Antinociceptive Activity of Methanol Extract of *Dioscorea Pentaphylla* Linn. Leaves in Mice

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Abstract

**Background:** *Dioscorea pentaphylla* L. a common plant of Dioscorea family commonly called five leaves in Bangladesh. The plant contains the alkaloid, carbohydrate, tannin, gum protein, steroid, glycoside, flavonoids. It is used as an analgesic, anti-inflammatory, and Powder of plant extract given orally in abdominal pain after delivery. The aim of the study: The present study was designed to evaluate chemical constituents and to investigate the antinociceptive activity of methanol extracts of *Dioscorea pentaphylla* (MEDP). **Methods:** The antinociceptive activity of MEDP was investigated using heat-induced (hot-plate and tail-immersion test) and chemical-induced (acetic acid, Formalin-induced) nociception models in mice at 200 & 400mg/kg doses. **Result:** Oral administration of the methanolic extract of leaves of *Dioscorea pentaphylla* L. (200 & 400 kg/mg) dose-dependently reduced nociceptive response to acute pain in acetic acid induced writhing. For acetic acid-induced writhing test highest inhibition (55.40 %) was found in case of highest dose (400 mg/kg) for leaf extract. Whereas standard drug diclofenac sodium causes (46.93%) writhing inhibition. Formalin-induced nociception test showed the significant effect in (200 and 400 mg/kg) for both dosages. On the other hand, MEDP showed the significant effect in hot plate, tail immersion test, at high dose (400mg/kg). **Conclusion:** MEDP showed significant antinociceptive activity via a multifactorial mechanism of action, indicating that the extract may be useful in the development of a new analgesic drug.

**Keywords:** *Dioscorea pentaphylla*, Dioscorea, Antinociceptive, Pain, Hot Plate Test, Tail Immersion Test.

1. Introduction

Pain is one of the most important health problems because of its prevalence and the disabilities. It is believed that acute pain serves as alarm and deserves to protect the organism against noxious stimuli, while chronic pain, in contrast, is an entire disease and may result from tissue injuries. Indeed, chronic pains may be a consequence of sustained inflammatory diseases or tissue damages such as nerve injury in the case of neuropathic pain. Chronic pain affects about 17–45% of the population and it is believed that the same range or even more may be found in developing countries where the population cannot afford manufactured drugs. Moreover, chronic pain is often resistant to existing therapy. So, there is a great need to search for new and better drugs.

Medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, especially to treat pain and inflammatory processes. Dioscorea yam is a member of the Yam family [1]. The yams are vining plants with 600 known species, 71 of which are native to North America (67 species in Mexico) [2]. It is native to Southeast Asia and is distributed throughout India, China, and southwards from Malaysia to Australia. In many species of yam, the rhizome (tuber) serves as both food and medicine [3]. Many native Americans and South Asians used a syrup of the root to relieve labor pain and later physicians gave wild yam to patients with colic pain, morning sickness, asthma, hiccough, rheumatism and gastritis related to alcoholism [4]. Modern herbalists value wild yam to treat intestinal colic, biliary colic, and flatulence as well as menstrual cramps and rheumatoid arthritis [5]. Herbalists combine wild yam with black cohosh (and sometimes burdock root and motherwort) for rheumatic complaints. Chinese herbalists use wild yam as a tonic [6]. The decoction of the tuber is given to animals for early recovery of fractured bones. The raw tuber is given to cattle to cure Diphtheria. Cough, cold, asthma, tuberculosis [7].

It has reported that Dioscorea species contains steroidal saponin, spiroconazole A, a phenanthrene, 2,7-dihydroxy-4-methoxyphenanthrene, flavonoids as quercitin, quercetin-3-O-β-D-glucopyranoside, and quercetin-3-O-β-D-galactopyranoside, and seven clerodane diterpenoids namely, bafoudiosbulbins A, B, C,
D. E, F, and G have been isolated from the methanol extract of the bulb of Dioscorea bulbifera var sativa [8]. Due to the lack of knowledge, we designed the present investigation in methanolic extract of *D. pentaphylla*. In this paper, we describe the chemical and heat induced antinociceptive activity of wild yams *D. pentaphylla* in mice model.

2. Materials and Methods

2.1. Plant Collection and Identification

*Dioscera pentaphylla* leaves collected during November, 2015 from the Jahangir Nagar University campus savar, Dhaka, Bangladesh. The plant's leaves verification done by the senior scientific officer of National Herbarium, Mirpur, Dhaka, Bangladesh. Additionally, the voucher specimen number (Accession No. 42052) deposited for further reference.

2.2. Preparation of Plant Extract

The Powdered leave of *D. pentaphylla* of about 400 g was taken in a clean and flat-bottomed glass beaker and soaked in 5000 mL methanol (Merck, Germany). The solvent extraction of plant leaves powder stirred at 25 ± 2 °C for 5 days. The solvent mixture filtrated by a piece of sterile and white cotton material and finally using Whatman No. 1 filter paper. The solvent completely removed by the operation of the rotary evaporator (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.) and obtained 30.07 g extract (Yield 75.78%). The prepared extract used for the phytochemical screening as well as antinociceptive studies.

2.3. Chemicals and Drugs

The following chemicals and drugs in our recent study: Acetic acid (Merck, Germany), methanol (Merck, Germany), formalin (Merck, Germany), morphine sulfate (Gonoshasthaya pharmaceuticals Ltd.) and diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh). MEDP at the doses of 200 and 400mg/kg dissolved in double distilled water and administered orally 30 minutes before to the test. The standard drug morphine sulphate 5 mg/kg (Manufactured by Square Pharmaceuticals Ltd., Bangladesh) used in hot-plate and tail immersion pain models. Diclofenac sodium 10 mg/kg (Square Pharmaceuticals Ltd., Bangladesh) used in writhing and licking tests prepared with saline water (containing 0.9% NaCl) which administered intraperitoneally 15 minutes before the experiments. The control group received saline water (containing 0.9% NaCl) 0.1 mL/mouse 30 minutes before the experiments.

2.4. Experimental Animals

Swiss albino mice (20-25 g) collected from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animal kept under standard laboratory conditions as well as maintained room temperature, relative humidity: 55-65%, 12 h light/dark cycle with standard diet and clean water ad libitum during the adaption period. The mice fasted overnight before the experiments. We followed Ethical Principles and Guidelines for mice observations which provided by Scientific Experiments on Animals (1995). This guideline created by Swiss Academy of Medical Sciences and Swiss Academy of Science. Our research program approved by the Institutional Animal Ethical Committee (SUB/IAEC/ 15.04) of Stamford University Bangladesh.

2.5. Phytochemical Screening

The crude methanol extract of *D. pentaphylla* leaves qualitatively tested for the detection of alkaloids, glycosides, flavonoids, tannins, reducing sugar, carbohydrates, steroids and saponins following standard procedures [9].

2.6. Measurement of Antinociceptive Activity

2.6.1. Hot Plate Test

The hot plate test (Heat-induced pain) performed to measure the possible nociceptive response using Eddy’s hot plate apparatus [10]. Experimental mice divided into five groups (n=10). Each mouse of the control group treated with saline water (0.9% NaCl; 0.1 mL/mouse) and the positive control group received morphine (5 mg/kg, i.p.) as a reference drug or MEDP (200, and 400 mg/kg, p.o.). During observation Eddy’s hot plate metal surface temperature maintained at 52 ± 2 °C. The doses of MEDP administered 30 minutes before the experimental observation while reference drug as positive control (morphine sulphate 5 mg/kg, i.p.) administered 15 minutes before the experimental observation. The nociceptive response in the form of fore paw licking, withdrawal of the paws or jumping was recorded at 0, 15, 30, 45, and 60 minutes of the following treatment. A cut-off period of the 20 seconds was maintained to avoid paw tissue damage. The hot plate experimental value expressed as the maximal possible effect (%MPE), [11] which was calculated using the following formula:

\[ \% \text{MPE} = \left( \frac{\text{Post drug latency} - \text{Pre drug latency}}{\text{Cut-off time} - \text{Pre drug latency}} \right) \times 100. \]

2.6.2. Tail Immersion Test

Tail immersion test (Heat-induced pain) in mice is performed to determine the central mechanism of analgesic
activity according to the method described by [12]. The antinociceptive response based on particularly the morphine-like drug selectively prolongs the typical tail withdrawal reflex in mice. The painful reaction consider as the tail dipping into the hot water which produced by thermal incentive. During experimental observation 1 to 2 cm of the tail of mice allowed to immerse warm water bath kept constant at 52 ± 1°C. Experimental mice divided into five groups (n=10). Each mouse of the control group treated with saline water (0.9% NaCl; 0.1 mL/mouse) and the positive control group received morphine (5 mg/kg, i.p.) as a reference drug or MEDP (200, and 400 mg/kg, p.o.). During observation Eddy’s hot plate metal surface temperature maintain at 52 ± 2°C. The doses of MEDP administered 30 minutes before the experimental observation while reference drug as positive control (morphine sulphate 5 mg/kg, i.p.) administered 15 minutes before the experimental observation. The nociceptive response in the form of fore paw licking, withdrawal of the paws or jumping was recorded at 0, 15, 30, 45, and 60 minutes of the following treatment. A cut-off period of the 20 seconds was maintained to avoid paw tissue damage. The %MEP was calculated by the same formula used in hot plate test.

2.6.3. Acetic Acid-induced Writhing Test

The peripheral antinociceptive response of MEDP measured by acetic acid-induced test (Chemical-induced pain). Experimental mice divided into five groups (n=10). Each mouse of the control group treated with saline water (0.9% NaCl; 0.1 mL/mouse) and the positive control group received Diclofenac sodium (10 mg/kg, i.p.) as a reference drug or MEDP (200, and 400 mg/kg, p.o.). The doses of MEDP administered 30 minutes whereas, the reference drug as positive control (diclofenac sodium 10 mg/kg, i.p.) administered 15 minutes before 0.7% acetic acid injection [13]. The writhing observation has done after 5 minutes of acetic acid injection. The visualization cue of each writhing appeared like strong abdominal muscle contractions, followed by trunk twisting and extension of the hind limbs as well as contact of the abdomen with the floor considered as complete writhing. Antinociceptive response expressed as writhing inhibition (%) and calculated by using the following formula:

\[
\text{Writhing inhibition} (%) = \left(\frac{Wc - Ws}{Wc}\right) \times 100,
\]

Where, Wc is the mean number of writhing of control and Ws is the mean number of writhing of the test sample.

2.6.4. Formalin-induced Paw Licking Test

The formalin-induced nociception response observed according to licking of hind paw [14]. Each mouse of the control group treated with physiological saline water (0.9% NaCl; 0.1 mL/mouse) and the positive control group received Diclofenac sodium (10 mg/kg, i.p.) as a reference drug or MEDP (200, and 400 mg/kg, p.o.). The doses of MEDP administered 60 minutes whereas, the reference drug as positive control (diclofenac sodium 10 mg/kg, i.p.) administered 15 minutes before 20 µl of 2.5% formalin made up in saline water. The formalin injected subcutaneously in the right hind paw of each mouse by the 30-gauge needle. The licking and biting considered as the nociceptive response which recorded for 5 minutes during formalin injection. Formalin-induced licking behavior determined the initial phase (First phase, neurogenic) 0-5 minutes whereas, late phase (Second phase, inflammatory) 15-30 minutes [15]. Antinociceptive activity calculated as the percentage of licking time inhibition.

3. Results

3.1. Phytochemical Screening

Preliminary phytochemical screening of the crude extract of D. pentaphylla revealed the presence of alkaloids, glycosides, flavonoids, tannins, steroids and saponins.

3.2. Measurement of Antinociceptive Activity

3.2.1. Hot plate test

The antinociceptive activity of MEDP at the doses (200, and 400 mg/kg) in hot plate significantly (\( p < 0.001 \)) increased the reaction time to the thermal stimulus and the antinociceptive effects was dose-dependent (Figure 1). The standard drug morphine sulphate showed highest %MPE values at all the observation periods 15 to 60 minutes (Table 1).

![Figure 1. Antinociceptive effect of D. pentaphylla leaves extract and morphine in hot plate test.](image)

3.2.2. Tail immersion test

The antinociceptive activity of MEDP at the doses (200, and 400 mg/kg) in tail immersion test significantly increased the latency period to hot-water induced thermal stimuli (\( p < 0.001 \)) and the antinociceptive effects was dose-dependent (Figure 2). The standard drug morphine sulphate showed highest %MPE values at all the observation periods 15 to 60 minutes (Table 2).
Antinociceptive Activity of Methanol Extract of *Dioscorea Pentaphylla* Linn. Leaves in Mice

### 3.2.3. Acetic acid-induced writhing test

The oral administration of MEDP at 200, and 400 mg/kg doses significantly \( p < 0.001 \) reduced the writhing episodes which induced by acetic acid compared with the control group (Figure 3). The percentage inhibition of abdominal contraction demonstrated the antinociceptive activity. Diclofenac sodium as the standard drug, inhibited 46.39% writhing in comparison to the control group. MEDP showed very strong writhing inhibitory effect at 400 mg/kg which value close to the effect like diclofenac sodium (55.40%). MEDP at the dose of 200 mg/kg also produced antinociceptive activity 28.37% (Table 3).

### 3.2.4. Formalin-induced paw licking test

The antinociceptive effect of MEDP at the doses of 200, and 400 mg/kg caused a significant \( p < 0.001 \) inhibition of the formalin-induced paw licking test when compared with the control group (Figure 4). The effects were dose-dependent inhibition of both neurogenic (0-5) minutes and inflammatory (15-30) minutes phases (Table 4). However, the antinociceptive effect was more precise in the second phase of this pain model. The nociception significantly reduced at 200, and 400 mg/kg doses in late phase. Diclofenac sodium significantly \( p < 0.001 \) reduced formalin-induced nociception in early phase (71.67%) and late phase (96.11%).

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Table 1. Antinociceptive effect of *D. pentaphylla* leaves extract and morphine in hot plate test.

| Treatment | Dose (mg/kg) | 0 min | 15 min | 30 min | 45 min | 60 min |
|-----------|--------------|-------|--------|--------|--------|--------|
| Control   | 0.1 ml/mouse | 7.00±.316 | 6.84±.21 | 7.09±.10 | 7.46±.28 | 7.98±.31 |
| Morphine  | 5            | 8.86±.23 | 12.67±.76*** | 14.30±2.48** | 10.91±52** | 12.65±.46*** |
| MEDP 200  | 6.67±.16 | 6.79±.09 | 8.16±.42 | 9.49±.75 | 9.72±.36* |
| MEDP 400  | 7.65±.31 | 10.23±31*** | 11.21±.29*** | 11.63±.39*** | 12.22±.53*** |

Each value is expressed as the mean ± SEM (n=10). MEDP = Methanol extract of *Dioscorea pentaphylla* leaves. *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \) compared with the control group (Dunnett’s test).

Table 2. Antinociceptive effect of *D. pentaphylla* leaves extract and in tail immersion test.

| Treatment | Dose (mg/kg) | 0 min  | 15 min | 30 min | 45 min | 60 min |
|-----------|--------------|--------|--------|--------|--------|--------|
| Control   | 0.1 ml/mouse | 2.31±.14 | 2.2±.09 | 2.34±.33 | 2.41±.30 | 2.59±.067 |
| Morphine  | 5            | 3.14±.28 | 5.16±.38** | 6.08±.38*** | 6.09±.84* | 4.46±.22** |
| MEDP 200  | 3.00±.34 | 4.02±.82* | 4.34±1.49* | 3.59±.19 | 3.34±.19 |
| MEDP 400  | 3.19±.45 | 4.36±.18* | 4.79±.19** | 4.46±.44* | 4.27±.29** |

Each value is expressed as the mean ± SEM (n=10). MEDP = Methanol extract of *Dioscorea pentaphylla* leaves. *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \) compared with the control group (Dunnett’s test).
Table 3. Antinociceptive effect of D. pentaphylla leaves extract in acetic acid-induced writhing test in mice

| Treatment        | Dose (mg/kg) | Number of writhing | Inhibition (%) |
|------------------|--------------|--------------------|----------------|
| Control          | 0.1 ml/mouse | 44.4±1.77          | -              |
| Diclofenac Sodium| 10           | 23.8±1.46***       | 46.39%         |
| MEDP 200         | 31.8±1.59*** | 28.37%             |
| MEDP 400         | 19.8±0.37*** | 55.40%             |

Each value is expressed as the mean ± SEM (n=10). MEDP = Methanol extract of Dioscera pentaphylla leaves. * p < 0.001 compared with the control group (Dunnett’s test).

Table 4. Antinociceptive effect of D. pentaphylla leaves extract in formalin test in mice

| Treatment        | Dose (mg/kg) | Early Phase (0-5)min | Inhibition (%) | Late Phase (15-30) | Inhibition (%) |
|------------------|--------------|----------------------|----------------|---------------------|----------------|
| Control          | 0.1 ml/mouse | 94.6±1.50            | -              | 41.2±1.15          | -              |
| Diclofenac Sodium| 10           | 26.80±1.2***         | 71.67%         | 1.60±.24***        | 96.11%         |
| MEDP 200         | 12±1.94***   | 87.31%               | 2.60±.67***    | 93.68%             |
| MEDP 400         | 17.6±1.50*** | 81.39%               | 1.60±.24***    | 96.11%             |

Each value is expressed as the mean ± SEM (n=10). MEDP = Methanol extract of Dioscera pentaphylla leaves. * p < 0.001 compared with the control group (Dunnett’s test).

4. Discussion

Our present study about the oral administration of MEDP elicits as a promising alternative candidate against central and peripheral painful condition. The earlier study has reported by the researchers that chemistry of D. pentaphylla contains new pharmacologically active agents, mainly to improve the treatment of pain. The hot plate and tail immersion tests are widely accepted methods for understanding the centrally acting antinociceptive activities. In our study, MEDP at 200 and 400 mg/kg doses exhibited antinociceptive effects in the hot plate and tail immersion tests by increasing hot plate latency as well as tail withdrawal response. The present findings endorsed that the central antinociceptive activity of MEDP may due to its effect on µ-opioid receptors of spinal as well as the supraspinal system. MEDP containing compounds may minimize the activity of adenylyl cyclase which responsible for the Ca²⁺ influx at axon terminal of afferent nerve. The antinociceptive effect exerts by decreasing cAMP level, K⁺ efflux, and subsequently hyperpolarization of the nerves.

The acetic acid-writhing test in mice extensively used for measuring the central and peripheral antinociceptive response to visceral pain. Intraperitoneal administration of acetic acid administration increase in the release of pro-inflammatory mediators such as cyclooxygenase (COX), prostaglandins (PGs), lipooxygenase (LOX), histamine, serotonin, bradykinin, substance P, and cytokines (IL-1β, IL-8, TNF-α) in peripheral tissue fluid [16]. The expression of these mediators causes the excitation of primary afferent nociceptors entering in the dorsal horn of the spinal cord as well as the thalamus at central and peripheral nervous system. Our experimental findings also provide the supporting evidences about the use of D. pentaphylla in painful and inflammatory conditions.

Formalin-induced paw licking test, the antinociceptive property of MEDP observed through two distinct phases. MEDP activity observed as significant and dose-dependent at both neurogenic (early phase) and inflammatory (late phase) pain responses caused by formalin in mice. Sensory afferent fibers, specifically C-fibers (nociceptive nerve) stimulation release neuropeptides such as substance P which induced neurogenic pain in case of early phase. Contrary, various inflammatory mediators like prostaglandins (PGs), histamine, bradykinin, and serotonin responsible for induced inflammatory pain in the late phase [17]. The earlier study reported that peripherally acting agents inhibit both first and second phases of the formalin response and that many centrally acting drugs only modulate the second phase of the formalin trial via spinal and supraspinal mechanisms. In our viewpoint, the finding data convinced us MEDP produces antinociception in both phases of the formalin-induced nociception test. The late phase response observed in the acetic acid-induced writhing test is due to the inhibition of the inflammatory mediators. Although thermal models revealed the centrally acting antinociceptive action of MEDP through the possible involvement of opioids receptors. As our expectation, MEDP expressing a quite different result. In case of the early phase, nerve ending directly involved the production of nociceptive responses. Nevertheless, it might be possible that MEDP produces less efficacy in the early phase of the formalin test. It is quite possible due to the different mechanisms with the thermal models where the antinociceptive activity of MEDP more prominent.

The phytochemical screening of MEDP revealed the presence of alkaloids, glycosides, and flavonoids, tannins, reducing sugar, carbohydrates, steroids and saponins. A considerable amount of alkaloids and flavonoids also quantified from the leaves of MEDP. Flavonoids and alkaloids interact directly with the prostaglandin system.
Thereby, flavonoids and alkaloids are possible candidates for the antinociceptive agent. The entire experimental observation convinced us to assume that the antinociceptive effects of MEDP due to its high flavonoid contents. Additionally, flavonoids able to inhibit eicosanoid biosynthesis (such as prostaglandins) which has the involvements in various immunological responses and the end product of cyclooxygenase (COX) and lipoxygenase (LOX) pathway. Hence, the COX inhibitory of EETP may reduce the production of the chemical compounds (phospholipids) which is responsible for the prostaglandins synthesis and ultimately relieve the pain sensation.

5. Conclusions

These above findings convinced us MEDP possesses significant antinociceptive effects in mice model. The antinociceptive activity of MEDP is prominent in both Chemical-induced pain and Heat-induced pain models. We assumed that the antinociceptive activity of MEDP may involve in participation ATP-sensitive K⁺ channel and cyclic adenosine monophosphate pathway at nociceptors. Further studies required to determine MEDP mechanism and inhibition pathway as well as isolate the potential compounds that may apply as the lead for drug development.

List of Abbreviations

MEDP = methanolic extracts of Dioscorea pentaphylla Linn.
ICDDR, B = International Center for Diarrhoeal Disease and Research, Bangladesh
COX = cyclooxygenase
LOX = lipoxygenase
PG = prostaglandins
cAMP = cyclic Adenosine Monophosphate

Declarations

Ethics approval and consent to participate All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/15.04) of Stamford University Bangladesh approved all experimental rules.

Availability of Data and Material

Not applicable

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Competing Interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Authors' Contributions

Md. Shahed-Al-Mahmud coordinated all laboratory experiments, analyzed and interpreted results. Md. Towhidul Islam did phytochemical analysis, Hot plate test, Tail immersion test, Acetic acid-induced writhing test, Formalin test and interpreted all experiments. Senior lecturer Anawara Begum supervised our research. Md. Shahed-Al-Mahmud did statistical analysis and drafted the manuscript. All authors read and approved the manuscript.

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