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Anxiolytic and Antidepressant-like Effects of *Desmodium triflorum* Extract Against Chronic Restraint Stress in Wistar Rats: Behavioral, Neurochemical, and Antioxidant Evidence

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Background: Anxiety and depression are among the most prevalent psychiatric disorders worldwide and are associated with dysregulation of monoaminergic neurotransmission, hypothalamic-pituitary-adrenal (HPA) axis hyperactivity, and oxidative stress. Conventional pharmacotherapies, including benzodiazepines and tricyclic antidepressants, are limited by dependence, withdrawal effects, and poor long-term tolerability, highlighting the need for safer plant-derived alternatives such as *Desmodium triflorum*.

Objective: This study evaluated the anxiolytic and antidepressant-like potential of aqueous *Desmodium triflorum* extract in a chronic restraint stress (CRS) model in Wistar rats and investigated the neurochemical, neuroendocrine, and antioxidant mechanisms underlying its effects.

Methods: Adult Wistar rats were exposed to 3 h of daily immobilization stress for 30 days and treated with vehicle, *Desmodium triflorum* extract (400 or 800 mg/kg BW, orally), diazepam (2 mg/kg), or imipramine (15 mg/kg). Behavioral assessments included the elevated plus-maze, forced swim, and open field tests. Blood cortisol, brain dopamine (DA), serotonin (5-HT), oxidative stress markers, and hepatic histopathology were evaluated.

Results: CRS induced significant anxiety- and depressive-like behaviors, elevated cortisol, altered cortical and hippocampal DA and 5-HT levels, and reduced antioxidant defenses. *Desmodium triflorum* extract produced dose-dependent improvements, with the 800 mg/kg dose significantly increasing open-arm exploration, reducing immobility, lowering cortisol, restoring monoamine levels, and enhancing glutathione, superoxide dismutase, and catalase activities. Hepatic histopathology showed no treatment-related abnormalities.

Conclusion: Aqueous *Desmodium triflorum* extract exerts dose-dependent anxiolytic and antidepressant-like effects through monoaminergic modulation, HPA-axis normalization, and antioxidant restoration, with a favorable hepatic safety profile. These findings support further mechanistic and translational investigation of *Desmodium triflorum* as a potential phytotherapeutic for stress-related psychiatric disorders.

Keywords: *Desmodium triflorum*; Anxiolytic; Antidepressant; Chronic restraint stress; Monoamine neurotransmitters; Oxidative stress; Hypothalamic-pituitary-adrenal axis; Wistar rats

1. INTRODUCTION

Anxiety and depressive disorders represent two of the most prevalent and disabling categories of psychiatric illness worldwide, characterized by excessive fear and

worry, anhedonia, cognitive impairment, and behavioral disturbance [1,2]. These conditions frequently co-occur, compounding disease burden and complicating treatment response [3]. Despite their high global prevalence, anxiety and depression remain substantially underdiagnosed and

undertreated, particularly in low- and middle-income settings, owing to persistent stigma, shortages of trained mental health professionals, and inadequate healthcare infrastructure [1]. Current first-line pharmacotherapy relies on antidepressants, anxiolytics, antipsychotics, and mood stabilizers targeting the serotonergic (5-HT), γ -aminobutyric acid (GABA), noradrenergic, and dopaminergic (DA) systems [4]. Although effective for many patients, these agents are frequently associated with adverse effects, dependence potential, withdrawal phenomena, and limited long-term efficacy, resulting in suboptimal adherence and treatment resistance in a substantial proportion of individuals [4]. These limitations have renewed scientific interest in medicinal plants as adjunctive or alternative therapeutic strategies, particularly within ethnopharmacological traditions where botanical remedies for mood disorders have long-established use [5]. Mechanistically, stress-related psychiatric disorders are underpinned by an interconnected network of neurobiological disturbances, including monoaminergic dysregulation, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, and heightened oxidative and neuroinflammatory burden [6,7]. Chronic activation of the HPA axis elevates circulating glucocorticoids, which in turn promote oxidative stress and disrupt monoaminergic neurotransmission within limbic structures such as the cerebral cortex and hippocampus [6]. Plant-derived bioactive compounds capable of modulating one or more of these interconnected pathways neurotransmitter systems, oxidative stress cascades, and neuroendocrine signaling are therefore of particular pharmacological interest for the management of anxiety and depression.

Desmodium triflorum (L.) DC., a leguminous plant used traditionally in several Asian ethnomedical systems with well-documented phytochemical diversity and pharmacological potential [8], contains flavonoid and polyphenolic constituents, including quercetin, that have documented neuroprotective, antioxidant, and central nervous system-modulating properties [9,10]. Related *Desmodium* species have demonstrated notable free-radical scavenging and antioxidant activity in preclinical models [11], suggesting that *D. triflorum* may possess comparable bioactivity relevant to stress-related neuropsychiatric dysfunction. However, the anxiolytic and antidepressant potential of *D. triflorum* has not been systematically evaluated using validated behavioral, neurochemical, and neuroendocrine endpoints in a chronic stress paradigm.

The chronic restraint stress (CRS) paradigm is a well-validated rodent model that reproduces core behavioral, neurochemical, and physiological features of human chronic stress exposure, including anxiety- and depressive-like

behavior, HPA-axis hyperactivity, and monoaminergic and oxidative disturbances [12,13]. A recent systematic review and meta-analysis specifically confirmed that CRS reliably induces anhedonic- and depressive-like phenotypes across rodent species, supporting its continued use as a translationally relevant stress model [13]. Accordingly, the present study employed a 30-day CRS protocol in Wistar rats to evaluate the anxiolytic and antidepressant-like efficacy of aqueous *D. triflorum* extract at two dose levels (400 and 800 mg/kg BW), benchmarked against the standard anxiolytic diazepam and the standard antidepressant imipramine. Behavioral outcomes (EPMT, FST, OFT) were integrated with biochemical assessment of blood cortisol, cortical and hippocampal DA and 5-HT levels, and oxidative stress biomarkers, together with hepatic histopathological evaluation, to provide a mechanistically grounded assessment of the therapeutic potential of *D. triflorum* and to validate its traditional use in the management of stress-related mood disorders.

2. MATERIALS AND METHODS

2.1. Plant Material and Chemicals

A standardized aqueous extract of *Desmodium triflorum* Linn. was procured from Shivay Herbs and Healthcare (Jaipur, Rajasthan, India) and stored at -20°C until use. The extract was stored at -20°C to preserve the integrity of its bioactive constituents, specifically its flavonoid and polyphenolic compounds. Aqueous plant extracts are susceptible to microbial growth, enzymatic degradation, and oxidative processes at room temperature or even 4°C ; freezing at -20°C is a standard practice to maintain the stability of these sensitive phytochemicals. The experimental protocol required 30 consecutive days of daily administration during the chronic restraint stress (CRS) period. Diazepam (Sun Pharmaceutical Industries Ltd., India), imipramine (Intas Pharmaceuticals Ltd., India), and carboxymethylcellulose (CMC; HiMedia Laboratories, India) were procured for experimental use. All other reagents used for biochemical assays were of analytical grade.

2.2. Experimental Animals

Adult Wistar rats (200–250 g) were housed in standard polypropylene cages under controlled environmental conditions (12 h light/dark cycle, $22 \pm 2^{\circ}\text{C}$, 50–60% relative humidity) with free access to a standard pellet diet and water ad libitum. All experimental procedures were approved by the Institutional Animal Ethics Committee in accordance with the

guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India (approval no. ITS/02/IAEC/2024) [14].

2.3. Experimental Design and Grouping

Rats were randomly allocated into six experimental groups (n = 6 per group), as summarized in Table 1: (I) normal control receiving 0.9% normal saline vehicle without stress exposure;

(II) stress control (negative control) receiving vehicle under CRS; (III) positive control I, receiving imipramine (15 mg/kg, p.o.) under CRS; (IV) positive control II, receiving diazepam (2 mg/kg, p.o.) under CRS; (V) treatment group I, receiving *D. triflorum* extract (400 mg/kg BW, p.o.) under CRS; and (VI) treatment group II, receiving *D. triflorum* extract (800 mg/kg BW, p.o.) under CRS.

Table 1. Experimental grouping and treatment protocol.

Group	Treatment / Drug Dose	n
Control	0.9% normal saline (vehicle), no stress	6
Negative control (NC)	Chronic restraint stress + vehicle	6
Positive control I	Chronic restraint stress + imipramine (15 mg/kg, p.o.)	6
Positive control II	Chronic restraint stress + diazepam (2 mg/kg, p.o.)	6
Treatment group I	Chronic restraint stress + <i>D. triflorum</i> extract (400 mg/kg, p.o.)	6
Treatment group II	Chronic restraint stress + <i>D. triflorum</i> extract (800 mg/kg, p.o.)	6

Data are group identifiers as originally designated (n = 6 animals/group); total N = 36.

2.4. Induction of Chronic Restraint Stress

Anxiety- and depressive-like phenotypes were induced using a chronic restraint stress (CRS) protocol over 30 consecutive days [12]. All treatments (saline, imipramine, diazepam, or *D. triflorum* extract) were administered once daily by oral gavage between 09:00 and 10:00 h. Thirty minutes after drug administration, animals in all stress-exposed groups (Groups II–VI) were subjected to immobilization stress for exactly 3 h per day at a consistent time, using well-ventilated restrainers, for 30 consecutive days without interruption; the normal control group was handled identically but not restrained. This fixed-schedule immobilization protocol reproduces the behavioral, neurochemical, and neuroendocrine alterations characteristic of chronic stress exposure in humans [12]. Food and water intake were monitored daily in all groups to track changes in metabolic status. Behavioral assessments (EPMT, FST, OFT) were performed on Days 1, 15, and 30 of the protocol.

2.5. Behavioral Assessment

2.5.1. Elevated Plus-Maze Test (EPMT)

Anxiolytic-like activity was assessed using the EPMT, comprising two enclosed arms (50 × 10 × 40 cm), two open arms (50 × 10 cm), and a central platform (10 × 10 cm) elevated 50 cm above the floor [15]. Each rat was placed at the center facing an open arm and allowed to explore freely for 5 min under video recording. An arm entry was defined as placement of all four paws within that arm. The number of open-arm entries and time spent in the open arms were recorded, with higher values indicating reduced anxiety-like behavior. The apparatus was cleaned with 70% ethanol between trials to eliminate olfactory cues.

2.5.2. Forced Swim Test (FST)

Depressive-like behavior was evaluated using the FST, originally described by Porsolt et al. [16], a validated model for antidepressant screening [17]. Rats were individually placed in a glass cylinder (50 cm height × 22 cm diameter) containing water to a depth of 33 cm at 25 °C and allowed to swim for 5 min under video recording. Immobility was

defined as floating without struggling, with only movements necessary to keep the head above water; climbing behavior was defined as vigorous forepaw movements against the cylinder wall. Reduced immobility time and increased climbing time relative to the stress control group were interpreted as antidepressant-like activity.

2.5.3. Open Field Test (OFT)

General locomotor and exploratory activity were assessed using the OFT [18], employing a clear Plexiglas arena (40 × 40 × 40 cm) with the floor divided into 16 equal squares. Each rat was placed at the center and allowed to explore freely for 5 min. Crossing was defined as movement of all four paws into an adjacent square, and rearing was defined as the animal standing on its hind limbs. This test enabled discrimination between genuine anxiolytic/antidepressant activity and non-specific sedative or psychostimulant effects.

2.6. Biochemical Estimations

Following completion of behavioral testing on Day 30, animals were sacrificed by cervical dislocation under light anesthesia. Whole brains were rapidly excised, rinsed in ice-cold saline, blotted, weighed, and homogenized (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4) using a glass-Teflon homogenizer. Homogenates were centrifuged at 10,000 rpm for 15 min at 4 °C, and the resulting supernatant was used for the estimation of oxidative stress markers.

2.6.1. Oxidative Stress Markers

Lipid peroxidation was quantified by measuring malondialdehyde (MDA) formation using the thiobarbituric acid reactive substances (TBARS) assay, in which homogenate was reacted with thiobarbituric acid, acetic acid, and sodium dodecyl sulfate at 95 °C for 60 min [19]. Reduced glutathione (GSH) content was determined using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB); homogenate was deproteinized with 5% trichloroacetic acid, and the supernatant was reacted with DTNB and phosphate buffer, with the resulting yellow chromophore measured spectrophotometrically at 412 nm [20]. Superoxide dismutase (SOD) activity was assessed by its capacity to inhibit pyrogallol autoxidation [21]. Catalase (CAT) activity was determined by monitoring the rate of decomposition of hydrogen peroxide (H₂O₂), recorded as the decline in absorbance at 240 nm over 1 min [22].

2.7. Measurement of Brain Monoamine Neurotransmitter Levels

After behavioral testing on Day 30, animals were sacrificed and brains rapidly collected and stored at –80 °C. The cerebral cortex and hippocampus were dissected, weighed, and homogenized in phosphate-buffered saline (tissue weight [g]:PBS volume [mL] = 1:9) using a glass homogenizer on ice, followed by centrifugation at 5000 × g for 5 min at 4 °C. Dopamine (DA) and serotonin (5-HT) concentrations in the resulting supernatants were quantified by enzyme-linked immunosorbent assay (ELISA); intra-assay coefficients of variation for DA and 5-HT were both <10%, with assay sensitivities of 18.75 pg/mL and 9.38 ng/mL, respectively.

2.8. Blood Cortisol Estimation

Blood samples were collected on Day 30 to determine plasma cortisol concentrations as a biomarker of HPA-axis activation, given the well-established role of cortisol in mediating the physiological stress response [23,24]. Blood was collected from the heart and centrifuged (at 1500 × g for 15 min) to obtain the supernatant, which was then stored at –80 °C. These samples were analyzed within 30 days of storage. Cortisol levels were determined in duplicate using a 1:100 serum dilution and a commercially available kit (GENLISA Rat Cortisol ELISA Kit).

2.9. Histopathological Evaluation

At the end of the study, livers and brain were harvested from all animals to evaluate the systemic safety of *D. triflorum* extract. The right hepatic lobe from each rat was fixed in 4% paraformaldehyde for 7 days and processed for paraffin embedding. Sections (5 µm) were cut using a microtome and stained with hematoxylin and eosin (H&E) for 10 min at room temperature. Liver and brain morphology was examined under a Carl Zeiss AxioScope fluorescence microscope (Rushmore Precision Co., Ltd.) equipped with ZEN 2.3 (blue edition) software at 10× magnification.

2.10. Statistical Analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). Group differences were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc test, using SPSS Statistics version 22 and GraphPad Prism version 10. A probability value of $p < 0.05$ was considered statistically significant.

3. RESULTS

Daily monitoring of physiological parameters confirmed that food and water intake remained stable across all experimental groups throughout the 30-day study period. No significant differences in metabolic status were observed between the normal control, stress control, or treatment groups ($p > 0.05$), indicating that the reported behavioral and biochemical alterations were not confounded by systemic metabolic distress.

As specified in the experimental design, all six experimental groups (Normal Control, Stress Control, Imipramine, Diazepam, and *D. triflorum* extract at 400 and 800 mg/kg) were subjected to the behavioral and biochemical assessments. To facilitate a clear comparison with established clinical standards, results for the Elevated Plus-Maze Test (EPMT) emphasize the benchmark effects of diazepam (Positive Control II), the primary anxiolytic standard in Tables 2 and 3 and Figure 1. Conversely, results for the Forced Swim Test (FST) prioritize comparisons with imipramine (Positive Control I), the standard antidepressant, in Tables 4 and 5 and Figure 2. All six groups were consistently analyzed for the Open Field Test, neuroendocrine markers, neurochemical levels, and oxidative stress biomarkers to provide a holistic view of the extract's multi-modal therapeutic profile Tables 6–9 and Figures 3–6.

3.1. Effect of *Desmodium triflorum* Extract on Anxiolytic Activity (EPMT)

Anxiolytic-like activity was assessed using the EPMT, in which the percentage of open-arm entries and time spent in the open arms serve as validated indices of reduced anxiety. Outcomes were evaluated on Days 1, 15, and 30 to characterize the time-course of extract efficacy. Chronic restraint stress significantly reduced both the percentage of open-arm entries and time spent in the open arms at every time point relative to the normal control group ($p < 0.001$), confirming successful induction of anxiety-like behavior (Tables 2 and 3). Diazepam produced a robust and sustained reversal of this phenotype, significantly increasing open-arm entries and open-arm time relative to the stress group at all time points ($p < 0.001$). *D. triflorum* extract produced a clear dose- and time-dependent anxiolytic effect. The 400 mg/kg dose increased open-arm entries progressively, from a modest effect on Day 1 ($p < 0.05$) to a marked increase by Day 30 ($p < 0.001$); its effect on open-arm time followed a similar delayed trajectory, reaching significance only from Day 15 onward ($p < 0.05$, then $p < 0.01$ on Day 30), suggesting a cumulative rather than acute mechanism of action at this dose. In contrast, the 800 mg/kg dose exhibited a more rapid onset, significantly

increasing both open-arm entries and open-arm time as early as Day 1 ($p < 0.01$) and sustaining peak efficacy through Day 15 and Day 30 ($p < 0.001$), with a magnitude of effect approaching that of diazepam by the end of the study.

3.2. Effect of *Desmodium triflorum* Extract on Antidepressant Activity: Immobility Time in the FST

The antidepressant-like potential of *D. triflorum* extract was evaluated using the FST, in which immobility time is a validated index of behavioral despair. Chronic restraint stress produced a progressive increase in immobility time across all testing intervals relative to control, with severity escalating from Day 1 ($p < 0.05$) to Day 30 ($p < 0.001$), confirming successful induction of depressive-like behavior (Table 4). Imipramine treatment robustly reduced immobility time from Day 1 ($p < 0.01$), achieving maximal reduction by Day 30 ($p < 0.001$).

D. triflorum extract at both 400 and 800 mg/kg produced highly significant reductions in immobility time from Day 1 through Day 30 ($p < 0.001$ at all time points), indicating a rapid-onset antidepressant-like effect at both doses. The response was clearly dose-dependent: the 800 mg/kg group showed a markedly steeper decline in immobility over the course of the study, reaching 90.83 ± 0.95 sec. by Day 30, value closely approaching that of imipramine (80.00 ± 1.06 sec) whereas the 400 mg/kg group plateaued at a comparatively higher immobility time of 121.00 ± 1.24 sec.

3.3. Effect of *Desmodium triflorum* Extract on Climbing Time in the FST

Climbing behavior in the FST reflects active coping and is particularly sensitive to agents with noradrenergic/dopaminergic activity. Chronic restraint stress significantly decreased climbing time on Days 1 and 15 relative to control ($p < 0.001$), reflecting suppressed active escape behavior (Table 5). Imipramine markedly increased climbing time across all time points relative to the stress group ($p < 0.001$), consistent with its catecholamine reuptake-inhibiting mechanism.

Both doses of *D. triflorum* extract significantly reversed the stress-induced deficit in climbing behavior from Day 1 through Day 30 ($p < 0.001$). The 800 mg/kg dose demonstrated superior efficacy, sustaining elevated climbing times (approximately 71–74 s) that approached the performance of the imipramine group (approximately 75–80 s), suggesting engagement of catecholaminergic pathways at the higher dose.

Table 2. Effect of *Desmodium triflorum* extract on anxiolytic activity: percentage of open-arm entries in the EPMT.

Group	Day 1 (% , Mean $\pm$ S.E.M.)	Day 15 (% , Mean $\pm$ S.E.M.)	Day 30 (% , Mean $\pm$ S.E.M.)
Control	41.8 $\pm$ 1.15	33.6 $\pm$ 1.60	26.3 $\pm$ 1.39
Chronic restraint stress	21.7 $\pm$ 1.01	16.4 $\pm$ 1.54	13.1 $\pm$ 1.02
Diazepam	43.4 $\pm$ 1.62	38.8 $\pm$ 1.23	39.3 $\pm$ 1.05
<i>D. triflorum</i> (400 mg/kg)	31.1 $\pm$ 1.17	25.7 $\pm$ 1.19	35.3 $\pm$ 1.27
<i>D. triflorum</i> (800 mg/kg)	39.2 $\pm$ 1.15	36.2 $\pm$ 1.12	38.8 $\pm$ 0.94

$p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

Table 3. Effect of *Desmodium triflorum* extract on anxiolytic activity: percentage of time spent in the open arms in the EPMT.

Group	Day 1 (% , Mean $\pm$ S.E.M.)	Day 15 (% , Mean $\pm$ S.E.M.)	Day 30 (% , Mean $\pm$ S.E.M.)
Control	24.2 $\pm$ 1.18	28.3 $\pm$ 1.60	21.3 $\pm$ 1.39
Chronic restraint stress	17.5 $\pm$ 1.03	16.4 $\pm$ 1.54	11.1 $\pm$ 1.02
Diazepam	39.4 $\pm$ 1.65	34.8 $\pm$ 1.23	41.4 $\pm$ 1.05
<i>D. triflorum</i> (400 mg/kg)	19.3 $\pm$ 1.15	23.1 $\pm$ 1.19	26.1 $\pm$ 1.27
<i>D. triflorum</i> (800 mg/kg)	28.5 $\pm$ 1.13	31.6 $\pm$ 1.12	36.83 $\pm$ 0.94

$p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

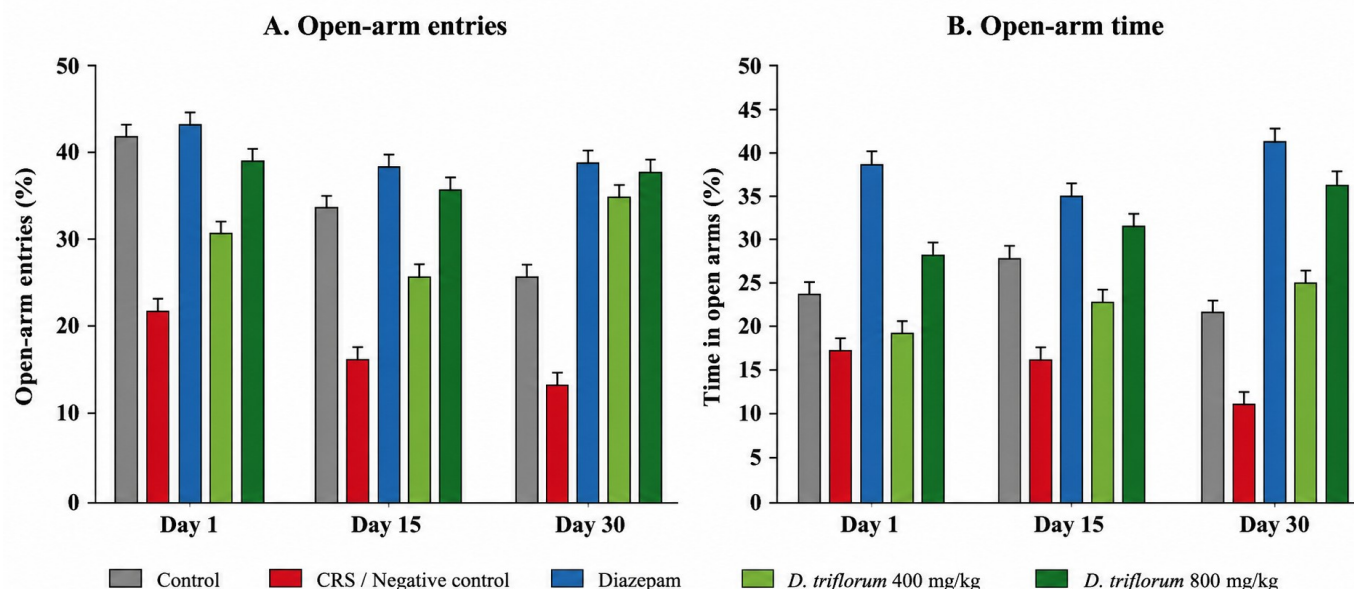


Fig. 1. Effect of *Desmodium triflorum* extract on anxiolytic-like activity in the elevated plus-maze test (EPMT) across Days 1, 15, and 30. (A) Percentage of open-arm entries. (B) Percentage of time spent in the open arms. Bars represent mean $\pm$ S.E.M. ($n = 6/\text{group}$). $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. control; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

3.4. Effect of *Desmodium triflorum* Extract on Spontaneous Locomotor Activity (OFT)

The OFT was used to distinguish genuine anxiolytic/antidepressant activity from non-specific sedative or stimulant effects by assessing exploratory rearing and horizontal crossing. Chronic restraint stress significantly decreased both rearing ($p < 0.01$) and crossing ($p < 0.001$) relative to control, indicating suppressed exploratory drive and general locomotor activity (Table 6). Both diazepam and imipramine reversed these deficits, significantly increasing rearing and crossing relative to the stress group.

The 400 mg/kg dose of *D. triflorum* extract restored rearing and crossing to levels statistically comparable with the normal control group ($p < 0.01$), while the 800 mg/kg dose produced a more pronounced increase in both parameters ($p < 0.001$), approaching the activity levels of the positive control groups. Critically, extract-treated groups did not exceed control-level activity, indicating that the behavioral improvements observed in the EPMT and FST reflect genuine anxiolytic/antidepressant activity rather than non-specific psychostimulation.

3.5. Effect of *Desmodium triflorum* Extract on Blood Cortisol Levels

Cortisol was assessed as a peripheral biomarker of HPA-axis activation. Chronic restraint stress significantly elevated blood cortisol relative to control (41.6 ± 1.41 vs. 24.2 ± 0.71 $\mu\text{g/dL}$, $p < 0.001$), confirming marked HPA-axis hyperactivation (Table 7). Both diazepam and imipramine significantly attenuated this rise, restoring cortisol toward baseline ($p < 0.001$).

D. triflorum extract mitigated the stress-induced cortisol surge in a dose-dependent manner: the 400 mg/kg dose significantly reduced cortisol (36.2 ± 1.12 $\mu\text{g/dL}$, $p < 0.05$), while the 800 mg/kg dose produced a stronger regulatory effect (32.7 ± 1.03 $\mu\text{g/dL}$, $p < 0.01$), consistent with a dose-dependent normalization of HPA-axis activity.

3.6. Effect of *Desmodium triflorum* Extract on Brain Monoamine Neurotransmitter Levels

DA and 5-HT are key monoamines governing mood, stress reactivity, and emotional regulation; their dysregulation is strongly implicated in anxiety and depression. Chronic restraint stress severely disrupted monoaminergic balance ($p < 0.001$), producing paradoxical elevations of both DA and 5-HT within the cerebral cortex, while significantly depleting hippocampal DA (Table 8), a regional dissociation consistent with stress-induced redistribution of monoaminergic tone between cortical and limbic structures.

Diazepam strongly normalized these disturbances across both brain regions ($p < 0.01$ to $p < 0.001$). Imipramine significantly normalized cortical and hippocampal DA and elevated hippocampal 5-HT, consistent with its serotonergic/noradrenergic reuptake-inhibiting mechanism; however, cortical 5-HT remained numerically higher under imipramine than in the stress group and did not reach statistical significance, indicating that imipramine did not normalize this specific parameter. This regional dissociation restoration of hippocampal but not cortical 5-HT is an unexpected finding that may reflect differential serotonin transporter density or turnover between cortical and

hippocampal terminals and warrants confirmation in future studies.

The 400 mg/kg dose of *D. triflorum* extract moderated the cortical monoamine surge and elevated depleted hippocampal DA levels. The 800 mg/kg dose exerted more potent regulatory activity, significantly suppressing the abnormal cortical elevations of DA ($p < 0.001$) and 5-HT ($p < 0.01$), while stabilizing both hippocampal DA and 5-HT toward control values, indicating a dose-dependent bidirectional normalization of monoaminergic tone across both brain regions.

Table 4. Effect of *Desmodium triflorum* extract on antidepressant activity: duration of immobility time (s) in the FST.

Group	Day 1 (s, Mean $\pm$ S.E.M.)	Day 15 (s, Mean $\pm$ S.E.M.)	Day 30 (s, Mean $\pm$ S.E.M.)
Control	170.2 $\pm$ 1.20	181.3 $\pm$ 1.61	189.3 $\pm$ 1.36
Chronic restraint stress	180.7 $\pm$ 1.05	209.3 $\pm$ 1.54	219.2 $\pm$ 1.08
Imipramine	130.0 $\pm$ 1.63	120.3 $\pm$ 1.28	80.00 $\pm$ 1.06
<i>D. triflorum</i> (400 mg/kg)	149.3 $\pm$ 1.05	141.3 $\pm$ 1.15	121.00 $\pm$ 1.24
<i>D. triflorum</i> (800 mg/kg)	139.5 $\pm$ 1.18	129.8 $\pm$ 1.20	90.83 $\pm$ 0.95

$p < 0.05$, $p < 0.01$, $p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

Table 5. Effect of *Desmodium triflorum* extract on climbing time (s) in the FST.

Group	Day 1 (s, Mean $\pm$ S.E.M.)	Day 15 (s, Mean $\pm$ S.E.M.)	Day 30 (s, Mean $\pm$ S.E.M.)
Control	59.83 $\pm$ 0.94	53.80 $\pm$ 1.01	49.50 $\pm$ 1.17
Chronic restraint stress	39.50 $\pm$ 0.76	30.17 $\pm$ 0.14	29.17 $\pm$ 1.07
Imipramine	75.50 $\pm$ 0.76	78.33 $\pm$ 1.05	79.83 $\pm$ 1.40
<i>D. triflorum</i> (400 mg/kg)	60.33 $\pm$ 1.05	62.33 $\pm$ 1.05	65.50 $\pm$ 0.76
<i>D. triflorum</i> (800 mg/kg)	71.50 $\pm$ 1.20	74.17 $\pm$ 0.94	71.33 $\pm$ 1.05

$p < 0.001$ vs. control group; * $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

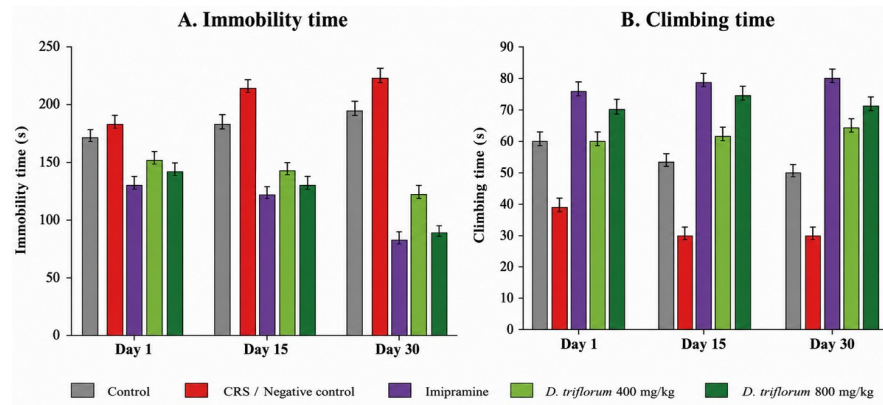


Fig. 2. Effect of *Desmodium triflorum* extract on antidepressant-like activity in the forced swim test (FST) across Days 1, 15, and 30. (A) Immobility time. (B) Climbing time. Bars represent mean $\pm$ S.E.M. (n = 6/group). $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. control; * $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

Table 6. Effect of *Desmodium triflorum* extract on rearing and crossing behavior in the OFT (Day 30).

Group	No. of rearings (Mean $\pm$ S.E.M.)	No. of crossings (Mean $\pm$ S.E.M.)
Control	18.50 $\pm$ 0.76	43.67 $\pm$ 0.88
Chronic restraint stress	9.5 $\pm$ 0.76	24.83 $\pm$ 1.88
Diazepam	27.83 $\pm$ 0.60	56.83 $\pm$ 0.94
Imipramine	24.01 $\pm$ 0.57	58.50 $\pm$ 1.20
<i>D. triflorum</i> (400 mg/kg)	18.52 $\pm$ 0.78	41.67 $\pm$ 1.14
<i>D. triflorum</i> (800 mg/kg)	21.51 $\pm$ 0.76	52.67 $\pm$ 1.31

$p < 0.01$, $p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

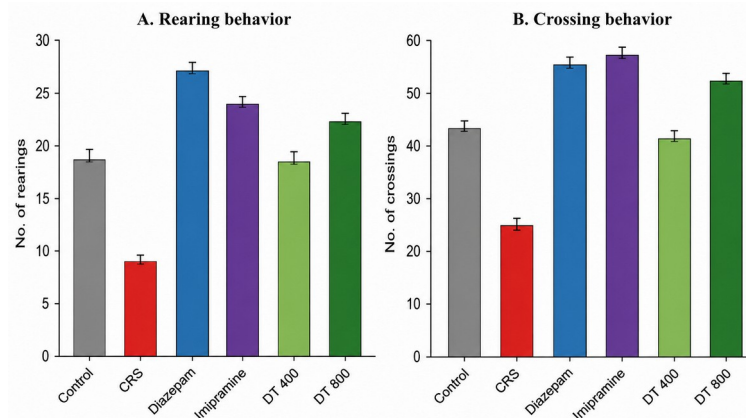


Fig. 3. Effect of *Desmodium triflorum* extract on spontaneous locomotor activity in the open field test (OFT, Day 30). (A) Number of rearings. (B) Number of crossings. Bars represent mean $\pm$ S.E.M. (n = 6/group). $p < 0.01$, $p < 0.001$ vs. control; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

Table 7. Effect of *Desmodium triflorum* extract on blood cortisol levels (Day 30).

Group	Blood cortisol ($\mu\text{g/dL}$, Mean $\pm$ S.E.M.)
Control	24.2 ± 0.71
Chronic restraint stress	41.6 ± 1.41
Diazepam	27.9 ± 0.98
Imipramine	29.1 ± 0.87
<i>D. triflorum</i> (400 mg/kg)	36.2 ± 1.12
<i>D. triflorum</i> (800 mg/kg)	32.7 ± 1.03

$p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

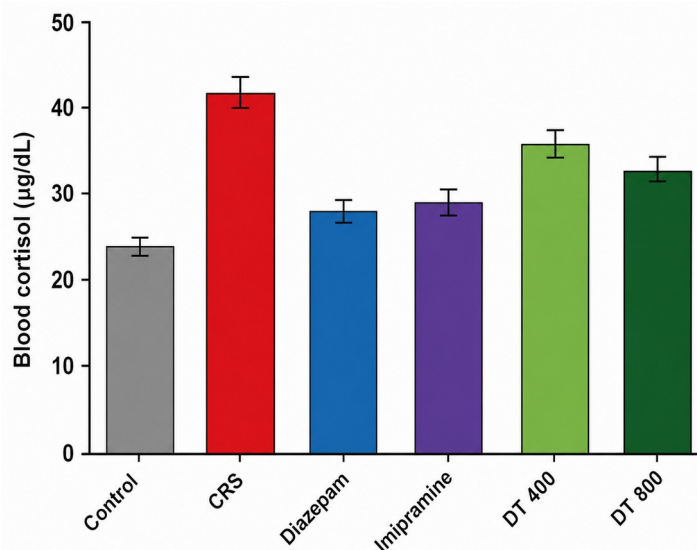


Fig. 4. Effect of *Desmodium triflorum* extract on blood cortisol levels (Day 30). Bars represent mean $\pm$ S.E.M. ($n = 6/\text{group}$). $p < 0.001$ vs. control; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

3.7. Effect of *Desmodium triflorum* Extract on Oxidative Stress Markers

Biochemical analysis demonstrated that *D. triflorum* extract possesses marked antioxidant activity, significantly attenuating lipid peroxidation and restoring endogenous antioxidant enzyme activity in a dose-dependent manner (Table 9), supporting an antioxidant-mediated component of its anxiolytic and antidepressant efficacy.

The stress control group exhibited a marked surge in lipid peroxidation (LPO) accompanied by pronounced depletion of GSH, SOD, and CAT relative to normal control ($p < 0.01$), consistent with stress-induced oxidative injury. Both

diazepam and imipramine robustly counteracted this oxidative burden, restoring LPO, GSH, SOD, and CAT toward homeostatic levels ($p < 0.01$).

The 400 mg/kg dose of *D. triflorum* extract significantly mitigated the stress-induced oxidative burden ($p < 0.01$), lowering LPO and elevating free-radical scavenging enzyme activity. The 800 mg/kg dose exerted a stronger protective effect ($p < 0.01$), restoring GSH, SOD, and CAT activity to levels closely matching those of the normal control and standard-drug groups, indicating a dose-dependent antioxidant mechanism contributing to the neuroprotective and behavioral effects of the extract.

Table 8. Effect of *Desmodium triflorum* extract on dopamine and 5-HT levels (ng/g) in the cerebral cortex and hippocampus (Day 30).

Group	DA Cortex	DA Hippocampus	5-HT Cortex	5-HT Hippocampus
Control	329.5 ± 14.2	131 ± 3.21	412.2 ± 16.5	211 ± 10.6
Chronic restraint stress	563.8 ± 21.4	70 ± 5.09	735.4 ± 11.2	395 ± 12.4
Diazepam	356.6 ± 13.1	142 ± 9.76	475.7 ± 14.8	226 ± 13.6
Imipramine	394.6 ± 12.9	152 ± 10.4	799.8 ± 14.6	424 ± 15.9
<i>D. triflorum</i> (400 mg/kg)	469.7 ± 13.3	116 ± 7.51	625.3 ± 15.4	296 ± 9.4
<i>D. triflorum</i> (800 mg/kg)	361.5 ± 10.8	136 ± 8.72	522.7 ± 11.3	258 ± 10.6

$p < 0.001$ vs. control group; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). DA, dopamine; 5-HT, serotonin. Exact per-comparison significance levels (which specific group/time-point differs at $p < 0.05$, $p < 0.01$, or $p < 0.001$) are reported in the corresponding narrative results (Section 3).

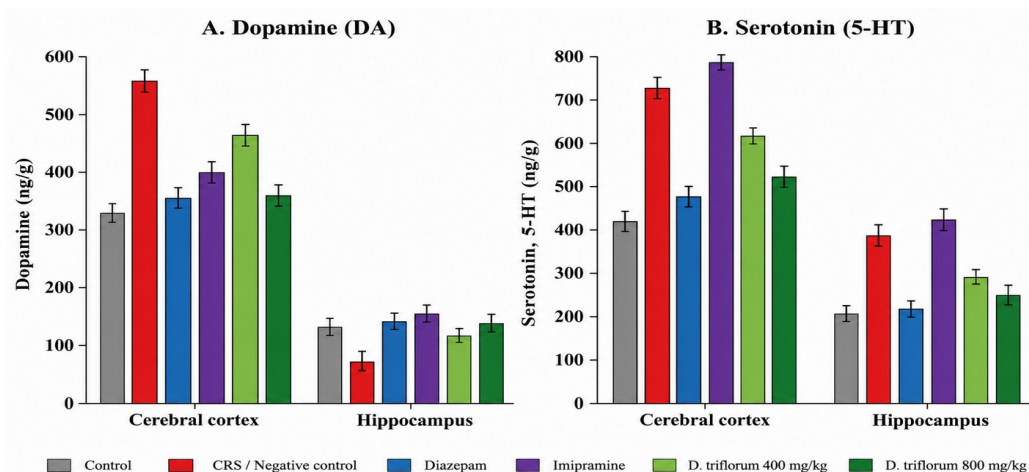


Fig. 5. Effect of *Desmodium triflorum* extract on cortical and hippocampal dopamine and serotonin levels (Day 30). (A) Dopamine (DA). (B) Serotonin (5-HT). Bars represent mean ± S.E.M. ($n = 6$ /group). $p < 0.001$ vs. control; $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

Table 9. Effect of *Desmodium triflorum* extract on brain oxidative stress markers (Day 30).

Group	LPO (nmol MDA/mg protein)	GSH (μmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Control	1.95 ± 0.13	4.12 ± 0.24	6.45 ± 0.18	52.11 ± 2.14
Chronic restraint stress	4.82 ± 0.22	2.13 ± 0.19	3.24 ± 0.20	28.65 ± 1.82
Diazepam	2.05 ± 0.11	4.05 ± 0.20	6.30 ± 0.15	50.72 ± 2.31
Imipramine	2.17 ± 0.16	4.15 ± 0.29	6.22 ± 0.11	49.37 ± 1.38
<i>D. triflorum</i> (400 mg/kg)	2.48 ± 0.17	3.82 ± 0.18	5.82 ± 0.21	46.90 ± 1.75

Group	LPO (nmol MDA/mg protein)	GSH (μmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
<i>D. triflorum</i> (800 mg/kg)	2.12 ± 0.14	4.35 ± 0.22	6.12 ± 0.19	51.44 ± 1.92

p < 0.01 vs. control group; *p* < 0.01 vs. chronic restraint stress group (one-way ANOVA, LSD post hoc test). LPO, lipid peroxidation; MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase. Exact per-comparison significance levels (which specific group/time-point differs at *p*<0.05, *p*<0.01, or *p*<0.001) are reported in the corresponding narrative results (Section 3).

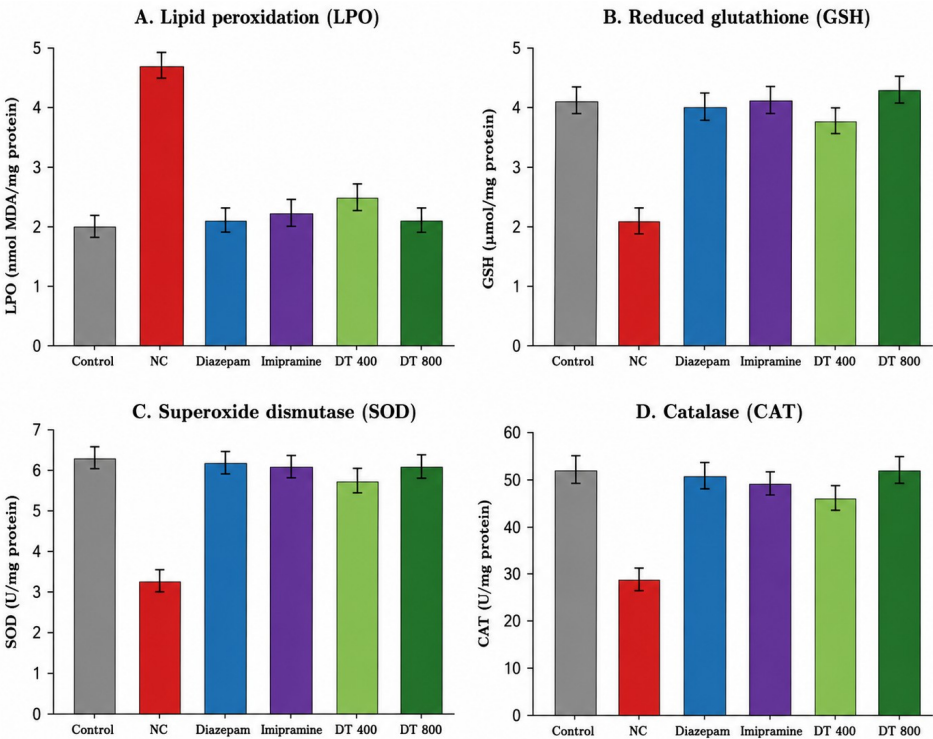


Fig. 6. Effect of *Desmodium triflorum* extract on brain oxidative stress markers (Day 30). (A) Lipid peroxidation (LPO). (B) Reduced glutathione (GSH). (C) Superoxide dismutase (SOD). (D) Catalase (CAT). Bars represent mean ± S.E.M. (*n* = 6/group). *p* < 0.01 vs. control; *p* < 0.01 vs. chronic restraint stress (CRS) group. DT, *D. triflorum* extract.

3.8. Histopathological Evaluation

Representative H&E-stained sections of the hippocampal dentate gyrus are shown in Fig. 7. The normal control group exhibited normal neuronal morphology with well-organized molecular, granule cell, and polymorphic layers. In contrast, the chronic restraint stress (CRS) group showed marked neuronal degeneration, cytoplasmic vacuolation, and disrupted cellular organization. Treatment with imipramine

markedly preserved neuronal architecture, while diazepam restored normal neuronal morphology. *D. triflorum* (400 mg/kg) produced partial neuroprotection with reduced neuronal degeneration, whereas *D. triflorum* (800 mg/kg) showed near-normal neuronal architecture with only minimal histopathological alterations, indicating dose-dependent neuroprotection against CRS-induced neuronal damage. (Figure 7).

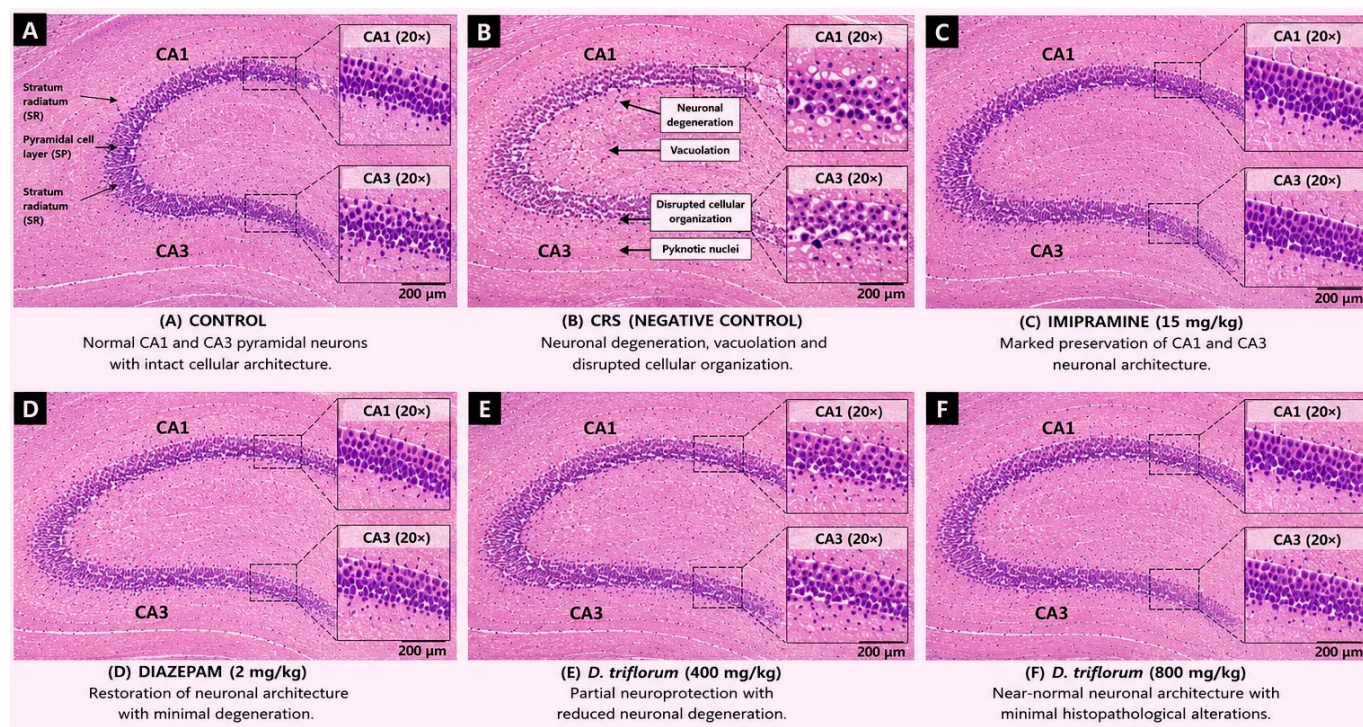


Fig. 7. Representative photomicrographs of H&E-stained sections of the hippocampal dentate gyrus. (A) Normal control showing normal neuronal morphology; (B) CRS group showing neuronal degeneration, vacuolation, and disrupted cellular organization; (C) Imipramine-treated group showing marked preservation of neuronal architecture; (D) Diazepam-treated group showing restoration of neuronal morphology; (E) *D. triflorum* (400 mg/kg) showing partial neuroprotection with reduced neuronal degeneration; (F) *D. triflorum* (800 mg/kg) showing near-normal neuronal architecture with minimal histopathological alterations. ML, molecular layer; GCL, granule cell layer; PL, polymorphic layer; DG, dentate gyrus. Scale bar = 50 µm.

4. DISCUSSION

The present study provides an integrated behavioral, neurochemical, neuroendocrine, and antioxidant characterization of the anxiolytic and antidepressant-like effects of *Desmodium triflorum* extract in a chronic restraint stress (CRS) model in Wistar rats. The extract produced consistent, dose-dependent efficacy across all behavioral paradigms the EPMT, FST, and OFT while concurrently normalizing HPA-axis activity, cortical and hippocampal monoamine levels, and oxidative stress markers, without evidence of hepatotoxicity. The pathophysiology of anxiety and depression is closely linked to disturbances in monoaminergic neurotransmission. DA and 5-HT are critical modulators of mood, emotional processing, and stress resilience [25,26,27], and dysfunction within these systems is strongly implicated in the onset of anxiety and depressive disorders. Given the dense dopaminergic and serotonergic innervation of the cerebral cortex and hippocampus, stress-induced alterations in regional DA and 5-HT concentrations would be expected to disrupt emotional regulation, memory consolidation, and motivational drive [26,27]. Chronic activation of the HPA axis, culminating in sustained

glucocorticoid elevation, is a well-established driver of anxiety- and depression-like phenotypes in both rodents and humans [23,24,28]. A recent mechanistic review further indicates that HPA-axis dysregulation interacts with neuroinflammatory and oxidative pathways to drive hippocampal structural and functional alterations underlying chronic stress-associated depression [28], consistent with the combined neuroendocrine and oxidative disturbances observed in the present study. Consistent with this framework, rats subjected to 30 days of restraint stress in the present study exhibited pronounced anxiety-like behavior evidenced by reduced open-arm exploration in the EPMT together with marked elevation of blood cortisol, confirming robust HPA-axis activation. *D. triflorum* extract, particularly at 800 mg/kg BW, produced a rapid-onset and sustained increase in open-arm entries and open-arm time, together with a significant reduction in circulating cortisol, indicating that the extract's anxiolytic action is mechanistically linked to attenuation of HPA-axis hyperactivity rather than to non-specific sedation a distinction reinforced by the absence of psychostimulant or sedative artifacts in the OFT.

The FST findings similarly support a genuine antidepressant-like action of *D. triflorum* extract. The dose-dependent

reduction in immobility time, together with a concurrent increase in climbing time, is consistent with amelioration of behavioral despair through engagement of catecholaminergic pathways, given that climbing behavior in the FST is preferentially sensitive to agents with noradrenergic/dopaminergic activity [26]. At 800 mg/kg BW, the extract's efficacy in reducing immobility time approached that of imipramine, a standard tricyclic antidepressant, suggesting that at the higher dose the extract engages antidepressant-relevant mechanisms with a magnitude comparable to established pharmacotherapy.

At the neurochemical level, chronic restraint stress produced a regionally dissociated pattern of monoamine dysregulation paradoxical cortical elevation alongside hippocampal depletion of DA that likely reflects stress-induced redistribution of monoaminergic outflow between cortical and limbic circuits rather than uniform depletion. *D. triflorum* extract normalized this dissociated pattern in a dose-dependent manner, suppressing the abnormal cortical surge while restoring hippocampal DA and 5-HT toward control levels. Notably, unlike *D. triflorum* extract, imipramine did not significantly reduce the stress-elevated cortical 5-HT concentration in the present dataset, indicating that its normalization of monoaminergic tone was regionally incomplete. This suggests that *D. triflorum*'s action is not simply a phenocopy of monoamine reuptake inhibition but may involve additional, extract-specific regulatory mechanisms a distinction supporting therapeutic rather than merely symptomatic modulation of mood circuitry, and one that merits direct mechanistic follow-up.

Oxidative stress is increasingly recognized as a converging mechanism linking chronic HPA-axis activation to neuronal dysfunction in anxiety and depression [6]. In the present study, chronic restraint stress produced a marked increase in lipid peroxidation accompanied by depletion of GSH, SOD, and CAT activity, consistent with impaired endogenous antioxidant defense. *D. triflorum* extract significantly attenuated lipid peroxidation and restored antioxidant enzyme activity in a dose-dependent manner, findings that align with the documented antioxidant activity of related *Desmodium* species [11], the antioxidant potential documented across other Indian medicinal plants [29], and with the broader literature on flavonoid-mediated neuroprotection [10]. These antioxidant effects likely act synergistically with monoaminergic and neuroendocrine modulation to underlie the extract's overall anxiolytic and antidepressant efficacy.

Quercetin, a flavonoid constituent identified in *D. triflorum* extract [8], has documented neuroprotective, anxiolytic, and

antidepressant properties, including modulation of glutamatergic NMDA receptor signaling in models of stress-induced depressive behavior [9] and broader antidepressant-relevant activity attributed to flavonoids as a class [10]. Notably, combined in vivo and in silico evidence indicates that quercetin also exerts anxiolytic-like effects through direct interaction with GABA-A receptor subunits, potentiating diazepam-like GABAergic transmission [30], offering a plausible complementary mechanism to the monoaminergic and antioxidant pathways discussed above and directly relevant to the anxiolytic (EPMT) findings of the present study. The presence of quercetin therefore represents a plausible, though not directly confirmed in this study, phytochemical basis for the behavioral, neurochemical, and antioxidant effects observed. Nonetheless, *D. triflorum* extract is a complex phytochemical mixture, and the relative contribution of quercetin versus other constituents (e.g., additional flavonoids, phenolic acids, or triterpenoids) to the observed effects, including any GABAergic component, cannot be determined from the present data and would require receptor-binding or antagonist-challenge studies (e.g., flumazenil co-administration) to confirm directly.

Histopathological evaluation revealed no abnormalities in liver morphology across any treatment group, including at the higher 800 mg/kg dose, supporting a favorable hepatic safety profile for *D. triflorum* extract over the 30-day exposure period. This is consistent with its long-standing traditional use and supports its further preclinical development as a candidate phytotherapeutic agent.

4.1. Strengths and Limitations

This study integrates behavioral, neuroendocrine, neurochemical, biochemical, and histopathological endpoints within a single validated chronic restraint stress paradigm, providing a mechanistically coherent picture of the pharmacological activity of *Desmodium triflorum* and directly benchmarking its efficacy against two clinically established reference drugs. In addition to behavioral and biochemical improvements, representative H&E-stained photomicrographs of the hippocampal dentate gyrus demonstrated dose-dependent preservation of neuronal architecture in *D. triflorum*-treated animals, thereby providing morphological evidence supporting its neuroprotective effects against chronic stress-induced neuronal damage. Several limitations should nonetheless be acknowledged. First, behavioral outcomes across Days 1, 15, and 30 were analyzed by one-way ANOVA at each time point separately; a two-way repeated-measures ANOVA (or mixed-effects model) treating time and treatment as within- and between-subject factors,

respectively, would more appropriately account for the repeated-measures structure of the data and is recommended for any reanalysis prior to submission. Second, the study evaluated only two dose levels; a broader dose-response characterization, including doses below 400 mg/kg, would better define the minimum effective and maximum tolerated doses. Third, group sizes ($n = 6$) were relatively modest, and no a priori power calculation was performed; although this sample size is consistent with many preclinical behavioral pharmacology studies, future investigations should include statistical power estimation to strengthen the robustness of the findings. Fourth, although histopathological examination demonstrated neuroprotective changes in the hippocampus, quantitative histomorphometric analysis or neuronal cell counting was not performed, which would have provided additional objective evidence of tissue preservation. Fifth, the manuscript does not describe randomization of animals to groups or blinding of investigators during behavioral scoring and histopathological assessment, both of which are recommended under the ARRIVE 2.0 guidelines and should be explicitly reported in future studies. Sixth, oxidative stress markers, cortisol, and monoamine neurotransmitter levels were assessed only as terminal (Day 30) endpoints, precluding evaluation of their temporal relationship with the progressive behavioral improvements observed throughout the study. Seventh, the specific bioactive constituent(s) responsible for the observed pharmacological effects have not been isolated or mechanistically confirmed, and the extract's actions on GABAergic, glutamatergic, serotonergic, and dopaminergic signaling pathways remain to be investigated using receptor-level and molecular approaches. Finally, as with all rodent models, the chronic restraint stress paradigm cannot fully reproduce the complexity and heterogeneity of human anxiety and depressive disorders, and therefore further translational and clinical studies are required to establish the therapeutic potential of *D. triflorum* in humans.

4.2. Future Perspectives

Future work should prioritize bioassay-guided fractionation and isolation of the active constituents of *D. triflorum* extract, direct receptor-binding and electrophysiological studies to establish its interaction with GABAergic, serotonergic, and dopaminergic targets, and pharmacokinetic profiling to support dose selection for translational studies. Extended toxicological evaluation beyond hepatic histopathology, including renal, hematological, and reproductive safety endpoints, together with well-designed clinical trials, will be necessary to establish the therapeutic potential of *D. triflorum* as a novel phytotherapeutic for anxiety and depression.

5. CONCLUSION

Desmodium triflorum extract produced dose-dependent anxiolytic- and antidepressant-like effects in a chronic restraint stress model in Wistar rats, with the 800 mg/kg dose approaching the efficacy of the standard reference drugs diazepam and imipramine across behavioral, neurochemical, and neuroendocrine endpoints. These effects were mechanistically associated with normalization of cortical and hippocampal dopamine and serotonin levels, attenuation of HPA-axis hyperactivity as reflected by reduced blood cortisol, and restoration of endogenous antioxidant defenses, in the absence of detectable hepatotoxicity. Collectively, these findings support the traditional use of *D. triflorum* in stress-related conditions and identify it as a promising candidate for further phytopharmaceutical development. Mechanistic studies targeting active-compound isolation, receptor-level interactions, and eventual clinical evaluation are warranted to translate these preclinical findings toward human application.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), I.T.S College of Pharmacy, Ghaziabad, Uttar Pradesh, India, in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India (Approval No. ITS/02/IAEC/2024). Ethical approval was obtained prior to the commencement of the study, and all experimental procedures were conducted in accordance with applicable national guidelines and institutional regulations for the care and use of laboratory animals.

RESEARCH INVOLVING ANIMALS

All animal experiments were conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, and complied with the principles outlined in the Guide for the Care and Use of Laboratory Animals (8th Edition, National Research Council, USA). The study was designed and reported in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines.

RESEARCH INVOLVING PLANTS

Desmodium triflorum used in this study was obtained as a commercially available standardized aqueous extract from

Shivay Herbals and Healthcare, Jaipur, Rajasthan, India. Therefore, no wild plant collection or field study was conducted, and no special permits or permissions were required.

CONSENT FOR PUBLICATION

Not applicable. This manuscript does not contain any identifiable personal data, images, audio, or video recordings of human participants requiring consent for publication.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

FUNDING

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CONFLICT OF INTEREST

The authors declare that they have no competing interests and no financial or personal relationships that could have influenced the work reported in this manuscript.

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