



Neuropharmacological Evaluation of *Grewia hirsuta* Vahl. Fruit Extract for Anxiolytic and Antidepressant-Like Effects in Experimental Mice

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Account Information	ABSTRACT
Acceptance: Aug 2026 Published: September 2026 Volume 1 • Issue 1 • 2026 Keywords: <i>Grewia hirsuta</i> Vahl; hydroalcoholic extract; anxiolytic-like activity; antidepressant-like activity; elevated plus-maze; light-dark box; forced swim test; tail suspension test.	Background: Anxiety and depressive disorders are major neuropsychiatric conditions for which the search for safer and pharmacologically useful plant-derived agents remains relevant. <i>Grewia hirsuta</i> Vahl. is a medicinal plant whose fruit has not been extensively characterized for anxiolytic-like and antidepressant-like behavioural activity. Objective: The present study evaluated the neuropharmacological effects of a hydroalcoholic fruit extract of <i>G. hirsuta</i> (HEGH) in Swiss albino mice using complementary behavioural models. Methods: Fruits collected from Indore, Madhya Pradesh, were authenticated and extracted by sequential petroleum ether defatting followed by hydroalcoholic extraction. Preliminary phytochemical screening and acute oral toxicity assessment were performed. HEGH at 200 and 400 mg/kg, p.o. was evaluated in the elevated plus-maze (EPM) and light-dark box (LDB) tests for anxiolytic-like activity and in the forced swim test (FST) and tail suspension test (TST) for antidepressant-like activity. Diazepam and imipramine were used as reference standards for the respective endpoints. Results: The extract yield was 28% and phytochemical screening indicated terpenoids, flavonoids, tannins/phenolics, saponins and proteins/amino acids. No mortality or apparent acute toxicity was observed at 2000 mg/kg. HEGH 400 mg/kg increased EPM open-arm time from 40 ± 2.1 to 62 ± 3.9 s and head dips from 14 ± 1.02 to 19 ± 0.7, while LDB light-compartment time increased from 52 ± 3.2 to 111 ± 6.4 s and crossings from 10 ± 1.2 to 19 ± 3.1. In the FST and TST, immobility decreased from 125 ± 1.5 to 89 ± 3.3 s and from 105 ± 2.8 to 60 ± 4.1 s, respectively. The 200 mg/kg dose produced weaker responses. Conclusion: HEGH demonstrated a dose-related anxiolytic-like and antidepressant-like behavioural profile, with 400 mg/kg showing the most consistent activity. These findings support further phytochemical, neurochemical and mechanistic investigation, but do not establish clinical efficacy or a specific molecular mechanism.

1. INTRODUCTION

Anxiety and depressive disorders are common and clinically important conditions associated with substantial functional impairment and reduced quality of life. Pharmacological treatment is effective for many patients, but limitations related to delayed onset, adverse effects, treatment resistance and incomplete response continue to stimulate research into new therapeutic leads. Medicinal plants remain an important source of structurally

diverse secondary metabolites and bioactive molecules that may influence central nervous system pathways. Behavioural models are widely used for the initial preclinical screening of candidate anxiolytic and antidepressant agents. The elevated plus-maze (EPM) quantifies avoidance of exposed open arms and has been pharmacologically validated for detecting anxiolytic-like effects [1]. The light-dark box (LDB) similarly exploits the conflict between exploratory drive and aversion to brightly illuminated areas; increased exploration of the light compartment is commonly interpreted as an anxiolytic-like response [2]. For antidepressant-like screening, the forced swim test (FST) and tail suspension test (TST) measure stress-coping behaviour and immobility and have been extensively used for identifying compounds with antidepressant-like profiles [3,4]. Importantly, these tests are screening paradigms rather than complete models of human anxiety or depressive disorders.

Grewia hirsuta Vahl. belongs to the family Malvaceae and is used in traditional medicinal practices. Plant-derived phenolics, flavonoids, terpenoids and related constituents are of particular interest because several classes of such compounds have demonstrated neuroactive and antioxidant properties in experimental systems. However, preliminary phytochemical detection alone cannot establish which constituents are responsible for a behavioural effect. Therefore, systematic evaluation using more than one behavioural model is necessary. The present study was undertaken to characterize the hydroalcoholic fruit extract of *G. hirsuta* (HEGH), assess its acute oral tolerability, and investigate its anxiolytic-like and antidepressant-like effects in Swiss albino mice. The working hypothesis was that HEGH would modify anxiety- and stress-coping-related behaviours in a dose-dependent manner.

2. MATERIALS AND METHODS

2.1 Plant material and authentication

The fruits of *G. hirsuta* Vahl. were collected from a garden near Indore, Madhya Pradesh, India. The plant material was authenticated by Dr Sandeep K. Verma, Botanist, Government Institute of Science, with voucher number J/Bot/SLF-049. Fruits were dried, powdered and stored until extraction.

2.2 Extraction

The powdered fruit was first defatted by maceration with petroleum ether for 10 days with intermittent agitation. After filtration and removal of the petroleum ether fraction, the marc was extracted by cold maceration with a hydroalcoholic solvent containing ethanol and water (70:30, v/v) for 15 days. The mixture was filtered through Whatman filter paper and the filtrate was concentrated with controlled heating/magnetic stirring and subsequently dried. The dried hydroalcoholic extract was designated HEGH. Percentage yield was calculated as extract weight relative to starting plant-material weight. The extraction approach was based on conventional pharmacognostic procedures [5].

2.3 Preliminary phytochemical screening

Qualitative phytochemical screening was performed using conventional pharmacognostic procedures for alkaloids, terpenoids, flavonoids, tannins/phenolic compounds, saponins, proteins/amino acids, glycosides and fats/lipids [6,7].

2.4 Acute oral toxicity

Acute oral toxicity was assessed according to OECD Test Guideline 425, the Acute Oral Toxicity—Up-and-Down Procedure [8]. Animals were observed closely during the first 4 h after dosing and daily thereafter for 14 days for mortality, changes in behaviour, locomotor activity, posture, respiration, skin/fur and food/water consumption. Body weight and gross pathological findings were also considered. A single oral dose of 2000

mg/kg was reported to produce no mortality or apparent treatment-related toxicity. Lower doses were therefore selected for behavioural studies.

2.5 Experimental animals and study design

Swiss albino mice weighing approximately 20–40 g were randomly allocated to treatment groups (n=5/group). A vehicle-treated group served as control. Diazepam was used as the reference standard for anxiolytic experiments and imipramine was used as the reference standard for antidepressant-like experiments. HEGH was administered orally at 200 and 400 mg/kg. The source document reports imipramine as 20 mg/kg in the study-design section and 15 mg/kg in the antidepressant results table; the present article retains the dose reported with the outcome table (15 mg/kg) and this protocol discrepancy should be verified against the laboratory record before submission.

2.6 Elevated plus-maze

The EPM consisted of two open arms (50×10 cm), two closed arms (50×10×40 cm) and a central platform (10×10 cm), elevated approximately 50 cm above the floor. Each mouse was placed on the central platform and observed for 5 min. Open-arm time, closed-arm time and head-dip behaviour were recorded. Increased open-arm exploration was interpreted as an anxiolytic-like response [1].

2.7 Light-dark box

The LDB consisted of interconnected illuminated and dark compartments with an approximately 6×6 cm opening. Animals were placed in the illuminated compartment and observed for 5 min. Time spent in the light compartment and the number of crossings were recorded. Increased light-compartment exploration was interpreted as an anxiolytic-like response [2].

2.8 Forced swim test

The FST followed an established mouse protocol with a 15-min pre-test and a 6-min test session separated by 24 h [3]. Immobility was scored during the test session, with immobility defined as the absence of active escape-directed movement other than minimal movement necessary to keep the head above water. Reduced immobility relative to vehicle control was interpreted as antidepressant-like activity.

2.9 Tail suspension test

In the TST, mice were suspended individually by adhesive tape positioned near the tail tip and observed for 6 min. Total immobility time was recorded. Reduced immobility compared with vehicle control was interpreted as an antidepressant-like behavioural response [4].

2.10 Statistical analysis

Results are expressed as mean±SD (n=5/group). One-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test was reported for behavioural comparisons. Statistical significance was interpreted at p<0.05, p<0.01 and p<0.001. The statistical approach and significance labels should be checked against the original statistical output before journal submission.

3. RESULTS

3.1 Pharmacognostic and extract characteristics Qualitative screening showed terpenoids, flavonoids, tannins/phenolic compounds, saponins and proteins/amino acids, while alkaloids, glycosides and fats/lipids were not detected.

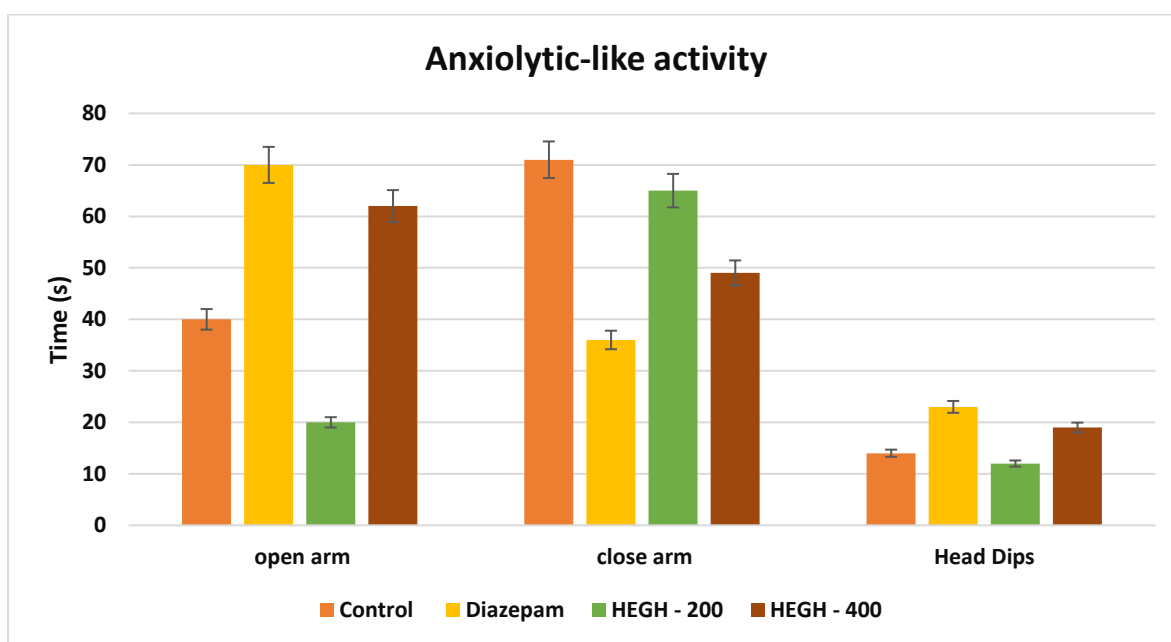
Parameter	Observation
Colour	Reddish brown
Odour	Aromatic
Texture	Smooth
Foreign matter	2.9%
Loss on drying	0.70%
Total ash	7.1–7.5%
HEGH yield	28%
HEGH appearance	Light brown, aromatic, smooth

3.2 Acute oral toxicity

No mortality or apparent treatment-related clinical toxicity was observed after oral administration of HEGH at 2000 mg/kg during the observation period. Animals maintained normal general behaviour, locomotor activity, feeding, drinking and appearance. The finding supports selection of lower doses for pharmacological screening; however, it should be interpreted as an acute tolerability observation rather than proof of long-term safety [8].

3.3 Anxiolytic-like activity

Group	EPM open-arm time (s)	EPM closed-arm time (s)	Head dips
Control	40±2.1	71±2.3	14±1.02
Diazepam	70±3.4**	36±2.6**	23±1.09**
HEGH 200 mg/kg	20±6.5	65±5.9	12±1.2
HEGH 400 mg/kg	62±3.9*	49±3.5*	19±0.7**

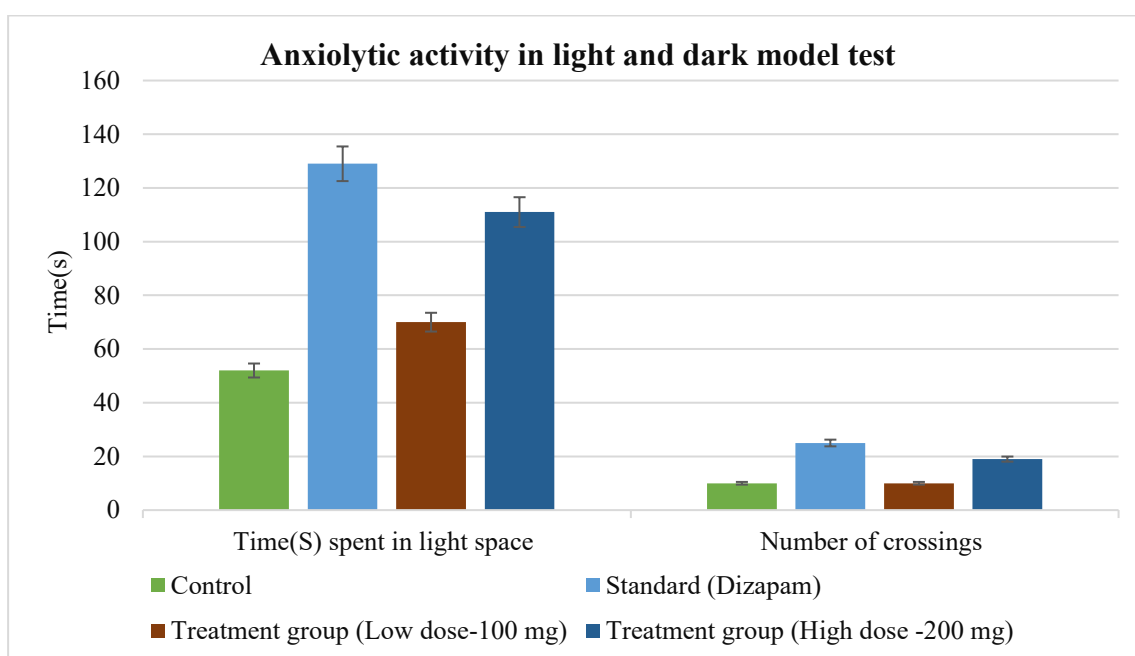


In the EPM, HEGH 400 mg/kg increased open-arm time and head-dip behaviour while reducing closed-arm time relative to control. The 200 mg/kg dose did not reproduce the higher-dose response. The magnitude of

the 400 mg/kg response approached diazepam for open-arm time and head dips, although the standard remained more active.

Group	Light time (s)	Crossings
Control	52±3.2	10±1.2
Diazepam	129±4.6	25±1.9
HEGH 200 mg/kg	70±4.5	10±3.8
HEGH 400 mg/kg	111±6.4	19±3.1

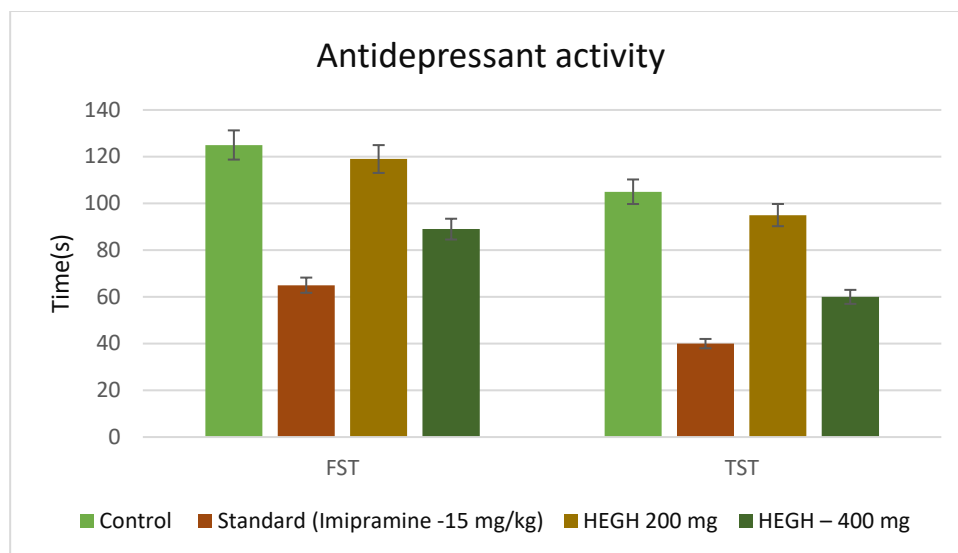
In the LDB, HEGH 400 mg/kg increased time spent in the light compartment and the number of transitions compared with control. The 200 mg/kg dose produced a smaller change in light-compartment time and little change in crossings. Concordant responses in the EPM and LDB support an anxiolytic-like behavioural profile at the higher dose.



3.4 Antidepressant-like activity

Group	FST immobility (s)	TST immobility (s)
Control	125±1.5	105±2.8
Imipramine 15 mg/kg	65±2.7	40±3.1
HEGH 200 mg/kg	119±6.4	95±6.7
HEGH 400 mg/kg	89±3.3	60±4.1

HEGH 400 mg/kg reduced immobility in both the FST and TST compared with vehicle control, whereas the 200 mg/kg dose produced only modest reductions. The reported statistical analysis indicated significance for the 400 mg/kg dose in both tests, while the lower dose was not significant. The standard drug produced the largest reduction in immobility.



4. DISCUSSION

The present study provides preliminary evidence that HEGH possesses anxiolytic-like and antidepressant-like behavioural activity in mice. The pharmacological signal was dose dependent, with 400 mg/kg consistently outperforming 200 mg/kg across the major behavioural endpoints. Importantly, the activity was reproduced in two independent anxiety paradigms and two antidepressant-like screening paradigms, reducing the likelihood that the finding is confined to a single test. The EPM results are consistent with the established interpretation that increased open-arm exploration reflects reduced anxiety-like avoidance [1]. HEGH 400 mg/kg increased open-arm time from 40 to 62 s and head dips from 14 to 19, while closed-arm time declined from 71 to 49 s. The LDB provided convergent evidence: light-compartment time increased from 52 to 111 s and crossings from 10 to 19. Because locomotor activation can influence performance in anxiety paradigms, future studies should include an open-field or equivalent locomotor assessment before attributing all changes exclusively to anxiolysis. The antidepressant-like findings were also internally consistent. HEGH 400 mg/kg decreased FST immobility from 125 to 89 s and TST immobility from 105 to 60 s. Both tests are established screening tools, and decreased immobility is a common response to clinically effective antidepressant drugs [3,4]. Nevertheless, modern interpretation emphasizes that FST and TST assess stress-coping behaviour and should not be equated directly with human depression. The current results therefore support the more cautious terminology 'antidepressant-like activity'. The phytochemical profile offers a plausible starting point for future mechanistic work. Flavonoids and phenolic compounds are particularly relevant because plant polyphenols have been investigated for neuroprotective and antidepressant-like effects in experimental models [9]. However, the present qualitative screening does not identify individual compounds or establish exposure-response relationships. Chemical fingerprinting by HPLC/LC-MS, isolation of active constituents, and bioactivity-guided fractionation are consequently important next steps. The absence of mortality or obvious acute toxicity at 2000 mg/kg provides supportive evidence for acute tolerability and is consistent with the purpose of OECD TG 425, which is designed to characterize acute oral toxicity while minimizing animal use [8]. It does not establish repeated-dose safety, reproductive safety, genotoxicity or clinical safety. Additional toxicological studies would be required before any translational conclusions.

Mechanistically, the current study cannot determine whether the behavioural effects arise from GABAergic, serotonergic, noradrenergic, dopaminergic, glutamatergic, antioxidant or other pathways. Such hypotheses should therefore remain provisional. Receptor-antagonist studies, neurotransmitter estimation, enzyme assays, oxidative-stress biomarkers and molecular investigations would provide a stronger mechanistic basis. The study should also be strengthened by preregistered/randomized allocation, blinded behavioural scoring,

explicit treatment-to-test intervals, environmental standardization and reporting consistent with contemporary animal-research reporting principles. The principal limitations are the small group size (n=5), qualitative rather than quantitative phytochemical characterization, absence of a dedicated locomotor-control experiment, and lack of mechanistic or repeated-dose toxicity studies. In addition, the source document contains protocol inconsistencies concerning some doses (notably imipramine and the lower EPM/LDB extract dose) and these should be reconciled with the original laboratory records before submission. The present article therefore treats the reported results as preliminary rather than definitive evidence.

5. CONCLUSION

The hydroalcoholic fruit extract of *G. hirsuta* Vahl. demonstrated a promising, dose-related anxiolytic-like and antidepressant-like behavioural profile in Swiss albino mice. HEGH 400 mg/kg produced the most consistent effects in the EPM and LDB and reduced immobility in both the FST and TST. The extract showed a 28% yield and contained several classes of phytoconstituents, while acute oral administration at 2000 mg/kg produced no mortality or apparent acute toxicity. The findings justify further investigation of *G. hirsuta* fruit using quantitative phytochemical profiling, bioactivity-guided fractionation, locomotor controls, neurochemical studies and receptor-specific mechanistic experiments.

6. REFERENCES

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Ethics statement

The source document describes an animal behavioural study and indicates that experiments were conducted under an approved institutional animal-ethics framework. CCSEA-IAEC approval was taken from swami Vivekanad college of Pharmacy, Indore

Conflict of interest

The authors should declare any financial or personal relationships that could influence the work. No specific conflict-of-interest statement was provided in the supplied manuscript.

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