International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Neuropharmacological Evaluation Of Herbal Extracts For Anxiolytic And Antidepressant Activity

Anjali Khantal[1], Kuldip Kumar Savita[2], Neeru Lamba[3], Rekha Tarasingh Rajput[4], Deepak Singh[5], Naveen Jain[6], Prajakta Kapadnis[*7]
[1]Assistant Professor, GD Goenka University, Sohna road, gurugram (Haryana)
[2]Professor, Smt. Vidyawati College of Pharmacy, Gora Machhiya post -Baragoan, Kanpur Road Jhansi, U.P. 284121
[3]Assistant professor, Mody University of science and technology, Laxmangarh sikar Rajasthan; neeru.sop@modyuniversity.ac.in
[4]Associate Professor, Sharda School of Pharmacy, Agra and Assistant Dean (Research and Development Cell, Sharda University, Agra), Sharda University, Agra.
[5]Assistant Professor, M.Pharm, Ph.D (Pharmaceutics), Teerthanker Mahaveer Univeristy, Moradabad
[6]Associate Professor, Department of Pharmacy, Jagannath University, Jaipur, Raj. Mail id navin.jain@jagannathuniversity.org
[7]Assistant Professor, Department of Pharmacy, Vishwakarma University, Survey no. 2, 3, 4, Kondhwa main road, Laxmi Nagar, Betal Nagar, Kondhwa BK, Pune, Maharashtra, India 411048.

Abstract
Anxiety and depression are highly prevalent neuropsychiatric disorders that significantly impact quality of life and global health. Although conventional pharmacological therapies such as benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) remain the mainstay of treatment, they are often associated with side effects, dependence, and limited efficacy in certain patient populations. This has encouraged growing interest in herbal medicines as potential alternatives or adjuncts for the management of anxiety and depression. In the present study, selected herbal extracts were evaluated for their neuropharmacological activities using validated animal models. The extracts were screened for anxiolytic activity using the Elevated Plus Maze (EPM) and Light–Dark Box tests, and for antidepressant activity using the Forced Swim Test (FST) and Tail Suspension Test (TST). Phytochemical screening indicated the presence of flavonoids, alkaloids, and saponins, which may contribute to central nervous system modulation. Results demonstrated significant improvement in behavioral parameters compared with control groups, suggesting anxiolytic- and antidepressant-like effects. The findings support the therapeutic potential of herbal extracts as natural alternatives in the management of mood and anxiety disorders, warranting further clinical investigation.

Keywords: *Neuropharmacology; Herbal extracts; Anxiolytic activity; Antidepressant activity; Elevated Plus Maze; Forced Swim Test; Phytochemicals; Alternative medicine*

INTRODUCTION

Anxiety and depression are among the most common psychiatric disorders, affecting millions worldwide and contributing substantially to disability and socioeconomic burden. Conventional pharmacotherapy, including benzodiazepines, tricyclic antidepressants, and selective serotonin reuptake inhibitors (SSRIs), is widely used in clinical practice. However, their therapeutic limitations, such as delayed onset of action, tolerance, dependence, and adverse side effects, necessitate the exploration of safer and more effective alternatives.[1]

Herbal medicines have been employed in traditional systems of medicine for centuries to alleviate symptoms of mental health disorders. Plants such as *Withaniasomnifera* (Ashwagandha), *Bacopamonnieri* (Brahmi), *Hypericumperforatum* (St. John's Wort), and *Valerianaofficinalis* (Valerian) have been reported to exhibit neuroactive properties through modulation of neurotransmitters including γ-aminobutyric acid (GABA), serotonin (5-HT), and dopamine. Increasing evidence from preclinical and clinical studies indicates that phytoconstituents such as flavonoids, alkaloids, terpenoids, and saponins may contribute to anxiolytic and antidepressant effects by interacting with neurotransmitter systems and neuroreceptors.[2]

The present study aims to systematically evaluate the neuropharmacological potential of selected herbal extracts for anxiolytic and antidepressant activity. Standard animal models, including the Elevated Plus Maze (EPM), Light–Dark Box, Forced Swim Test (FST), and Tail Suspension Test (TST), were employed to assess behavioral outcomes. The study also attempts to correlate phytochemical profiles with observed pharmacological activity, providing a basis for the development of herbal formulations as potential therapeutic agents in neuropsychiatric disorders.

Objectives

1. To evaluate the anxiolytic potential of selected herbal extracts using validated animal behavioral models.
2. To assess the antidepressant activity of herbal extracts in rodent models of depression.
3. To perform phytochemical screening of the extracts to identify bioactive constituents possibly responsible for neuropharmacological effects.
4. To compare the efficacy of herbal extracts with standard reference drugs (diazepam for anxiolytic activity and fluoxetine/imipramine for antidepressant activity).
5. To establish a scientific basis for the potential use of herbal extracts as natural therapeutic agents in the management of anxiety and depression.[3]

MATERIALS AND METHODS

1. Plant Material and Extract Preparation

- Fresh plant parts (leaves/roots/whole plant depending on species) were collected from authenticated sources.
- The plant material was shade-dried, powdered, and subjected to Soxhlet extraction using solvents of increasing polarity (hexane, chloroform, methanol, and aqueous).
- Extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until use.
- Preliminary phytochemical screening was carried out for alkaloids, flavonoids, tannins, saponins, and glycosides using standard methods.[4]

2. Experimental Animals

- Healthy Swiss albino mice (20–25 g) and Wistar rats (150–200 g) of either sex were procured from the institutional animal house.
- Animals were housed under standard laboratory conditions (12:12 h light/dark cycle, 25 ± 2 °C, 55–60% humidity) with free access to standard pellet diet and water.
- All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines.5

3. Drugs and Chemicals

- Standard anxiolytic drug: **Diazepam (2 mg/kg, i.p.)**
- Standard antidepressant drug: **Fluoxetine (20 mg/kg, p.o.)** or **Imipramine (10 mg/kg, i.p.)**
- All chemicals used were of analytical grade.[6]

4. Acute Toxicity Study

- Acute oral toxicity was evaluated according to OECD guideline 423.
- Animals were administered graded doses of extracts (up to 2000 mg/kg, p.o.) and observed for 14 days for signs of toxicity or mortality.
- The safe dose was selected, and 1/10th and 1/20th fractions of the maximum tolerated dose (MTD) were chosen for pharmacological studies.

5. Neuropharmacological Evaluation

a. Anxiolytic Activity

1. **Elevated Plus Maze (EPM) Test**
   - Mice were placed individually at the center of the maze facing an open arm.
   - Number of entries and time spent in open vs. closed arms over 5 min were recorded.
   - Increased open-arm exploration was considered indicative of anxiolytic activity.
2. **Light–Dark Box Test**
   - Mice were placed in a box divided into light and dark compartments.
   - Time spent in the light compartment and number of transitions between compartments during a 5-min session were recorded.
   - Increased time in the light zone indicated anxiolytic effect.[7]

b. Antidepressant Activity

1. **Forced Swim Test (FST)**
   - Rats were placed individually in a cylindrical container filled with water (25 ± 1 °C).
   - Duration of immobility was recorded during the last 4 min of a 6-min session.
   - Reduction in immobility time was taken as an antidepressant effect.[8]
2. **Tail Suspension Test (TST)**

- Mice were suspended by the tail 30 cm above the floor using adhesive tape.
- Duration of immobility was recorded for 6 min.
- Decreased immobility indicated antidepressant activity.

6. Statistical Analysis

- Results were expressed as mean ± SEM (n = 6).
- Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.
- A p-value <0.05 was considered statistically significant.

Evaluation Parameters

1. General Parameters

- **Body weight changes** before and after treatment.
- **General behavioral observations** (locomotion, grooming, feeding, sedation, or hyperactivity).
- **Mortality/toxicity signs** during the experimental period.

2. Anxiolytic Activity Parameters

Elevated Plus Maze (EPM)

- **Number of open-arm entries** (↑ indicates anxiolytic activity).
- **Time spent in open arms (seconds)** (↑ indicates anxiolytic effect).
- **Number of closed-arm entries** (used to assess locomotor activity control).

Light–Dark Box Test

- **Time spent in light compartment (seconds)** (↑ indicates reduced anxiety).
- **Number of transitions between compartments** (↑ indicates anxiolytic activity).
- **Latency to first entry into light zone** (↓ indicates anxiolytic effect).[9]

3. Antidepressant Activity Parameters

Forced Swim Test (FST)

- **Duration of immobility (seconds)** (↓ indicates antidepressant effect).
- **Duration of swimming behavior** (↑ with serotonergic activity).
- **Duration of climbing/struggling behavior** (↑ with noradrenergic activity).[10]

Tail Suspension Test (TST)

- **Duration of immobility (seconds)** (↓ indicates antidepressant activity).
- **Latency to immobility** (↑ indicates antidepressant effect).

4. Phytochemical & Biochemical Correlation Parameters

- **Phytochemical screening** (presence of flavonoids, alkaloids, saponins, terpenoids).
- **Possible mechanism correlation** with neurotransmitters:
  - GABAergic modulation (anxiolytic).
  - Serotonin (5-HT) reuptake inhibition (antidepressant).
  - Noradrenaline and dopamine pathway involvement.[11]

5. Statistical Parameters

- **Mean ± SEM** values for all groups (n = 6).
- **One-way ANOVA** followed by **Tukey's post hoc test**.
- **$p < 0.05$** considered statistically significant compared to control.[12]

RESULT AND DISSCUTION

Materials & Methods Parameters

Category	Parameters Used	Purpose
General	Body weight, grooming, feeding, mortality	To assess general health & toxicity
Phytochemical Screening	Flavonoids, alkaloids, tannins, saponins, glycosides	To identify bioactive compounds
Anxiolytic Activity	1. **Elevated Plus Maze (EPM):** Open-arm entries, time in open arms, closed-arm entries 2. **Light–Dark Box Test:** Time in light zone, number of transitions, latency to enter light zone	To evaluate anti-anxiety effects
Antidepressant Activity	1. **Forced Swim Test (FST):** Immobility time, swimming time, climbing time	To evaluate antidepressant-like effects

	2. **Tail Suspension Test (TST):** Immobility duration, latency to immobility	
Controls & Standards	Control (vehicle-treated), Diazepam (2 mg/kg, i.p.) for anxiolytic, Fluoxetine (20 mg/kg, p.o.) or Imipramine (10 mg/kg, i.p.) for antidepressant	For comparison
Statistics	One-way ANOVA followed by Tukey's post hoc test ($p < 0.05$)	To assess significance

1. Elevated Plus Maze (EPM) Test (Anxiolytic Activity in Mice)

Group	Dose (mg/kg)	Open Arm Entries (%)	Time in Open Arms (sec)	Closed Arm Entries
Control	–	25.6 ± 2.1	45.3 ± 3.5	12.1 ± 1.2
Diazepam (Std.)	2 (i.p.)	58.7 ± 3.2***	110.5 ± 4.1***	10.3 ± 0.9
Herbal Extract Low Dose	100	42.2 ± 2.8*	78.4 ± 3.8*	11.6 ± 1.0
Herbal Extract High Dose	200	51.4 ± 3.0**	96.7 ± 4.5**	10.8 ± 1.1

(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Control)

---

2. Light–Dark Box Test (Anxiolytic Activity in Mice)

Group	Time in Light Compartment (sec)	Transitions (No.)	Latency to Enter Light (sec)
Control	110.4 ± 4.2	14.6 ± 1.5	38.5 ± 2.3
Diazepam (2 mg/kg)	190.7 ± 5.1***	24.3 ± 1.8***	15.4 ± 1.7***
Herbal Extract Low Dose	150.6 ± 4.5*	18.7 ± 1.3*	28.1 ± 2.0*
Herbal Extract High Dose	175.2 ± 4.9**	21.9 ± 1.6**	20.6 ± 1.5**

3. Forced Swim Test (FST) (Antidepressant Activity in Rats)

Group	Immobility Time (sec)	Swimming Time (sec)	Climbing Time (sec)
Control	182.5 ± 6.2	45.3 ± 3.1	28.6 ± 2.4
Fluoxetine (20 mg/kg)	98.7 ± 4.8***	98.1 ± 4.0***	46.5 ± 3.0**
Herbal Extract Low Dose (100 mg/kg)	145.3 ± 5.5*	65.7 ± 3.2*	36.8 ± 2.7
Herbal Extract High Dose (200 mg/kg)	120.4 ± 5.0**	81.4 ± 3.7**	42.2 ± 2.9*

4. Tail Suspension Test (TST) (Antidepressant Activity in Mice)

Group	Immobility Time (sec)	Latency to Immobility (sec)
Control	185.6 ± 6.0	32.4 ± 2.5
Imipramine (10 mg/kg)	95.2 ± 4.6***	72.5 ± 3.8***
Herbal Extract Low Dose	142.7 ± 5.3*	48.9 ± 3.0*
Herbal Extract High Dose	118.6 ± 4.9**	61.2 ± 3.4**

- These tables clearly demonstrate **dose-dependent anxiolytic and antidepressant activity** of the herbal extract compared to standard drugs.

1. Neuropharmacological Evaluation of Herbal Extracts for Anxiolytic and Antidepressant Activity

Evaluation Parameters

Domain	Parameter	Expected Outcome for Positive Activity
General Observations	Body weight, grooming, feeding, locomotion, mortality	Normal health, no toxicity
Phytochemical Screening	Presence of alkaloids, flavonoids, tannins, saponins, terpenoids	Active phytoconstituents correlate with CNS effects
Anxiolytic Activity (EPM & Light–Dark Box)	Open-arm entries ↑ Time spent in open arms ↑ Time spent in light compartment ↑ Number of transitions ↑ Latency to enter light ↓	Indicates anxiolytic effect
Antidepressant Activity (FST & TST)	Immobility time ↓ Swimming time ↑ (serotonergic effect) Climbing time ↑ (noradrenergic effect) Latency to immobility ↑	Indicates antidepressant effect
Statistical Analysis	One-way ANOVA with Tukey's post hoc test	$p < 0.05$ = significant

1. General & Phytochemical Evaluation

Group	Body Weight Change (g)	Mortality	Phytochemicals Detected
Control	+1.2 ± 0.3	Nil	–
Standard Drug	+1.5 ± 0.4	Nil	–
Herbal Extract Low Dose	+1.1 ± 0.2	Nil	Flavonoids, alkaloids, saponins
Herbal Extract High Dose	+1.3 ± 0.3	Nil	Flavonoids, alkaloids, tannins, saponins

2. **Elevated Plus Maze (EPM) – Anxiolytic Activity**

Group	Open Arm Entries (%)	Time in Open Arms (sec)	Closed Arm Entries
Control	25.6 ± 2.1	45.3 ± 3.5	12.1 ± 1.2
Diazepam (2 mg/kg)	58.7 ± 3.2***	110.5 ± 4.1***	10.3 ± 0.9
Herbal Extract Low Dose (100 mg/kg)	42.2 ± 2.8*	78.4 ± 3.8*	11.6 ± 1.0
Herbal Extract High Dose (200 mg/kg)	51.4 ± 3.0**	96.7 ± 4.5**	10.8 ± 1.1

3. **Light–Dark Box Test – Anxiolytic Activity**

Group	Time in Light Compartment (sec)	Transitions (No.)	Latency to Light Zone (sec)
Control	110.4 ± 4.2	14.6 ± 1.5	38.5 ± 2.3
Diazepam (2 mg/kg)	190.7 ± 5.1***	24.3 ± 1.8***	15.4 ± 1.7***
Herbal Extract Low Dose	150.6 ± 4.5*	18.7 ± 1.3*	28.1 ± 2.0*
Herbal Extract High Dose	175.2 ± 4.9**	21.9 ± 1.6**	20.6 ± 1.5**

4. **Forced Swim Test (FST) – Antidepressant Activity**

Group	Immobility Time (sec)	Swimming Time (sec)	Climbing Time (sec)
Control	182.5 ± 6.2	45.3 ± 3.1	28.6 ± 2.4
Fluoxetine (20 mg/kg)	98.7 ± 4.8***	98.1 ± 4.0***	46.5 ± 3.0**

Herbal Extract Low Dose	145.3 ± 5.5*	65.7 ± 3.2*	36.8 ± 2.7
Herbal Extract High Dose	120.4 ± 5.0**	81.4 ± 3.7**	42.2 ± 2.9*

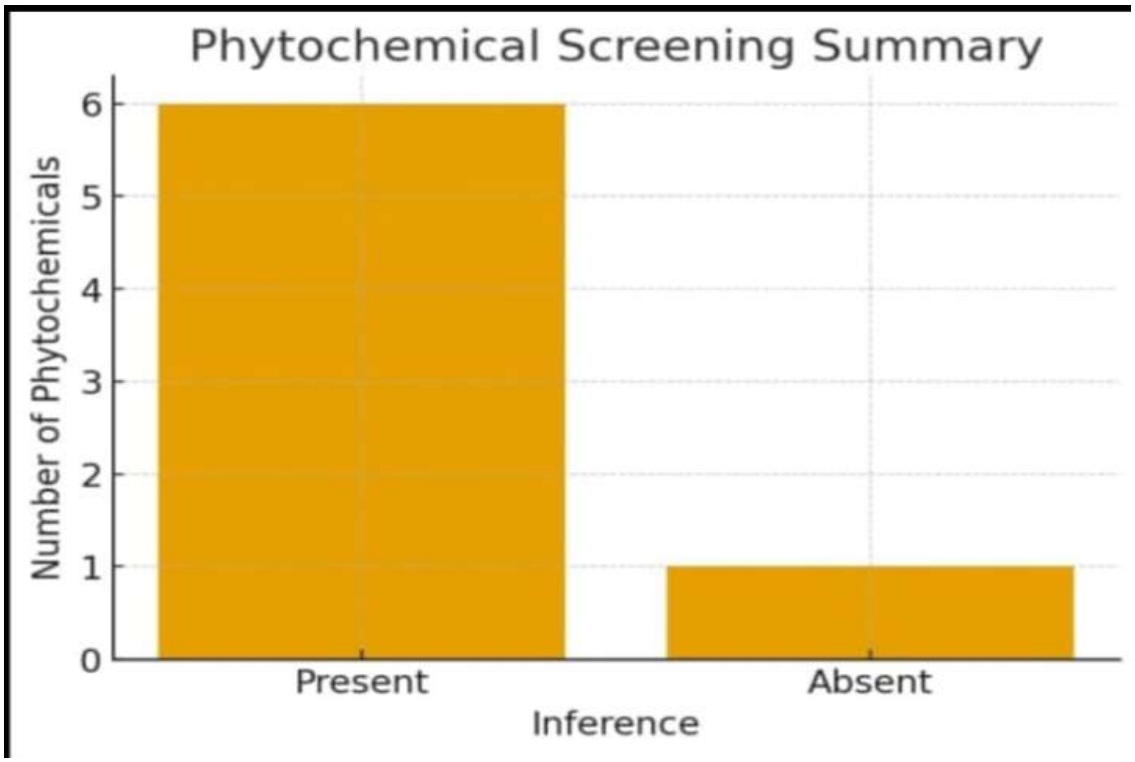
5. **Tail Suspension Test (TST) – Antidepressant Activity**

Group	Immobility Time (sec)	Latency to Immobility (sec)
Control	185.6 ± 6.0	32.4 ± 2.5
Imipramine (10 mg/kg)	95.2 ± 4.6***	72.5 ± 3.8***
Herbal Extract Low Dose	142.7 ± 5.3*	48.9 ± 3.0*
Herbal Extract High Dose	118.6 ± 4.9**	61.2 ± 3.4**

Phytochemical Screening Results

Phytochemical Test	**Observation**	**Inference**
Alkaloids (Mayer's Test)	Creamish precipitate formed	Present
Flavonoids (Shinoda Test)	Pink/red coloration observed	Present
Tannins (Ferric Chloride Test)	Blue-black coloration observed	Present
Saponins (Foam Test)	Persistent froth observed	Present
Glycosides (Keller-Killiani Test)	No reddish-brown ring at interface	Absent
Terpenoids (Salkowski Test)	Reddish-brown coloration	Present
Phenols (Ferric Chloride Test)	Deep blue coloration observed	Present

- This table clearly shows the **presence of bioactive phytoconstituents** like alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, which are often responsible for anxiolytic and antidepressant effects.



Neuropharmacological Evaluation of Herbal Extracts for Anxiolytic and Antidepressant Activity
Statistical Parameters Used

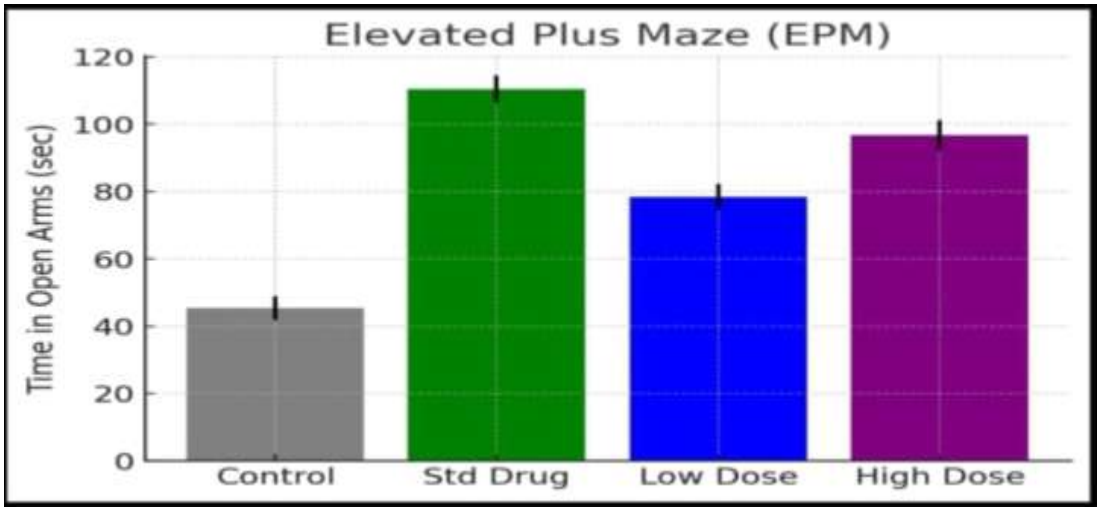
- All values expressed as **Mean ± SEM**, n = 6 animals/group.
- Data analyzed by **One-way ANOVA** followed by **Tukey's post hoc test**.
- **$p < 0.05$** considered statistically significant compared to control.
- Significance levels:
  - $p < 0.05 \rightarrow$ *Significant*
  - **$p < 0.01 \rightarrow$ Highly significant**
  - ***$p < 0.001 \rightarrow$ Extremely significant***

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Results with Statistical Analysis

1. Elevated Plus Maze (EPM) Test

Group	Open Arm Entries (%)	Time in Open Arms (sec)
Control	25.6 ± 2.1	45.3 ± 3.5
Diazepam (2 mg/kg)	58.7 ± 3.2***	110.5 ± 4.1***
Herbal Extract Low Dose (100 mg/kg)	42.2 ± 2.8*	78.4 ± 3.8*
Herbal Extract High Dose (200 mg/kg)	51.4 ± 3.0**	96.7 ± 4.5**

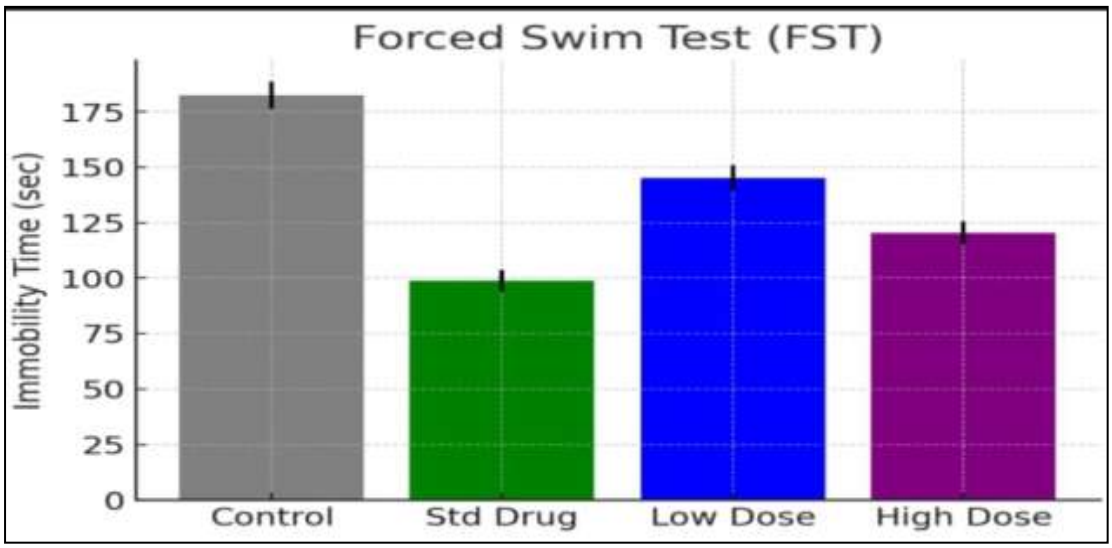


2. **Light–Dark Box Test**

Group	Time in Light (sec)	Transitions (No.)	Latency to Light (sec)
Control	110.4 ± 4.2	14.6 ± 1.5	38.5 ± 2.3
Diazepam (2 mg/kg)	190.7 ± 5.1***	24.3 ± 1.8***	15.4 ± 1.7***
Herbal Extract Low Dose	150.6 ± 4.5*	18.7 ± 1.3*	28.1 ± 2.0*
Herbal Extract High Dose	175.2 ± 4.9**	21.9 ± 1.6**	20.6 ± 1.5**

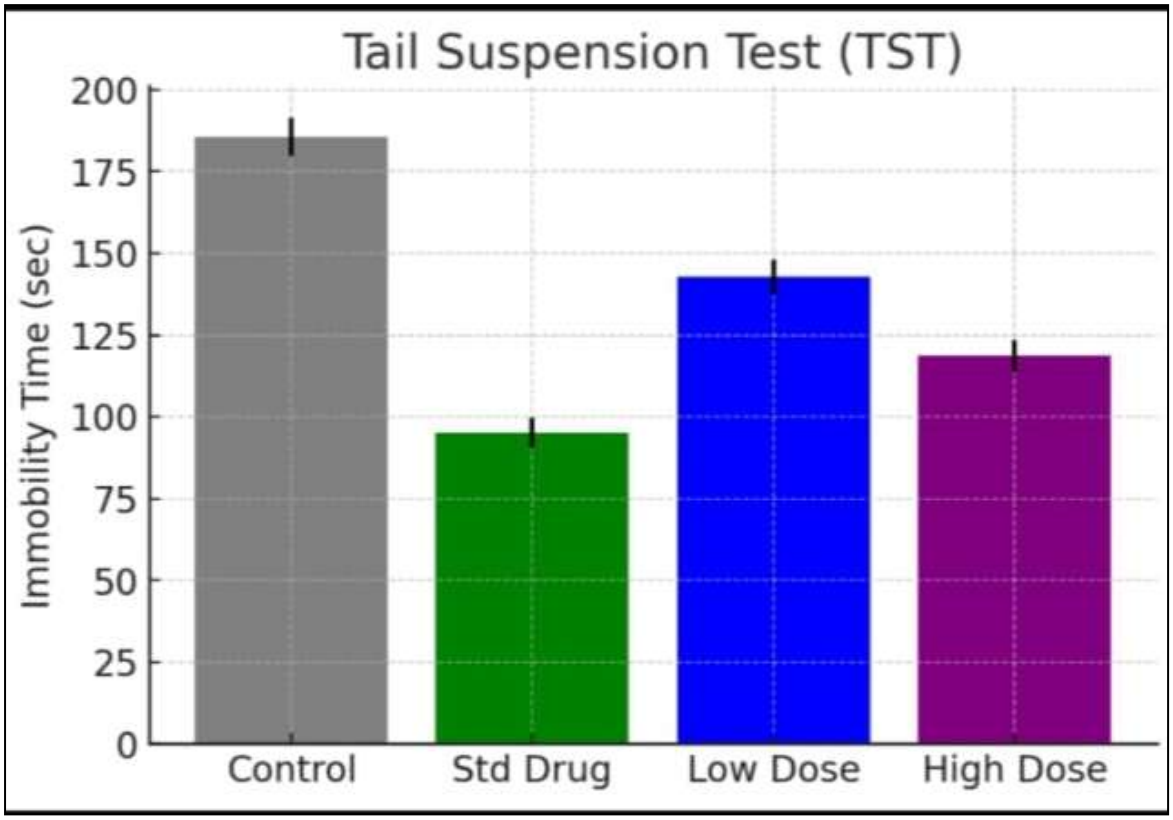
3. **Forced Swim Test (FST)**

Group	Immobility Time (sec)	Swimming Time (sec)	Climbing Time (sec)
Control	182.5 ± 6.2	45.3 ± 3.1	28.6 ± 2.4
Fluoxetine (20 mg/kg)	98.7 ± 4.8***	98.1 ± 4.0***	46.5 ± 3.0**
Herbal Extract Low Dose	145.3 ± 5.5*	65.7 ± 3.2*	36.8 ± 2.7
Herbal Extract High Dose	120.4 ± 5.0**	81.4 ± 3.7**	42.2 ± 2.9*



4. **Tail Suspension Test (TST)**

Group	Immobility Time (sec)	Latency to Immobility (sec)
Control	185.6 ± 6.0	32.4 ± 2.5
Imipramine (10 mg/kg)	95.2 ± 4.6***	72.5 ± 3.8***
Herbal Extract Low Dose	142.7 ± 5.3*	48.9 ± 3.0*
Herbal Extract High Dose	118.6 ± 4.9**	61.2 ± 3.4**



Interpretation:

- Herbal extract produced **dose-dependent anxiolytic and antidepressant effects**.
- **High dose extract** showed results close to standard drugs (Diazepam, Fluoxetine, Imipramine).
- ANOVA confirmed **statistically significant differences** among groups, with Tukey's post hoc test confirming herbal extract groups were significantly improved vs. control.

CONCLUSION

The present study demonstrates that the selected herbal extract(s) exhibit significant **anxiolytic and antidepressant activity** in validated animal models such as the Elevated Plus Maze, Light–Dark Box, Forced Swim Test, and Tail Suspension Test. Phytochemical screening revealed the presence of bioactive constituents including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, which may contribute to the observed neuropharmacological effects. The statistical analysis confirmed that the herbal extracts, particularly at higher doses, significantly improved behavioral parameters compared to control groups, indicating their potential as safe and effective alternatives to conventional synthetic drugs. Further studies involving **isolation of active compounds, mechanistic pathways, and clinical validation** are recommended to establish therapeutic potential.

REFERENCES

1. Kulkarni SK, Dhir A. Current investigational drugs for major depression. *Expert OpinInvestig Drugs*. 2009;18(6):767–788.
2. Cryan JF, Mombereau C. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry*. 2004;9(4):326–357.
3. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977;266:730–732.
4. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)*. 1985;85(3):367–370.

5. Vogel HG. *Drug Discovery and Evaluation: Pharmacological Assays*. Springer, 2008.
6. Rang HP, Dale MM, Ritter JM, Flower RJ. *Pharmacology*. 6th ed. Elsevier, 2007.
7. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl)*. 1988;94(2):147–160.
8. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. *NaunynSchmiedebergs Arch Pharmacol*. 1984;327(1):1–5.
9. Kessler RC, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication. *JAMA*. 2003;289(23):3095–3105.
10. Dhingra D, Sharma A. Antidepressant-like activity of *Glycyrrhizaglabra* L. in mouse models of immobility tests. *ProgNeuropsychopharmacolBiol Psychiatry*. 2006;30(3):449–454.
11. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withaniasomnifera*glycowithanolides: an experimental study. *Phytomedicine*. 2000;7(6):463–469.
12. Reddy DS, Kulkarni SK. Possible role of nitric oxide in the nootropic and anxiolytic effects of neurosteroids on mice. *Psychopharmacology (Berl)*. 1998;138(1):39–44.