

To Evaluate The Antidepressant Activity Of Aqueous Extract Of Oleo Gum Resin Of 'Ferula Asafoetida', On Socially Induced Stress In Mice

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ABSTRACT

Objective:To evaluate the antidepressant-like effects of the aqueous extract of oleo gum resin of *Ferula asafoetida* (FAAE) in mice exposed to socially induced stress using behavioral and biochemical parameters. **Materials and Methods:** Adult Swiss mice were divided into four groups (n=6/group): Animals were subjected to a 14-day treatment regimen. Behavioral parameters including Forced Swim Test (FST), Tail Suspension Test (TST), and Open Field Test (OFT) were performed. Biochemical estimations included plasma corticosterone and nitrite levels. **Results:** Plasma Corticosterone Levels: FAAE significantly reduced corticosterone levels in both stressed and unstressed mice compared to control ($p<0.05$ to $p<0.001$). Forced Swim Test: FAAE decreased immobility times significantly across the 14-day period, though the effect was less than that of Imipramine. Tail Suspension Test: FAAE (300 and 600 mg/kg) significantly reduced immobility time compared to positive control ($p<0.001$), indicating antidepressant-like activity. Open Field Test: FAAE and Imipramine both showed a reduction in locomotor activity compared to control ($p<0.05$), suggesting possible CNS depressant or sedative effects. **Conclusion:**The aqueous extract of oleo gum resin of *Ferula asafoetida* exhibits significant antidepressant-like activity in socially stressed mice. This effect may be mediated through the modulation of stress hormones (corticosterone), nitric oxide levels, and behavioral despair. Although less effective than the standard antidepressant Imipramine, FAAE demonstrated notable potential for further pharmacological exploration.

INTRODUCTION

Depression is a prevalent neuropsychiatric disorder characterized by persistent low mood, anhedonia, cognitive dysfunction, and behavioral despair. According to the World Health Organization, depression is a leading cause of disability worldwide, affecting over 280 million people of all ages globally¹. While conventional antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) are widely used, they often present limitations including delayed onset of action, adverse side effects, and low efficacy in treatment-resistant cases². These limitations have prompted the exploration of alternative therapies, particularly those derived from natural sources.

Ferula asafoetida, commonly known as asafoetida, is a perennial herb belonging to the Apiaceae family. Its

oleo gum resin has been traditionally used in Ayurvedic and Unani medicine for its anti-inflammatory, antispasmodic, carminative, and neuroprotective properties^{3,4}. Recent phytochemical studies reveal that the resin contains various bioactive compounds including ferulic acid, sesquiterpene coumarins, and sulfur-containing constituents, which may contribute to its CNS-modulating effects⁵. Moreover, some studies suggest its potential in modulating neurotransmitter systems, oxidative stress, and inflammatory pathways—all of which are implicated in the pathophysiology of depression^{6,7}.

Stress is a major contributor to the development and exacerbation of depressive disorders. Chronic social stress in rodents has been extensively used as an experimental model to study depression and screen antidepressant activity of drugs⁸. Behavioral paradigms such as the Forced Swim Test (FST), Tail

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Suspension Test (TST), and Open Field Test (OFT) are reliable and widely accepted models for assessing antidepressant-like activity in rodents⁹.

Given the traditional use and pharmacological potential of *Ferula asafoetida*, the present study was designed to evaluate the antidepressant-like activity of the aqueous extract of its oleo gum resin in mice subjected to socially induced stress. Both behavioral and biochemical assessments—including corticosterone and nitrite levels—were conducted to investigate the extract's potential mechanisms of action.

MATERIAL AND METHOD

Chemicals and equipment requirement:

- Water (for Soxhlet extraction).
- p-Nitroso-N, N-dimethylaniline (for corticosterone estimation).
- Sodium hydroxide (for corticosterone estimation and Biuret method).
- Buffer solution (pH 9.8, for corticosterone estimation).
- Phenol in ethanol (for corticosterone estimation).
- Potassium ferricyanide (for corticosterone estimation).
- Sulphanilamide in m-phosphoric acid (for plasma nitrite estimation).
- N-(1-Naphthyl) ethylene diamine dihydrochloride (for plasma nitrite estimation).
- Bovine serum albumin (BSA) solution (for total protein concentration estimation).
- Surfactant (for total protein concentration estimation).

Plant Material: Oleo gum resins of *Ferula asafoetida* were obtained from Shreedha Phyto Extracts, Jaipur.

Methodology:

• Standard drug:

In this study, Imipramine was administered orally at a dose of 15 mg/kg body weight once daily to the animals in Group IV, which served as the standard group. The administration continued for 14 days, coinciding with the period of depression induction using the Chronic Unpredictable Mild Stress (CUMS) model. The dose and duration were selected based on previous studies demonstrating the efficacy of Imipramine in reversing depressive-like behaviors in rodents.

• Test component:

In this study, the aqueous extract of *Ferula asafoetida* (FAAE) 300mg/kg(orally) and 600 mg/kg (orally) daily upto 14 days was used as the test drug to evaluate its potential antidepressant and oxidative stress-modulating effects.

• Extraction Procedure of the Test Plant (*Ferula asafoetida*):

The extraction of oleo gum resin from “*Ferula asafoetida*” was conducted using the Soxhlet extraction method with water as the solvent. The procedure involved the following steps:

Preparation of Plant Material:

The oleo gum resin of *Ferula asafoetida* was obtained from a local Ayurvedic store and air-dried to remove moisture. The dried resin was then coarsely powdered using a mechanical grinder to facilitate effective extraction¹⁰.

Soxhlet Extraction:

- Approximately 50 g of the powdered resin was placed in a thimble within the Soxhlet apparatus.
- About 500 ml of distilled water was added to the round-bottom flask of the Soxhlet apparatus.
- The extraction was performed at 50°C, allowing the water to continuously cycle through the plant material. This process ensures that the bioactive compounds are efficiently leached from the



powdered resin into the solvent (Khandelwal, 2008). The extraction was continued until the solvent in the siphon tube was clear, indicating that the extraction process was complete.

- The aqueous extract was collected and concentrated using a rotary evaporator at a reduced temperature (below 50°C) to remove excess water while preserving the active constituents.
- The concentrated extract was then dried in a vacuum oven to obtain a semi-solid mass of the aqueous extract¹¹.

4.3 Procurement of Animal:

Swiss mice were procured from procured from Lovely Professional University, Phagwara, and kept in Animal House Facility, St. Soldier institute of Pharmacy, Jalandhar, Punjab, India. All animals were housed in clean polypropylene cages under controlled environmental conditions, with a 12-hour light/dark cycle, a temperature of 22±2°C, and relative humidity of 55±10%. The mice were provided with a standard pellet diet and water *ad libitum*. Animal care and use were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, ensuring ethical treatment and welfare of the animals throughout the study.

4.4 Experimental Protocol:

4.4.1 Experimental Design:

The study involved 24 Swiss mice, which were divided into four groups, each consisting of six animals. Group I, the control group, consisted of naïve animals that received a standard pellet diet and tap water *ad libitum*. This group was treated with normal saline (0.9% NaCl) at a dose of 10 ml/kg, administered orally, to serve as a baseline. Group II the test group I, received *Ferula asafoetida* aqueous extract (FAAE) at a dose of 300 mg/kg orally for 14 consecutive days. Group III, the test group II, received *Ferula asafoetida* aqueous extract (FAAE) at a dose of 600 mg/kg orally for 14 consecutive days. Group IV, the standard group, was administered Imipramine, a known antidepressant, at a dose of 15 mg/kg orally once daily. This experimental design was established to evaluate the effects of the *Ferula asafoetida* extract in comparison to the standard antidepressant and control groups, providing insights into its potential therapeutic properties.

4.4.2 Experimental protocol in In Vivo Studies:

Duration of study 14 days

EXPERIMENTAL DESIGN

Groups	Treatment	No. of animal
Group-I	Naive animal, received standard pellet diet and water <i>ad libitum</i> daily. Control group, normal saline 0.9% NaCl (10 ml/kg, or 1 ml of/100 g body weight orally.)	06
Group-II	Test group-I, received FAAE 300mg/kg (orally) daily upto 14 days.	06
Group-III	Test group-II, received FAAE 600mg/kg (orally) daily upto 14 days.	06
Group-IV	Standard group received 15mg/kg Imipramine orally daily	06

FAAE- *Ferula Asafoetida* Aqueous Extract (Test Drug)

Imipramine (Standard drug)



Total No. of animals required:

No. of the animal in each group(n) = 6

No. of groups (N) = 4

Total no. of animals required = 24

4.5 Experimental Parameter**4.5.1 Biochemical Estimation: -**

- Collection of Blood Samples**

On 15th day, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10 min using refrigerated centrifuge (Paramount scientific works, Ambala cantt, India) to separate the plasma, which was used for estimation of corticosterone levels.

- Estimation of plasma corticosterone levels:**

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartosand Pesez, 1979. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10 % solution of p-nitroso-N, N-dimethyl aniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and were let to stand at 0°C for 5 h, protected against light. To the above solution, 2.0ml of buffer for Ph9.8, 5.0 ml of Solution of phenol in ethanol and 0.50 ml of 1.0% aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20±2°C for 10 min. The solution was read at 650 nm using UV-visible spectrophotometer (UV3200 UV-VIS Spectrophotometer, Somajiguda, Hyderabad).

- Estimation of plasma nitrite levels –**

Plasma nitrite was measured by using the method of Green et al, 1982. A mixture of 1% w/v sulphanilamide in 5% aqueous solution of m-phosphoric acid (1 part) and 0.1% w/v N-(1-Naphthyl) ethylene di amine di hydrochloride (1 part) was prepared and kept at 0°C for 60 min. 0.5 ml plasma was mixed with 0.5 ml of the above mixture and kept in dark for 10 min at room temperature. The

absorbance was read at 546 nm using UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, Netherland CHRIST)¹².

4.5.2 Behavioural Estimation:

The depressant activity was examined by using the:

1. Forced swim test (FST) For the forced swim test (FST), Mice of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25±1°C. Treatment was given 60 min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect¹³

2. Tail suspension method (TST) The tail suspension method used in this study was similar to those described by Steru et al., (1985). Treatment was given 60 min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless¹⁴.

3. Open field test (OFT): The locomotor activity test was conducted using the Open Field Test (OFT) as described by Herrera-Ruiz et al., to explore potential correlations between locomotor activity and the antidepressant effects. Mice that had received treatments were subjected to the OFT 60 minutes prior to assessment in the Forced Swim Test (FST) or Tail Suspension Test (TST) to measure immobility time. Each mouse was placed individually in a 30×30×15 cm box, with the floor divided into 9 equal squares. Following



a 5-minute habituation period, the number of squares crossed with all paws, as well as grooming and rearing behaviors, were recorded for 6 minutes. The box was cleaned with 10% ethanol between trials to maintain consistency^{15,16}.

RESULT AND DISCUSSION

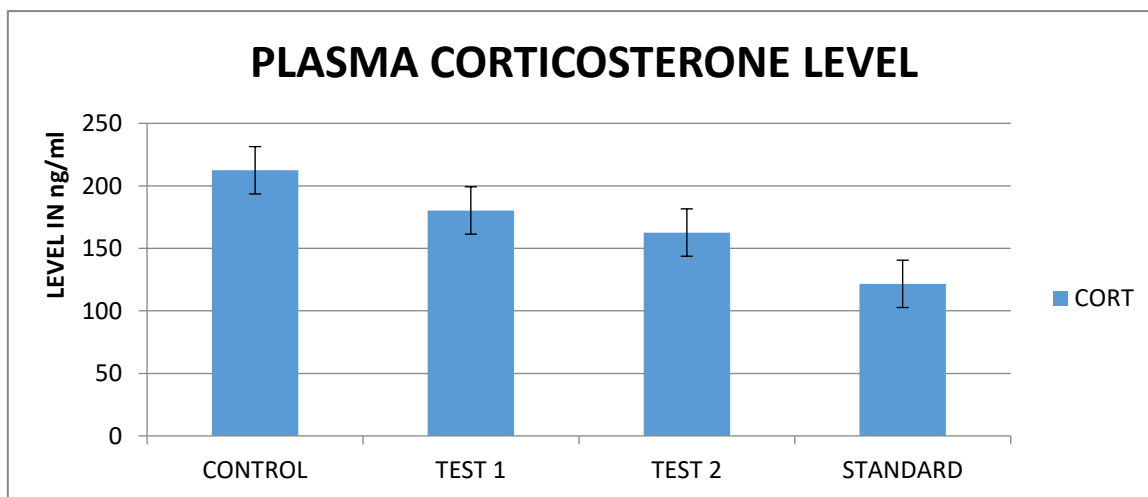
5.1 ESTIMATION OF PLASMA CORTICOSTERONE LEVELS:

Administration of the aqueous extract of *Ferula Asafoetida* for 14 consecutive days significantly reduced corticosterone levels in both unstressed and stressed mice. The reductions were statistically significant at $p < 0.05$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively, when compared to their respective vehicle-treated controls.

Table 5.1 ESTIMATION OF PLASMA CORTICOSTERONE LEVELS

Group	Plasma Corticosterone Levels ($\mu\text{g/ml}$) (Mean $\pm$ SD)
Group I (Normal)	212.45 $\pm$ 1.12
Group II (Test Drug I)	180.32 $\pm$ 0.17 ^b
Group III (Test Drug II)	162.65 $\pm$ 0.29 ^b
Group IV (Standard)	121.63 $\pm$ 0.22 ^a

Values are expressed as mean $\pm$ S.E.M. ^a denotes $p < 0.05$ as compared with to normal control group and ^b denotes $p < 0.05$ as compared to Standard drug treated group (One way ANOVA followed by Tukey's test).



Graph 5.1 Plasma Corticosterone Level

5.2 ESTIMATION OF PLASMA NITRITE LEVELS:

Plasma nitrite levels were significantly increased ($p < 0.001$) in mice exposed to chronic unpredictable mild

stress. Administration of the aqueous extract of *Ferula Asafoetida* for 14 consecutive days significantly reduced plasma nitrite levels in unstressed mice ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

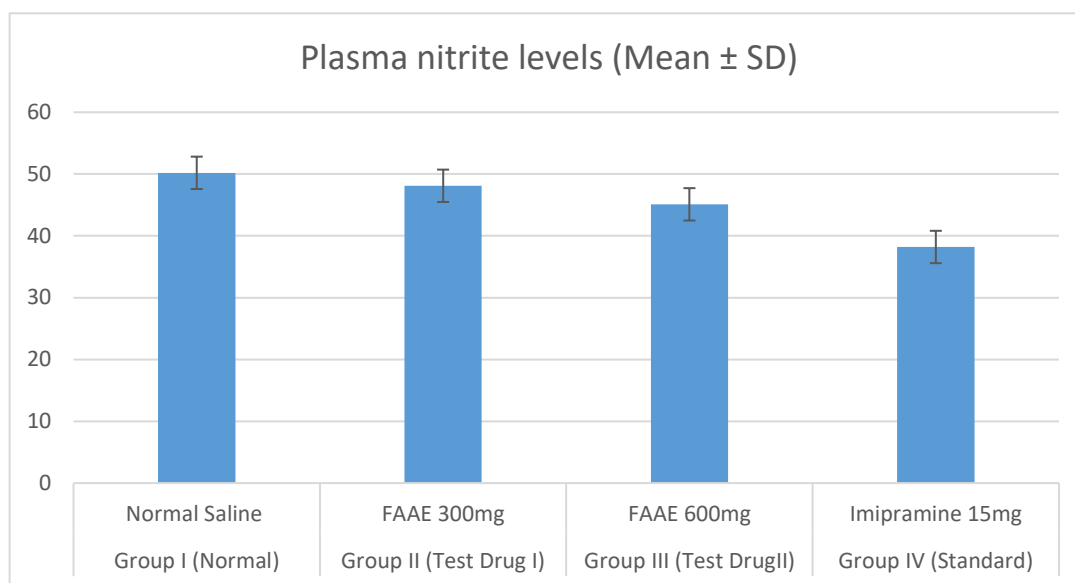
Table 5.2 Estimation of Plasma Nitrite Levels

Group	Treatment	Plasma nitrite levels (Mean $\pm$ SD)
Group I (Normal)	Normal Saline	50.2 $\pm$ 2.34
Group II (Test Drug I)	FAAE 300mg	48.1 $\pm$ 0.22 ^b
Group III (Test Drug II)	FAAE 600mg	45.1 $\pm$ 1.64 ^b
Group IV (Standard)	Imipramine 15mg	38.2 $\pm$ 1.45 ^a



Values are expressed as mean $\pm$ S.E.M. ^a denotes $p < 0.05$ as compared with to normal control group and

^b denotes $p < 0.05$ as compared to Standard drug treated group (One way ANOVA followed by Tukey's test).



Graph 5.2 Plasma Nitrite levels

5.3 ESTIMATION OF BEHAVIOUR PARAMETERS:

5.3.1 Forced Swim Test (FST): The table presents the effect of FAAE (Ferula Asafoetida Aqueous Extract) on the duration of immobility in the Forced Swim Test (FST) over 14 days. Group I (normal control) treated with normal saline showed minimal immobility, whereas Group II (Test Drug I) FAAE at 300 mg/kg

Group III (Test Drug II) FAAE at 600 mg/kg demonstrated a greater efficacy. Group IV (standard) treated with Imipramine (15 mg/kg) showed a marked reduction in immobility, confirming its antidepressant effect. The statistical significance (indicated by superscripts) suggests that FAAE possesses antidepressant-like activity, though it is less effective than Imipramine.

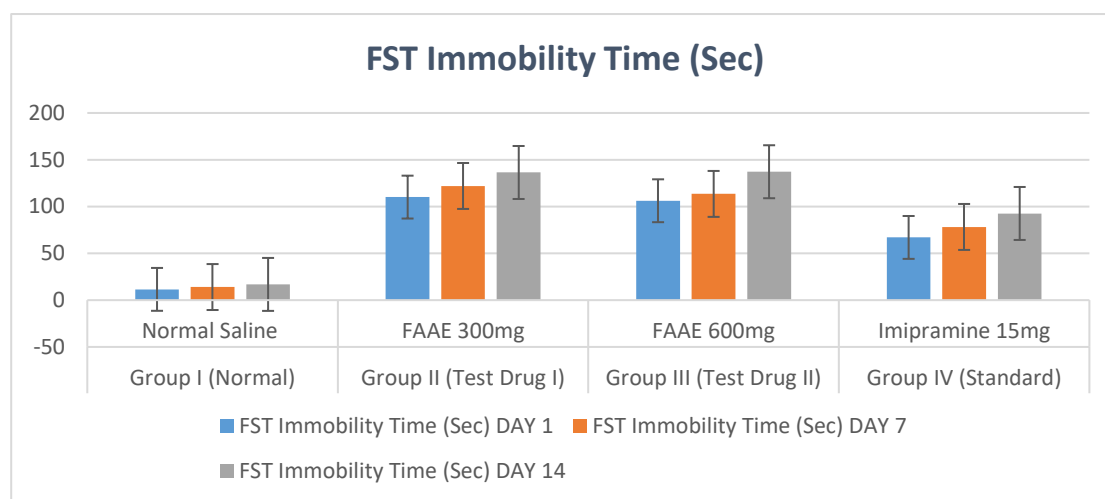
Table 5.3 Forced Swim Test

Groups	Dose (per kg b.w)	FST Immobility Time (Sec)		
		DAY 1	DAY 7	DAY 14
Group I (Normal)	Normal Saline	11.5 $\pm$ 3.725	14 $\pm$ 3.312	16.8 $\pm$ 2.393
Group II (Test Drug I)	FAAE 300mg	110.1 $\pm$ 10.54 ^b	122 $\pm$ 12.33 ^b	136.4 $\pm$ 14.55 ^b
Group III (Test Drug II)	FAAE 600mg	106.2 $\pm$ 10.77 ^b	113.5 $\pm$ 15.20 ^b	137.2 $\pm$ 12.68 ^b
Group IV (Standard)	Imipramine 15mg	67 $\pm$ 10.215 ^a	78.2 $\pm$ 25.532 ^a	92.6 $\pm$ 10.716 ^a

Values are expressed as mean $\pm$ S.E.M. ^a denotes $p < 0.05$ as compared with to normal control group and

^b denotes $p < 0.05$ as compared to Standard drug treated group (One way ANOVA followed by Tukey's test).





Graph 5.3 Forced Swim Test

5.3.2 Tail Suspension Test (TST): The table presents the effect of FAAE on immobility duration in the Tail Suspension Test (TST) over 14 days. Group I (normal control) treated with normal saline showed the lowest immobility times, while Group II FAAE at 300 mg/kg and Group III FAAE at 600 mg/kg showed a more pronounced antidepressant-like activity. Group IV

(Standard) standard) treated with Imipramine (15 mg/kg) significantly reduced immobility times across all days, confirming its antidepressant effect. Statistical significance (denoted by superscripts) suggests that FAAE, exhibits potential antidepressant effects, though it remains less effective than Imipramine.

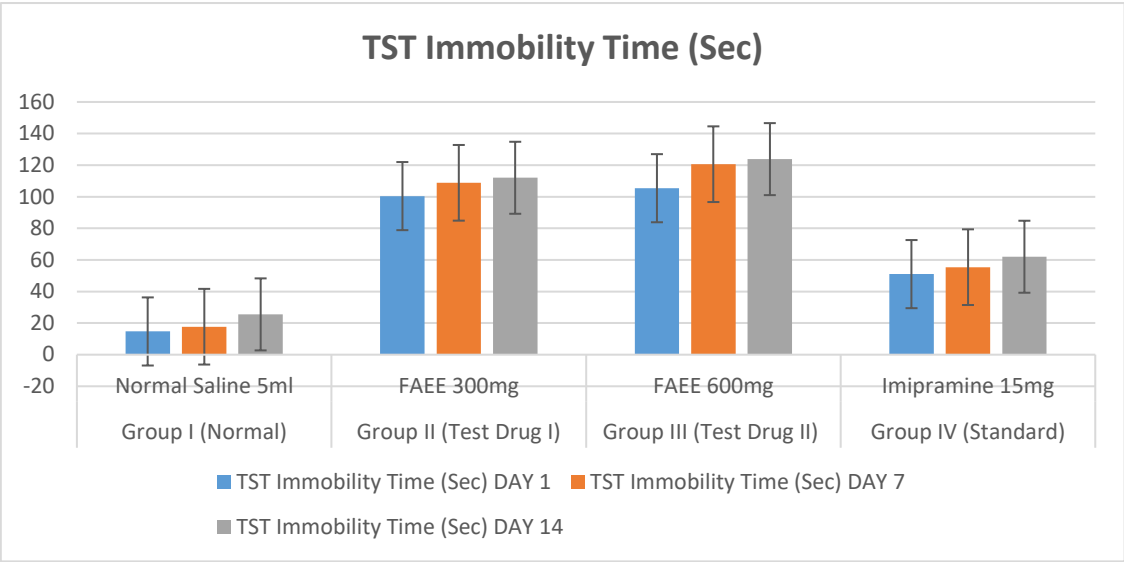
Table 5.4 Tail Suspension Test (TST)

Groups	Dose (per kg b.w)	TST Immobility Time (Sec)		
		DAY 1	DAY 7	DAY 14
Group I (Normal)	Normal Saline 5ml	14.7± 3.307	17.7± 1.818	25.5 ± 2.282
Group II (Test Drug I)	FAAE 300mg	100.4 ± 3.068 ^b	108.8 ± 6.034 ^b	112.0± 5.321 ^b
Group III (Test Drug II)	FAAE 600mg	105.4 ± 9.044 ^b	120.6 ± 4.522 ^b	123.8± 4.542 ^b
Group IV (Standard)	Imipramine 15mg	51 ± 6.588 ^a	55.4 ± 3.694 ^a	62 ± 2.121 ^a

Values are expressed as mean ± S.E.M. ^a denotes $p < 0.05$ as compared with to normal control group and

^b denotes $p < 0.05$ as compared to Standard drug treated group (One way ANOVA followed by Tukey's test).





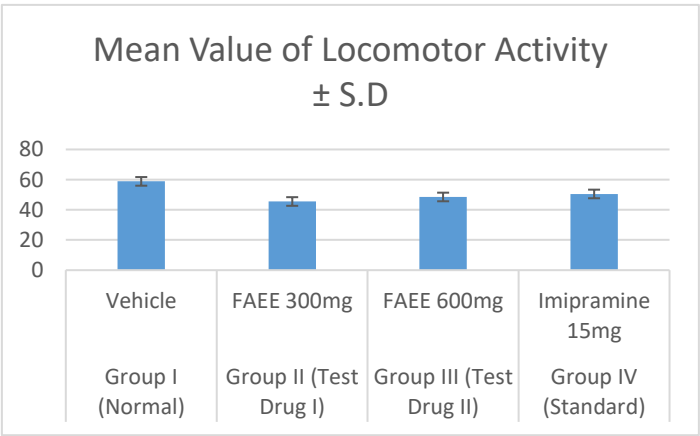
Graph 5.4 Tail Suspension Test (TST)

5.3.3 Open Field Test(OFT): The table presents the effect of FAAE and Imipramine on spontaneous locomotor activity in mice using the open field test. The control group (vehicle) showed the highest locomotor activity, while the standard group (Imipramine 15 mg/kg) also reduced locomotor activity, indicating its central nervous system depressant effect. FAAE at 300 mg/kg and 600mg/kg further decreased locomotor activity, suggesting a potential sedative-like effect. The statistical significance indicates that FAAE at the tested dose significantly altered locomotor behavior compared to both the control and standard groups.

Sample	Dose (mg/kg)	Mean Value of Locomotor Activity ± S.D
Group I (Normal)	Vehicle	58.83 ± 6.066
Group II (Test Drug I)	FAAE 300mg	45.5 ± 3.635 ^b
Group III (Test Drug II)	FAAE 600mg	48.5 ± 3.989 ^b
Group IV (Standard)	Imipramine 15mg	50.5 ± 3.730 ^a

Values are expressed as mean ± S.E.M. ^a denotes $p < 0.05$ as compared with to normal control group and ^b denotes $p < 0.05$ as compared to Standard drug treated group (One way ANOVA followed by Tukey’s test).

Table 5.5 Open Field Test (OFT)



Graph 5.5 Open Field Test (OFT)

CONCLUSION

In conclusion, the findings from this study highlight the potential of FAAE as a natural therapeutic agent for stress-related disorders. Its ability to lower corticosterone levels, reduce oxidative stress, and improve behavioral parameters suggests that FAAE may serve as an effective alternative or adjunct to conventional antidepressants. Further pharmacological and toxicological studies are necessary to fully understand its therapeutic potential and ensure its safety for clinical application.

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CONFLICTS OF INTERESTS

There are no conflicts of interest.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

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