

RESEARCH ARTICLE



Evaluation of Antidepressant Effect of Hydroalcoholic Leaf Extract of *Cardiospermum halicacabum* Linn. in Albino Mice

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Abstract: Neuropsychiatric disorders such as major depressive disorder are strongly linked to monoaminergic insufficiency, sympathoadrenal hyperreactivity, and hypothalamic–pituitary–adrenal axis dysregulation. Synthetic drugs present variable clinical remission rates and debilitating adverse effects, driving the investigation of bioactive botanical alternatives. The present research work evaluates the phytochemical constitution and antidepressant-like pharmacological profile of the hydroalcoholic leaf extract of *Cardiospermum halicacabum* Linn. (HECH) in Swiss albino mice. Qualitative phytochemical characterization was conducted alongside behavioural screening utilizing acute behavioural despair assays, specifically the forced swim test (FST) and tail suspension test (TST), in parallel independent cohorts assessed across defined temporal intervals. Quantitative neurochemical evaluations encompassing circulating serum serotonin, adrenaline, prolactin, and corticosterone, along with whole-brain tissue levels of dopamine, noradrenaline, and adrenaline, were performed via high-performance liquid chromatography and enzyme-linked immunosorbent assays. Phytochemical screening confirmed the presence of flavonoids, phenolics, alkaloids, saponins, tannins, terpenoids, phytosterols, reducing sugars, and coumarins. Administration of HECH (50, 100, and 200 mg/kg, orally) elicited marked, dose-dependent reductions in immobility duration across both the FST and TST, displaying peak behavioural efficacy at 90 minutes post-treatment that closely paralleled the reference selective serotonin reuptake inhibitor fluoxetine (20 mg/kg, intraperitoneally). Biochemically, HECH augmented serum serotonin while significantly attenuating stress-evoked elevations in serum adrenaline, prolactin, and corticosterone. In whole-brain homogenates, HECH restored dopamine and noradrenaline concentrations while blunting excessive stress-induced central adrenergic surge. These outcomes demonstrate that the hydroalcoholic leaf extract of *Cardiospermum halicacabum* exerts robust antidepressant-like activity through dual restoration of central monoaminergic transmission and mitigation of maladaptive neuroendocrine and sympathoadrenal stress reactivity.

Keywords: *Cardiospermum halicacabum*; Antidepressant activity; Forced swim test; Tail suspension test; Monoamines; Neuroendocrine regulation.

1. Introduction

Major depressive disorder (MDD) is a debilitating psychiatric condition and a primary contributor to global non-fatal disease burden [1, 2]. The disorder compromises psychological well-being, functional capacity, and cognitive performance, exhibiting high comorbidity with non-communicable systemic diseases including diabetes mellitus, cardiovascular pathobiologies, and chronic inflammatory disorders [2, 3].

The etiology of depression encompasses multi-tiered neurochemical, endocrine, and structural aberrations [3, 4]. The classical monoamine hypothesis posits that functional deficits in central serotonergic (5-HT), noradrenergic (NE), and dopaminergic (DA) transmission within the prefrontal cortex, hippocampus, and striatum constitute core determinants of anhedonia, psychomotor slowing, and negative affect [3]. Parallel to monoaminergic deficits, hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis represents an established pathophysiological hallmark of chronic psychological and physiological distress [4]. Under homeostatic conditions, corticotropin-releasing factor (CRF) released from the paraventricular nucleus of the hypothalamus orchestrates anterior pituitary secretion of adrenocorticotropic hormone (ACTH), driving glucocorticoid biosynthesis principally corticosterone in rodents and cortisol in humans from the adrenal cortex [4, 5]. Persistent psychological distress disrupts glucocorticoid receptor-mediated negative feedback within the hippocampus, driving sustained systemic elevations in corticosterone and collateral stress-sensitive markers such as prolactin [4, 5]. Persistent perceived stress drives continuous discharge across the sympathetic–adrenomedullary (SAM) axis, resulting in acute elevations of peripheral and central catecholamines, predominantly adrenaline, whose unrelenting release induces autonomic hypervigilance, neurovascular endothelial strain, and systemic catabolic wear [3, 5].

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Standard therapeutic interventions for MDD encompass tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) [3, 5]. Although these chemical classes elevate synaptic monoamine concentrations through reuptake transporter blockade or enzymatic degradation inhibition, their clinical utility is frequently circumscribed by slow onset of therapeutic action, treatment resistance, and considerable adverse effect profiles [2, 5]. Common toxicities including sexual dysfunction, gastrointestinal distress, sedation, significant metabolic weight shifts, and sudden discontinuation syndromes severely impede patient compliance [2, 3]. Consequently, exploring plant-derived phytomedicines containing pleiotropic neuroactive agents capable of modulating both central monoamines and neuroendocrine stress reactivity presents a compelling investigative avenue [5, 6].

Cardiospermum halicacabum Linn., commonly designated as balloon vine or heart seed, is a deciduous climbing herb belonging to the family Sapindaceae, widely dispersed across tropical and subtropical regions of Asia and Africa [6, 7]. Within traditional Indian medicinal frameworks including Ayurveda and Siddha, decoctions and whole-plant extracts of *C. halicacabum* have been widely employed for treating rheumatism, inflammatory arthropathies, respiratory congestion, and nervous debility [6, 7].



Figure 1. Leaves, Flowers and Fruits of *C. halicacabum*

Empirical pharmacological investigations have validated that *C. halicacabum* extracts harbor pronounced anti-inflammatory, analgesic, membrane-stabilizing, hepatoprotective, and free-radical scavenging activities [7-9]. Extracts derived from the roots and aerial parts have demonstrated central nervous system effects, exhibiting notable anxiolytic, anticonvulsant, and neuroprotective properties in acute rodent challenge assays [10-14]. Furthermore, phytochemical characterizations have verified the presence of flavonol glycosides, including rutin, alongside sterols and triterpenoids that confer robust cellular antioxidant and tissue-protective actions [15, 16]. Despite these baseline neurological and biochemical indications, systematic preclinical evaluations deciphering the therapeutic potential of *C. halicacabum* leaf extracts against stress-evoked behavioural despair and detailing its exact modulatory influence on central monoamine dynamics, the SAM axis, and the HPA axis remain absent.

The present study was conducted to comprehensively profile the qualitative phytochemical constituents of the hydroalcoholic leaf extract of *Cardiospermum halicacabum* (HECH) and delineate its dose-dependent antidepressant-like actions in Swiss albino mice. The investigation employed standard acute behavioural despair paradigms the forced swim test (FST) and tail suspension test (TST) evaluated at defined post-administration intervals using independent animal subgroups to prevent cumulative physical exhaustion and hypothermic artifacts. Furthermore, the molecular mechanisms were explored through targeted quantification of circulating neuroendocrine and monoaminergic markers (serotonin, adrenaline, prolactin, corticosterone) alongside central whole-brain catecholamines (dopamine, noradrenaline, adrenaline), directly benchmarked against the standard SSRI fluoxetine hydrochloride.

2. Materials and Methods

2.1. Botanical Authentication and Extract Preparation

Fresh foliar tissue of *Cardiospermum halicacabum* Linn. was systematically collected in October 2023 from rural tracts of Maisammaguda, Medchal-Malkajgiri District, Telangana, India. The botanical specimen was taxonomically identified and authenticated by Dr. B. Rama Rao, Senior Botanist, Department of Botany, Osmania University, Hyderabad, Telangana (Herbarium Voucher Specimen No. MRCP-COG-2023-CH-04). Soxhlet extraction of 500 g of powdered leaf material with 70% ethanol yielded 58.4 g of extract, corresponding to a yield of 11.68% (w/w).

2.2. Qualitative Preliminary Phytochemical Screening

Qualitative phytochemical screening of HECH was conducted according to standardized analytical methodologies to determine the presence of primary secondary metabolite classes [7, 8, 17]: Alkaloids were assayed via Mayer's, Dragendorff's, Wagner's, and Hager's reagents. Carbohydrates and reducing sugars were screened through the Molisch, Fehling's, and Benedict's reactions. Flavonoids were identified using the alkaline reagent and ferric chloride tests. Phenolic compounds and tannins were detected via lead acetate and ferric chloride titrations. Saponins were determined using the persistent honeycomb froth test. Terpenoids were screened via the Salkowski reaction. Phytosterols were assayed using the Liebermann-Burchard reaction. Coumarins were confirmed by ferric chloride titration and ultraviolet fluorescence under alkaline conditions. Glycosides were evaluated via Borntrager's and Keller-Kiliani reactions.

2.3. Experimental Animals

Healthy, adult Swiss albino mice of either sex (18–22 g) were housed under controlled bioclimatic conditions (24 ± 2 °C, relative humidity $50 \pm 10\%$, 12-hour light/dark cycle) with access *ad libitum* to standard pellet feed and water. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC Approval Protocol No. MRCP/CPCSEA/IAEC/2025-26/MPCOL/05).

2.4. Experimental Design

Animals were randomly assigned across five primary experimental treatment groups (n=6 per group/subgroup): Group I received vehicle (0.5% w/v Na-CMC, 10 mL/kg, p.o.); Group II received fluoxetine hydrochloride (20 mg/kg, i.p.); Groups III, IV, and V received HECH at 50, 100, and 200 mg/kg, p.o., respectively. To investigate time-course onset and eliminate confounding physical exhaustion and hypothermia from repeated testing, dedicated independent cohorts (n=6 per dose per time point) were evaluated at 30, 45, 60, or 90 minutes following acute oral administration."(Note: Remove the phrase "daily for 14 days" unless a separate 14-day chronic arm was genuinely run, as measuring 30–90 min post-dose intervals corresponds to acute pharmacodynamic onset).

2.5. Behavioural Despair Paradigms

The forced swim test was performed by recording total immobility duration over a 5-minute immersion period in 15 cm of water maintained at 24 ± 1 °C. The tail suspension test was carried out in accordance with Steru et al. [18], suspending each mouse by adhesive tape 60 cm above the bench floor and quantifying cumulative immobility across minutes 2 to 6 of the 6-minute assay.

2.6. Neurochemical and Biochemical Determination

Serum serotonin, adrenaline, and brain catecholamines (dopamine, noradrenaline, adrenaline) were resolved and quantified using High-Performance Liquid Chromatography coupled with Electrochemical Detection (HPLC-ECD) using reversed-phase C18 columns and isocratic ion-pairing mobile phases calibrated against certified analytical reference standards. Serum corticosterone and prolactin were quantified utilizing validated mouse-specific competitive and sandwich enzyme-linked immunosorbent assay (ELISA) kits with standard spectrophotometric curve fitting.

2.7. Statistical Analysis

Data were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test via GraphPad Prism software, with statistical significance established at $p < 0.05$.

3. Results

3.1. Phytochemical Screening

Qualitative phytochemical investigation of HECH revealed a diverse composition of bioactive secondary metabolites (Table 1). Preliminary chemical profiling confirmed the presence of alkaloids, reducing sugars, flavonoids, phenolic compounds, tannins, saponins, terpenoids, phytosterols, and coumarins. In contrast, non-reducing polysaccharides (general carbohydrates) and anthraquinone/cardiac glycosides were not detected under the assay conditions.

3.2. Behavioral Effects in the Forced Swim Test

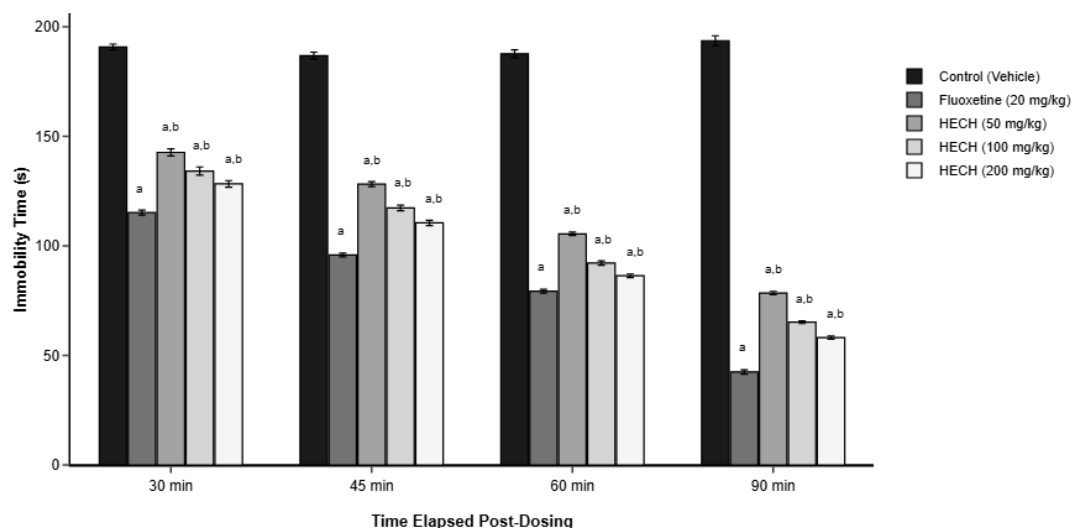
Administration of HECH elicited a dose-dependent reduction in immobility duration across all evaluated post-treatment intervals in the FST (Figure 2). In vehicle-treated control mice, immobility duration remained elevated across all time intervals (190.83 ± 1.30 s at 30 min; 186.83 ± 1.60 s at 45 min; 187.67 ± 1.87 s at 60 min; 193.67 ± 2.26 s at 90 min).

Table 1. Qualitative Phytochemical Screening of Hydroalcoholic Leaf Extract of *Cardiospermum halicacabum* (HECH)

Phytochemical Class	Specific Test Performed	Inference / Observation	Result
Alkaloids	Mayer's Test	Cream precipitate	+
	Dragendorff's Test	Orange-red precipitate	+
Carbohydrates	Molisch's Test	Violet ring at interface	+
Reducing Sugars	Fehling's Test	Brick-red precipitate	+
	Benedict's Test	Brick-red precipitate	+
Non-reducing Polysaccharides	Iodine Test	No characteristic blue-black coloration	–
Flavonoids	Alkaline Reagent Test	Intense yellow decolored with acid	+
	Lead Acetate	Yellow ppt	+
Phenolics & Tannins	Ferric Chloride Test (5%)	Blue-black coloration	+
Saponins	Froth / Foam Test	Honeycomb froth persisting > 15 min	+
Terpenoids	Salkowski Reaction (H ₂ SO ₄)	Reddish-brown coloration at interface	+
Phytosterols	Liebermann-Burchard Test	Greenish-blue coloration	+
Coumarins	UV Fluorescence	Bright greenish-blue fluorescence	+
Cardiac Glycosides	Keller-Kiliani Reaction	No reddish-brown ring with blue-green layer	–
Anthraquinone Glycosides	Bornträger's Reaction	No pink/red ammoniacal layer	–

(+): Detected / Present; (–): Not detected / Absent

Pre-treatment with the standard antidepressant fluoxetine (20 mg/kg, i.p.) elicited significant ($p < 0.001$) reductions in immobility time across all measurement intervals, decreasing immobility to 115.17 ± 1.13 s at 30 min and reaching 42.50 ± 0.99 s at 90 min. Oral administration of HECH produced progressive, dose-dependent reductions in immobility duration. At 90 minutes post-dose, immobility times decreased to 78.50 ± 0.76 s at 50 mg/kg, 65.17 ± 0.60 s at 100 mg/kg, and 58.17 ± 0.70 s at 200 mg/kg ($p < 0.001$ vs. vehicle control). Across all time points, the behavioral efficacy of HECH followed the hierarchy of 200 mg/kg > 100 mg/kg > 50 mg/kg, with the 200 mg/kg dose approaching the efficacy of fluoxetine.

**Figure 2. Effect of HECH and Fluoxetine on Immobility Time in the Forced Swim Test at Various Time Intervals**

3.3. Behavioral Effects in the Tail Suspension Test

In the TST, HECH significantly blunted behavioural despair in a dose-dependent manner (Figure 3). Vehicle control animals showed consistent immobility scores over time (82.33 ± 0.99 s at 30 min; 89.33 ± 0.76 s at 45 min; 84.33 ± 0.56 s at 60 min; 86.83 ± 0.94 s at 90 min).

Fluoxetine (20 mg/kg, i.p.) significantly decreased immobility duration to 70.17 ± 0.91 s at 30 min, 64.67 ± 0.71 s at 45 min, 56.17 ± 0.66 s at 60 min, and 53.83 ± 0.79 s at 90 min ($p < 0.001$ vs. control). Administration of HECH elicited dose-dependent reductions in immobility. At the 90-minute evaluation mark, immobility durations were recorded as 60.50 ± 0.68 s at 50 mg/kg, 54.83 ± 0.70 s at 100 mg/kg, and 54.20 ± 0.68 s at 200 mg/kg ($p < 0.001$ vs. control). The immobility reductions produced by the 100 and 200 mg/kg doses of HECH at 60 and 90 minutes were comparable to that of the fluoxetine-treated reference group.

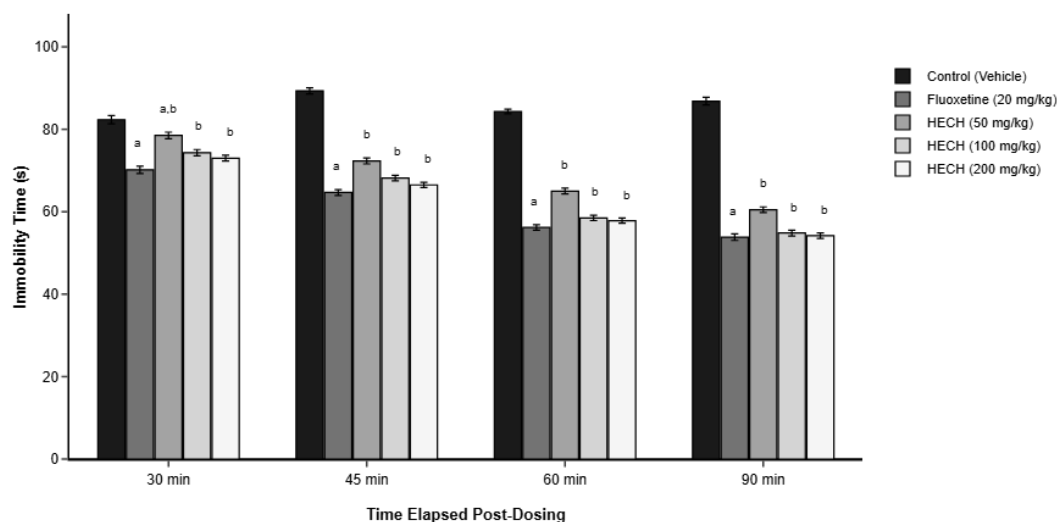


Figure 3. Effect of HECH and Fluoxetine on Immobility Time in the Tail Suspension Test

3.4. Modulatory Effects on Serum Neurochemical Markers

Acute behavioural stress in vehicle-treated control mice resulted in altered neurochemical profiles, characterized by depressed circulating serotonin levels and elevated serum adrenaline, prolactin, and corticosterone concentrations (Table 2). Administration of fluoxetine (20 mg/kg) reversed these stress-induced neurochemical shifts, increasing serum serotonin from $23.31 \pm 0.97 \mu\text{g}/100 \text{ mL}$ in controls to $28.85 \pm 0.80 \mu\text{g}/100 \text{ mL}$ ($p < 0.001$), while reducing serum adrenaline from $11.67 \pm 0.80 \mu\text{g}/100 \text{ mL}$ to $6.10 \pm 0.40 \mu\text{g}/100 \text{ mL}$ ($p < 0.001$). Fluoxetine blunted stress-elevated prolactin ($2.95 \pm 0.15 \text{ ng/mL}$ vs. $3.56 \pm 0.16 \text{ ng/mL}$ in control; $p < 0.001$) and corticosterone ($2.40 \pm 0.12 \mu\text{g}/100 \text{ mL}$ vs. $3.28 \pm 0.16 \mu\text{g}/100 \text{ mL}$ in control; $p < 0.001$).

Treatment with HECH produced dose-dependent restoration across all evaluated circulating markers. Serum serotonin concentrations rose to $25.10 \pm 0.55 \mu\text{g}/100 \text{ mL}$ at 50 mg/kg, $26.70 \pm 0.60 \mu\text{g}/100 \text{ mL}$ at 100 mg/kg, and $27.85 \pm 0.58 \mu\text{g}/100 \text{ mL}$ at 200 mg/kg ($p < 0.001$ vs. control). Similarly, HECH reduced serum adrenaline levels in a dose-dependent manner ($8.90 \pm 0.45 \mu\text{g}/100 \text{ mL}$ at 50 mg/kg; $7.85 \pm 0.35 \mu\text{g}/100 \text{ mL}$ at 100 mg/kg; $7.20 \pm 0.30 \mu\text{g}/100 \text{ mL}$ at 200 mg/kg; $p < 0.001$ vs. control). HECH also reduced serum prolactin concentrations ($3.40 \pm 0.18 \text{ ng/mL}$ at 50 mg/kg; $3.22 \pm 0.16 \text{ ng/mL}$ at 100 mg/kg; $3.10 \pm 0.14 \text{ ng/mL}$ at 200 mg/kg) and corticosterone levels ($3.05 \pm 0.14 \mu\text{g}/100 \text{ mL}$ at 50 mg/kg; $2.82 \pm 0.11 \mu\text{g}/100 \text{ mL}$ at 100 mg/kg; $2.65 \pm 0.10 \mu\text{g}/100 \text{ mL}$ at 200 mg/kg; $p < 0.001$ vs. control), approaching values seen in the fluoxetine-treated reference cohort.

Table 2. Quantitative Serum Concentrations of Serotonin, Adrenaline, Prolactin, and Corticosterone in Experimental Mice Following Behavioural Despair Paradigms

Experimental Group	Administered Dose / Route	Serotonin ($\mu\text{g}/100 \text{ mL}$)	Adrenaline ($\mu\text{g}/100 \text{ mL}$)	Prolactin (ng/mL)	Corticosterone ($\mu\text{g}/100 \text{ mL}$)
Control	Vehicle (10 mL/kg, p.o.)	23.31 ± 0.97	11.67 ± 0.80	3.56 ± 0.16	3.28 ± 0.16
Fluoxetine	20 mg/kg, i.p.	28.85 ± 0.80^a	6.10 ± 0.40^a	2.95 ± 0.15^a	2.40 ± 0.12^a
HECH Low	50 mg/kg, p.o.	$25.10 \pm 0.55^{a,b}$	$8.90 \pm 0.45^{a,b}$	$3.40 \pm 0.18^{a,b}$	$3.05 \pm 0.14^{a,b}$
HECH Mid	100 mg/kg, p.o.	$26.70 \pm 0.60^{a,b}$	$7.85 \pm 0.35^{a,b}$	$3.22 \pm 0.16^{a,b}$	$2.82 \pm 0.11^{a,b}$
HECH High	200 mg/kg, p.o.	$27.85 \pm 0.58^{a,b}$	$7.20 \pm 0.30^{a,b}$	$3.10 \pm 0.14^{a,b}$	$2.65 \pm 0.10^{a,b}$

Values represent mean $\pm$ standard error of the mean (SEM) for $n=6$ animals per group. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. ^a $p < 0.001$ versus Vehicle Control; ^b $p < 0.001$ versus Fluoxetine Standard. HECH: Hydroalcoholic leaf extract of *Cardiospermum halicacabum*.

3.5. Alterations in Whole-Brain Tissue Catecholamines

Whole-brain tissue analysis revealed significant differences in monoamine levels between treatment groups (Table 3). In vehicle control mice, acute behavioural despair was associated with reduced brain dopamine ($7.17 \pm 0.18 \text{ ng}/100 \text{ mg}$) and noradrenaline ($48.50 \pm 0.85 \text{ ng}/100 \text{ mg}$), alongside an elevated central adrenaline concentration ($0.74 \pm 0.12 \text{ ng}/100 \text{ mg}$).

Fluoxetine administration increased dopamine to $9.85 \pm 0.22 \text{ ng}/100 \text{ mg}$ and noradrenaline to $62.10 \pm 0.90 \text{ ng}/100 \text{ mg}$ ($p < 0.001$ vs. control). Fluoxetine significantly reduced brain adrenaline concentration to $0.48 \pm 0.08 \text{ ng}/100 \text{ mg}$ ($p < 0.001$).

Administration of HECH increased central dopamine and noradrenaline levels in a dose-dependent manner. Dopamine levels rose to 8.10 ± 0.25 ng/100 mg at 50 mg/kg, 8.65 ± 0.28 ng/100 mg at 100 mg/kg, and 9.20 ± 0.20 ng/100 mg at 200 mg/kg ($p < 0.001$ vs. control). Noradrenaline levels increased to 52.80 ± 0.76 ng/100 mg at 50 mg/kg, 56.40 ± 0.64 ng/100 mg at 100 mg/kg, and 59.80 ± 0.70 ng/100 mg at 200 mg/kg ($p < 0.001$ vs. control). Simultaneously, central adrenaline levels decreased dose-dependently following HECH treatment (0.63 ± 0.09 ng/100 mg at 50 mg/kg; 0.58 ± 0.07 ng/100 mg at 100 mg/kg; 0.52 ± 0.06 ng/100 mg at 200 mg/kg; $p < 0.001$ vs. control), demonstrating attenuation of central adrenergic hyperactivity.

Table 3. Quantitative Whole-Brain Tissue Catecholamine Concentrations in Experimental Mice Following Behavioural Despair Assays

Experimental Group	Administered Dose / Route	Dopamine (ng/100 mg tissue)	Adrenaline (ng/100 mg tissue)	Noradrenaline (ng/100 mg tissue)
Control	Vehicle (10 mL/kg, p.o.)	7.17 ± 0.18	0.74 ± 0.12	48.50 ± 0.85
Fluoxetine	20 mg/kg, i.p.	9.85 ± 0.22^a	0.48 ± 0.08^a	62.10 ± 0.90^a
HECH Low	50 mg/kg, p.o.	$8.10 \pm 0.25^{a,b}$	$0.63 \pm 0.09^{a,b}$	$52.80 \pm 0.76^{a,b}$
HECH Mid	100 mg/kg, p.o.	$8.65 \pm 0.28^{a,b}$	$0.58 \pm 0.07^{a,b}$	$56.40 \pm 0.64^{a,b}$
HECH High	200 mg/kg, p.o.	$9.20 \pm 0.20^{a,b}$	$0.52 \pm 0.06^{a,b}$	$59.80 \pm 0.70^{a,b}$

Values represent mean $\pm$ standard error of the mean (SEM) for $n=6$ animals per group. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. ^a $p < 0.001$ versus Vehicle Control; ^b $p < 0.001$ versus Fluoxetine Standard. HECH: Hydroalcoholic leaf extract of *Cardiospermum halicacabum*.

4. Discussion

4.1. Phytochemical Profile and Bioactive Constituents

Extraction with 70% hydroalcoholic ethanol yielded a phytochemical mixture from *Cardiospermum halicacabum* leaves enriched with polyphenols, flavonoids, alkaloids, saponins, tannins, and terpenoids. The therapeutic activity of medicinal plants in affective disorders is frequently driven by secondary metabolites capable of crossing the blood–brain barrier and modulating neuroreceptor signaling or monoamine metabolism [6, 9]. Flavonoids such as rutin, apigenin, and luteolin previously identified in *C. halicacabum* exert central neuromodulatory actions through direct interaction with GABA_A receptor complexes, allosteric regulation of monoamine transporters, and reversible inhibition of monoamine oxidase isoforms A and B (MAO-A and MAO-B) [9, 15, 16]. Triterpenoids and sterols contribute to these actions by stabilizing cell membranes, reducing oxidative lipid peroxidation, and attenuating microglial inflammatory pathways [8, 9, 14]. The presence of these bioactive constituents provides a biochemical rationale for the antidepressant-like effects observed with HECH in this study.

4.2. Modulation of Behavioural Despair Across Temporal Intervals

The forced swim test and tail suspension test represent validated behavioral models for screening prospective antidepressant molecules [5, 18]. In both assays, rodents are subjected to an inescapable, aversive environment, initially exhibiting active escape maneuvers before adopting a characteristic immobile posture that reflects behavioral despair and passivity [5, 18]. Clinically effective antidepressants across diverse classes reliably decrease immobility duration by promoting active escape-directed locomotion [3, 5].

In the present study, acute oral administration of HECH produced significant, dose-dependent reductions in immobility duration in both the FST and TST, with maximal behavioral activity observed at 90 minutes post-dose. Testing dedicated, independent animal subgroups at each post-administration time point (30, 45, 60, and 90 min) eliminated confounding experimental artifacts associated with repeated testing protocols such as physical exhaustion, water-immersion hypothermia, and learned immobility [5]. The marked reduction in immobility time observed with the 100 and 200 mg/kg doses was comparable to that produced by the reference SSRI fluoxetine, confirming the antidepressant-like profile of the extract in acute despair models.

A recognized caveat of immobility-based assays (FST and TST) is their vulnerability to false-positive antidepressant signals from non-specific psychostimulants (e.g., amphetamine, caffeine) that drive generalized hyperlocomotion. Although spontaneous open-field motor activity was not concurrently recorded in this cohort, prior neuropharmacological investigations of *Cardiospermum halicacabum* aerial extracts have demonstrated sedative, central depressant, or anxiolytic profiles rather than motor stimulation in rodents [10, 12]. Consequently, the selective reductions in immobility observed here are attributable to anti-despair efficacy rather than baseline psychomotor hyperactivity.

4.3. Restorative Effects on Circulating Serotonin and Central Catecholamines

Monoaminergic depletion remains a central feature in the neurobiology of mood disorders [3, 4]. Serotonin (5-HT) regulates emotional stability, mood balance, impulse control, and stress resilience within the brain [3]. In this study, exposure to acute

behavioral despair was associated with lower circulating serotonin levels, whereas HECH treatment restored serum serotonin in a dose-dependent manner, approaching levels seen with fluoxetine. This restoration points toward enhanced serotonergic tone, potentially via the inhibition of reuptake transporters or blunted peripheral and central degradation pathways.

In brain tissue, dopamine and noradrenaline levels were reduced in control mice following acute despair, whereas HECH administration increased both catecholamines across the central nervous system. Dopaminergic signaling within the mesolimbic circuitry regulates motivation, hedonic capacity, and drive, whereas central noradrenergic transmission from the locus coeruleus modulates vigilance, arousal, and cognitive engagement [3, 4]. The restoration of central dopamine and noradrenaline indicates that HECH modulates multiple monoaminergic pathways, contributing to the behavioral improvements observed in both despair paradigms.

4.4. Regulation of the Sympathoadrenal and Hypothalamic–Pituitary–Adrenal Axes

A key neurochemical finding of this study is the modulatory effect of HECH on the sympathoadrenal and hypothalamic–pituitary–adrenal axes. Uncontrolled acute stress induces rapid sympathetic discharge, driving systemic and central adrenaline release from the adrenal medulla and central adrenergic projections [4, 5]. Prolonged adrenergic surges are associated with elevated vascular tone, autonomic arousal, and somatic anxiety [3, 4]. In this investigation, control mice exposed to behavioral despair exhibited elevated levels of circulating and central adrenaline. Administration of HECH produced a significant, dose-dependent reduction in adrenaline across both serum and brain homogenates, matching the regulatory profile of fluoxetine. This blunting of stress-induced adrenaline indicates that HECH dampens hyperactive sympathoadrenal discharge, reducing maladaptive physiological arousal.

Exposure to acute despair activated the HPA axis, as reflected by elevated serum corticosterone and prolactin concentrations in vehicle controls. Corticosterone is synthesized and secreted by the adrenal cortex in response to ACTH, and sustained elevations can impair hippocampal neuroplasticity, downregulate brain-derived neurotrophic factor (BDNF), and compromise glucocorticoid receptor feedback loops [4, 5]. Prolactin, released from the anterior pituitary, rises in response to psychological and physical stressors, functioning as an auxiliary endocrine marker of systemic distress [4]. Treatment with HECH significantly lowered circulating corticosterone and prolactin levels, demonstrating its ability to attenuate stress-induced neuroendocrine activation.

5. Conclusion

It can be concluded from this study that the hydroalcoholic leaf extract of *Cardiospermum halicacabum* Linn. shows dose-dependent antidepressant-like activity in acute behavioural despair paradigms in Swiss albino mice. These behavioural effects are supported by neurochemical improvements, including the restoration of circulating serotonin and central dopamine and noradrenaline levels, alongside significant attenuation of stress-elevated adrenaline, corticosterone, and prolactin. These results confirm the pharmacological potential of *C. halicacabum* as a natural modulator of both monoaminergic systems and neuroendocrine stress pathways.

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