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CENTRAL AND PERIPHERAL ANALGESIC EFFECTS OF *TRACHYSPERMUM ROXBURGHIANUM* SEED

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ABSTRACT

Background: *Trachyspermum roxburghianum* (Radhuni), a culinary spice traditionally used in Bangladesh for pain management, lacks thorough experimental validation of its analgesic properties. **Objective:** This study investigated the central and peripheral analgesic activities of the methanolic extract of *Trachyspermum roxburghianum* seeds (METRS) in Swiss albino mice. Peripheral analgesic activity was evaluated using the acetic acid-induced writhing test, while central analgesic activity was assessed via the tail immersion method. **Result:** METRS exhibited significant, dose-dependent analgesic effects in both models. In the writhing test, doses of 50, 100, and 200 mg/kg body weight produced inhibitions of 33.69%, 50.00%, and 58.69%, respectively. In the tail immersion test, the 200 mg/kg dose significantly prolonged reaction time and increased percentage elongation, demonstrating central analgesic activity comparable to the standard drug diclofenac sodium. **Conclusion:** The findings indicate that METRS possesses notable analgesic properties, likely mediated through the inhibition of inflammatory mediators and modulation of nociceptive pathways, thereby providing scientific validation for the traditional use of this culinary spice in pain management.

KEYWORDS: *Trachyspermum roxburghianum*, Culinary spice, Analgesic activity, Acetic acid–induced writhing, Tail immersion test.

INTRODUCTION

Spices are an integral component of human diets worldwide, valued not only for their ability to enhance flavor, aroma, and food preservation but also for their health-promoting properties. Increasing scientific evidence indicates that many commonly used culinary spices act as dietary sources of bioactive phytochemicals capable of modulating oxidative stress, inflammatory responses, nociception, and microbial growth.[1,2] Consequently, spices are now widely recognized as important contributors to functional foods and nutraceuticals, bridging the gap between traditional dietary practices and modern pharmacological research.

Several epidemiological observations and experimental studies indicate that regular dietary intake of spices rich in phenolic compounds, flavonoids, and essential oils is associated with a reduced risk of chronic conditions, including inflammatory disorders, metabolic syndromes, and neurodegenerative diseases.[1,3] These beneficial effects are primarily attributed to secondary metabolites such as terpenoids, phenylpropanoids, coumarins, and alkylbenzenes, which modulate multiple molecular pathways involved in inflammation, oxidative stress, and nociception.[1,4] Consequently, growing scientific evidence has renewed interest in traditional culinary spices as promising sources of analgesic and anti-inflammatory agents.[2]

Bangladesh has a rich culinary heritage in which spices are integral to everyday food practices as well as traditional healthcare systems. Notably, spices from the Apiaceae family have been reported to exhibit diverse pharmacological activities, including effects on the central nervous, cardiovascular, and respiratory systems, among others.[5,6] These biological properties are primarily attributed to the presence of bioactive constituents such as polysaccharides, alkaloids, phenylpropanoids (including simple phenylpropanoids and coumarins), flavonoids, and polyacetylenic compounds.[5-7]

*Trachyspermum roxburghianu*m (syn. *Psammogeton involucratus*), locally known as Radhuni, is a widely used aromatic spice in Bangladesh and neighboring regions. The dried fruits (“seeds”) are a key ingredient in Bengali cuisine and are commonly included in the traditional *panch phoron* spice mixture along with cumin, fennel, fenugreek, black cumin, and mustard. Radhuni is valued for its strong aroma, resembling parsley, and its characteristic

flavor similar to celery, and is frequently used to improve palatability and reduce undesirable odors in food preparations.

Beyond its culinary importance, *T. roxburghianum* has a long history of use in traditional medicine as a digestive stimulant, carminative, antispasmodic, and remedy for respiratory ailments, including cough, cold, asthma, and bronchitis.[8] Phytochemical investigations have revealed that the seeds and their essential oil are rich in biologically active constituents such as thymol, sabinene, limonene, β-caryophyllene, flavonoids, and other phenolic compounds.[9-11] These constituents are known to exert antioxidant and anti-inflammatory effects, which may contribute to the spice's traditional use in alleviating pain and inflammatory conditions.[12]

Recent pharmacological studies have reported that extracts and essential oils of *T. roxburghianum* exhibit antimicrobial, antioxidant, wound-healing, and analgesic activities in experimental models, providing scientific support for its ethnomedicinal applications. However, systematic evaluation of its central and peripheral analgesic effects, particularly using crude methanolic seed extracts, remains limited. Therefore, the present study was designed to investigate the central and peripheral analgesic activities of the methanolic extract of *T. roxburghianum* seeds (METRS), aiming to establish a scientific correlation between its traditional culinary-medicinal use as a spice and its experimentally validated pharmacological effects.

MATERIALS AND METHOD

Drugs and chemicals

The reference standard morphine sulfate (Gonoshasthaya Pharmaceuticals Ltd., Bangladesh) and diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh) were used as positive controls for the central and peripheral analgesic models, respectively. Glacial acetic acid was procured from Merck KGaA (Germany) for the writhing test. All chemicals were of analytical grade. The METRS was prepared in-house using absolute methanol (Merck KGaA, Germany), and subsequent doses were freshly prepared by suspending the dried extract in a 1% (v/v) Tween-80 aqueous solution immediately before administration. All drug and extract solutions were prepared to ensure the administered volume was 10 mL per kg of body weight.

Collection and preparation of plant material

Mature seeds of *T. roxburghianum* were procured from a local market in Dhaka, Bangladesh (**Fig. 1**). The seeds were initially sun-dried for multiple days to reduce moisture content, followed by oven-drying at a temperature below 40 °C for 24 hours to achieve uniform dryness and facilitate milling. The thoroughly dried material was then pulverized into a coarse powder using an industrial-grade grinder at the Phytochemical Research Laboratory, University of Information Technology and Sciences (UITS), Bangladesh.

Extract preparation

Approximately 300 g of the powdered seeds were macerated in 2 L of absolute methanol within a sealed, amber-colored glass container (2.5 L capacity) to prevent photodegradation. The mixture was maintained at ambient temperature for a period of 15 days, with intermittent manual agitation to optimize compound extraction. Following maceration, the mixture was sequentially filtered through a cotton plug and Whatman No. 1 filter paper. The resulting filtrate was concentrated under ambient conditions using evaporation until approximately 70% of the solvent had been removed, yielding METRS.[13]

Design of experimental animals

Swiss albino male mice, weighing 25–30 g and aged 4–5 weeks, were obtained from the animal research branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). The animals were housed in polyvinyl cages under standard laboratory conditions and were provided with a pelleted diet recommended by ICDDR, B. To maintain uniform hydration and physiological conditions, food and water were withheld for 12 hours before the experiments. All experimental procedures were conducted in strict accordance with established ethical guidelines for the care and use of laboratory animals. A total of 50 mice were used in the study, with 25 animals allocated for peripheral analgesic evaluation and 25 for central analgesic assessment. The animals were randomly divided into five groups (Group I–V), each consisting of five mice, and each group received a specific treatment. Groups I, II, and III were administered the test extract at doses of 50, 100, and 200 mg/kg body weight, respectively. Group IV received the standard drug diclofenac sodium, while Group V served as the control and was treated with distilled water. Before treatment, each mouse was individually weighed, and all administered doses were adjusted accordingly. For accurate observation and identification during the experiments, individual animals within each group were marked and labeled sequentially as M1 (mice 1), M2, M3, M4, and M5.

Evaluation of analgesic activity

Acetic acid-induced writhing test

The peripheral analgesic effect of the METRS was evaluated using the acetic acid–induced writhing model, as previously described.[14] Mice were pretreated orally with ME (methanolic extract) at 50, 100, and 200 mg/kg or with diclofenac sodium (100 mg/kg, reference drug). Pain was induced 30 minutes later via intraperitoneal injection of 1% acetic acid (0.1 mL/10 g). Each animal was observed individually, and the number of writhes (abdominal constrictions accompanied by stretching or licking) was recorded over a 10-minute period beginning 5 minutes post-injection. Percent inhibition of writhing was calculated relative to the vehicle-treated control group. The percentage inhibition of writhing was calculated using the following equation:

$$\%\ Inhibition = \frac{Control\ licking\ response - Test\ licking\ response}{Control\ licking\ response} \times 100$$

Tail immersion test

The central analgesic activity of METRS was assessed using the tail immersion method, as previously described.[15] Central analgesic activity was evaluated using the tail immersion assay. Mice received oral administration of ME (50, 100, 200 mg/kg) or a subcutaneous injection of morphine sulfate (5 mg/kg, reference drug). After 40 minutes, the proximal third of the tail was immersed in a water bath maintained at 55 ± 0.5 °C. The latency to a rapid tail-flick response was recorded as the reaction time. The experiment was conducted at 30, 60, and 90 minutes post-treatment. Central analgesic activity was quantified as the percentage elongation of reaction time relative to the vehicle-treated control group. The percentage of time elongation was calculated using the following equation:

$$\%\ Time\ Elongation = \frac{T - t}{t} \times 100$$

Where,

T = mean time of the treated group

t = mean time of the control group

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical comparisons between treatment groups and the control were performed using one-way analysis of variance

(ANOVA) followed by Dunnett's post-hoc test. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Acetic acid-induced writhing test

Subcutaneous administration of acetic acid (1%) induced a significant writhe response in the control group. Pre-treatment with the methanolic extract produced a dose-dependent reduction in this nociceptive behavior. At doses of 50, 100, and 200 mg/kg body weight, the extract significantly ($p < 0.05$) decreased the mean number of writhes to 15.25 ± 1.25, 11.5 ± 0.65, and 9.5 ± 1.55, respectively, compared to 23 ± 0.41 in the control group (**Table 1**). This corresponded to percent inhibitions of writhing of 33.69%, 50.00%, and 58.69%. The standard drug (diclofenac sodium, 10 mg/kg) exhibited 76.08% inhibition, with a mean writhe count of 5.5 ± 0.87 **(Fig. 2).**

Tail immersion test

The METRS significantly increased the average tail withdrawal time in the tail immersion test in a dose-dependent manner (**Table 2**). The effect was evident at all observation periods (30, 60, and 90 minutes), with the highest prolongation of immersion time observed at 200 mg/kg, which showed highly significant analgesic activity compared to the control group *(***$p < 0.001$*)*. Lower doses (50 and 100 mg/kg) also produced significant increases in withdrawal latency, though to a lesser extent.

Also, the percentage elongation of tail withdrawal latency increased progressively with dose and time. The 200 mg/kg dose produced the greatest percentage elongation, particularly at 60 min (66.57%), indicating maximal central analgesic efficacy. The standard drug showed the highest percentage elongation at all time points (93.73%, 124.72%, and 77.59% at 30, 60, and 90 min), confirming the dose-dependent central analgesic effect of the extract (**Fig. 3**).

DISCUSSION

The analgesic potential of the METRS was evaluated using experimental models designed to assess both central (narcotic) and peripheral (non-narcotic) mechanisms of pain modulation. Peripheral analgesic activity was investigated employing the acetic acid–induced writhing test in mice, a well-established model for screening compounds that interfere with inflammatory pain pathways.[16] Intraperitoneal administration of dilute glacial acetic acid

(1%) is known to induce localized inflammatory responses characterized by increased vascular permeability and the release of endogenous pain mediators.[17]

This inflammatory insult triggers the biosynthesis of prostaglandins via the cyclooxygenase (COX) pathway and leukotrienes through the lipoxygenase (LOX) pathway.[18] Among these mediators, prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) play a pivotal role in the sensitization of peripheral nociceptors, thereby contributing to pain perception.[19] Suppression of these mediators is a recognized mechanism underlying the analgesic effects of non-steroidal anti-inflammatory drugs (NSAIDs).[20]

In the present study, administration of the METRS resulted in a moderate and dose-dependent reduction in acetic acid–induced writhing, indicating notable peripheral analgesic activity. The observed inhibition of nociceptive responses suggests that the extract may exert its analgesic effect through attenuation of prostaglandin synthesis or release, thereby reducing inflammatory pain signaling. Statistical analysis confirmed that the analgesic effects at all tested doses were significant compared to the control group ($p < 0.05$).

Among the tested doses, the extract exhibited maximum analgesic efficacy at 200 mg/kg body weight, demonstrating a pain-relieving effect comparable to that of the standard reference drug, diclofenac sodium (100 mg/kg, oral). This comparable activity strongly supports the presence of pharmacologically active secondary metabolites within the extract.

The METRS produced a significant, dose-dependent increase in tail withdrawal latency, indicating notable central analgesic effects. The highest dose (200 mg/kg) demonstrated highly significant activity ($***p < 0.001$) at all observation periods, with maximal percentage elongation at 60 minutes (66.57%), suggesting peak central action at this time point. Although the standard drug exhibited superior analgesic efficacy, the extract showed comparable trends in both time- and dose-response patterns. The progressive increase in latency and percentage elongation may be attributed to the modulation of nociceptive transmission and possible interaction with central pain inhibitory mechanisms.

The analgesic activity observed may be attributed to bioactive constituents such as phenolic compounds, flavonoids, and terpenoids previously reported in *T. roxburghianum*, which are known to modulate inflammatory mediators, inhibit COX enzymes, and reduce oxidative stress. Collectively, these findings suggest that *T. roxburghianum* seeds possess promising

analgesic properties mediated through both peripheral inflammatory pathways and potentially central mechanisms, thereby validating its traditional use in pain management.

Table

Table 1: Peripheral analgesic activity of *Trachyspermum roxburghianum* methanolic seed extract in the acetic acid-induced writhing test in mice.

Group	Average writhing (Mean ± SEM)
STD	5.5 ± 0.87***
ME 50	15.25 ± 1.25*
ME 100	11.5 ± 0.65***
ME 200	9.5 ± 1.55*

Here, ME = Methanolic extract; STD = Standard; CTL = Control; SEM = Standard error of the mean. Data are presented as Mean ± SEM (n = 5). *$p < 0.05$, ***$p < 0.001$, compared to the control.

Table 2: Central analgesic effect of methanolic extract of seeds of *Trachyspermum roxburghianum* in the tail immersion test.

Group	Average time of immersion (Mean ± SEM)		
	30 minutes	60 minutes	90 minutes
CTL	3.19 ± 0.19	3.56 ± 0.14	3.48 ± 0.15
STD	6.18 ± 0.05***	8.00 ± 0.08***	6.18 ± 0.08***
ME 50	3.59 ± 0.09*	4.13 ± 0.15*	4.01 ± 0.14
ME 100	4.62 ± 0.08***	4.98 ± 0.10***	4.36 ± 0.27*
ME 200	5.10 ± 0.10***	5.93 ± 0.12***	5.05 ± 0.11***

Here, ME = Methanolic extract; STD = Standard; CTL = Control; SEM = Standard error of the mean. Data are presented as Mean ± SEM (n = 5). *$p < 0.05$, ***$p < 0.001$, compared to the control.

Figure

Fig. 1: Seeds of *Trachyspermum roxburghianum*.

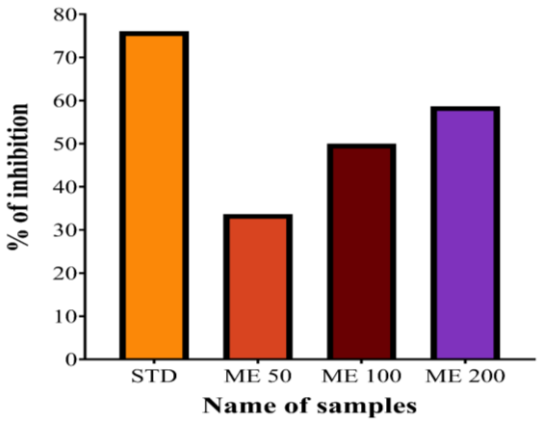

Fig. 2: % of inhibition of the methanolic extract of *T. roxburghianum* seeds (METRS) using acetic acid-induced writhing responses. ME = Methanolic extract; STD = Standard.

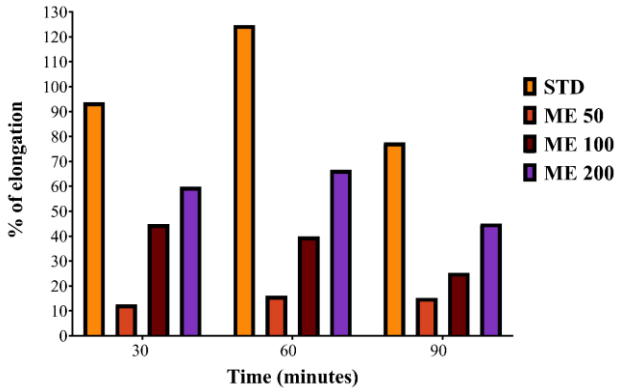

Fig. 3: % of inhibition of the methanolic extract of *T. roxburghianum* seeds (METRS) on tail immersion test in mice. ME = Methanolic extract; STD = Standard.

CONCLUSION

The present investigation demonstrates that the METRS exhibits significant central and peripheral analgesic activities in experimental animal models. The observed dose-dependent prolongation of pain reaction time and increased inhibition of nociceptive responses suggest involvement of both central and peripheral mechanisms, possibly through suppression of prostaglandin synthesis and modulation of inflammatory pathways. The analgesic efficacy at higher doses was comparable to that of the standard NSAIDs, indicating strong pharmacological potential. These effects may be attributed to the presence of bioactive secondary metabolites such as phenolic compounds, flavonoids, and terpenoids. Overall, the findings provide scientific validation for the traditional use of *T. roxburghianum* as a pain-relieving spice and warrant further studies to isolate active constituents and elucidate their precise molecular mechanisms.

ABBREVIATIONS

METRS: methanolic extract of *Trachyspermum roxburghianum* seeds; ICDDR, B: International Centre for Diarrheal Diseases and Research, Bangladesh; LOX: lipoxygenase; PGE_2: prostaglandin E_2; PGI_2: prostacyclin; NSAIDs: non-steroidal anti-inflammatory drugs, COX: cyclooxygenase.

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Conflicts of interest

The authors declare no conflicts of interest.

Author Contribution

Joy Sarker: Conceptualization, Validation, Resources, Methodology, Data curation, Formal analysis, Writing.

Mahfuza Rahman: Investigation, Formal analysis, Writing – review and editing

Md. Al Amin: Data curation, Methodology, Writing – review and editing

ABM Ashraful: Software, Validation, Visualization, Writing – review and editing; Correspondence

Ethical statements

The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), UITS, Bangladesh (**Approval No. UITS/PHARM/IEC/2025/21**), following the internationally accepted guidelines for the care and use of laboratory animals.

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