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Pharmacological Evaluation of Anti - depressant Activity of Ethanolic Extract of *Feronia limonia* Leaves in Swiss *Albino* Mice

Rohit Jatav[1], Rekha Gour[2], Anant K. Patel[3]

[1]PG Student, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

[2]Professor, Swami Vivekanand College of Pharmacy, Indore (M. P.), India
Email: *gourrekha4[at]gmail.com*

[3]Professor, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

Abstract: ***This research was aimed to evaluate the potential anti - depressant activity of Ethanolic Extract of Feronia limonia (EEFL) leaves in Swiss Albino mice. Materials and Methods: Animal group was divided as control (n=2) and received normal saline and negative group (n=3) where no treatment required and six animals were equally divided in standard and low dose of test extract (100mg/kg) and high dose of test extract (200mg/kg). Antidepressant potential of EEFL was evaluated by submitting the mice to Forced Swim Test (FST) and Tail Suspension Test (TST) and Open Field Test. Result: The yield was found to be 29.14% and the EEFL leaves and alkaloids, flavonoids, glycosides, saponins, amino acids, fats, and oils were identified in the extract. The duration of immobility in the FST was reduced in mice treated with the EEFL at 100mg/kg immobility time was 104.3 sec. while at dose 200mg, it was 92.7 sec. this was higher compared to the standard drug fluoxetine (72.9 sec.). In the TST, the immobility time was significantly higher in EEFL at dose 100mg/kg was 104.92 sec. and EEFL at dose 200mg/kg was 94.9 sec. while compared with standard drug fluoxetine at dose 20mg/kg was 84.52 sec. In open field test the EEFL doses of 100 and 200 mg/kg increased peripheral squares crossed by mice but did not significantly increase central square crossings compared to control. Standard drug showed a significant increase in central square crossings. Fluoxetine resulted in a significant increase in animal rearing compared to control. Conclusion: The present study suggested that PF extracts possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depressive disorders.***

Keywords: *Feronia limonia*, antidepressant activity, tail suspension test, forced swim test, open field test

1. Introduction

In present scenario every third person is suffering from depression, dementia, low mental health or behavioral disorder due to low quality life, work pressure and multiple factor lead of depression and in now a days in market to treat depression number of drugs are present with their toxic effects Psychiatric illness is also often associated with suicide and there are between 10 and 20 million attempts at suicide are made annually. On the basis of the above information, the leaves of *Feronia limonia* was selected for evaluating its antidepressant activity due to its traditional use in the management of anxiety, stress, insomnia, hysteria, skin inflammation, cough and fever. Chemical constituents in *Feronia limonia* include essential oil, flavonoids, alkaloids, saponins, sesquiterpene. [1 - 7]

2. Materials & Methods

2.1 Collection and Identification

The plant material was collected from local area in Indore and authenticated by Botanist as a *Limonia acidissima* Leaves (Family - Rutaceae) and Voucher Specimen Number: J/Bot. /2023 - 0114.

2.2 Preparation of Extract

Procedure:

Soxhlet apparatus was used for the extraction procedure. The apparatus was placed on a heating mantle and the flask containing the solvent was heated at around 55°C. The extraction process was carried out for 24 hours until the color of the thimble became colorless or clear. Take 36.80grms air - dried fine powder of the leaves of *Feronia limonia* were taken and same cycle repeat again. subsequently ethanolic extract was poured into a porcelain evaporating dish. The concentrated extract was boiling at 55°C at water bath to achieve a semi - solid dark reddish - brown color mass. The residue was dried, weighed and dried in desiccator and used for subsequent experiments. The weight of the dried residue was found to be 22.38 grams. The % yield was determined using the formula below.

Preliminary Phytochemical studies:
The present phytoconstituents in the leaves of *Feronia limonia* was alkaloids, flavonoids, fats and oils, amino acids, saponins, and glycosides,. [9 - 10]

2.3 Pharmacological Evaluation:

Experimental Animals: -
Healthy Swiss Albino mice weighing about 25 - 30 gm of either sex was obtained from the animal house of Swami Vivekananda College of pharmacy, Indore. The animals were housed in well - ventilated standard polypropylene cages at controlled temperature (22ºC ± 3ºC) and relative humidity ranging between 50 - 70%. The animals had 12 hrs light: 12 hrs dark cycle. The animals were kept individually

in the large spacious hygenic cages during the course of experimental period. The animals had free access to standard laboratory pellets and drinking water. The animals were allowed to acclimatize for seven days before being used for the studies. [1, 11 - 12]

The experimental protocol was approved by the Institutional Animal Ethical Committee of our institute. (Approval No: IAEC/SVCP/2023/10) and were strictly in accordance with the norms of CPCSEA. New Delhi (CPCSEA stands for Committee for the Purpose of Control & Supervision of Experiments on Animals, India).

Experimental design

Table 1: A total number of 20 healthy Swiss Albino mice were divided into 5 groups each

S. No.	Groups	No. of Animals
1.	Normal Control [13]	2
2.	Negative Control	3
3.	Low dose of test extract (100mg/kg) [1, 14 - 15]	5
4.	High dose of test extract (200mg/kg) [1, 14 - 15]	5
5.	Standard Drug Fluoxetine Hydrochloride (20mg/kg) [1, 16]	5
	Total	20

Forced Swim Test (FST):
In FST mice were individually forced to swim in open glass chamber (25×15× 25cm) containing freshwater to a height of 15 cm and maintained at 26°±1°C. At this height of water, an animal wasn't able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Water in the chamber was changed after subjecting each animal to FST because "used water" was show to alter the behavior. Each animal was shown vigorous movement during initial 2 min period of the test. The duration of immobility were manually recorded during the next 4 min of the total 6 min testing period. Mice were consider to be immobile when they can struggling and remain floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel was dried and return to their housing conditions. After the initial 2 min period of the test mice were treated with low dose and high dose of test drug also treated with standard drug and returned to cages for 30 min Then the animals was individually forced to swim in open glass chamber for further record of next 4 min test. . [1, 12, 14]

Tail Suspension Test (TST):
In TST each mouse was individually suspended to the edge of a table, 50 cm above the floor, by adhesive tape place approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolate from other animals during the test. For six minutes, the entire immobility duration was carefully recorded. Animals were considered to be immobile when it didn't show any body movement, hung passively and completely motionless. Every mouse was utilised just once throughout the test, which was carried out in a room with low lighting. When documenting the immobility of the animals, the observer was blind to the medication treatments administered to the subjects of the study. After the initial 6 min period of the test mice were treated with low dose and high dose of test drug also treated with standard drug and returned to cages for 30 min, then the animals was individually suspended to the edge of a table then further record immobility for next 6 min test. [1, 12, 14]

Open Field Test: -
This test utilizes behavioral changes in mice was expose to novel environments and is use to confirm that the observe anti - depressant effect is not due to stimulation of general motor activity. Various types of Open field apparatus was use to test the mice. The open field test was carried out and the dark grey floor subdivides into 16 equal parts in a wooden box (100cm x 100 cm x30 cm). Respective treatment was given to the animals and 30 min later, the animals was individually place in the corner square of the open field.

The following parameters were observed for 5 min 10:

- Activity in the centrepiece (count of crossed central squares)
- Spontaneous ambulation (number of squares crossed). [1]

3. Results

% yield extract of compound and Phytochemical screening: The yield was found to be 29.14% and the EEFL leaves and alkaloids, flavonoids, glycosides, saponins, amino acids, fats, and oils were identified in the extract.

Table 2: Qualitative phytochemical analysis of ethanolic *Feronia limonia* extract

Phytochemicals	Name of test	Inference	Result
Alkaloids	Dragendroff's test	Orange brown Precipitate	Positive
	Hager's Test	Yellow coloration	Positive
Flavonoids	Sulphuric acid test	Brownish - red precipitate	Positive
Glycoside	Legal's test	Pink coloration	Positive
Tannin and phenolic content	Ferric chloride test	Deep blue coloration	Negative
Saponin	Foam test	Persistent foam not form	Positive
	Froth test	Formation of 1cm layer of foam	Positive
Amino acid	Cystein test	Black precipitation	Positive
Fat and oil	Saponification test	Clear blue solution	Positive

Evaluation of Anti- depressant Activity:

Forced Swim Test:

The result of the effect of ethanolic leaves extracts of *Feronia limonia* on the duration of immobility is shown in Table 1. The animals were treated with distilled water 10ml/kg P. O as control, 100mg/kg, p. o of EEFL and 200mg/kg, P. O. of EEFL and as standard drug Fluoxetine HCL 20mg/kg, P. O.

Table 3: Ethanolic extract of *Feronia limonia*. leaves Immobility time in average value in Forced swim test.

Group Name	Dose	Immobility time (sec)
Normal Control	0.28	179.3
Standard group Fluoxetine 20mg/kg	0.56	72.9
Low dose of ethanolic extract (100mg/kg)	2.86	104.3
High dose of ethanolic extract (200mg/kg)	5.72	92.7

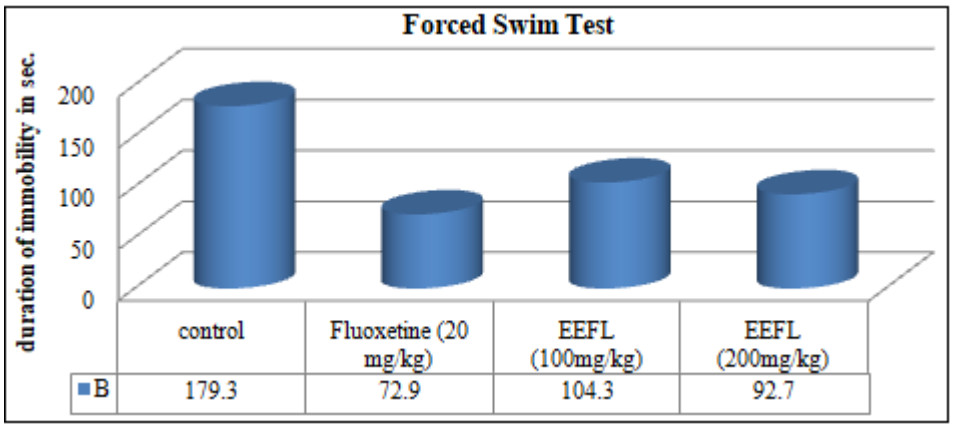

Figure 1: Effects of EEFL and Fluoxetine on duration of immobility in the FST. Results are expressed as mean±S. E. M. compared to respective control group

Tail Suspension Test: -

The result of the effect of ethanolic leaves extracts of *Feronia limonia* on the duration of immobility is shown in Table 5. The animals were treated with distilled water 10ml/kg p. o as control, 100mg/kg, p. o of EEFL and 200mg/kg, p. o of EEFL, Fluoxetine 20mg/kg, p. o as standard.

Table 4: Ethanolic extract of *Feronia limonia*. leaves Immobility time in average value in Tail Suspension Test

Group Name	Dose	Immobility time (sec.)
Normal Control	0.27	163.6
Standard group Fluoxetine 20mg/kg	0.58	84.52
Low dose of ethanolic extract (100mg/kg)	2.82	104.92
High dose of ethanolic extract (200mg/kg)	5.56	94.9

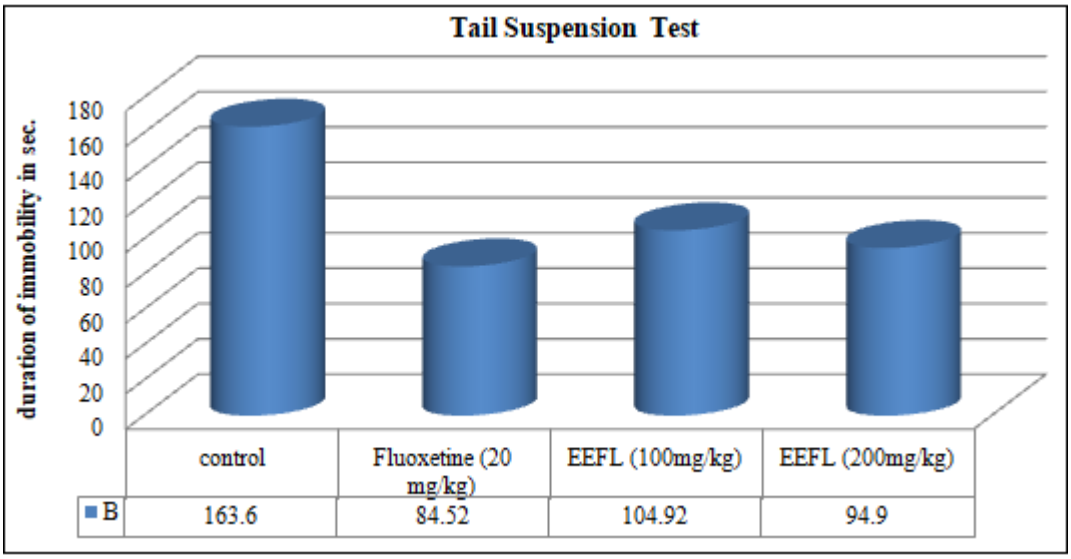

Figure 2: Effects of EEFL and Fluoxetine on duration of immobility in the TST. Results are expressed as mean±S. E. M. compared to respective control group.

Open Field Test

Though there was slight increase in the number of squares crossed (peripheral) by mice in EEFL treated groups (100 and 200 mg/kg, p. o.) as compared to control, the number of central Squares crossed in the control and standard (Fluoxetine) group were 10.17 ± 0.9 and 31.16±0.4 sec. respectively. There was a significant increase in no. of crossings in Fluoxetine group as compared to control group. But when different doses of EEFL were used alone the increase in no. of central square crossings was not statistically significant. There was significant increase in the rearing of animals with Fluoxetine in comparison to the control group. There was also increased number of rearing in test drug treated groups which was not statistically significant.

Figure 5: Effects of Fluoxetine and EEFL on behaviour of mice in Open field test.

No. of squares crossed (Mean±SEM)			No. of Rearings (Mean±SEM)	
Treatments	N	Centre	Periphery	Total
Control	2	10.17±1.33	82.35±0.8	92.52±2.13
Fluoxetine (20mg/kg)	5	31.16±0.4	109.06±0.3	140.39±3.97
EEFL (100mg/kg)	5	15.24±0.3	88.15±0.3	103.39±0.6
EEFL (200mg/kg)	5	19.16±0.3	88.95±0.4	108.11±0.7

Values are expressed as mean±SEM, EEFL - Ethanolic Extract of *Feronia limonia*

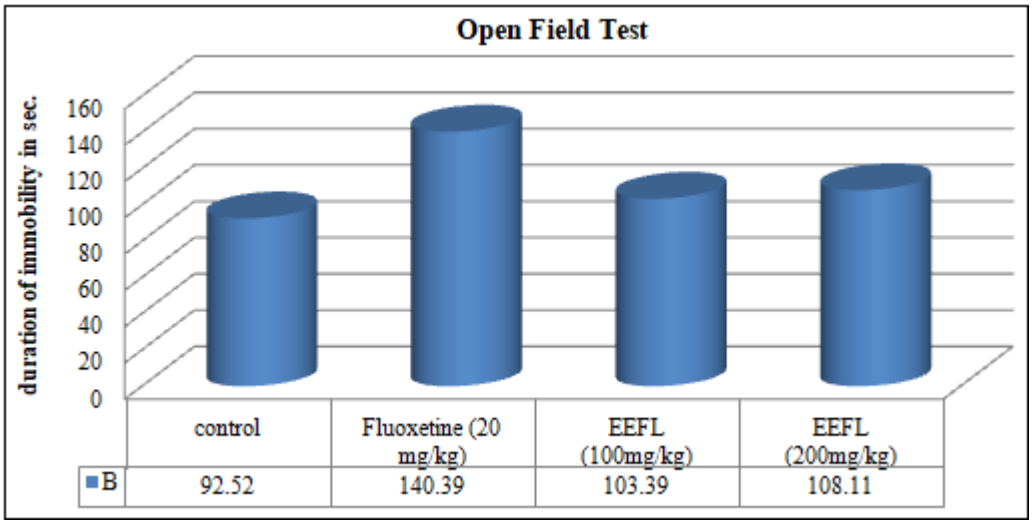

Figure 3: Effects of EEFL and Fluoxetine on duration of immobility in the OFT. Results are expressed as mean±S. E. M. compared to respective control group.

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