

Ethanol Extract of *Solanum campylacanthum* Mitigates Hypoxia-Induced Neurobehavioral and Biochemical Alterations in Male Wistar Rats [†]

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Abstract

Hypoxic stress disrupts homeostasis, provoking neuroendocrine and oxidative responses that manifest as behavioral impairments and biochemical alterations, with elevated corticosterone and oxidative damage as hallmark features. *Solanum campylacanthum*, traditionally used as a pest deterrent, lacks empirical validation, though its synonym *S. incanum* exhibits antioxidant activity. This study evaluated ethanol extract of *S. campylacanthum* leaves (EESC) in male Wistar rats subjected to repeated hypoxic stress by daily 20 min exposures in sealed transparent chambers over 14 days, creating a hypoxic–hypercapnic environment that induced neurobehavioral and biochemical alterations. EESC significantly alleviated anxiety, depressive-like behaviors, locomotor deficits, and memory decline, while reducing corticosterone and malondialdehyde, enhancing antioxidant defenses, and preventing adrenal hypertrophy. These findings highlight EESC’s adaptogenic and neuroprotective potential under hypoxic stress.

Keywords: *Solanum campylacanthum*; hypoxia; neurobehavioral; biochemical; oxidative damage; adaptogenic; adrenal hypertrophy; antioxidant; stress-induced



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1. Introduction

Hypoxic stress represents a critical neuropathological state characterized by insufficient oxygen availability, leading to disruption of neuronal homeostasis, dysregulation of neuroendocrine function, and excessive generation of reactive oxygen species (ROS) [1,2].

These processes culminate in neurobehavioral impairments—including anxiety, depression-like phenotypes, and cognitive deficits—as well as biochemical disturbances such as elevated corticosterone levels and oxidative damage markers [1,2]. Despite its established role in exacerbating neurological and stress-related disorders, therapeutic options for hypoxia-induced neurotoxicity remain limited, thereby intensifying interest in plant-derived neuroprotective agents.

Medicinal plants have historically served as vital sources of therapeutic compounds, particularly in developing regions where over 80% of populations rely on herbal remedies for primary healthcare [3]. *Solanum* genres (family *Solanaceae*) have demonstrated diverse biological and pharmacological activities, including antioxidant, anti-inflammatory, and neuroprotective effects, largely attributed to their rich content of flavonoids, phenolic compounds, steroidal alkaloids, saponins, and other bioactive secondary metabolites [4]. Consequently, *Solanum* species have emerged as promising candidates for mitigating oxidative stress-mediated neurodegenerative conditions.

Solanum campylacanthum Hochst. ex A. Rich, a close relative of *S. incanum* (commonly known as ‘Sodom apple’) [5] is a perennial shrub indigenous to Africa, commonly found in disturbed habitats, overgrazed lands, and roadside environments. It is extensively employed in traditional medicine across Eastern and Southern Africa for the management of diverse ailments, ranging from dermatological infections to pain syndromes, with these applications largely attributed to its pronounced antioxidant and anti-inflammatory properties arising from an abundant repertoire of bioactive phytochemicals [6,7]. Despite its ethnomedicinal relevance, *S. campylacanthum* remains relatively understudied compared to other bioactive members of the genus.

Phytochemical investigations have revealed that the fruits and leaves of *S. incanum* contain a wide spectrum of metabolites, including terpenes, flavonoids, bioflavonoids, xanthines, tannins, saponins, cyanates, oxalates, anthraquinones, and steroidal glycoalkaloids such as solanine and solasonine [6,7]. Additionally, its foliage is enriched with essential minerals, notably potassium (K) and calcium (Ca) [8,9]. These bioactive constituents underscore the potential of *S. campylacanthum* extracts as adaptogenic agents capable of attenuating hypoxia-induced neurobehavioral and biochemical perturbations. Accordingly, systematic evaluation of its ethanol extract may provide critical insights into the development of novel plant-based therapeutics targeting hypoxia-associated neuropsychological disorders.

2. Materials and Methods

2.1. Plant Material

The leaves of *S. campylacanthum* were harvested from the farmland in Kampala International University, Tanzania, and plant samples were validated by the Department of Botany at the University of Dar es Salaam, Tanzania. A voucher specimen was deposited in the herbarium with the voucher number JJK01.

2.2. Plant Extraction Procedure

Fresh leaves of *S. campylacanthum* were collected, air-dried for two weeks, and pulverized into fine powder. The ethanol extract was prepared using the cold maceration technique [10], whereby 1000 g of powdered material was soaked in 15.6 L of 80% ethanol for 72 h with intermittent stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated in a water bath maintained at 50 °C for 72 h. The dried residue yielded 150 g of ethanol extract of *S. campylacanthum* leaves (EESC), corresponding to 15% *w/w* of the initial plant material. The extract was stored at 4 °C until use.

2.3. Drugs and Chemicals

5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was procured from Sigma Aldrich (St. Louis, MO, USA), and thiobarbituric acid (TBA) and sodium phosphate salts were obtained from Sigma Aldrich (Steinheim, Germany). Enzyme-linked immunosorbent assay (ELISA) kits were sourced from Elabscience® (Wuhan, China) for corticosterone (Cat. No. E-OSL-R0002). All other solvents were reagents of analytical grade.

2.4. Extract Administration and Acute Toxicity Assessment

The EESC was reconstituted in distilled water, which served as the vehicle control. Both extract and vehicle were administered orally to the experimental animals. The dosing regimen for EESC was determined based on findings from the acute oral toxicity test conducted as earlier described [11] with modifications. Briefly, the test was conducted in two phases. In the first phase, groups of three rats each received graded doses of 10, 100, and 1000 mg/kg orally. In the second phase, individual rats were administered higher doses (1600, 2900, and 5000 mg/kg) to refine the LD₅₀ estimate. Animals were observed continuously for the first 4 h and periodically for 24 h, with monitoring extended up to 72 h post-administration. Parameters assessed included mortality, changes in posture, grooming, locomotor activity, food and water intake, and overt signs of toxicity. No mortality or toxic signs were observed at any dose tested, and the oral LD₅₀ was therefore estimated to be greater than 5000 mg/kg p.o. These findings confirm a wide margin of safety and justify the use of 400 mg/kg as the highest dose in the present study. This procedure established the safe dose range and guided the selection of experimental doses subsequently employed in behavioral and biochemical assays.

2.5. Experimental Animals

Male Wistar rats (150–200 g) were obtained from the Central Animal House, College of Medicine, University of Ibadan. Animals were housed under standard conditions with free access to food and water, and acclimatized for two weeks prior to experimentation. All procedures were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/087-0923/22, from 20 May 2024), and the experiments complied with the ARRIVE guidelines and the principles of laboratory animal care.

2.6. Experimental Design

Following Olugbemide et al. [12] with minor modifications, thirty rats were randomly assigned to five orally treated groups ($n = 6$) using a manual lottery method, in which group numbers were written on slips of paper, shuffled, and drawn sequentially until all animals were allocated. All animals received a standardized treatment volume of 10 mL/kg. Group 1: vehicle (distilled water) without hypoxia; Group 2: distilled water; Groups 3–5: EESC (100, 200, and 400 mg/kg), respectively. Animals in Groups 2–5 were exposed to hypoxic conditions one hour after pretreatment. The rats (150–200 g) were individually placed in sealed transparent chambers (internal volume: 450 mL) for 20 min daily over 14 consecutive days. The 20 min exposure duration was guided by a preliminary anoxic tolerance test in rats and is consistent with published rat studies demonstrating reproducible hypoxic responses without mortality [13]. Each chamber was sealed without external gas inflow or outflow during the exposure period. Under these conditions, progressive oxygen depletion and carbon dioxide accumulation occur, creating a repeated hypoxic–hypercapnic environment. Treatment solutions were prepared and coded by an independent researcher not involved in behavioral scoring, and all behavioral assessments were performed by trained observers blinded to the treatment codes. On day 14, the elevated plus maze, open field, sucrose splash test, and Y maze were administered consecutively, with approxi-

mately 30–40 min intervals between each assay, during which animals were returned to their home cages. The sequence began with an anxiety-like behavior test (elevated plus maze), followed by a locomotor/exploratory test (open field), then the sucrose splash test (depressive-like behavior), concluding with the Y maze (cognitive performance). On day 15, blood samples were collected for the serum corticosterone assay, and whole-brain homogenates were analyzed for oxidative stress markers.

2.7. Neurobehavioural Assessments

2.7.1. Test for Anxiety-like Behavior (Elevated Plus Maze)

Anxiety-like behavior was assessed using the elevated plus maze [14]. The maze consisted of two open arms ($50 \times 10 \times 30$ cm), two closed arms ($50 \times 10 \times 30$ cm), and a central platform (10×10 cm), elevated 50 cm above the floor. Each rat was placed at the edge of an open arm and allowed to explore for 5 min. Entries and time spent in each arm were recorded. The index of open arm avoidance (IOAA) was calculated as described by [15].

2.7.2. Test for Locomotor and Exploratory Activity (Open Field Test)

Locomotor and exploratory activity were evaluated in the open field test [16,17]. Rats were placed individually in a Plexiglas arena ($100 \times 100 \times 40$ cm) subdivided into 16 squares. Rearing frequency was recorded over 30 min, and line crossings were quantified during the first 5 min. The arena was cleaned with 5% ethanol between trials [18].

2.7.3. Test for Depressive like Behavior (Sucrose Splash Test)

The sucrose splash test (SST) was conducted according to the protocol outlined by [19] with slight modifications. Each rat was sprayed with 10% sucrose solution on the head and dorsal coat, and total time spent grooming was recorded for 5 min. Grooming activity was defined as self-cleaning behaviors, including licking or scratching of the fur [20].

2.7.4. Test for Spatial Memory (Y-Maze Test)

Spatial working memory was assessed using the Y-maze test with dimensions of ($75 \times 15 \times 15$ cm) [21,22]. Each rat was placed in arm A and allowed to explore freely for 5 min. Arm entries were recorded, and alternation percentage was calculated [23]. The maze was cleaned with 5% ethanol between trials [18].

2.8. Blood Collection, Euthanization and Tissue Collection

At the conclusion of the behavioral paradigms, the animals were anesthetized via intraperitoneal injection of ketamine (50 mg/kg) combined with diazepam (5 mg/kg). Retro-orbital blood sampling was selected for its efficiency in obtaining sufficient plasma volumes and was performed under anesthesia in strict accordance with institutional ethical guidelines to minimize animal distress. Blood was collected using heparinized capillary tubes, centrifuged at 3000 rpm for 15 min, and plasma was harvested for subsequent biochemical analysis. Following blood collection, the animals were humanely euthanized by cervical dislocation to ensure cessation of vital signs. The whole brains were rapidly excised on ice, rinsed in ice-cold saline to remove residual blood, and processed immediately for subsequent biochemical analysis.

2.9. Determination of Serum Corticosterone

Serum corticosterone level was analyzed using an ELISA kit (Elabscience®, Wuhan, China) according to manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader.

2.10. Preparation of Brain Tissue for Biochemical Assays

Harvested brains were homogenized in 10% *w/v* phosphate buffer (0.1 M, pH 7.4) using a glass–Teflon homogenizer. Homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C, and the resulting supernatants were aliquoted and stored at –20 °C until analysis.

2.11. Biochemical Assays

Assays of Antioxidant/Oxidative Stress Biomarkers

Reduced Glutathione (GSH): GSH levels were quantified using the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) assay, as described by [24,25]. Briefly, deproteinized tissue supernatants were incubated with DTNB, and the resulting chromophore was measured spectrophotometrically at 412 nm. Values were expressed as $\mu\text{mol}/\text{mg}$ protein.

Superoxide Dismutase (SOD): SOD activity was determined according to the method of [26], which relies on the enzyme's ability to inhibit adrenaline autooxidation. Diluted supernatants were combined with carbonate buffer and adrenaline, and absorbance was monitored at 495 nm at 60 s intervals over 240 s. Enzyme activity was expressed as U/mg protein.

Catalase (CAT): CAT activity was measured using the procedure described by [27], based on the decomposition of hydrogen peroxide (H_2O_2). Supernatants were incubated with H_2O_2 , and the reaction was terminated with ammonium molybdate to form a stable complex with residual H_2O_2 . Absorbance was recorded at 405 nm, and activity was expressed as U/mg protein.

Lipid Peroxidation (MDA): Lipid peroxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) assay, following [28]. Brain tissue supernatants were diluted, deproteinized with trichloroacetic acid, centrifuged, and reacted with thiobarbituric acid under heat. After cooling, absorbance was measured at 532 nm (LT4500 microplate reader; Hertfordshire, UK). Malondialdehyde (MDA) concentrations were expressed as nmol/mg protein.

2.12. Statistical Analysis

Data are expressed as mean $\pm$ SEM. Group differences were analyzed using one-way or two-way ANOVA, followed by Tukey's post hoc test (GraphPad InStat[®], v8.4.2; GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Acute Toxicity Test

Acute oral toxicity testing revealed no mortality or toxic signs up to 5000 mg/kg *p.o.*, indicating an LD_{50} greater than 5000 mg/kg and supporting the safety of the doses employed.

3.2. Effects of EESC on Anxiety-like, Exploratory and Motor Behaviors Under Hypoxia

Repeated hypoxic exposure (20 min/day for 14 days) significantly reduced open-arm entries and duration, rearing, and line crossings, while increasing the index of open-arm avoidance compared to non-stressed controls. Oral administration of EESC markedly reversed these deficits, increasing open-arm frequency [$F(4, 25) = 41.76$, $p < 0.0001$], duration [$F(4, 25) = 81.42$, $p < 0.0001$], rearing [$F(4, 25) = 60.72$, $p < 0.0001$], and line crossing [$F(4, 25) = 69.16$, $p < 0.0001$], while reducing avoidance index [$F(4, 25) = 25.16$, $p < 0.0001$] relative to stress controls (Table 1). The effects of EESC were non-linear across doses, with moderate doses (100–200 mg/kg) producing greater efficacy than the highest dose (400 mg/kg). These findings indicate a robust protective effect of EESC against hypoxia-induced anxiety, exploratory and motor impairments.

Table 1. Effects of EESC on parameters of anxiety, exploration, and motor function in hypoxic rats using the elevated plus maze and open field tests.

Treatment Groups	Frequency of Open Arm Entry	Duration (s) in Open Arm	Index of Open Arm Avoidance	Frequency of Rearing	Number of Line Crossing
Control	2.00 ± 0.26	108.30 ± 4.75	51.39 ± 3.06	16.33 ± 0.56	34.17 ± 0.54
Stress control	1.00 ± 0.00 *	41.60 ± 3.25 *	76.49 ± 2.33 *	9.67 ± 0.42 *	25.83 ± 1.85 *
EESC (100 mg/kg, p.o.) + Stress	4.50 ± 0.2 #	63.00 ± 1.63 #	53.11 ± 1.58 #	23.17 ± 1.01 #	47.17 ± 0.75 #
EESC (200 mg/kg, p.o.) + Stress	4.17 ± 0.31 #	62.67 ± 1.93 #	52.89 ± 1.84 #	20.00 ± 0.58 #	45.83 ± 0.70 #
EESC (400 mg/kg, p.o.) + Stress	2.50 ± 0.22 #	100.50 ± 3.03 #	52.97 ± 1.46 #	17.17 ± 0.48 #	34.17 ± 1.01 #

Values are expressed as mean ± SEM, (n = 6). * $p < 0.05$ vs. control, # $p < 0.05$ vs. stress control (one-way ANOVA followed by Tukey's post hoc test).

3.3. Effects of EESC on Depressive-like Behavior and Dose-Dependent Memory Enhancement Under Hypoxia

Hypoxic stress controls exhibited a significant reduction in grooming duration and spatial memory performance ($p < 0.05$) relative to non-stressed controls, consistent with enhanced depressive-like features. Oral EESC treatment (200 and 400 mg/kg) significantly increased grooming duration [$F(4, 25) = 48.85$, $p < 0.0001$]. In the Y-maze, EESC produced a dose-dependent increase in spatial memory performance, with 100–400 mg/kg significantly improving correct alternation percentages [$F(4, 25) = 22.79$, $p < 0.0001$] compared to stress controls. These findings demonstrate that EESC mitigates hypoxia-induced behavioral despair and confers a dose-responsive enhancement of memory function. The corresponding results are presented in Figure 1, which includes both panels: (a) sucrose splash test (grooming duration) and (b) Y-maze test (percentage correct alternation).

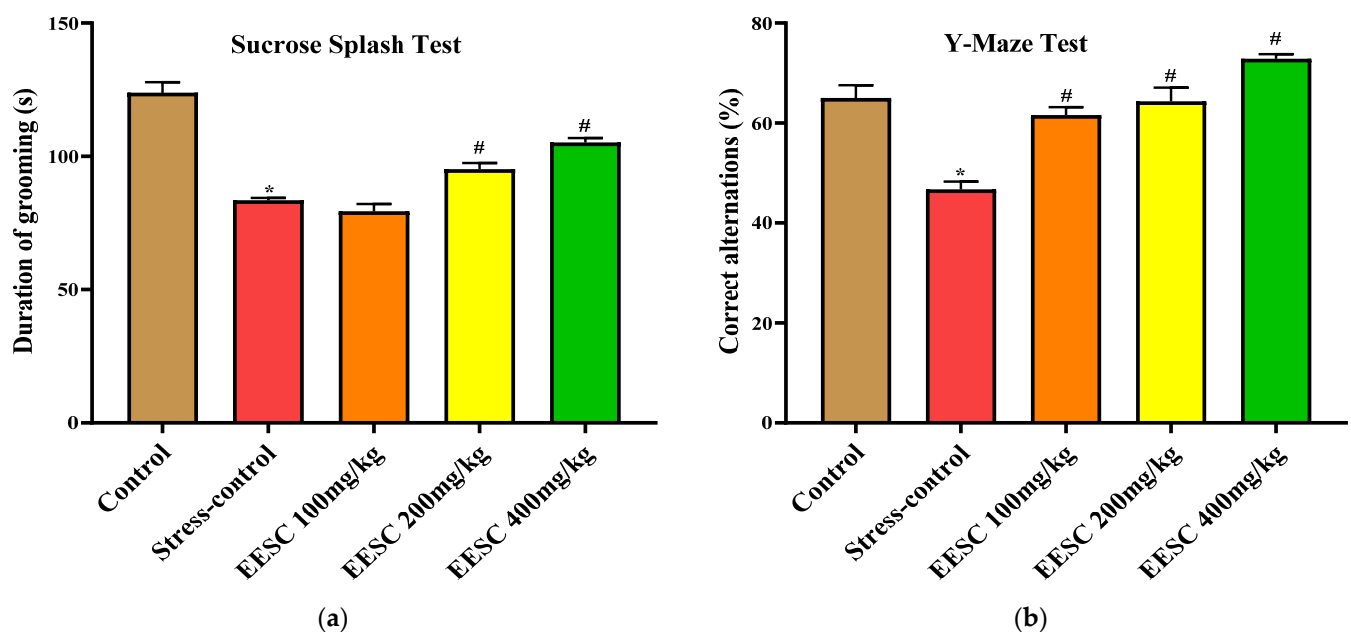


Figure 1. Effects of EESC on depressive-like behavior and memory function in hypoxic rats: (a) duration of grooming; (b) percentage correct alternations. Each bar indicates the mean ± SEM (n = 6). * $p < 0.05$ vs. control; # $p < 0.05$ relative to stress control (one-way ANOVA followed by Tukey's post hoc test).

3.4. Effects of EESC on Hypothalamic–Pituitary–Adrenal Axis Activity Under Hypoxia

Hypoxic stress controls exhibited a significant increase in serum corticosterone levels and relative adrenal weight ($p < 0.05$) compared to non-stressed controls, consistent with hypothalamic–pituitary–adrenal (HPA) axis hyperactivity. Oral administration of

EESC (100–400 mg/kg) produced a dose-dependent reduction in serum corticosterone [F (4, 25) = 27.18, $p < 0.0001$] and relative adrenal weight (%) [F (4, 25) = 25.52, $p < 0.0001$]. These findings demonstrate that EESC effectively mitigates hypoxia-induced HPA axis dysregulation (Figure 2).

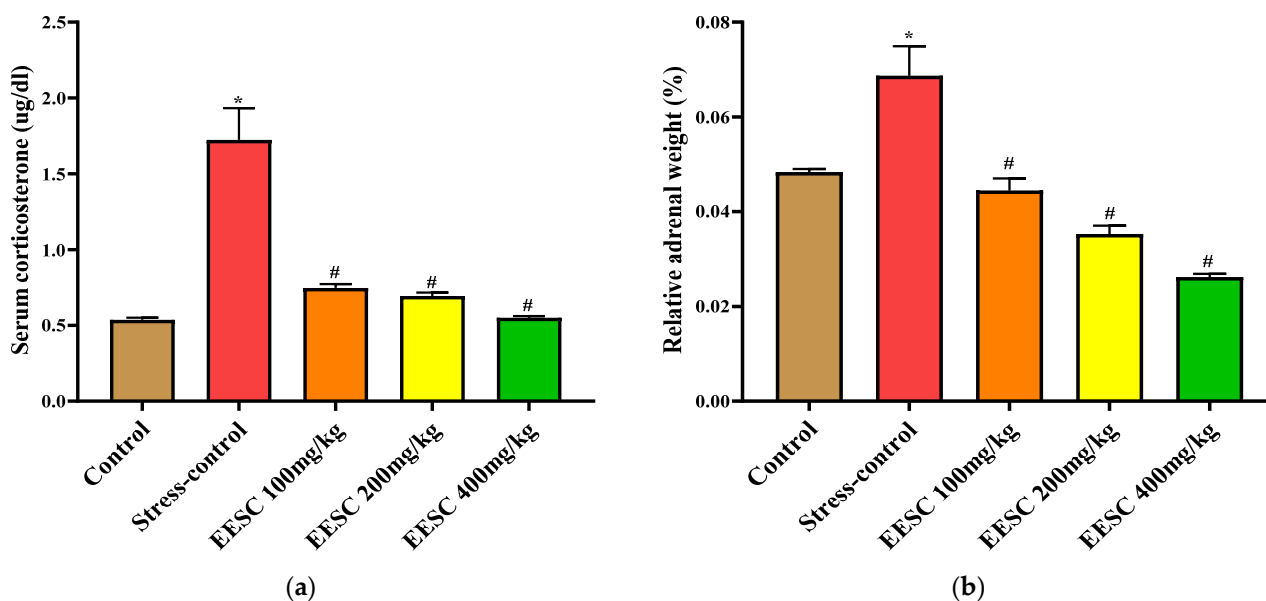


Figure 2. The EESC reduces corticosterone and adrenal hypertrophy in hypoxic rats. (a) Serum corticosterone level. (b) Relative adrenal weight. Each bar indicates the mean $\pm$ SEM (n = 6). * $p < 0.05$ vs. control; # $p < 0.05$ relative to stress control (one-way ANOVA followed by Tukey's post hoc test).

3.5. Effects of EESC on Oxidative Stress Markers Under Hypoxia

Repeated hypoxic exposure (20 min/day for 14 days) significantly increased whole-brain malondialdehyde (MDA) content and correspondingly reduced superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels relative to non-stressed controls, indicating pronounced oxidative stress. Oral administration of EESC (100–400 mg/kg) markedly reversed these deficits in a dose-dependent manner, elevating SOD [F (4, 25) = 427.70, $p < 0.0001$], CAT [F (4, 25) = 1760.00, $p < 0.0001$], and GSH [F (4, 25) = 112.00, $p < 0.0001$], while reducing MDA levels [F (4, 25) = 730.70, $p < 0.0001$] relative to stress controls (Table 2). These findings demonstrate a robust protective effect of EESC against hypoxia-induced oxidative damage.

Table 2. Effects of EESC on brain oxidative stress markers in hypoxic rats.

Treatment Groups	Stress Biomarkers			
	MDA (nmoles/mg Protein)	SOD (U/mg Protein)	CAT (U/mg Protein)	Reduced GSH (μ mol/mg Protein)
Control	44.55 $\pm$ 0.63	8.30 $\pm$ 0.07	83.76 $\pm$ 0.51	1.85 $\pm$ 0.03
Stress control	98.96 $\pm$ 0.72 *	3.12 $\pm$ 0.13 *	39.10 $\pm$ 0.32 *	1.10 $\pm$ 0.01 *
EESC (100 mg/kg, p.o.) + Stress	68.73 $\pm$ 0.31 #	6.75 $\pm$ 0.09 #	66.60 $\pm$ 0.55 #	1.29 $\pm$ 0.04 #
EESC (200 mg/kg, p.o.) + Stress	53.20 $\pm$ 1.68 #	7.63 $\pm$ 0.09 #	79.46 $\pm$ 0.25 #	1.50 $\pm$ 0.03 #
EESC (400 mg/kg, p.o.) + Stress	44.44 $\pm$ 0.51 #	8.16 $\pm$ 0.10 #	84.13 $\pm$ 0.55 #	1.85 $\pm$ 0.03 #

Values are expressed as mean $\pm$ SEM, (n = 6). * $p < 0.05$ vs. control, # $p < 0.05$ vs. stress control (one-way ANOVA followed by Tukey's post hoc test).

4. Discussion

Rodent models consistently demonstrate that hypoxia initiates a cascade of neurobiological insults culminating in circuit-level dysfunction and measurable behavioral and

biochemical impairments [29]. In the present study, oral administration of EESC significantly attenuated hypoxia-induced disruptions in male Wistar rats. Hypoxic exposure impaired exploratory drive, emotional regulation, cognitive performance, and locomotor activity, corroborating its established role as a potent inducer of oxidative and behavioral stress [30,31]. Treatment with EESC ameliorated these deficits, highlighting its antioxidant, adaptogenic, and neuroprotective potential. Biochemical assays confirmed attenuation of oxidative stress markers, underscoring the therapeutic promise of EESC in mitigating hypoxia-related dysfunction.

Both chronic intermittent and sustained hypoxia elicit maladaptive changes characterized by heightened anxiety, depressive-like phenotypes, and cognitive decline [32,33]. These effects persist beyond re-oxygenation and are mechanistically linked to oxidative stress, neuroinflammation, and dysregulated neurotransmitter and orexin signaling [32,34]. The resultant imbalance within limbic circuits underpins anxiety-like behaviors. Consistent with this, hypoxic rats demonstrated reduced open-arm exploration. Notably, the magnitude of difference in the avoidance index likely reflects its heightened sensitivity to concurrent reductions in both time and entries, thereby amplifying apparent group differences. This phenotype was reversed by EESC, implicating its antioxidant activity. Such findings align with prior evidence supporting the anxiolytic potential of antioxidant-rich botanicals such as *Withania somnifera* and *Hibiscus sabdariffa* [35,36]. Importantly, the behavioral improvements did not follow a strictly linear dose–response; moderate doses (100–200 mg/kg) produced greater efficacy than the highest dose (400 mg/kg), suggesting a non-linear pattern likely attributable to non-specific sedative effects at higher doses.

Novelty-induced rearing (NIR), a robust marker of exploratory drive, is orchestrated by hypothalamic melanin-concentrating hormone neurons, locus coeruleus activity, and hippocampal–dopaminergic circuits that integrate arousal and novelty detection [37]. Locomotor activity, quantified by line crossings in the open-field test, complements NIR as an index of motor output and motivational state. Both behaviors are highly vulnerable to hypoxic stress, which elevates reactive oxygen species (ROS), perturbs neuronal excitability, and impairs synaptic plasticity, thereby suppressing rearing and locomotor performance [29,38,39]. In this study, hypoxia markedly reduced NIR and locomotor activity, whereas EESC significantly reversed these deficits. Restoration of rearing and locomotor activity reflects antioxidant-mediated adaptogenic properties likely linked to preservation of dopaminergic and hippocampal pathways. These findings align with prior evidence that plant-derived antioxidants, including *S. incanum*, modulate dopaminergic transmission critical for exploratory and motor functions [40], and are further supported by reports that phytochemicals mitigate hypoxia-induced behavioral impairments by sustaining redox homeostasis and protecting synaptic integrity [30,38,41]. Collectively, these results substantiate the antioxidant potential of *S. campylacanthum* in counteracting hypoxia-related neurobehavioral dysfunction.

Chronic or intermittent hypoxia disrupts monoaminergic (serotonin, dopamine) and cholinergic signaling through elevated acetylcholinesterase activity, while excessive ROS generation and antioxidant depletion exacerbate neurochemical imbalance, driving depressive-like behaviors and cognitive decline [12,31]. These alterations manifest as reduced grooming in the sucrose splash test and impaired spatial working memory in the Y-maze, linked to hippocampal oxidative damage and diminished brain-derived neurotrophic factor (BDNF). Consistent with these findings, hypoxic rats in the present study exhibited shortened grooming duration and reduced alternation performance, both significantly ameliorated by EESC. Restoration of behavioral indices and attenuation of oxidative stress parallel reports on its synonym *Solanum incanum*, which demonstrates similar neuroprotective and cognition-supportive properties [40,42,43]. Moreover, chronic hy-

poxia induced adrenal hypertrophy and elevated corticosterone, markers of hypothalamic–pituitary–adrenal (HPA) axis hyperactivity [44], which correlated with oxidative damage and hippocampal-dependent memory decline, implicating glucocorticoid-mediated neurotoxicity as a key driver [31,45,46]. Notably, EESC mitigated adrenal enlargement and suppressed corticosterone elevation, suggesting that attenuation of HPA axis hyperactivity underlies its neuroendocrine protective effect. By reducing systemic glucocorticoid load and preserving hippocampal integrity, EESC improved Y-maze alternation and grooming behavior, positioning it as a promising candidate for managing hypoxia-related cognitive and emotional dysfunction via modulation of the endocrine–oxidative stress axis.

Biochemical profiling further revealed profound oxidative stress after 14 days of hypoxia, evidenced by depletion of endogenous antioxidants and elevated lipid peroxidation markers [31]. Specifically, hypoxia reduced superoxide dismutase (SOD) and catalase (CAT) activities and depleted reduced glutathione (GSH), permitting unchecked ROS accumulation and neuronal membrane damage, as indicated by elevated malondialdehyde (MDA) levels [12]. EESC administration reversed this pathological profile, restoring SOD and CAT activities, replenishing GSH stores, and lowering MDA concentrations. These effects are consistent with activation of the Nrf2/ARE signaling pathway, a master regulator of antioxidant defense [36], and corroborate prior evidence that phytochemicals mitigate hypoxia-induced oxidative damage by sustaining redox homeostasis. Collectively, these findings position EESC as a potent neuroprotective agent whose efficacy in hypoxic conditions is driven by its capacity to neutralize ROS, preserve neuronal membrane integrity, and suppress biochemical markers of oxidative injury.

5. Conclusions

This study demonstrates that EESC confers significant neuroprotective benefits against hypoxia-induced behavioral and biochemical impairments in male Wistar rats. By restoring antioxidant defenses and suppressing hypothalamic–pituitary–adrenal axis hyperactivity, EESC effectively reversed deficits in exploratory drive, emotional regulation, and spatial memory. Collectively, these findings highlight the adaptogenic and neuroprotective potential of *S. campylacanthum* as a promising candidate for therapeutic intervention in hypoxia-related neuropsychological disorders through modulation of the endocrine–oxidative stress axis.

6. Limitations

This study is limited by the absence of chamber O₂/CO₂ monitoring during hypoxic exposure, the lack of detailed phytochemical profiling of EESC—which restricts attribution of effects to specific bioactive constituents—and the use of only male rats, precluding generalization across sexes. In addition, beginning the behavioral sequence with the elevated plus maze, an anxiogenic procedure, may have introduced stress carryover effects influencing subsequent tasks. These factors collectively constrain mechanistic interpretation and underscore the need for future studies incorporating real-time gas monitoring, comprehensive phytochemical characterization, counterbalanced test orders, and inclusion of both sexes to enhance translational relevance.

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Institutional Review Board Statement: The study was conducted in accordance with the ARRIVE guidelines and the principles of laboratory animal care. The study protocol was reviewed and approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/087-0923/22, from 20 May 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are not publicly available due to ethical restrictions related to animal experimentation.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EESC	Ethanol extract of <i>Solanum campylacanthum</i> leaves
SOD	Superoxide dismutase
CAT	Catalase
GSH	Glutathione
MDA	Malondialdehyde
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
TBA	Thiobarbituric acid
ELISA	Enzyme-linked immunosorbent assay
Nrf2/ARE	Nuclear factor erythroid 2-related factor 2/Antioxidant Response Element
HPA	Hypothalamic-pituitary-adrenal
NIR	Novelty-induced rearing

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