RESEARCH PAPER

Evaluation of Central and Peripheral Analgesic Activity of Hydroalcoholic Root Extract of *Cynodon dactylon* (L.) Pers. in Experimental Rats

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Abstract

Background: Pain is a common clinical symptom and a major cause of reduced quality of life. Conventional analgesics such as NSAIDs and opioids are effective but may cause gastrointestinal irritation, renal or cardiovascular toxicity, tolerance, and dependence. *Cynodon dactylon* (L.) Pers., traditionally used for pain and inflammation, was investigated for its analgesic potential.

Objective: To evaluate the pharmacognostic, physicochemical, phytochemical, acute-toxicity, and analgesic activities of the hydroalcoholic extract of *Cynodon dactylon* roots (HECD) in experimental animals.

Methods: Authenticated *C. dactylon* roots were dried, powdered, and extracted with 90% ethanol using Soxhlet extraction. The extract was subjected to preliminary phytochemical screening and acute oral toxicity testing at 2,000 mg/kg in Wistar rats. Analgesic activity was evaluated using the hot-plate test for central analgesia and the acetic acid-induced writhing test for peripheral analgesia, with diclofenac sodium (10 mg/kg) as the standard.

Results: The hydroalcoholic extract yield was 9.72% w/w. Phytochemical screening detected carbohydrates, flavonoids, phenols/tannins, triterpenoids, proteins, and amino acids. HECD at 2,000 mg/kg produced no mortality or visible toxic signs. In the hot-plate test, HECD at 200 and 400 mg/kg significantly increased thermal nociceptive latency compared with the control. In the acetic acid-induced writhing test, HECD reduced writhing by 32.54% at 200 mg/kg and 48.11% at 400 mg/kg, whereas diclofenac sodium produced 75.11% inhibition.

Conclusion: The hydroalcoholic root extract of *Cynodon dactylon* exhibited favourable preliminary safety and significant central and peripheral analgesic activity. These effects may be associated with flavonoids, phenolic compounds, tannins, and triterpenoids. Further phytochemical isolation, mechanistic studies, chronic toxicity evaluation, and clinical studies are recommended.

Keywords: *Cynodon dactylon*; analgesic activity; hot-plate test; acetic acid-induced writhing; herbal medicine; flavonoids

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Conventional analgesics, particularly non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, remain the mainstay of pain management. NSAIDs act mainly by inhibiting cyclooxygenase enzymes and reducing prostaglandin synthesis, while opioids modulate pain through central opioid receptors. However, long-term use of NSAIDs is associated with gastrointestinal irritation, ulceration, bleeding, renal impairment, and cardiovascular risk. Opioids may cause sedation, constipation, respiratory depression, tolerance, dependence, and addiction. These limitations have increased interest in medicinal plants as alternative or complementary sources of analgesic agents with multi-target actions and potentially better safety profiles.4

Herbal medicines have been used for centuries in Ayurveda, Unani, Traditional Chinese Medicine, and folk medicine for the management of pain, fever, inflammation, wounds, and other ailments.

Plant-derived constituents such as flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, and saponins can modulate pain pathways by inhibiting inflammatory mediators, reducing oxidative stress, or acting on receptors involved in nociception. Several medicinal plants, including *Curcuma longa*, *Zingiber officinale*, *Salix alba*, and *Capsicum* species, have demonstrated analgesic or anti-inflammatory effects in experimental and clinical studies . Nevertheless, traditional use alone is insufficient to establish efficacy or safety; scientific standardization, phytochemical characterization, toxicity evaluation, and pharmacological validation are essential.6

Cynodon dactylon (L.) Pers., commonly known as Bermuda grass, Doob grass, or Durva, is a perennial grass belonging to the family Poaceae. It is widely distributed in tropical and subtropical regions and occupies an important place in Indian traditional medicine. In Ayurveda and folk practice, different parts of the plant, including roots, leaves, and whole plant, have been used for pain, inflammation, wounds, fever, urinary disorders, and digestive complaints. Previous investigations have reported antioxidant, anti-inflammatory, antimicrobial, antipyretic, antidiabetic, hepatoprotective, wound-healing, and central nervous system activities of *C. dactylon* extracts [8–10]. Phytochemical studies have identified flavonoids, phenolic compounds, tannins, triterpenoids, carbohydrates, proteins, carotenoids, and other constituents that may contribute to these biological effects.8

Although several studies have evaluated the aerial parts or whole plant of *C. dactylon*, evidence specifically supporting the analgesic potential of its roots remains limited. The root is traditionally used in various herbal preparations, but its pharmacological activity has not been sufficiently validated using standardized extracts and experimental pain models. Therefore, the present study was designed to evaluate the pharmacognostic, physicochemical, phytochemical, acute-toxicity, and analgesic properties of the hydroalcoholic extract of *Cynodon dactylon* roots in experimental animals. The analgesic effect was assessed using the hot-plate test, which detects centrally mediated thermal nociception, and the acetic acid-induced writhing test, which evaluates peripherally mediated inflammatory pain. This study may provide scientific support for the traditional use of *C. dactylon* roots and contribute to the development of safer plant-based analgesic agents [11].

2. Materials and Methods

2.1 Plant material collection and authentication

Roots of *Cynodon dactylon* (L.) Pers. were collected from a natural habitat during the appropriate season. The roots were separated from the plant, washed thoroughly with running water to remove soil and other adhering impurities, and shade-dried at room temperature. The plant material was taxonomically identified and authenticated by Dr. Alok Srivastav, Associate Professor, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh, India. A voucher specimen was deposited in the departmental herbarium for future reference.vikki-plag-file.docx

2.2 Pharmacognostic evaluation

Macroscopic evaluation of the dried roots was performed by observing colour, odour, taste, size, shape, surface characteristics, and fracture. For microscopic analysis, free-hand transverse sections of the root were prepared, stained with safranine to highlight lignified tissues, and examined under a light microscope. Powder microscopy was also carried out to identify diagnostic features such as parenchyma cells, fibres, vessel elements, and starch grains .

2.3 Physicochemical evaluation

Physicochemical parameters of the powdered root were determined according to standard pharmacognostic procedures. These included loss on drying, total ash value, water-soluble ash, acid-insoluble ash, alcohol-soluble extractive value, and water-soluble extractive value. The percentage values were calculated on a dry-weight basis and used as preliminary quality-control parameters for the crude drug.2

2.4 Preparation of hydroalcoholic extract

The shade-dried roots were coarsely powdered using a mechanical grinder and passed through a suitable sieve to obtain uniform particle size. The

powdered material was extracted with 90% ethanol using a Soxhlet apparatus until the solvent in the siphon tube became colourless. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further dried to obtain a solid residue, designated as the hydroalcoholic extract of *Cynodon dactylon* roots (HECD), and stored in an airtight container at low temperature until further use. The percentage yield was calculated using the following formula:

Percentage yield=Weight of dried extractWeight of crude drug×100\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of crude drug}} \times 100Percentage yield=Weight of crude drugWeight of dried extract×100

2.5 Preliminary phytochemical screening

The hydroalcoholic and aqueous extracts were subjected to qualitative phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenols and tannins, steroids, triterpenoids, saponins, proteins, and amino acids. Standard reagents and colour reactions were used, including Mayer's, Wagner's, and Dragendorff's reagents for alkaloids; ferric chloride and gelatin tests for tannins; alkaline reagent and lead acetate tests for flavonoids; and the frothing test for saponins.3

2.6 Drugs and chemicals

Diclofenac sodium was used as the standard analgesic drug. Acetic acid, ethanol, carboxymethylcellulose (CMC), gum acacia, and phytochemical screening reagents were of analytical grade. Distilled water was used throughout the study.4

2.7 Experimental animals

Healthy albino Wistar rats of either sex, weighing 180–200 g, were used for acute toxicity and analgesic activity studies. Female rats used in the toxicity study were nulliparous and non-pregnant. Animals were acclimatized for at least five days before experimentation and maintained under standard laboratory conditions at 22 ± 3°C, 50–60% relative humidity, and a 12-hour light/dark cycle. Standard pellet diet and water were provided ad libitum. The experimental protocol followed institutional animal-ethics requirements and OECD principles of humane animal care.6

2.8 Acute oral toxicity study

Acute oral toxicity of HECD was evaluated using the OECD up-and-down procedure/limit-test approach. HECD was suspended in 0.5% CMC and administered orally at a limit dose of 2,000 mg/kg body weight after an overnight fast of approximately 18 hours. Food was withheld for 1–2 hours after dosing. Animals were observed during the first 30 minutes, periodically during the first 24 hours with special attention during the first 4 hours, and then daily for 14 days. Mortality, behavioural changes, skin and fur condition, salivation, diarrhoea, tremors, convulsions, lethargy, sleep, coma, and other signs of toxicity were recorded.7

2.9 Evaluation of analgesic activity

Animals were randomly divided into treatment groups, each containing six rats. The groups included vehicle control, diclofenac sodium 10 mg/kg, and HECD at 200 and 400 mg/kg. The test extract and standard drug were administered as a single dose before the respective pain tests.9

2.9.1 Hot-plate test

Central analgesic activity was evaluated using Eddy's hot-plate method. The hot plate was maintained at 55 ± 0.5°C, and a cut-off time of 15 seconds was used to prevent tissue injury. Each rat was placed on the hot plate, and the reaction time was recorded when the animal licked its fore- or hind-paw or jumped. The reaction time was recorded at 0 (pre-drug), 30, 60, 90, 120, 150, and 180 minutes after treatment. An increase in reaction latency compared with the control group indicated central analgesic activity.9

2.9.2 Acetic acid-induced writhing test

Peripheral analgesic activity was evaluated using the acetic acid-induced writhing test. One hour after administration of the test extract or standard drug, 0.2 mL of 0.8% acetic acid solution was administered intraperitoneal to induce visceral pain. Each rat was placed in an individual observation chamber, and the number of writhing responses—characterized by abdominal constriction and hind-limb stretching—was

counted from 5 to 30 minutes after acetic acid injection. The percentage inhibition of writhing was calculated as follows:

Percentage inhibition=Mean writhes of control−Mean writhes of treated groupMean writhes of control×100\text{Percentage inhibition} = \frac{\text{Mean writhes of control} - \text{Mean writhes of treated group}}{\text{Mean writhes of control}} \times 100Percentage inhibition=Mean writhes of controlMean writhes of control−Mean writhes of treated group×100

2.10 Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM), with six animals per group. Data were analysed using one-way analysis of variance (ANOVA), followed by comparison of treated groups with the control group. Differences were considered statistically significant at $p<0.05p < 0.05p<0.05$, $p<0.01p < 0.01p<0.01$, and $p<0.001p < 0.001p<0.001$.

3. Results and Discussion

3.1 Pharmacognostic and physicochemical evaluation

The roots of Cynodon dactylon were fibrous, cylindrical, cream-coloured, and up to approximately 4 mm thick. Transverse sections showed an intact epidermis, broad cortex, continuous sclerenchymatous cylinder, and wide dictyostele. Powder microscopy revealed parenchyma strands, fibres, vessel elements, and abundant starch grains, supporting the authenticity of the crude drug.



Figure 1. Roots of Cynodon dactylon.



Figure 2. Transverse section of C. dactylon root—entire view.

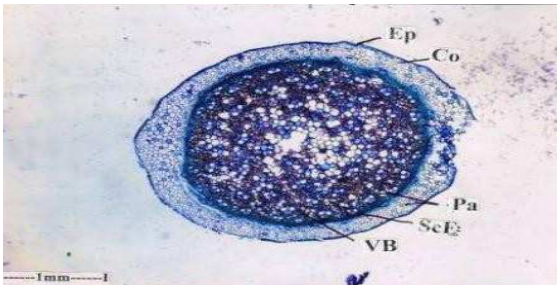


Figure 3. Vascular bundle on the inner part of the sclerenchyma cylinder.

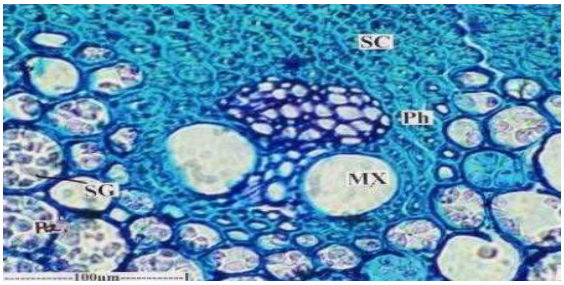


Figure 4. Vascular strand on the outer part of the sclerenchyma cylinder.

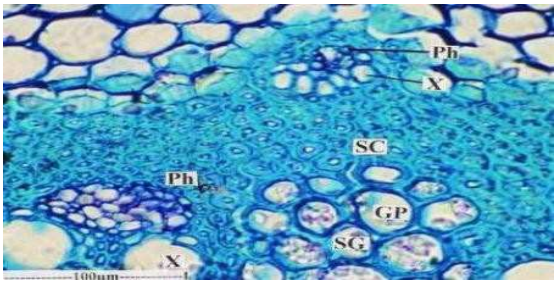


Figure 5. Medullary vascular bundle—enlarged view.

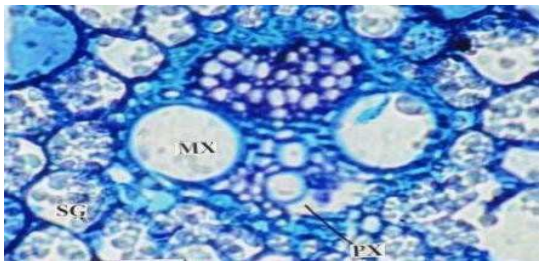


Figure 6. Ground parenchyma cells with starch grains.

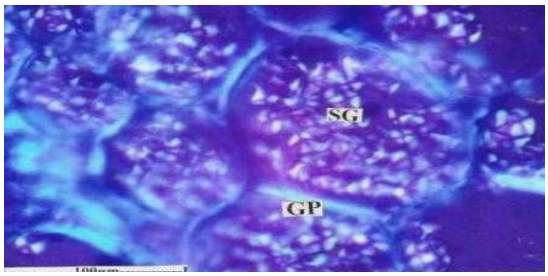


Figure 7. Powder microscopy: parenchyma strand.

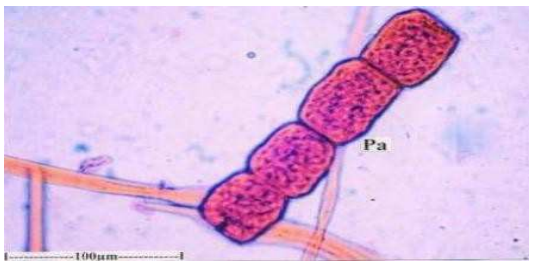


Figure 8. Powder microscopy: narrow fibres and parenchyma.

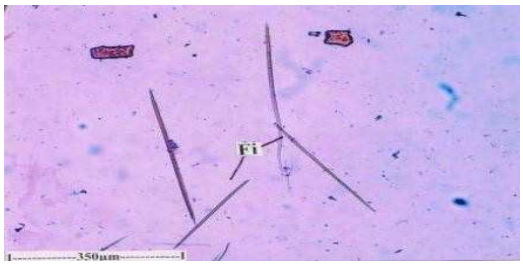


Figure 9. Powder microscopy: narrow fibre—enlarged view.

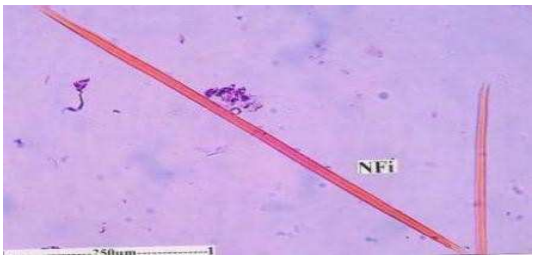


Figure 10. Powder microscopy: narrow and wide fibres.

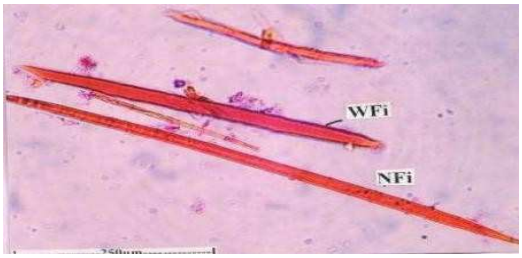


Figure 11. Powder microscopy: vessel elements with simple end-wall perforation.

The physicochemical constants are presented in Table 1 and Figure 12. Loss on drying was 7.23% w/w, total ash 8.49% w/w, and acid-insoluble ash 1.11% w/w, indicating low moisture and minimal siliceous contamination. The alcohol-soluble extractive value (9.11% w/w) exceeded the water-soluble extractive value (5.46% w/w), suggesting a higher proportion of moderately polar constituents.

Table 1. Physicochemical constants of C. dactylon root

Parameter	Mean (% w/w)
Loss on drying	7.23
Total ash	8.49
Acid-insoluble ash	1.11
Water-soluble ash	3.23
Alcohol-soluble extractive	9.11
Water-soluble extractive	5.46

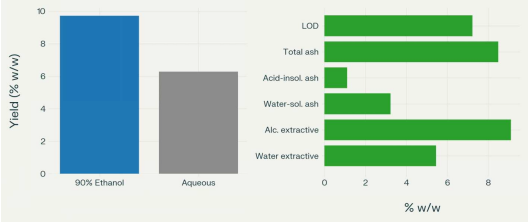


Figure 12. Extraction yield and physicochemical constants of C. dactylon root.

3.2 Extraction yield and phytochemical screening

Soxhlet extraction of 50 g powdered root with 90% ethanol yielded 4.86 g hydroalcoholic extract (9.72% w/w), whereas aqueous extraction yielded 3.14 g (6.28% w/w). The higher hydroalcoholic yield indicates more efficient extraction of polar and moderately polar phytoconstituents.

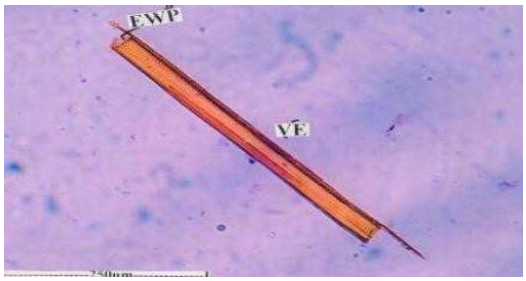


Figure 13. Soxhlet extraction of powdered C. dactylon root.

Both hydroalcoholic and aqueous extracts contained carbohydrates, flavonoids, phenols/tannins, and triterpenoids. Proteins were detected in the aqueous extract and amino acids in the hydroalcoholic extract. Alkaloids, glycosides, steroids, and saponins were not detected. These constituents may contribute to the observed analgesic and anti-inflammatory activity.

3.3 Acute oral toxicity

Oral administration of HECD at 2,000 mg/kg produced no mortality or observable toxic manifestations during 14 days. Animals showed no abnormal behaviour, salivation, diarrhoea, tremors, convulsions, lethargy, or other signs of toxicity. Thus, HECD passed the acute oral toxicity limit test and was preliminarily safe at the tested dose.

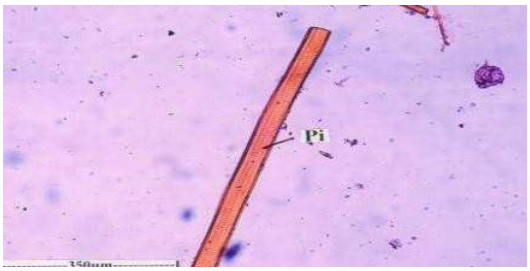


Figure 14. Oral dosing of experimental animal.

3.4 Hot-plate test

In the hot-plate test, the control group showed reaction times of 4.34–4.46 s. Diclofenac sodium (10 mg/kg) produced the greatest increase in latency. HECD at 200 and 400 mg/kg significantly increased thermal nociceptive latency compared with control, indicating central analgesic activity.

Table 2. Effect of HECD on hot-plate reaction time in rats

Treatment	Dose (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
Control	—	4.34 ± 0.02	4.41 ± 0.06	4.46 ± 0.05	4.42 ± 0.05	4.38 ± 0.04	4.35 ± 0.03
Diclofenac sodium	10	9.11 ± 0.54**	9.14 ± 0.02**	9.19 ± 0.06**	8.72 ± 0.07**	7.96 ± 0.06**	6.84 ± 0.05**
HECD	200	6.12 ± 0.05*	6.15 ± 0.75	6.19 ± 0.98*	5.94 ± 0.06*	5.61 ± 0.05*	5.24 ± 0.04*
HECD	400	5.53 ± 0.08	5.56 ± 0.46**	5.58 ± 0.98**	5.42 ± 0.07**	5.21 ± 0.06*	4.96 ± 0.05*

Values are mean ± SEM (n = 6). *p < 0.05; **p < 0.01 versus control.



Figure 15. Effect of HECD in rats on the hot-plate test.

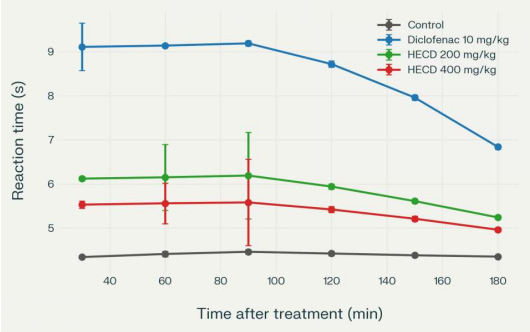


Figure 16. Effect of HECD on hot-plate reaction time in rats. Data are mean ± SEM (n = 6).

The increase in thermal latency suggests modulation of central pain-processing pathways. However, the effect was not consistently dose-dependent, as the 200 mg/kg dose showed slightly higher latency than 400 mg/kg at several time points. Diclofenac sodium remained more effective throughout the observation period.

3.5 Acetic acid-induced writhing test

The control group produced 62.11 ± 1.65 writhes per 30 min. Diclofenac sodium reduced writhing by 75.11%. HECD produced 32.54% inhibition at 200 mg/kg and 48.11% inhibition at 400 mg/kg, demonstrating dose-related peripheral analgesic activity.

Table 3. Effect of HECD on acetic acid-induced writhing in rats

Treatment	Dose (mg/kg)	Writhes per 30 min	Inhibition (%)
Control	—	62.11 ± 1.65	—
Diclofenac sodium	10	14.68 ± 0.59	75.11
HECD	200	45.62 ± 1.53	32.54
HECD	400	38.66 ± 1.54	48.11

Values are mean ± SEM (n = 6).

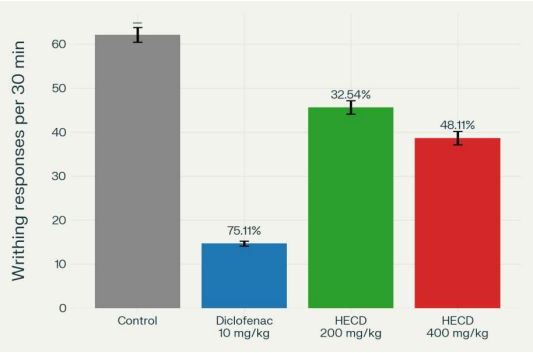


Figure 17. Effect of HECD on acetic acid-induced writhing in rats.

Suppression of acetic acid-induced writhing indicates peripheral analgesic activity, possibly through inhibition of inflammatory mediators such as prostaglandins, bradykinin, histamine, and serotonin. The greater inhibition at 400 mg/kg supports a dose-related effect.

3.6 Overall discussion

HECD exhibited both central and peripheral analgesic activity. The hot-plate results suggest central pain modulation, while reduction of acetic acid-induced writhing indicates peripheral anti-nociceptive activity. The activity may be attributed to flavonoids, phenolic compounds, tannins, and triterpenoids present in the extract. Further isolation of active constituents, mechanistic studies, chronic toxicity evaluation,

and clinical studies are required to confirm therapeutic potential and establish a definitive dose-response relationship.

3.6 Overall discussion

The present findings demonstrate that the hydroalcoholic root extract of Cynodon dactylon possesses appreciable central and peripheral analgesic activity in rats. In the hot-plate model, HECD increased thermal nociceptive latency, suggesting an effect on central pain modulation. In the acetic acid-induced writhing model, HECD significantly reduced writhing responses, particularly at 400 mg/kg, indicating inhibition of peripheral inflammatory pain mechanisms.

The analgesic activity may be associated with flavonoids, phenolic compounds, tannins, and triterpenoids detected in the extract. These phytochemical classes are known to modulate inflammatory mediators, oxidative stress, prostaglandin synthesis, and nociceptor sensitization. However, the present study evaluated a crude hydroalcoholic extract; therefore, the precise active constituents and molecular mechanisms remain to be established.

Further studies should include isolation and characterization of active compounds, quantitative phytochemical analysis, anti-inflammatory assays, receptor-based mechanistic studies, subacute and chronic toxicity evaluation, and clinical validation. Such studies would help confirm the therapeutic potential of C. dactylon root extract and establish a reliable dose-response relationship.

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