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# Antinociceptive Effect of Methanol Extract of *Diospyros malabarica* (Desr.) Kostel Leaves in Mice

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## Abstract

**Background:** *Diospyros malabarica* (Desr.) Kostel, a small to medium-sized tree in the Ebenaceae family, is known as "Deshi Gab" in Bangladesh. Fever, diabetes, snake bite, diarrhea, biliousness, and ulcer ailments are all treated with the herb. This study's goal was to examine in mouse models the antinociceptive properties of methanol extract of *Diospyros malabarica* leaves (MEDM). **Methods:** For the purpose of determining the antinociceptive activity in mice, five distinct pain models including hot plate, tail immersion, acetic acid-induced writhing, formalin and glutamate-induced nociception tests were used. The conventional medications were morphine sulphate (5 mg/kg, intraperitoneally) and diclofenac sodium (10 mg/kg, intraperitoneally). While the control group was expecting deionized water, MEDM was given orally at dosages of 200, 400, and 600 mg/kg (0.1 mL/mouse, orally). **Results:** According to the current research, MEDM strongly reflected the antinociceptive activity of all mouse models of chemical and heat-induced pain (*$p < 0.05$). 400 and 600 mg/kg demonstrated a considerable (*$p < 0.05$) ability to prolong the reaction of latency to pain in opposition to thermally produced nociception in hot plate and tail immersion tests. Inhibition levels in the acetic acid-induced writhing test were 11.57%, 37.77%, and 51.83%, respectively. The extract suppressed 20.78%, 45.48%, and 56.93% of licking during the initial stages of formalin-induced nociception. In the late phase, the extract showed higher rates of licking than the control group (13.14%, 50.28%, and 66.85%). The glutamate-induced nociception test was significantly (*$p < 0.05$) prevented by the plant extract. Compared to the control, it demonstrated an inhibition of licking of 22.85%, 47.32%, and 63.42%, respectively. **Conclusions:** It is evident that the plant extract has exceptional analgesic properties. To determine the precise processes behind antinociceptive effect and to identify the substances that produce this activity, more research

is required. The study's findings also support the long-standing use of MEDM in painful conditions.

## Keywords

*Diospyros malabarica*, Extract, Antinociceptive, Pain

## 1. Background

*Diospyros malabarica* (Desr.) Kostel is a large genus of trees or shrubs in the Ebenaceae family that are found all across both hemispheres. India is home to about 41 species that are native to the country, the majority of which may be found in the evergreen forests of the Deccan, Assam, and Bengal [1] [2]. It is also known as "Deshi Gab" locally in Bangladesh, is a small to medium-sized tree that is glabrous except for its younger sections and has numerous spreading branches that form an impenetrable gloomy crown [3]. Several Diospyros species are used medicinally in Indian systems of traditional medicine like Ayurveda and Unani to treat conditions including fever, diabetes, snake bites, diarrhea, biliousness, ulcers, and more [4]. The many portions of this plant have historically been utilised for various reasons. Bark is astringent, acrid, cooling, anti-inflammatory, constipating, depurative, and febrifuge. It is also useful for treating skin conditions, pruritus, dyspepsia, haemorrhages, burns, diabetes, fever, spermatorrhoea, and vaginal disorders. Diuretic, carminative, laxative, styptic, ophthalmic, scotomic, nyctalopia, ophthalmic, epistaxis, haemoptysis, burns, tubercular glands, scabies, and wounds are among conditions that the leaves can treat. Flowers are effective for treating leucorrhoea, urethrorrhea, splenomegaly, nyctalopia, anaemia, and scabies. They are also diuretic and aphrodisiac. Fruits are beneficial in aphthae, pharyngodynia, and vitiated states of pitta and vata because they are bitter, acrid, cooling, digestive, carminative, and oleaginous [1].

In addition to being used as a diuretic, carminative, laxative, ophthalmic, styptic, and diuretic, leaves are also helpful for burns, tubercular glands, scabies, wounds, strangury, dyspepsia, flatulence, scotoma, nyctalopia, epistaxis, and hemoptysis [5]. Unripe fruits are oleaginous, caustic, astringent, and bitter [6]. Unripe fruits are used to cure wounds, cholera, mouth ulcers, dysentery, and diarrhea [7]. Ripe fruits are very nourishing and help rural residents feel secure in their access to food. In traditional Indian medicine, mature fruits are important as tonics and aphrodisiacs [8]. Recently, it has been proposed that the gum from the fruits of *Diospyros malabarica* may be effective as a binder in tablet formation [9]. Lupeol, betulin, betulinic acid, oleanolic acid, sitosterol, myricyl alcohol, and tannins are all present in the bark [10]. Lupeol is present in heartwood [11]. Leucopelargonidin-3-O-L-rhamnopyranoside [12], nonadecan-7-ol-2-one [13], and -sitosterol are all found in stems. Betulin, oleanolic acid, peregrinol, and beta-sitosterol are all found in leaves [10]. Betulinic acid is present in seeds [14]. Tetrahydroxy-3,5,3-methoxyflavanone-4,O-L-rhamnopyranoside and

5,7,3,4-tetrahydroxyflavanone-3,O-D-glucopyranosyl-1,4-L-rhamnopyranoside are found in roots [10]. Fruits include roughly 50% pectin, protein, and glucose. Amongst secondary metabolites, Diospyros malabarica fruit include soluble tannins, furano-(2",3",7,8)-3',5'-dimethoxy-5-hydroxyflavone,4'-hydroxy-3,6,3',5'-tetramethoxy-7,8-pyranoflavone, peregrinol, hexacosane, hexacosanol, b-sitosterol, betulinic acid, and lupeol [15].

The methanol extracts of *Diospyros malabarica*'s bark and seeds have been researched in pharmacological trials as an antidiarrheal [16]. Numerous bacteria's development has been demonstrated to be inhibited by the methanol extract of *Diospyros malabarica* fruits [17]. A fruit extract from *Diospyros malabarica* that is aqueous has been shown to have hypoglycemic and antihyperglycemic properties [18]. A methanol extract of unripe *Diospyros malabarica* fruits has been shown to have anthelmintic action [19]. *Diospyros malabarica*'s fruits can help with elevated oxidative stress, hyperlipidemia, and hyperglycemia [15]. Methanolic extract of bark has exhibited significant antioxidant activity against Ehrlich ascites carcinoma bearing mice and Dalton's ascites lymphoma bearing swiss albino mice [5]. The ethanolic extract of leaves has a sizable antifertility effect [20], while the methanolic extract of leaves showed strong antihyperglycemic effects [21].

*Diospyros malabarica* has a high concentration of bioconstituents that are pharmacologically active, making it a potential candidate for use as a phytomedicine. In many traditional medical systems across the world, all of this plant's organs—especially its fruits, bark, and leaves are utilised to prepare medicines because of its antioxidant capacity and ability to treat a variety of ailments [22]. Additionally, the leaf extracts have strong antidiabetic [23], antimicrobial, anti-inflammatory, and antipyretic properties and are used to treat burns, diabetes, atherosclerosis, intermittent fever, and cancer [24]. The anticancer and antidiarrheal properties of the stem's alcoholic extract were discovered. Ripe *Diospyros malabarica* fruits have been extracted with methanol and have demonstrated antibacterial, anticancer, antioxidant, hepatoprotective, antidiabetic, and antidiarrheal properties [25] [26]. The following were the compounds' hypoglycemic and antihyperglycemic, antibacterial, and antiurolithiatic properties [27]. *Diospyros malabarica* has been used traditionally to treat diarrhoea, prostaglandin synthesis suppression, and ongoing gastrointestinal motility decrease [28].

Based on the investigations, we created and carried out the current study to examine the interaction between five mouse pain models and a methanol extract of *Diospyros malabarica* leaves. The mechanism of action of the methanol extract of *Diospyros malabarica* leaves on induced antinociception has to be further studied in the current research.

## 2. Methods

### 2.1. Plant Material and Extraction

At the month of July 2022, the fresh leaves of *Diospyros malabarica* were har-

vested at Manohorgonj, Cumilla, Bangladesh. Azim Uddin, Salar Khan Herbarium, Dhaka University, Bangladesh (Flora of Bangladesh), then identified and verified the plant samples. For further reference, the Herbarium has already received a voucher specimen number (DUSH: 10815). After being dark dried for a week, the fresh leaves were ground into a fine powder. A beaker was used to macerate 270 g of powdered dry leaves for 3 days at 25˚C ± 2˚C with periodic stirring. The extract was then filtered using sterilized cotton filters and Whatman No. 1 filter paper. The extract was then filtered using sterilized cotton filters and Whatman No. 1 filter paper. By using a rotary evaporator (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.), the solvent was entirely evaporated, yielding 25 g of extract. The research on acute toxicity and antinociceptive effects employed this extract.

### 2.2. Animals

Swiss albino mice, 3 - 4 weeks of age, weighing between 20 and 30 g, both sexes were used in this study. The International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) Animal Research Branch provided Swiss albino mice. During the adaption period, animals were housed with food and water under standard laboratory settings (room temperature: 25˚C ± 2.0˚C, relative humidity: 55% - 65%, and 12 h light/dark cycle). Prior to doing the studies, the animals spent two weeks becoming used to the lab setting. Prior to the studies, mice were fasted for the whole night. All experimental animals were handled in accordance with the Swiss Academy of Sciences' and the Swiss Academy of Medical Sciences' Ethical Principles and Guidelines for Scientific Experiments with Animals (1995). All experimental methods were approved by the Stamford University Bangladesh Ethics Committee (SUB/IAEC/23).

### 2.3. Drugs and Treatments

Before the tests, the control group was given 0.1 mL/mouse of deionized water orally. The positive control group received intraperitoneally (i.p.) the standard drug morphine in hot plate, tail immersion test, and acetic acid-induced writhing at the dose of 5 mg/kg and diclofenac sodium in formalin, glutamate, and licking tests at the dose of 10 mg/kg 15 minutes prior to the experiments. 30 minutes before to the trials, MEDM was given orally at dosages of 200, 400, and 600 mg/kg (b.w.). Deionized water was used to make each medication dosage and MEDM dose.

### 2.4. Phytochemical Screening

According to established protocols, alkaloids, flavonoids, glycosides, carbohydrate, steroid, tannin, reducing sugar, and saponin were qualitatively detected in the crude methanol extract of *Diospyros malabarica* (MEDM) [29].

### 2.5. Acute Toxicity Test

Three experimental groups (n = 5) and a control group of animals were each

given five animals. The experimental groups received oral administration of MEDM at doses of 1000, 2000, and 4000 mg/kg. The animals were housed in separate cages. Following gavage, animals were given unlimited access to food and drink. For 14 days, we looked for any allergic responses (skin rashes, itching), eye and mucous membrane discharges, behavioral abnormalities, food and drink refusal, salivation, convulsions, tremors, diarrhea, and animal death. The body weight of the animals that survived the monitoring period was noted. After that, animals were slaughtered so that the important organs could be examined for any abnormalities and major gross alterations [30] [31].

## 3. Antinociceptive Activity Test

### 3.1. Hot Plate Test

The hot plate test method was used to examine potential centrally mediated analgesic effects in a preferential manner [32]. Five groups of five mice each were created out of the animals. Mice were put on Eddy's hot plate, which was maintained at a temperature of 52˚C ± 1˚C, and were given one of three treatments: control (Equivalent volume of deionized water, 0.1 mL/mouse, p.o.), morphine as a standard medication (5 mg/kg, i.p.), or MEDM (200, 400, and 600 mg/kg, p.o.). To protect the paw tissue, a 20-second cutoff time was kept. At 0, 30, 60, 90, and 120 minutes after treatment, the reaction was observed as forepaw licking, paw withdrawal symptoms, or leaping. Then, using the following formula, the percentage of the maximum potential effect (% MPE) was determined:

% MPE = [(Post-drug latency − Pre-drug latency)/(Cut off time − Pre-drug latency)] × 100.

### 3.2. Tail Immersion Test

The tail immersion test is based on the discovery that morphine-like medications specifically lengthen the mouse tail withdrawal response' usual reaction time. This approach was utilized to assess the primary mechanism behind analgesic efficacy. Here, the thermal incentive—dipping by the tip of the tail in hot water—caused the painful reactions in the animals [33]. Five mice per group were used to split the mice into the five groups. According to the protocol, mice pretreated with morphine (5 mg/kg, i.p.) or MEDM (200, 400, and 600 mg/kg, p.o.) had 1 to 2 cm of their tails submerged in warm water that was consistently held at 54˚C. It was noted how long it took for the tail to deflect after it had submerged. To protect the mice's tail tissue, a 20-second latency interval was kept in place. After administering morphine and MEDM, the latency duration of the tail-withdrawal reaction was measured at 0, 30, 60, 90, and 120 min. This was done to test the analgesic's effectiveness. Then, using the same formula as the hot plate test, the percentage MPE was computed.

% MPE = [(Post-drug latency − Pre-drug latency)/(Cut off time − Pre-drug latency)] × 100.

### 3.3. Acetic Acid-Induced Writhing Test

This test was used to determine MEDM's peripheral analgesic activity in pain brought on by chemicals [34]. Five groups of the animals were created (n = 5). Diclofenac sodium, 10 mg/kg, intravenously; control; or MEDM, 200, 400, or 600 mg/kg, orally; on the other hand, the writhing was induced by the injection of 0.6% acetic acid 15 minutes after drug administration and 30 minutes after oral administration of MEDM, respectively. The mice were watched after 5 min following the injection of acetic acid, and for 30 min the number of writhing was tallied [35]. Complete writhing was defined as the stomach contracting, the body lengthening, the trunk and pelvis twisting, and the extension of the limbs. Inhibition of abdominal constriction as a percentage was used to calculate analgesic activity.

### 3.4. Formalin Test

Santos & Calixto and Santos *et al.* [36] [37] modified the procedure slightly to utilize it as narration. There were five groups of animals total (n = 5). Before the tests, the control group was given 0.1 mL/mouse of deionized water orally. The right hind paw of the mice was subcutaneously injected with 20 microliters of 2.5% formalin (in deionized water, subplantar) 1 hour after MEDM therapy (200, 400, and 600 mg/kg, p.o.) and 15 minutes after injection of diclofenac sodium (10 mg/kg, i.p.). As a gauge of pain response, the amount of time spent licking and biting the injected paw was counted. After injecting formalin, responses were monitored for 5 min (first phase, neurogenic) and for 15 - 30 min (second phase, inflammatory). The % inhibition of licking time was used to calculate antinociceptive activity.

### 3.5. Glutamate-Induced Nociception

The technique was used in a manner similar to that which Beirith *et al.* [38] previously described. Five groups of five mice each were created from the group of mice. The mice were treated with MEDM (200, 400, and 600 mg/kg, p.o.) for 30 min. after that, then with diclofenac sodium (10 mg/kg, i.p.) for 15 min. before receiving a volume of 20 μL of glutamate solution (10 mol, per paw). Before the tests, the control group was given 0.1 mL/mouse of deionized water orally. Following injection of glutamate, the mice were monitored independently for 15 minutes. The frequency with which it licked its injected paw was a sign of nociception.

### 3.6. Statistical Analysis

The findings are shown as mean SEM. Using the SPSS 18.00 program, one-way analysis of variance (ANOVA) was used for the statistical analysis, followed as necessary by Dunnett's post hoc test. At a threshold of ***$p < 0.001$, differences between groups were deemed significant.

## 4. Results

### 4.1. Phytochemical Screening

*Diospyros malabarica*'s crude extract underwent phytochemical screening, and the results showed the presence of tannin, reducing sugar, flavonoid, glycoside, carbohydrate, steroid, and saponin (Table 1).

### 4.2. Acute Toxicity

During the observation period, oral treatment of MEDM at dosages of 1000 - 4000 mg/kg did not result in any mortality, allergic responses, salivation, convulsion, tremors, diarrhea, or behavioral abnormalities. Furthermore, there were no statistically significant macroscopic alterations or abnormalities of the mice's important organs between the control and experimental groups.

### 4.3. Hot-Plate Test

At the dosages of 400 mg/kg and 600 mg/kg, MEDM had a statistically significant antinociceptive effect (*$p < 0.05$), as shown in Figure 1 and Table 2. Additionally, as compared to the control group (Deionized water), the administration of morphine at a dosage of 5 mg/kg demonstrated a substantial antinociceptive effect (**$p < 0.01$). Values are presented as mean ± SEM ($n = 5$). **$p < 0.01$ compared with the control group (ANOVA followed by post hoc Dunnett's test).

### 4.4. Tail Immersion Test

At dosages of 200 mg/kg, 400 mg/kg, and 600 mg/kg, the tail-immersion test findings claimed a substantial antinociceptive effect (***$p < 0.001$) compared to control. 200 mg/kg, 400 mg/kg, and 600 mg/kg of MEDM had antinociceptive effects that were equivalent to those of the reference medication (Figure 2 and Table 3). Morphine significantly reduced pain as compared to the control group, which consumed deionized water (***$p < 0.001$). Values are presented as

Table 1. *Diospyros malabarica* methanol extracts preliminary qualitative phytochemical screening (MEDM).

| Extract | MEDM |
|---|---|
| Alkaloid | − |
| Flavonoid | + |
| Glycoside | + |
| Carbohydrate | + |
| Steroid | + |
| Tannin | + |
| Reducing Sugar | + |
| Saponin | + |

[MEDM = Methanol Extract of *Diospyros malabarica*; (+): Present; (−): Absent].

**Table 2.** Antinociceptive effect of leaf extract of *Diospyros malabarica* on hot plate test.

| Treatment | Dose (mg/kg) | Latency of nociceptive response (in seconds) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 0.1 mL/mouse | 7.20 ± 0.583 | 10.20 ± 0.583 | 11.80 ± 0.735 | 12.80 ± 0.860 | 15.00 ± 1.000 |
| Standard (Morphine) | 5 | 13.80 ± 0.970 | 18.20 ± 1.562** | 21.40 ± 1.122* | 24.00 ± 1.095** | 27.40 ± 0.748** |
| MEDM | 200 | 11.60 ± 0.812 | 16.00 ± 2.025 | 17.20 ± 1.241 | 20.40 ± 0.678 | 21.80 ± 0.970 |
| MEDM | 400 | 12.20 ± 2.437 | 13.80 ± 1.020 | 18.60 ± 0.872 | 21.20 ± 1.200* | 24.80 ± 2.059* |
| MEDM | 600 | 14.60 ± 0.927 | 18.80 ± 0.490 | 21.40 ± 2.159* | 23.60 ± 1.600** | 29.00 ± 0.447** |

Values are presented as mean ± SEM (n = 5). MEDM = Methanol extract of *Diospyros malabarica*; **p < 0.01 compared with the control group (Dunnett's test). *p < 0.05 compared with the control group (Dunnett's test).

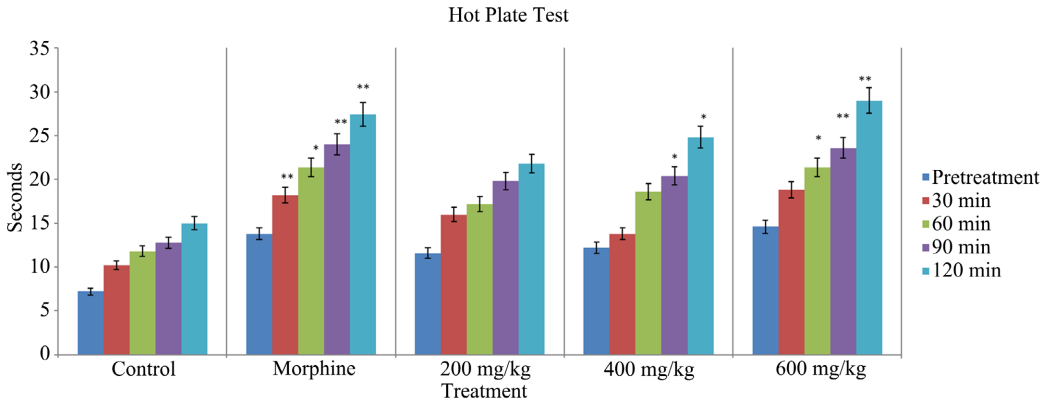


**Figure 1.** Antinociceptive effect of *Diospyros malabarica* leaves extract and morphine in hot plate test.

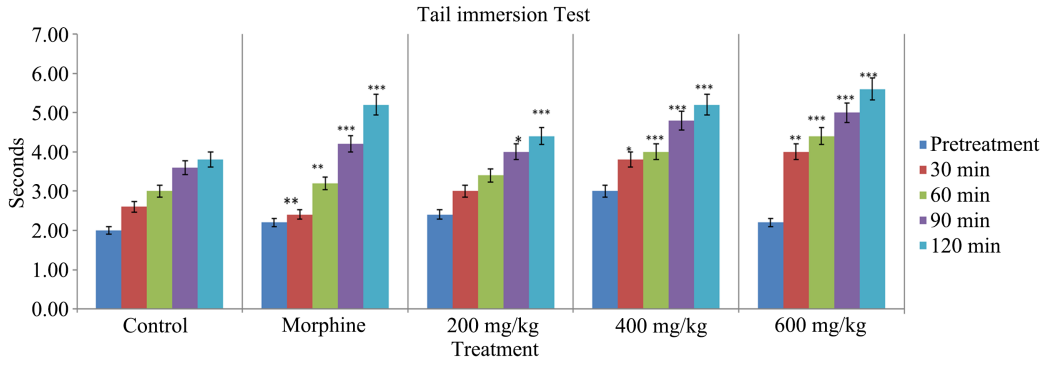


**Figure 2.** Antinociceptive effect of *Diospyros malabarica* leaves extract and morphine in tail immersion test.

**Table 3.** Effect of leaf extract of *Diospyros malabarica* extract on tail immersion test.

| Treatment | Dose | Response Time (in seconds) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 0.1 mL/mouse | 2.00 ± 0.00 | 2.60 ± 0.245 | 3.00 ± 0.447 | 3.60 ± 0.510 | 3.80 ± 0.374 |
| Standard | 5 mg/kg | 2.20 ± 0.200 | 2.40 ± 0.245** | 3.20 ± 0.374** | 4.20 ± 0.374*** | 5.20 ± 0.375*** |
| MEDM | 200 mg/kg | 2.40 ± 0.245 | 3.00 ± 0.316 | 3.40 ± 0.400 | 4.00 ± 1.049* | 4.40 ± 0.600*** |
| MEDM | 400 mg/kg | 3.00 ± 0.775 | 3.80 ± 0.374* | 4.00 ± 0.316*** | 5.00 ± 0.316*** | 5.20 ± 0.200*** |
| MEDM | 600 mg/kg | 2.20 ± 0.200 | 4.00 ± 0.316** | 4.40 ± 0.245*** | 4.80 ± 0.374*** | 5.60 ± 0.245*** |

Values are presented as mean ± SEM (n = 5). MEDM = Methanol extract of *Diospyros malabarica*; ***p < 0.001 compared with the control group (Dunnett's test), **p < 0.01 compared with the control group (Dunnett's test), *p < 0.05 compared with the control group (Dunnett's test).

mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

### 4.5. Acetic Acid-Induced Writhing Test

In Figure 3 and Table 4, the impact of MEDM treatment on the abdominal constriction test in mice is depicted. At dosages of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively, it was shown that MEDM was able to considerably suppress the nociceptive effects brought on by acetic acid when compared to the control group (Deionized water) (***p < 0.001). Diclofenac sodium, 10 mg/kg, 11.57% MEDM, 200 mg/kg, 37.77% MEDM, 400 mg/kg, and 51.83% MEDM, 600 mg/kg were determined to block constrictions to varying degrees (Table 4). All values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

### 4.6. Formalin Test

When compared to the control group (Deionized water), MEDM produced a dose-related inhibition of formalin-induced nociception and significantly inhibited both the neurogenic (0 - 5 min) and inflammatory (15 - 30 min) phases of the formalin-induced licking test (Figure 4 and Table 5). However, in the second stage of this pain model, its antinociceptive impact was more prominent. In all phases, formalin-induced nociception was considerably decreased by dic-

lofenac sodium (10 mg/kg, i.p.) (***p < 0.001). Values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

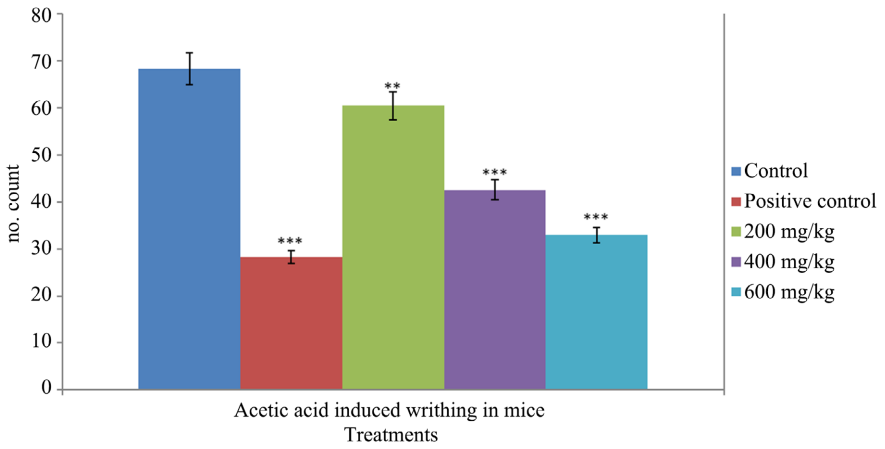


**Figure 3.** Antinociceptive effect of *Diospyros malabarica* leaves extract in acetic acid-induced writhing.

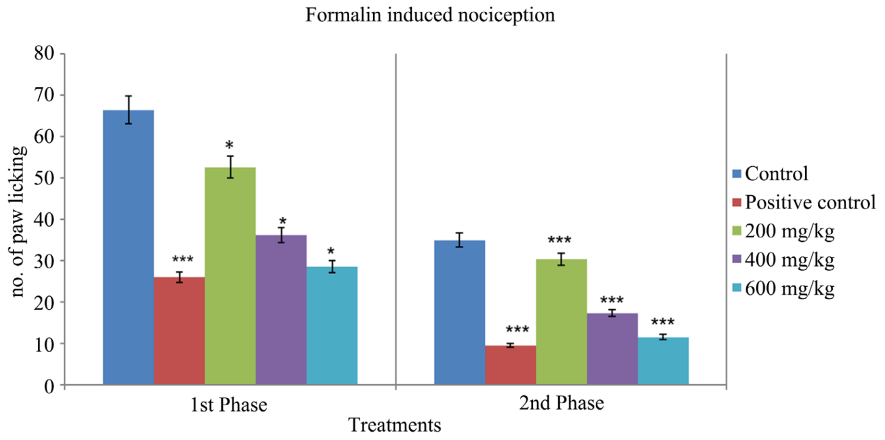


**Figure 4.** Antinociceptive effects of *Diospyros malabarica* leave extract in formalin-induced nociception.

**Table 4.** Effect of leaf extract of *Diospyros malabarica* extract on acetic acid -induced abdominal writhing test.

| Treatment | Dose (mg/kg) | Mean ± SEM | % of Inhibition |
|---|---|---|---|
| Control | 0.1 mL/mouse | 68.3 ± 2.338 | 0.00 |
| Diclofenac Sodium | 10 | 28.2 ± 2.488*** | 58.71 |
| MEDM | 200 | 60.4 ± 1.345** | 11.57 |
| MEDM | 400 | 42.5 ± 1.458*** | 37.77 |
| MEDM | 600 | 32.9 ± 0.980*** | 51.83 |

Values are expressed as Mean ± SEM (n = 5); MEDM = Methanol extract of *Diospyros malabarica*; ***p < 0.001 compared with the control group (Dunnett's test), **p < 0.01 compared with the control group (Dunnett's test).

**Table 5.** Antinociceptive effects of *Diospyros malabarica* leave extract in formalin-induced nociception.

| Treatment | Dose (mg/kg) | Licking of the hind paw | | | |
|---|---|---|---|---|---|
| | | Early phase | % of inhibition | Late phase | % of inhibition |
| Control | 0.1 mL/mouse | 66.40 ± 2.09 | 0.00 | 35.00 ± 1.61 | 0.00 |
| Diclofenac sodium | 10 | 26.00 ± 0.71** | 60.84 | 9.60 ± 0.93** | 72.57 |
| MEDM | 200 | 52.60 ± 3.01** | 20.78 | 30.40 ± 1.75** | 13.14 |
| MEDM | 400 | 36.20 ± 1.39** | 45.48 | 17.40 ± 1.12** | 50.28 |
| MEDM | 600 | 28.60 ± 0.93** | 56.93 | 11.60 ± 1.12** | 66.85 |

Values are presented as mean ± SEM (n = 5). MEDM = Methanol extract of *Diospyros malabarica*; ***p < 0.001 compared with the control group (Dunnett's test); *p < 0.05 compared with the control group (Dunnett's test).

### 4.7. Glutamate-Induced Nociception

MEDM administered orally produced dose-dependent antinociceptive effect. It was discovered that MEDM significantly prevented the glutamate-induced nociception test at dosages of 200 mg/kg, 400 mg/kg, and 600 mg/kg (Figure 5 and Table 6). As a conventional medication, diclofenac sodium (10 mg/kg) inhibited licking 65.14% less than the control group. When compared to the control group (Deionized water), all treatments showed considerable antinociceptive activity (***p < 0.001). All values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

## 5. Discussion

Pain is a physical, forceful, and defensive reaction. In general, painful stimuli cause significant withdrawal and avoidance reactions. Since central nociceptive pathways are sensitized and rearranged as a result of tissue injury and prolonged pain, this experience is extremely complex [39]. Nociceptors pick up the pain sense message, which is then sent to the brain via the pain sensory neuron and second row nerves via the sensation guidance route in the spinal dorsal horn. These nerves cross, ascend to the spinal cord, and descend to the thalamus at various locations throughout the brain stem and in particular relaying nuclei. From these nuclei, third row neurons carry sensory pain nerves to various regions of the brain membrane and limbic system. Long-term pain sufferers might experience negative mental repercussions, which is why people have been looking for ways to reduce or even eradicate pain for a very long time. There have also been several attempts to explain how pain works and how to manage it. Drugs of two different categories, synthetic and herbal, are used to relieve pain [40] [41].

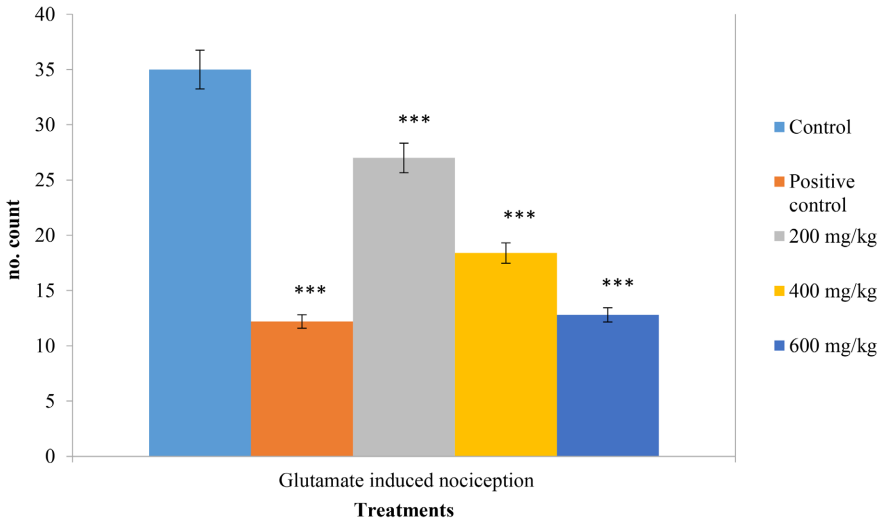


**Figure 5.** Antinociceptive effects of *Diospyros malabarica* leave extract in glutamate-induced nociception test.

**Table 6.** Antinociceptive effects of *Diospyros malabarica* leave extract in glutamate-induced nociception.

| Treatment | Dose (mg/kg) | Licking time (s) | % of inhibition |
|---|---|---|---|
| Control | 0.1 mL/mouse | 35.00 ± 1.14 | 0.00 |
| Diclofenac sodium | 10 | 12.20 ± 0.66** | 65.14 |
| MEDM | 200 | 27.00 ± 1.30** | 22.85 |
| MEDM | 400 | 18.40 ± 0.81** | 47.42 |
| MEDM | 600 | 12.80 ± 0.58** | 63.42 |

Values are presented as mean ± SEM (n = 5). MEDM = Methanol extract of *Diospyros malabarica*; ***p < 0.001 compared with the control group (Dunnett's test).

The current study shows that oral administration of MEDM decreased the threshold for heat and chemically generated pain and resulted in dose-dependent antinociceptive effects in several pain models. Since no mortality, allergic responses, salivation, convulsions, tremors, diarrhea, behavioral abnormalities, or physical alterations in important organs were observed in mice after receiving doses of MEDM greater than the maximum experimental dosage, this suggests that MEDM was not hazardous at the levels we used in our experiments.

The nociceptive response to heat stimuli in mice is a well-validated paradigm for opiate analgesic as well as various types of analgesic medications from spinal origin [42] [43] [44] detection in the hot-plate test and the tail immersion in hot water. A pain stimulus is acetic acid. Through the action of phospholipase A2 and other acyl hydrolases, intraperitoneal treatment of acetic acid promotes localized inflammation by liberating free arachidonic acid from tissue phospholipids [45]. The production of eicosanoids from arachidonic acid follows three main routes. The cyclooxygenase pathway is used for the synthesis of all eicosa-

noids having ring structures, including prostacyclines, thromboxanes, and prostaglandins. The lipooxygenase route is used to create the leucotrienes, HETE (hydroxy eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids), which are hydroxylated derivatives of straight-chain fatty acids. According to reports, the released prostaglandins, namely prostacyclines (PGI2) and prostaglandin-E, stimulate the A-fibers and cause pain perception. Sharp, localized pain is felt when the A-fibers are active [46].

By assessing the writhing effect that acetic acid injection caused and the prevention of writhing effect that the test samples produced, analgesic activity was evaluated. Any medication that reduces the writhing number exhibits analgesia by preventing the production of prostaglandins, a peripheral pain-inhibitory mechanism. This theory is in line with those writers' hypotheses that acetic acid-induced writhing test methods are helpful tools for assessing analgesics that operate both peripherally and centrally [47] [48]. At larger dosages, the *Diospyros malabarica* extract reduced the frequency of mice's acetic acid-induced writhing. Diclofenac sodium, which inhibits the production of prostaglandins, was employed as the positive control. By preventing the synthesis and release of prostaglandins, it lessens arthritic pain, edema, and inflammation [49] [50] [51]. In vitro polymorphonuclear leukocyte activity is also affected by the medication, which lowers chemotaxis, the creation of oxygen-derived free radicals, superoxide harmful radicals, and neutral proteases [52] [53]. Additionally, diclofenac has been shown to reduce inflammation brought on by several phlogistic agents in animal experiment models [54] [55]. In light of the antinociceptive activity demonstrated by the methanol extract of *Diospyros malabarica* in the hot-plate, hot tail-flick, and acetic acid-induced writhing tests, along with the responses of the positive control diclofenac, it was possible that this extract's antinociceptive property was mediated both centrally and peripherally.

In mouse paws, formalin causes pain that is mediated by two different routes. First, the immediate aftermath of formalin injection causes the early phase, which is characterized by neurogenic pain and brought on by both direct stimulation of sensory afferent fibers and activation of C-fibers. Inducing nociception during this phase also involves bradykinin and substance P. Second, the late phase (15 minutes after formalin injection), during which histamine-like inflammatory mediators, prostaglandins (PGs), bradykinin, serotonin, and other inflammatory mediators work in peripheral tissues to cause inflammation pain [56] [57]. The functional alterations in the dorsal horn of the spinal cord are another factor contributing to formalin-induced late phase pain [58]. According to the findings of the formalin-induced paw licking test (**Figure 4**), diclofenac and MEDM substantially ($*p < 0.001$) suppressed both stages of nociception. The inhibition increased in strength in the late phase and was dosage dependant. While peripheral analgesics (aspirin, hydrocortisone) mostly decrease the late phase of formalin-induced paw-licking in mice, central analgesics (opioids) suppress both stages [59] [60] [61]. The results of the hot plate and tail immersion tests are supported by the considerable suppression of both phase paw lick-

ings in the formalin test, which further points to the central pain prevention action of MEDM. Additionally, the paw-licking deterrent in the late phase shows that the inflammatory mediators were inhibited, as was seen in the acetic acid-induced writhing test.

N-methyl-D-aspartate (NMDA) receptors drive the glutamate-induced paw-licking nociception, although edema production is accompanied by non-NMDA receptors (AMPA, Kainate) at peripheral, supraspinal, and spinal regions. For the transfer of pain signals from the peripheral nervous system to the dorsal horn of the central nervous system, glutamate release substance P and IL-1, pro-inflammatory cytokines that resemble TNF, are necessary. Nitric oxide synthase (NOS) and reactive oxygen species (ROS) also stimulate the genes for TNF-, IL-1, and IL-6 as part of the pro-inflammatory signals [38] [62] [63]. MEDM considerably ($p = 0.001$) decreased paw licking and edema brought on by glutamate (**Figure 5**). The findings imply that MEDM is involved in the disruption of pro-inflammatory signals from ROS and NOS as well as the inhibition of NMDA and non-NMDA receptors.

The current study's findings showed that ethanol extract of *Diospyros malabarica* leaves greatly inhibited ear edoema during the first phases of inflammation, suggesting that the ethanolic extract may be reducing the generation of histamine and serotonin. This suggested that the ethanolic extract's ability to reduce inflammation could be due to its ability to suppress histamine generation, release, or activity. The antiinflammatory action of the *Diospyros malabarica* extract may potentially be related to the wound-healing function. *Diospyros malabarica* leaves' anti-inflammatory and wound-healing properties might be attributed to the ethanol extract's flavonoids or flavonoid glycosides. Collagen's primary job is to provide the skin tissue flexibility and strength. The tensile strength of repaired skin tissue can be used to indicate this strength. In the group of incision wounds treated with the extract as compared to the control group of animal wounds, a substantial rise in tensile strength suggests an increase in collagen. The quantity of protein in granulation tissue is thought to represent the rates of protein synthesis and cell proliferation. The amount of protein increases as collagen formation rises [64]. The results of the current investigation indicated that the extract treatment group's protein content had considerably higher levels. This increase in protein content suggests that the use of the appropriate extracts during therapy may be able to promote cell growth. *Diospyros malabarica* has already been found to have greater flavonoid content [65] [66], which may be the source of its anti-inflammatory and wound-healing properties.

## 6. Conclusion

The results of the current study make it abundantly evident that the plant extract has great analgesic potential. However, more investigation is needed to pinpoint the specific processes behind the antinociceptive action and to pinpoint the chemicals responsible for such activity.

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## Conflicts of Interest

The authors alone are responsible for the content and writing of the paper.

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