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EVALUATION OF CNS ANTI-DEPRESSANT ACTIVITY OF SEEDS OF *TRACHYSPERMUM ROXBURGHIANUM* IN SWISS ALBINO MICE

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ABSTRACT: The search for safer alternatives to synthetic neuroactive drugs has increased interest in medicinal spices with potential central nervous system (CNS) activity. *Trachyspermum roxburghianum*, a traditionally used aromatic spice, remains poorly explored for its neuropharmacological effects. This study evaluated the CNS depressant activity of the methanolic extract of *T. roxburghianum* seeds in mice. CNS depressant activity was assessed using forced swimming test (FST), tail suspension test (TST), and thiopental sodium-induced sleeping time assay in Swiss albino mice. The extract was administered orally at doses of 50, 100, and 200 mg/kg, while diazepam (1 mg/kg, i.p.) served as the reference standard. Acute oral toxicity was evaluated up to 2000 mg/kg. Qualitative phytochemical screening was performed, and statistical analysis was conducted using paired Student's t-test (one-tailed). The extract produced a dose-dependent increase in immobility time in both FST and TST. In the thiopental-induced sleeping time test, the extract significantly reduced sleep onset latency and prolonged sleep duration at higher doses. Phytochemical analysis confirmed the presence of flavonoids, terpenoids, saponins, phenolics, tannins, steroids, and cardiac glycosides. No mortality or severe toxicity was observed. *T. roxburghianum* seed extract exhibits significant CNS depressant activity in mice, supporting its traditional use and warranting further mechanistic and phytochemical investigations.

INTRODUCTION: Stress is a common problem in modern life and is linked to many psychiatric and neurological disorders, including depression, anxiety, epilepsy, schizophrenia, parkinsonism, and restlessness [1]. Drugs commonly used to treat these conditions, such as benzodiazepines and other sedative-hypnotics, are effective but often cause unwanted side effects. These include drowsiness, memory problems, weight gain, sexual dysfunction, and impaired motor and cognitive functions [2]. Long-term use of these drugs may also lead to tolerance, dependence, and addiction [3]. Because of these limitations, there is growing interest in finding safer and more natural alternatives for managing central nervous system (CNS) disorders.

Medicinal plants have been used for centuries to treat mental and neurological conditions. In Bangladesh, spices are an important part of daily life and are traditionally used not only for cooking but also for health benefits. Many spices contain bioactive compounds such as flavonoids, phenolics,

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and essential oils, which are known to show antioxidant, anti-inflammatory, and neuroactive properties [4–6]. The World Health Organization also recognizes the importance of traditional plant-based medicines, especially in developing countries, where they are affordable and culturally accepted [7]. *Trachyspermum roxburghianum* (Family: Apiaceae), commonly known as Radhuni, is a traditional spice used in some regions of South Asia. It has been used in folk and Ayurvedic medicine to treat digestive problems, pain, and inflammatory conditions [8]. The seeds of *T. roxburghianum* are widely employed in traditional medicine, where they are primarily appreciated for their antispasmodic, stimulant, tonic, and carminative activities [9]. Essential oil extracted from *T. roxburghianum* has been scientifically investigated, revealing promising biological activities such as central nervous system stimulation in murine models and inhibitory effects against *Entamoeba histolytica* [10]. However, scientific data on the neuropharmacological effects of *T. roxburghianum* seed extracts remain sparse.

Therefore, the present study was designed to evaluate the central nervous system depressant activity of the methanolic extract of *T. roxburghianum* seeds. Standard behavioral tests in Swiss albino mice, including forced swimming test, tail suspension test, and thiopental sodium-induced sleeping time, were used to assess its sedative and CNS depressant effects. This study aims to provide scientific support for the traditional use of this spice and explore its potential as a natural neuroactive agent.

MATERIALS AND METHODS:

Chemicals: Diazepam and thiopental sodium were sourced from Square Pharmaceuticals Ltd. and DBL Pharmaceuticals Ltd., respectively, both situated in Bangladesh. Analytical-grade methanol was procured from Sigma-Aldrich (USA). All remaining chemical reagents employed were of analytical grade. In behavioral evaluations, diazepam (1 mg/kg, i.p.) was utilized as the reference standard. To induce a soporific state in the sedative-hypnotic assay, thiopental sodium (40 mg/kg, i.p.) was administered.

Procurement and Processing of Botanical Specimens: Seeds of *Trachyspermum roxburghianum* were acquired from a regional market in Bangladesh. Foreign debris was removed from the plant material, which was subsequently desiccated under shade for a period of days. To protect heat-sensitive constituents, the seeds were then subjected to oven drying at a temperature below 40 °C. The desiccated seeds were mechanically comminuted into a coarse powder utilizing a grinder at the Phytochemical Research Laboratory, University of Information Technology and Sciences (UITS), Bangladesh.

Extract Preparation: A 300 g sample of the powdered seed material underwent maceration in 2.0 L of methanol within a light-protected glass vessel. The sealed mixture was maintained at ambient temperature for 15 days, with periodic agitation to optimize solute extraction. The resultant mixture was filtered sequentially through cotton and Whatman No. 1 filter paper. The filtrate was concentrated via solvent evaporation at room temperature until approximately 70% of the solvent was removed. The crude methanolic extract was preserved at 4 °C for subsequent investigation.

Experimental Subjects: Swiss albino mice of both sexes (aged 4–5 weeks; mean weight ~30 g) were procured from the Animal House at UITS, Bangladesh. Subjects were accommodated in polypropylene cages under controlled environmental parameters (temperature: 24±2 °C, humidity: 60–70%, 12-hour light/dark cycle) and provided with standard pelleted feed and water ad *libitum*. A 14-day acclimatization period preceded experimental procedures. All protocols adhered to the Federation of European Laboratory Animal Science Associations (FELASA) guidelines and received approval from the Institutional Animal Ethical Committee of UITS (Approval No: *UITS/PHARM/IEC/2025/21*) [11].

Acute Toxicity Assessment: A single-dose oral toxicity investigation was performed to evaluate the safety of the methanolic extract. Mice were segregated into four cohorts (n = 5). The control cohort received distilled water, whereas the experimental cohorts were orally administered the extract at dosages of 500, 1000, and 2000 mg/kg body weight. Subjects were monitored continuously for the initial 4 hours and intermittently for 72 hours post-administration for

manifestations of toxicity, alterations in behavior, hypersensitivity, or mortality [12].

Phytochemical Profiling: A qualitative analysis was conducted on the methanolic seed extract to identify principal secondary metabolites, including alkaloids, cardiac glycosides, steroids, tannins, saponins, phenolics, terpenoids, and flavonoids, employing established chemical assays as referenced in prior literature [13].

Experimental Design: For each pharmacological assay, animals were randomly distributed into five cohorts (n = 5 per group). Cohort I functioned as the control and received distilled water (0.1 mL/mouse, p.o.). Cohort II was administered diazepam (1 mg/kg, i.p.) as the positive control. Cohorts III–V received the *T. roxburghianum* methanolic extract at dosages of 50, 100, and 200 mg/kg body weight, respectively. Except for specific tests, the extract was delivered orally 30 minutes before behavioral assessment.

Pharmacological Tests:

Forced Swimming Test: This assay was employed to evaluate central nervous system (CNS) depressant activity. Individual mice were placed in a transparent cylindrical vessel (45 cm height × 20 cm diameter) containing water (depth: 17 cm; temperature: 25±1 °C). Following a 30-minute post-treatment interval, subjects underwent a 5-minute test session. Immobility duration was quantified, defined as the period during which the mouse remained passively buoyant, exhibiting only essential movements to maintain respiration [14].

Tail Suspension Test: This test was implemented to gauge behavioral despair and CNS depressant effects. Mice were individually suspended 50 cm above a surface using adhesive tape affixed approximately 1 cm from the distal tail end. After a 30-minute post-treatment interval, immobility time was recorded over a 6-minute observation window. Immobility was characterized by a complete absence of active movement, with the subject hanging inertly [15].

Thiopental Sodium-Induced Hypnosis Test: Sedative-hypnotic potency was determined via a thiopental sodium-induced sleep latency test. Thirty minutes after oral administration of the extract or vehicle, mice received an intraperitoneal injection of thiopental sodium (40 mg/kg). Sleep latency was defined as the interval between thiopental administration and the loss of the righting reflex. Total sleep duration was calculated as the period between the loss and subsequent recovery of this reflex [16].

Statistical Evaluation: All data are presented as mean ± standard error of the mean (SEM). Intergroup comparisons were performed using a paired, one-tailed Student's t-test. Statistical significance was established at thresholds of $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

RESULTS

Phytochemical Screening: Qualitative phytochemical analysis of the methanolic extract of *T. roxburghianum* seeds revealed the presence of several classes of secondary metabolites **Table 1**. Cardiac glycosides, steroids, tannins, saponins, phenolic compounds, terpenoids, and flavonoids were detected in the extract. However, alkaloids were not observed under the experimental conditions employed. The presence of these bioactive constituents suggests a broad phytochemical profile that may contribute to the observed neuropharmacological effects.

TABLE 1: QUALITATIVE ASSESSMENT OF PHYTOCHEMICAL COMPOUNDS IN THE CRUDE METHANOLIC EXTRACT OF SEEDS OF *T. ROXBURGHIANUM*

Name of the test	Observation	Result Present (+)/ Absent (-)
Alkaloids test	No brick red precipitation was found	-
Cardiac glycosides test	Reddish brown color was observed at the interface	+
Steroids test	Solution was not turned into black color	+
Tannins test	Dirty white precipitation was observed	+
Saponins test	Froth was appeared but not persisted on warming	+
Phenolic compounds test	Dark greenish precipitation was observed	+
Terpenoids test	Grayish color was observed	+
Flavonoids test	Yellow color was observed	+

Acute Toxicity: Oral administration of the methanolic seed extract at doses of 500, 1000, and 2000 mg/kg did not produce any mortality in mice during the 72 hour observation period.

Transient behavioral changes, including reduced locomotor activity, were noted within the first 30 minutes and persisted up to 4 hours post-administration, after which normal behavior was restored.

These findings indicate that the extract possesses a favorable safety profile, with an estimated LD_{50} value greater than 2000 mg/kg.

Forced Swimming Test: The results of the forced swimming test are summarized in **Table 2.** Administration of the methanolic extract induced a dose-dependent prolongation of immobility time. While the 50 mg/kg dose did not produce a statistically significant effect, significant increases were observed at 100 mg/kg ($p < 0.05$) and 200 mg/kg ($p < 0.0001$) when compared to the vehicle-treated control. The standard drug diazepam (1 mg/kg, i.p.) also elicited a pronounced and highly significant increase in immobility duration ($p < 0.0001$).

TABLE 2: EFFECTS OF *T. ROXBURGHIANUM* SEED EXTRACT AND DIAZEPAM ON FORCED SWIMMING TEST

Treatment	Dose (mg/kg)	Immobility time (s)
Control	0.1 mL/mouse	65.2±3.97
Diazepam	1	177±3.39****
ME50	50	71.2±2.82
ME100	100	84±3.49*
ME200	200	133.4±3.36****

Values are presented as mean ± SEM (n = 5). ME = Methanolic extract of *T. roxburghianum. *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001* vs. control (Student's t-test).

Tail Suspension Test: The tail suspension test outcomes are detailed in **Table 3.** Treatment with the methanolic extract led to a significant and dose-dependent increase in immobility time. This effect was particularly evident at the 100 mg/kg and 200 mg/kg doses, with the latter showing a highly significant increase ($p < 0.0001$) compared to the control. The 50 mg/kg dose did not yield a statistically significant change. The reference drug diazepam also produced a significant elevation in immobility duration ($p < 0.0001$).

TABLE 3: EFFECTS OF *T. ROXBURGHIANUM* SEED EXTRACT AND DIAZEPAM ON TAIL SUSPENSION TEST

Treatment	Dose (mg/kg)	Immobility time (s)
Control	0.1 mL/mouse	81.2±3.89
Diazepam	1	216.8±3.81****
ME50	50	83.2±3.84
ME100	100	108.2±5.13**
ME200	200	169±3.08****

Values are presented as mean ± SEM (n = 5). ME = Methanolic extract of *T. roxburghianum.*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001* vs. control (Student's t-test).

Thiopental Sodium-induced Sleeping Time Test: The effects of the methanolic extract on thiopental sodium-induced hypnosis are presented in **Table 4.** Pretreatment with the extract resulted in a dose-dependent modulation of sleep parameters, significantly shortening the latency to sleep onset and extending the total sleeping time. These effects were significant at the 100 mg/kg ($p < 0.001$) and 200 mg/kg ($p < 0.0001$) doses, with the highest dose demonstrating efficacy comparable to the reference drug diazepam (1 mg/kg, i.p.). Diazepam itself significantly reduced sleep latency and markedly prolonged sleep duration relative to the control ($p < 0.0001$).

TABLE 4: EFFECTS OF *T. ROXBURGHIANUM* SEED EXTRACT AND DIAZEPAM ON THIOPENTAL SODIUM-INDUCED SLEEPING TIME TEST

Treatment	Dose (mg/kg)	Time of onset of sleep (min.)	Total sleeping time (min)
Control	0.1 mL/mouse	6.4±0.51	30.2±2.20
Diazepam	1	3.8±0.37*	109.2±4.32****
ME50	50	14±1.52**	38.8±3.76

ME100	100	9.4±0.51	56.2±2.52***
ME200	200	6.6±0.51*	84±4.10****

Values are presented as mean ± SEM (n = 5). ME = Methanolic extract of *T. roxburghianum.*$*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$ vs. control (Student's t-test).

DISCUSSION: The present study systematically evaluated the central nervous system (CNS) depressant potential of the methanolic extract of *Trachyspermum roxburghianum* seeds using established behavioral and pharmacological models in Swiss albino mice. The findings demonstrate that the extract produces significant CNS depressant effects, as evidenced by increased immobility in the forced swimming and tail suspension tests, along with potentiation of thiopental sodium-induced sleep. These effects were observed in a dose-dependent manner and occurred without acute toxicity, indicating a favorable safety margin.

Behavioral despair models such as the forced swimming test (FST) and tail suspension test (TST) are widely used to assess neuroactive compounds affecting CNS function [17, 18]. Although these tests are commonly applied for screening antidepressant activity, increased immobility time is also indicative of reduced locomotor activity, sedation, or CNS depression. In the present investigation, the extract significantly prolonged immobility duration in both models, suggesting suppression of spontaneous motor activity and behavioral responsiveness. The concordant outcomes obtained from FST and TST strengthen the reliability of the observed CNS depressant effect, as these models differ in stress modality yet share sensitivity to sedative and depressant agents.

The thiopental sodium-induced sleeping time test further substantiated the CNS depressant activity of *T. roxburghianum*. Thiopental sodium, a barbiturate, induces sleep by enhancing GABA-mediated chloride ion influx through the $GABA_A$ receptor complex, leading to neuronal hyperpolarization and reduced excitability [19]. In this study, pretreatment with the extract significantly reduced sleep onset latency and prolonged total sleeping time, particularly at higher doses. This potentiation of thiopental-induced hypnosis suggests that the extract may enhance inhibitory neurotransmission or synergize with GABAergic pathways, similar to the action of benzodiazepines and other sedative-hypnotic agents. The observed CNS depressant effects can be plausibly attributed to the phytochemical composition of the extract. Qualitative screening revealed the presence of flavonoids, terpenoids, saponins, phenolic compounds, tannins, steroids, and cardiac glycosides. Among these, flavonoids and terpenoids have been extensively reported to exhibit neuropharmacological properties, including sedative, anxiolytic, and muscle-relaxant effects [20]. Several flavonoids are known to bind to the benzodiazepine site of the $GABA_A$ receptor, thereby producing CNS depressant activity [21]. Similarly, triterpenoids and saponins have been reported to modulate GABAergic neurotransmission and suppress CNS excitability [22].

In addition to direct receptor interactions, the antioxidant properties of phenolic and flavonoid compounds may indirectly contribute to CNS depressant effects. Oxidative stress has been implicated in neuronal hyperexcitability and mood disorders, and compounds capable of reducing oxidative burden may help stabilize neuronal function [23]. Although antioxidant parameters were not directly assessed in this study, the presence of phenolic constituents suggests a potential complementary mechanism that warrants further investigation.

Importantly, the acute toxicity study demonstrated that the extract was well tolerated up to a dose of 2000 mg/kg, with no mortality or severe behavioral abnormalities observed. The transient reduction in locomotor activity noted shortly after administration aligns with the CNS depressant profile observed in behavioral assays and supports the safety of the doses used for pharmacological evaluation.

Collectively, the findings of this study provide experimental evidence supporting the CNS depressant activity of *T. roxburghianum* seeds and justify its traditional use as a medicinal spice. However, the precise molecular mechanisms underlying these effects remain to be elucidated. Further studies involving receptor binding assays, neurotransmitter level estimation, and isolation of

active phytoconstituents are necessary to clarify the exact pathways involved and to identify the compounds responsible for the observed neuropharmacological activity.

CONCLUSION: The present study demonstrates the central nervous system depressant potential of *Trachyspermum roxburghianum* in experimental mouse models. The findings substantiate its traditional medicinal use and highlight the need for further pharmacological and mechanistic investigations to identify bioactive constituents with potential therapeutic value.

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