves of *Feronia limonia* were powdered in a mixer grinder.

Ethanolic Extract of *Feronia limonia*

The dried powdered *Feronia limonia* leaves weighed around 100g. It was wrapped in a muslin cloth and extracted using 95% ethanol v/v in Soxhlet apparatus maintained at around 60°C-70°C for period of 3 days. The extract was filtered, concentrated under reduced pressure and dried using Rota vapour at 60°C and subsequently in the water bath for evaporation of solvent for a period of 3 days. Dark greenish extract was obtained which yield of *Feronia limonia* leaves obtained was 13.65% w/w.[8-9]

EVALUATION METHODS

Elevated Plus maze: The plus maze apparatus consisted of two open arms, measuring (16x5 cm), and two closed arms, measuring (16x5x12 cm), and an open roof with the entire maze elevated (25 cm) from the floor. Swiss *albino* mice 25-30g was treated with normal saline, diazepam and test extract doses 30 min before being placed individually in the center of elevated plus maze, facing a closed arm. The spent in bath open and closed arms was recorded for 5 min. the time spent was measured in seconds. The numbers of entries into the open and closed arms were counted during the test. An entry was defined as having all four paws with the arm.[15]

Light and Dark Model: The Light and dark apparatus consisted of open top wooden box. Two distinct chambers, a black chamber (25 cm long×35 cm wide×35cm deep), painted black and made dark by covering its top with black plywood, and a bright chamber (25 cm long×35 cm wide×35cm deep), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. Two chamber were connected through a small open doorway (7.5 cm long x7.5 cm wide) situated on the floor level at the center of the partition. The mice were placed individually in the center of the light box after 30min of oral treatment and observed for 5 min.[16,17]

Statistical Analysis

Analysis of results was performed by ANOVA followed by *post-hoc* test. $P < 0.05$ was considered statistically significant.

3. RESULT

Elevated Plus Maze: The results in Table 2 revealed that mice given diazepam had a considerable increase in the number of open arm entries and time spent in open arms. They also exhibited a decrease in the amount of time spent in closed arms. FL-treated mice had more open arm entrances (6.5 mg/kg), more time spent in open arms (2.5, 4.5, and 6.5 mg/kg), and more rears in open arms (6.5 mg/kg), but less time spent in closed arms 2.5, 4.5, and 6.5 mg/kg) (Table 2).

Light and Dark Exploration Test: Table 3 showed that mice given the conventional drug diazepam spent more time in the light area and also retreated significantly. FL treated Mice increased their time spent in the light area by 2.5 mg/dl (P 0.05) and significantly by 4.5 and 6.5 mg/dl (P 0.01). They also demonstrated a reduction in immobility length at all three doses (Table 3).

Table 2: Effect of Administration of *Feronia Limonia* on Mice Behavior in Elevated Plus Maze.

Drug groups (*n*=6)	Number of open arms entries (s)	Number of total arm entries	Time spent in open arms (s)	Time spent in closed arms (s)	Number of rearsin open arms (s)
Control 1% Gum Acacia 10 ml/kg	1.2±0.86	3.63±0.55	16.55±1.66	255.86±6.66	2.0±0.53
Diazepam - 1 mg/kg	4.5±0.41*	6.83±0.42	80.0±2.57**	172.0±3.90**	3.0±0.56
FL - 2.5 mg/kg	1.78±0.35	4.86±0.5	46.5±2.25**	205.8±5.42**	2.9±0.55
FL - 4.5 mg/kg	3.40±0.36	6.1±0.22	59.36±7.50**	176.3±4.23**	3.88±0.40
FL - 6.5 mg/kg	5.0±0.52**	7.0±0.55**	76.65±6.70**	167.0±3.47**	4.2±0.50*

All values are mean ± SEM, Statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, **P*<0.05, ***P*<0.01, FL: *Feronia limonia*, SEM: Standard error mean

Table 3: Effect of Administration of *Feronia Limonia* on Mice Behavior in Bright and Dark Apparatus.

Drug groups (*n*=6)	Number of bright chamber entries (s)	Time spent in bright chamber (s)	Number of rears inbright chamber (s)	Duration of immobility (s)
Control 1% Gum Acacia- 10 ml/kg	1.5±0.33	16±0.16	3.2±0.33	75.16±3.80
Diazepam - 1 mg/kg	3.56±0.22*	30.0±3.22**	4.10±1.30	54.66±1.44
FL - 2.5 mg/kg	1.63±0.33	26.0±3.15*	2.88±0.22	65.5±2.16*
FL - 4.5 mg/kg	1.4±0.22	34.1±2.77**	3.5±0.32	55.22±2.43**
FL - 6.5 mg/kg	3.26±0.33	40.5±3.066**	4.00±0.30*	50.14±3.66**

All values are mean ± SEM, Statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, **P*<0.05, ***P*<0.01. FL: *Feronia Limonia*, SEM: Standard error mean

4. DISCUSSION

In this study, elevated plus maze test and light and dark exploration test were used to evaluate the anxiolytic activity of ethanolic extract of FL leaves in albino mice.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The reduction in entry and time spent in open arms are the indications of the high level of fear or anxiety. The number of entries and time spent in the open arms have been found to be increased by anxiolytic and reduced by anxiogenic agents. A significant increase in the time spent in open arms was observed after treatment with all three doses of FL.

After treatment with 8.5 mg/kg of FL extract, there is a significant increase in both time spent in open arms and entry into open arms, indicating anxiolytic action.

The light/dark exploration test is based on mice's inherent aversion to brightly lighted environments. Reduced frequency of entries, duration spent, and rearing behavior in the light box are all considered anxiety indicators.

Anxiolytics alleviate the natural aversion to light and increase the amount of time spent in the illuminated compartment. In this mouse, relative to vehicle, FL extract at doses of 4.5 and 6.5 mg/kg increased time spent in the lighted box and decreased immobility at all three levels, suggesting anxiolytic-like activity. FL has strong anti-stress activity, however the magnitude and efficacy for stress relief are lower when compared to the typical anxiolytic drug diazepam.

All of these behavioral changes in both paradigms imply that the animal's anxiety, aversion to light, and exploratory activity have diminished, which are comparable effects generated by the conventional medication diazepam.

5. CONCLUSION

The findings of this investigation imply that the extract of FL leaves has anti-anxiety properties. FL may therefore have a clinical use in the treatment of anxiety disorders. It is important to conduct more research on the plant extract's modes of action and the active ingredients that give rise to its biological effects. Recommendations in the conventional medical system provide a path to the creation of a novel antidepressant drug from well-known herbal remedies. After more research, it may be concluded that this impact may be related to the inhibition of norepinephrine uptake, which ultimately results in an increase in the availability of norepinephrine at synapses and can be taken into consideration for usage when thinking about the treatment of anxiety.

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