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ORIGINAL CONTRIBUTION

Central and peripheral antinociceptive activity of *D. linearis* ethanolic leaf extract

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ABSTRACT

Background: *Dicranopteris linearis,* a medicinal plant, is considered effective in relieving pain. Though previously it has been scientifically evaluated, very few of the studies aimed at differentiating the central and peripheral analgesia produced by this plant. The present study was designed to examine the efficacy of its leaf extract in antinociception and qualitatively assess its mode of activity.

Methods: Ethanol extract of the leaf at 400, 200 and 100 mg/kg were investigated on mice and compared with the standard(s). Tail flick, tail pressure, tail immersion and hot plate methods were employed to observe central acting potential whereas abdominal constriction and biphasic pain models were executed to understand its peripheral action.

Results: DLET 400 mg/kg confirmed moderate efficacy in comparison with the standard morphine in all centrally acting models and superseded (44.29% in formalin treatment, late phase) aspirin in peripherally acting models. Lower doses of the extract were able to produce mild effects in the experiments. Morphine inhibited the tail flick response up to 83.45% at 60-minute interval whereas aspirin exhibited similar efficacy in both writhing (41.06%) and biting (40.35% in late phase) tests.

Conclusion: Findings suggested that *D. linearis* leaf on ethanol extraction yielded in compounds that has potential to suppress nociception. The extract acted more like a peripheral inhibiting agent. However, further investigation is necessary to prescribe its safe and optimum use as an analgesic.

Keywords

Dicranopteris linearis, Antinociceptive, Central analgesic, Peripheral analgesic

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INTRODUCTION

A significant part of the world population is affected by pain which exerts a crucial challenge in clinical medicine [1,2]. As currently available antinociceptive drugs are associated with adverse effects like sedation, addiction, nausea, apnea, constipation etc., and the alternate choices of NSAIDs risks stroke, myocardial infarction, gastrointestinal ulceration and bleeding [1], Current studies are focused on finding safer and potent alternatives. The search includes natural sources like plant materials [3]. Among the many traditionally used plants for analgesic properties, *Dicranopteris linearis* L., is considered a potent one [4]. *D. linearis*, belonging to Gleicheniaceae family, is a medicinal plant, known for its efficacy over cough, hypersensitivity, respiratory distress, fever, ulcer, wound, women sterility, intestinal worms and many more ailments [5-7]. Scientifically, the plant has been confirmed as pharmacologically active against nociception, pyrexia, inflammation, infection, oxidation, hepatotoxicity and cytotoxicity whereas other potential activities are still under investigation [7-11].

As a traditionally used natural pain reliever, *D. linearis* was previously investigated at different solvent extraction (aqueous, methanol and chloroform) [8,12]. As selection of solvent extracts polar to non-polar plant material, the present study was attempted to fractionate the polar compounds of the plant leaf with ethanol and evaluate its analgesic activity in animal model [13]. No such study was reported before. Moreover, very few studies were found which explored both peripheral and central analgesia simultaneously. Thus, the study was also aimed to validate its appropriate use by comparing the central and peripheral analgesic action of the plant. Alongside the scientific evaluation of the efficacy, the study was also focused to draw optimum dose line for traditional use of the plant for antinociception.

METHODS

Collection and preparation of the extract

Fresh plant leaf (approximately 7 kg) was collected from Mymensingh district (24°45'14" N 90°24'11" E) of Bangladesh in June. 2014. A sample specimen was submitted to Bangladesh National Herbarium and preserved with an accession number DACB 42009. After a thorough wash, the leaves were sundried before crushed into powder. Approximately, 600g powder was obtained and soaked in 3L of ethanol (96%) and left for 72h with occasional shaking. At the end, the mixture was sieved with paper filter and concentrated using a Rotary Evaporator (Biobase RE-2010, China). Approximately 3 gm of crude extract was obtained and preserved in air-tight amber glass container [5].

Drugs and Reagents

Ethanol (RCI Labscan Limited, Thailand), Morphine (UniMed UniHealth Limited, BD), Acetyl Salicylic Acid (Square Pharmaceuticals Limited, BD), Paracetamol (Beximco Pharmaceuticals Limited, BD) were obtained for the experiments.

Grouping of Animal

Swiss Albino mice of both sexes, aged 45 days, weighed 24-30g, were selected for the experiments. Mice were kept in temperature-controlled room at 25±1 °C with 12h light/dark cycle and fed standard mice pellets and portable water *ad libitum*. For each experiment, mice were divided into five groups each containing six mice and designated as follows: Group 1: Control, administered with vehicle, water; Group 2: Positive Control/Standards, administered with Morphine (5mg/kg)/Acetyl Salicylic Acid (ASA) (100mg/kg), depending on the experiment; Group 3: Test Sample, *D. linearis* ethanolic leaf extract (DLET) 100 mg/kg; Group 4: Test Sample, DLET 200 mg/kg; Group 5: Test Sample, DLET 300 mg/kg.

Acute Toxicity Test

To investigate the immediate and short-term toxicity, healthy mice (n=5) were orally administered with high doses (100, 250, 500, 1000 mg/kg) of the plant extract and observed for the next 3 days for unusual behavior or any mortality [14].

Central Antinociceptive Tests

Tail pressure method

In this method, mechanical pain was induced at the base of mice tail through applying metal artery clip having its jaw covered with silicon to avoid tissue damage. The method was applied before and after the drug administration [15]. A cutoff time of 10s was considered and mice which did not struggle to get rid of the clip were not selected for further experiment. The time at which mice attempted to extricate the clip was recorded. The process was repeated at 30, 60, 90 and 120 minutes after the drug or test sample administration. Morphine (5mg/kg) served as positive control. From the comparison of pre- and post-treatment, percentage inhibition of pain was calculated. Increase in pain threshold in comparison with the control group was considered indication for antinociceptive activity.

$$\text{Percentage inhibition of pain (\%)} = \frac{T_{sample} - T_{Control}}{T_{Control}} \times 100$$

Where T = the time at which mice attempted to extricate its tail.

Tail flick response method

In this experiment, radiant heat was applied to mice tail using an analgesiometer (Orchid Scientific TFA01, India) to record the tail flicking latency before and after the drug or test sample administration [16,17]. The mice were kept individually in suitable restrainer keeping the tail free. 5A current was passed through a naked nichrome wire where the tail was placed at a distance of 1.5 cm and applied within 2 cm of the tail. The time between the onset of heat application and flicking of the tail was noted as reaction time. To avoid tissue injury, a cut-of time of 10s was considered. Paracetamol (10mg/kg) served as standard. The withdrawal time for the groups were compared with the negative control group where prolongation of the flicking response was considered as indication of antinociceptive activity. Percentage inhibition of pain was deduced from below formula:

$$\text{Percentage inhibition of pain (\%)} = \frac{T_{sample} - T_{Control}}{T_{Control}} \times 100$$

Where, T=time.

Tail Immersion Method

Mice tail at 2cm at the tip was dipped into warm water bath (55.0 ± 0.5 C) and the reaction time to withdraw the tail was recorded immediately before and in every 30 minutes interval after drug administration for 2 hours [18]. Longer sustained duration for tail withdrawal was considered an indication by the sample as pain reliever. Percentage inhibition was measured using following equation:

$$\text{Percentage inhibition of pain (\%)} = \frac{T_{sample} - T_{Control}}{T_{Control}} \times 100$$

Where, T=time.

Hot Plate Test

Thermal induced nociceptive stimulus was applied in this test using a hot plate, where mice were placed and observed for their escape-oriented behavior before and after the drug administration [19]. At first, a Ugo Basile 7280 hotplate, Italy was heated to 50 ± 0.2 °C and mice were placed and selected for the main test based on the cut-off latency of 5-7s. after selection, mice were challenged pre- and post- oral drug treatment at 30-, 60-, 90- and 120-minutes interval with the hotplate and response time was recorded. Percentage inhibition was calculated using the general formula:

$$\text{Percentage inhibition of pain (\%)} = \frac{T_{sample} - T_{Control}}{T_{Control}} \times 100$$

Where, T=time.

Peripheral Antinociceptive Tests

Acetic Acid-Induced Abdominal Constriction Test

The acetic acid induced abdominal constriction test was conducted to evaluate the peripheral antinociceptive activity of the plant extract [19]. 0.6% acetic acid (0.1ml/10g) was peritoneally injected to mice before they were observed for specific pattern of abdominal constriction, also known as writhing, for 25 minutes keeping the first 5 minutes excluded from the calculation. Standard ASA (100mg/kg) and the test samples were administered 1h prior commencing the experiment. Decrease in number of writhing was considered indicator of antinociceptive activity. Percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition of pain (\%)} = \frac{W_{Control} - W_{Sample}}{W_{Control}} \times 100$$

Where, W= number of writhing activities.

Formalin-Induced Paw Licking Test.

The formalin induced paw licking and biting test had been described as an appropriate model for assessing both central and peripheral analgesic properties of medicinal agents [18,19]. In this test, a centrally acting analgesic (ASA 100mg/kg) and a central and peripheral acting analgesic (Morphine 5mg/kg) was applied as standards. Sixty minutes after the drug and test sample administration, 20 µl of 5% v/v formalin was injected in subplantar surface of the left hind paw. The licking and biting responses were measured in seconds at two phases – early phase (0-5 min) and the late phase (16-30 min). reduction in biting and licking activity compared to the control group was considered the indication of analgesia. Percentage of pain inhibition was calculated as:

$$\text{Percentage inhibition of pain (\%)} = \frac{LT_{Control} - LT_{Sample}}{LT_{Control}} \times 100$$

Where, LT= total duration of paw licking activity.

Statistical Analysis

Maximum possible analgesia (MPA) was calculated in percentage and presented from data as mean ± standard error of the reaction time in all tests except for the abdominal constriction test where the data was based on the number of writhing. All groups were compared to the negative control group and in this regard, one way analysis of variance test (ANOVA) was performed

followed by Dunnett's T test via SPSS for windows software (version 24). Data were considered statistically significant when the confidence interval was found at 95% (p<0.05), 99% (p<0.01) or 99.99% (p<0.001), denoted with asterisk (*) sign.

RESULTS

Acute Toxicity Test

In the period of treatment and its following observation, no abnormal behavior or symptoms or death was recorded. However, indigestion was reported at higher doses like 1000 mg/kg. As a consequence, lower doses were adopted for the main experiments.

Tail pressure Test

In tail pressure test, maximum pendency was observed by morphine at 60 min (79.41%) followed by smooth fall in action (Figure 1a). On the other hand, DLET 400 demonstrated a linear increase in efficacy till 120 min. DLET 200 mimicked its higher dose though DLET 100 was associated with a sharp fall after its peak at 60 min (29.25%) as exhibited by morphine.

Tail flick response Test

Findings of the experiments were graphically presented to compare the test samples with the standard drug. In tail flick test, morphine at 5 mg/kg showed highest peak in

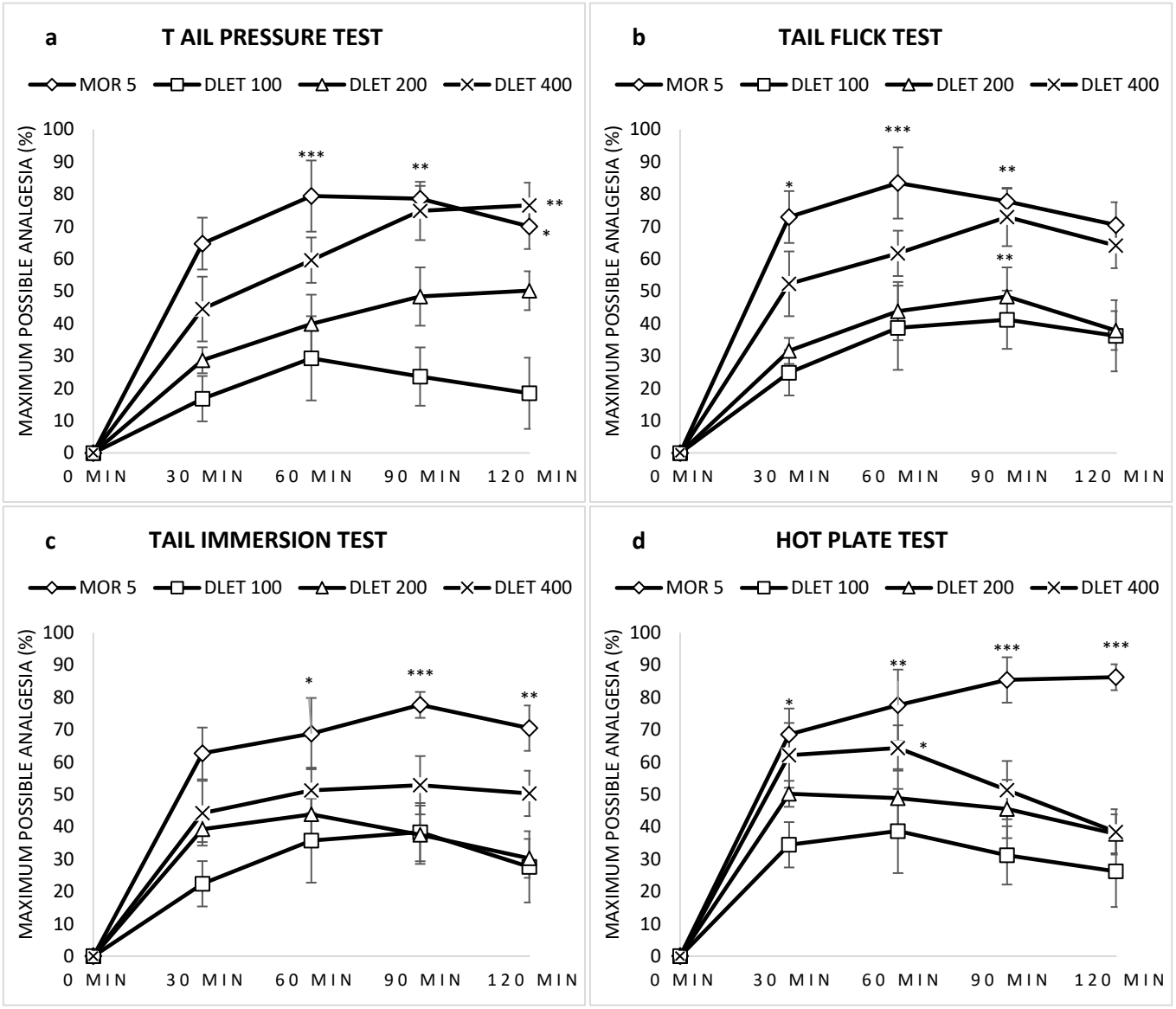

Figure 1 (a-d): Maximum possible analgesia (MPA) (%) representing the effect of the ethanol extract of the leaf of *D. linearis* compared to morphine sulfate (positive control) administered into mice, evaluated by centrally-acting models of (a) tail- pressure method (b) tail-flick method (c) tail immersion method and (d) hot plate method. MOR = Morphine Sulphate , DLET = *D. linearis* leaf ethanol extract. Data presented as mean ± standard error (n=6). and analyzed by one-way ANOVA followed by Dunnett t test where *, **, *** denoted p < 0.05, p < 0.01 and p < 0.001 respectively and statistically significant. All groups were compared to control.

efficacy at 60 min (83.45%) followed by a slight fall in next two intervals (Figure 1b). On the contrary, DLET 400 mg/kg exhibited a gradual increase in analgesia till 90 min (72.95%). DLET at lower doses showed mild action at initial intervals though shifted to moderate action over time.

Tail Immersion Test

Figure 1c depicted that, unlike tail flick and tail pressure methods, in tail immersion test, morphine was found to increase its analgesic action till 90 min (77.68%) whearas DLET 400 was observed with no significant increase in action after its first rise at 30 min (44.21%). Both DLET 200 and 100 was observed with increased efficacy till 60 min though could not produce significant differnces in action in late phases (90 min and 120 min).

Hot Plate Test

In hot plate method, morphine showed a linear sharp increase in analgesia from 30 min till 120 min (68.56% to 86.24%) (Figure 1d). On the contrary, other groups showed a mild increase in efficay after the first interval and ended in downward action in last two intervals (90 min and 120 min).

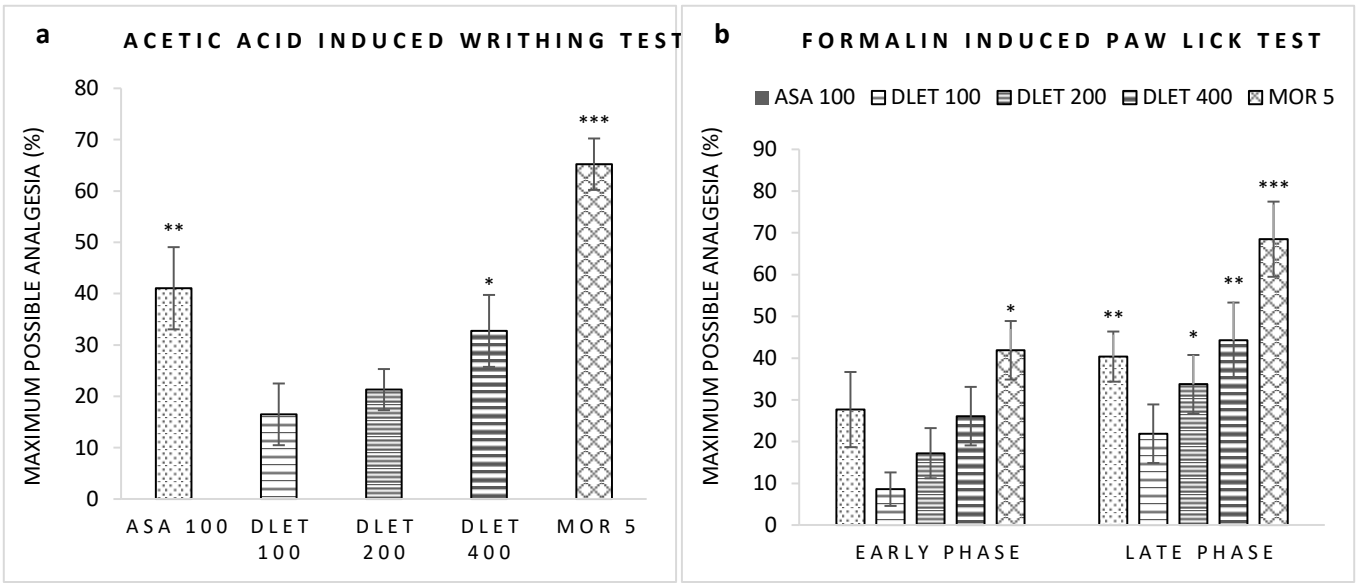

Figure 2 (a-b): Maximum possible analgesia (MPA) (%) representing the effect of the ethanol extract of the leaf of *D. linearis* compared to morphine sulfate and acetyl salicylic acid (positive controls) administered into mice, evaluated by peripherally-acting models of (a) acetic acid induced writhing test and (b) formalin induced biphasic pain test. MOR = Morphine Sulphate, ASA =Acetyl Salicylic Acid, DLET = *D. linearis* leaf ethanol extract. Data presented as mean ± standard error (n=6). and analyzed by one-way ANOVA followed by Dunnett t test where *, **, *** denoted $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively and statistically significant. All groups were compared to control.

Acetic Acid-Induced Abdominal Constriction Test

In acetic acid induced abdominal constriction test, the standard ASA 100 was able to reduce the pain by 41.06% (Figure 2a) whereas morphine suppressed the pain response to a great extent (65.23%). DLET 400 closely met the standard ASA by its action (32.75%). DLET 200 acted as a moderate analgesic (21.31%) whereas DLET 100 produced mild effect (16.49%).

Formalin-Induced Paw Licking Test

In formalin induced biphasic pain test, all groups generated mild action in early phases compared to that of their late phases except for morphine which significantly inhibited the pain in both phases (41.86% and 68.48% respectively) (Figure 2b). ASA 100 and DLET 400 inhibited the pain up to 27.66% and 26.09% respectively in early phase whereas in late phase, their action climbed up to 68.52% and 44.29% respectively. DLET 200 also produced a significant reduction of pain in late phase (33.74%).

DISCUSSION

Antinociceptive drugs act on central or peripheral nervous system in order to alleviate or relieve pain however, without significant alteration of consciousness [20]. While central analgesics raise the threshold for pain and alter physiological response towards it, peripheral analgesics inhibit the impulse generation at chemoreceptor sites of pain [21]. In this study, pain-state models were employed using thermal and pressure stimuli which illustrated the central analgesic responses

of drugs and test samples focusing above the spinal cord [22]. Tail flick, tail immersion and tail clip models mediated a spinal reflex to a nociceptive stimulus whereas hot plate method involved supraspinally organized brain functions of mice [23]. Except the pressure model, the other methods were acute thermic and phasic pain model which were subjected to selective attenuation of centrally-acting opioid-like analgesic compounds [18]. Regarding the peripheral analgesic models, acetic acid induced abdominal constriction was caused by peritoneal tissue damage and induced inflammation by peritoneal macrophages and mast cells released by TNF-α, IL-1β, IL-8, bradykinin, substance P, serotonin and histamine like mediators [24,25]. Analgesic activity expressed in this model were due to involvement of α2 and β1 adrenergic receptors [26]. On the contrary, formalin induced biphasic pain model was established through two phase nociceptive responses, firstly, by sensitizing sensory C-fibers and at prolonged phase by developing injury-induced spinal sensitization which eventually sensitized dorsal horn neuron for inflammation associated pain [18,27]. In general, centrally-acting drugs, like morphine, suppresses both phases of pain, while peripherally-acting drugs like aspirin only suppress the late phases [28].

Morphine sulphate and Acetyl Salicylic Acid were used as the standards as central and peripheral acting analgesics respectively. Morphine is a centrally-acting opioid-like analgesic compound whereas Aspirin is peripheral NSAIDs [4,24,29]. Being non-selective, aspirin irreversibly blocks cyclooxygenase isozymes- COX-1 and COX-2 that generates prostaglandin, a proinflammatory substance [30]. On the other hand, morphine binds to opioid receptors, inhibits transmission of pain signals, signals nociception-modulating neurons in the spinal cord, and also blocks primary afferent pain receptors to the dorsal horn sensory projection cells [31]. In all the experiments, morphine exhibited most significant antinociceptive response among all the groups at all intervals in comparison to control. In the central analgesic study through tail flick, tail pressure, tail immersion and hot plate methods, *D. linearis* leaf extract demonstrated significant pain reduction through prolonged reaction time by its high dose (400 mg/kg) however, failed to exhibit strong analgesic action by lower doses (200 & 100 mg/kg) as compared to morphine (5mg/kg). While in peripheral acting models, DLET 400 nearly produced similar level of action as aspirin (100 mg/kg) though the other two smaller doses could not produce directly comparable efficacy. None could reach the effectiveness of morphine in abdominal constriction as well as in the early phase licking activities by the mice.

The findings indicated that ethanol extract of *D. linearis* leaf acted more as a peripheral acting antinociceptive agent rather than its central action though data supports its moderate efficacy on central nervous system. The leaf contains high amount of phenol, flavonoids (as flavonol 3-Oglycosides), triterpenes, saponins and steroids [5]. Moreover, previous studies reported possible mechanisms for central and peripheral analgesic activity involving modulation of opioid receptors and TRPV1, bradykininergic and glutamatergic system, PKC activity and l-arginine/NO-dependent, cGMP-independent pathway [4]. Alongside, numerous volatile and non-volatile bioactive compounds was found in literature suggesting attenuation of nociception in mice [4].

CONCLUSION

In both peripheral and central acting models of antinociceptive studies, *D. linearis* leaf demonstrated efficacy though the degree of effectiveness were moderate. At this stage of study, responsible biological compounds were not investigated. Therefore, further investigations might be directed to determine optimum doses, best extraction solvent and phytochemical screening and fractionation.

Abbreviations

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; ASA: Acetyl Salicylic Acid; DLET: *D. linearis* leaf ethanol extract; TNF: Tumor Necrosis Factor; IL: Interleukins; TRPV1: The transient receptor potential cation channel subfamily V member 1; cGMP: Cyclic Guanosine Monophosphate.

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Authors' Contributions

This work was carried out in collaboration between all authors. Authors KN and MMB designed, coordinated and supervised the project. AH, MSUJ and RRT performed the experiments and prepared the graphical presentations. KN prepared the manuscript. MMB participated in the interpretation of data to reach a scientific discussion. All authors read and approved the final manuscript.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All experiments associated with animal handling were performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th ed.; The National Academies Collection adopted by the institutional guideline for animal handling (Ref. no. IPSDRLAB/AHCP/01/18). The experimental design was authorized by the Institutional Ethical Committee Clearance (Ref. No. IPSDRLAB/IECC/11/20) from the Institute for Pharmaceutical Skill Development and Research, Bangladesh.

Consent for Publication

Not applicable.

Competing Interests

All authors agreed on the article before submission and had no conflict of interests.

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