GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Analgesic activity of methanolic extract of *Heliotropium indicum* Linn leaves in mice

Shaikat Mallick [1], Ambia Khatun [1], Md. Abdul Mannan [1,*], Md. Tanvir Islam [2], Md. Azizul Haque [1], Paushi Sarker [2] and Ali Hasan Mujahid [1]

[1] *Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh.*
[2] *Department of Pharmacy, Daffodil International University, Daffodil Smart City, Birulia, Savar, Dhaka-1216, Bangladesh.*

GSC Biological and Pharmaceutical Sciences, 2026, 35(03), 151-161

Publication history: Received on 29 April 2026; revised on 12 June 2026; accepted on 15 June 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.35.3.0215

Abstract

Heliotropium indicum (Family: Boraginaceae) locally known as 'Hatishur', is a small annual or perennial herb distributed throughout Bangladesh, India, Srilanka, Nepal, Thailand, Tropical Asia, Africa and Philippines. The plant is used in different traditional and folklore systems of medicine for curing various diseases. This study was undertaken to evaluate the analgesic activity of methanolic extract of *Heliotropium indicum* (MEHI) in animal models with an aim to justify its traditional use. Five groups of five mice each were created out of the animals. Before the tests, the control group was given 0.1 mL/mouse of deionized water orally. The positive control group received intraperitoneally diclofenac sodium at the dose of 10 mg/kg 15 minutes prior to the experiments in hot plate, tail immersion, and acetic acid-induced writhing tests. Thirty minutes before to the trials, MEHI was given orally at dosages of 100, 200, and 400 mg/kg. Deionized water was used to make each medication dosage and MEHI dose. The hot plate and tail immersion tests are distinguished by their tendency to respond to the pain stimuli conducting through neuronal pathways. As our extract of *Heliotropium indicum* showed significant analgesic activity in thermal heat method, it can be assumed that the extract could act by a central antinociceptive mode like that of diclofenac. On the other hand, Acetic acid-induced writhing test represents pain sensation by triggering localized inflammatory response. Inhibition levels in the acetic acid-induced writhing test were 55.82%, 69.01% and 78.68%, respectively. Based on this result, the methanolic extract of *Heliotropium indicum* might possess analgesic activity in mice. Additional studies are necessary to further investigate and validate the safe traditional uses of *Heliotropium indicum*, facilitating its integration into broader research initiatives. Overall, the results give scientific support for the use of the plant in the management of pain.

Keywords: *Heliotropium indicum;* Analgesic; Diclofenac sodium; Phytochemistry; Traditional use

1. Introduction

Natural products play a substantial role in the discovery of new therapeutic agents because of their immense availability in nature, lead to the identification of bioactive molecules which allow the growth of new pharmaceutical agents. In the recent years, because of increasing interest in the use of pharmaceuticals, natural substances are the major source of complementary or alternative medicines, which are used in the treatment of many diseases [1]. In the recent years, because of increasing interest in the use of pharmaceuticals, natural substances are the major source of complementary or alternative medicines, which are used in the treatment of many diseases (1). From ancient times, plants are available to humans as a source of therapy. In the 19th century, due to the advancements in the field of pharmaceutical chemistry especially the medicinal chemistry more than 25% of drugs used in well developed countries are of plant origin and about 120 plant derived substances are used in modern system of medicines worldwide [2].

Heliotropium indicum Linn. (Family: Boraginaceae) locally known as 'Hatishur', is a small annual or perennial herb distributed throughout Bangladesh, India, Srilanka, Nepal, Thailand, Tropical Asia, Africa and Philippines. Its stem and

* Corresponding author: Md. Abdul Mannan

roots are covered with a fine hairy layer, and its flowers are small and grow in clusters which curve in on themselves at the tips [3]. *Heliotropium indicum* has been used in different traditional and folklore systems of medicine for curing various diseases such as skin diseases, poison bites, stomachache and nervous disorders [4]. Whereas Malasar tribes of Coimbatore district of Tamil Nadu, use leaf juice boiled with coconut oil to kill dandruff [5]. Different tribes of Cachar district, Assam, India use root juice of *Heliotropium indicum* to cure ophthalmia and fresh leaf extract is applied externally in fresh cuts and wounds [6]. The leaf paste is applied externally to cure rheumatism in Rayal Seema in Andhra Pradesh, India and skin infection in Nicaragua. The decoction of both leaf and root together is also used for treating whooping cough in children in Eastern Nicaragua [4]. In some African countries, another ethnopharmacological survey reports that *Heliotropium indicum* is believed to be useful in treating malaria, abdominal pain and dermatitis. The highest number of usages (22%) was reported for the treatment of malaria [7]. In Jamaica, the decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum while in Phillipines and Senegal, used orally as diuretic and for the treatment of kidney stone [8-10].

In Thailand, the dried inflorescence is believed to produce permanent sterilization when taken orally in females. One gram of the dried and powdered inflorescence mixed with milk or water is used for three days beginning with the fourth day of menses to achieve the desired result. Other folk remedies include use of decoction of the leaves for treatment of fever, insect bites, stings, diarrhea, skin rashes, menstrual disorder and urticaria. The decoction of the leaves is also credited to be useful in curing insect stings (macerated with sugar cane juice), scorpion stings and as abortive in large dose and emmenagouge in small dose. The decoction of both leaf and root together is also used for treating whooping cough in children in Eastern Nicaragua [11]. In Amazon, the paste of both leaf and root together is applied externally in scorpion stings, bug bites [12] while the paste is recommended for treating sores and warts in Taiwan [13]. In Malaysia, a paste made from the plant is applied to counteract putrefaction, to treat pyoderma and ringworm infection. In Burma, a decoction of the whole plant is used to treat gonorrhea while in Indonesia, an infusion of the leaves is used to soothe mouth sprue. A decoction of the dried roots is drunk in the Philippines to promote menses, while the seeds are used to treat cholera, malaria, and for wound-healing [14].

Aerial parts contain pyrrolizidine alkaloids, indicine (Principal), echinitine, supinine, heleurine, heliotrine, lasiocarpine, its N-oxide, acetyl indicine, indicinine and antitumour alkaloid, indicine-n-oxide. The plant also contains rapone and lupeol and an ester of retronecine. Roots contain high amount of estradiol [15]. Helindicine, a new pyrrolizidine alkaloid together with the known lycopsamine were isolated from the roots of *Heliotropium indicum* [16]. Presence of cynoglossine, europine-N-oxide, heleurine-N-oxide, heliotridine-N-oxide, heleotrine-N-oxide [17] and heliotrine [18] have been identified from the seeds. The essential oil of *Heliotropium indicum,* extracted by hydrodistillation was analyzed by Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Aldehydes (52.8%) occurred in the highest amount represented by phenylacetaldehyde (22.2%), (E)-2-nonenal (8.3%) and (E, Z)-2-nonadienal (6.1%), with a significant quantity of hexahydrofarnesylacetone (8.4%) [19]. In another experiment, the volatile oil isolated by hydrodistillation was analysed by a combination of GC–FID and GC–MS. The major constituents of the volatile oil were phytol (49.1%), 1-dodecanol (6.4%) and *β*-linalool (3.0%) [20]. *Heliotropium indicum* is accepted to be valuable in treating malaria, stomach agony, and dermatitis. The most elevated number of utilizations (22%) was accounted for its use against malaria. The mixture of the flower is taken orally for the treatment of menorrhagia in Jamaica [21]. In a concise review by, *Heliotropium indicum* is indicated to have antimicrobial [22], anti-tumour [23-24], anti-inflammatory [25], anti-fertility [21], anti-tuberculosis [26], analgesic, anti-plasmodial [24] wound healing [27-28], anti-diabetic [29], anti-hepatic and anti-cataract activity [30]. Medicinal plants of traditional clinical practices are not appropriately checked, resulting in an expanded danger of toxicity [31]. There is clear proof to implicate oxidative stress as a toxicity mechanism in various tissues. Oxidative stress mirrors lopsidedness in the degree of body oxidants and its capacity to detoxify them [32]. Oxidative stress has likewise been implicated in several disease conditions, including malignant growth [33], neurodegenerative sicknesses such as Alzheimer's [34], Parkinson's [35], Huntington [36], amyotrophic lateral sclerosis [37], diabetes [38], ulcer [39], among others.

This study was undertaken to evaluate the analgesic activity of methanolic extract of *Heliotropium indicum* in alleviating pain in animal model with an aim to justify its traditional use.

2. Methods

2.1. Materials

Fresh leaves of *Heliotropium indicum*, a milling machine, electronic balance, rotary evaporator, filter paper, water bath, distilled water, beaker, and oral gavage were utilized. Chemical such as methanol was sourced from a chemical laboratory.

2.2. Plant collection

Fresh leaves of the selected medicinal herb *Heliotropium indicum* were harvested from the local area of Dhaka District, Bangladesh in December, 2024 and were taxonomically identified at the Bangladesh National Herbarium, Dhaka. For further reference, the Herbarium has already received a voucher specimen number. The collected leaves were thoroughly washed with tap water to avoid specks of dust and other unwanted materials accumulated on the leaves from their natural environment. The dust-free leaves were shaded and dried at room temperature. After 4-5 days for obtaining aqueous extract, the properly dried leaves were then ground into the fine powder by using the grinding machine then the powder material was weighed properly. The fine powder was stored in a clean and tightly closed container for extraction.

2.3. Preparation and extraction of crude plant extract

Heliotropium indicum leaves were air-dried for three weeks. It was blended into a powdery form using an industrial blender. Exactly 280 g of the *Heliotropium indicum* leaves powder was measured with an electronic balance and was soaked in 1000 ml of methanol at room temperature for 72 hours with periodic stirring. The solvent was 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 28 g of extract. The extract was stored in the refrigerator until required for use. The research on acute toxicity and analgesic effects employed this extract.

2.4. Animals and husbandry

The International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) Animal Research Branch provided Swiss albino mice (20–30 g). During the adaption period, animals were housed with food and water under standard laboratory settings (room temperature: 25.0±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 Experiments with Animals (1995). All experimental methods were approved Academy by the Stamford University Bangladesh Ethics Committee (SUB/IAEC/25). All experimental animals were handled in accordance with the Swiss of Sciences' and the Swiss Academy of Medical Sciences' Ethical Principles and Guidelines for Scientific.

2.5. 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.) diclofenac sodium at the dose of 10 mg/kg 15 minutes prior to the experiments in hot plate, tail immersion, and acetic acid-induced writhing tests. Thirty minutes before to the trials, MEHI was given orally at dosages of 100, 200, and 400 mg/kg (b.w.). Deionized water was used to make each medication dosage and MEHI dose.

2.6. Phytochemical screening

Preliminary phytochemical screening of the methanolic extract of *Heliotropium indicum* was carried out to detect the possible presence or absence of different phytoconstituents such as alkaloid, flavonoid, glycoside, glucoside, carbohydrate, steroid, tannin, reducing sugar, gum and saponin [40].

2.7. Acute toxicity test

Five experimental groups and a control group (n=5) of animals were each given five animals. The experimental groups received oral administration of MEHI at doses of 500, 1000, 2000, 3000, 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 [41].

2.8. Analgesic activity test

2.8.1. Hot plate test

The hot plate test method was used to examine potential centrally mediated analgesic effects in a preferential manner [42]. 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±1 °C, and were given one of three treatments: control (Equivalent volume of

deionized water, 0.1 mL/mouse, p.o.), diclofenac sodium as a standard medication (10 mg/kg, i.p.), or MEHI (100, 200, and 400 mg/kg, p.o.). To protect the paw tissue, a 20-second cut off 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.

2.8.2. 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. Five mice per group were used to split the mice into the five groups. According to the protocol, mice pretreated with diclofenac sodium (10 mg/kg, i.p.) or MEHI (100, 200, and 400 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 diclofenac sodium and MEHI, 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 [43].

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

2.9. Acetic acid-induced writhing test

This test was used to determine MEHI peripheral analgesic activity in pain brought on by chemicals. Five groups of the animals were created (n = 5). Diclofenac sodium, 10 mg/kg, intravenously; control; or MEHI, 100, 200, or 400 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 MEHI, respectively. The mice were watched after 5 min following the injection of acetic acid, and for 30 min the number of writhing was tallied. Complete writhing was defined as the stomach contracting, the body lengthening, the trunk and pelvis twisting, and the extension of the limbs [44]. The analgesic activity was calculated using the following formula:

% Inhibition = [Mean no of writhes (control) - mean no of writhes (test)/Mean no of writhes (control)] × 100

2.10. 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<0.01 and *p<0.05 differences between groups were deemed significant.

3. Results

3.1. Phytochemical screening

From the results, the possible presence of alkaloid, flavonoid, glycoside, carbohydrate, steroid, tannin, reducing sugar, gum and saponin were confirmed through qualitative color changes of test reagents which will give a clue to the possible mechanisms of analgesic activity of the extract as indicated in Table 1.

Table 1 The preliminary qualitative phytochemical screening of the methanolic extract of *Heliotropium indicum* (MEHI).

Extract	**MEHI**
Alkaloid	+
Flavonoid	+
Glycoside	+
Carbohydrate	+
Steroid	+

Tannin	+
Reducing Sugar	+
Saponin	+
Gum	+
Glucoside	-

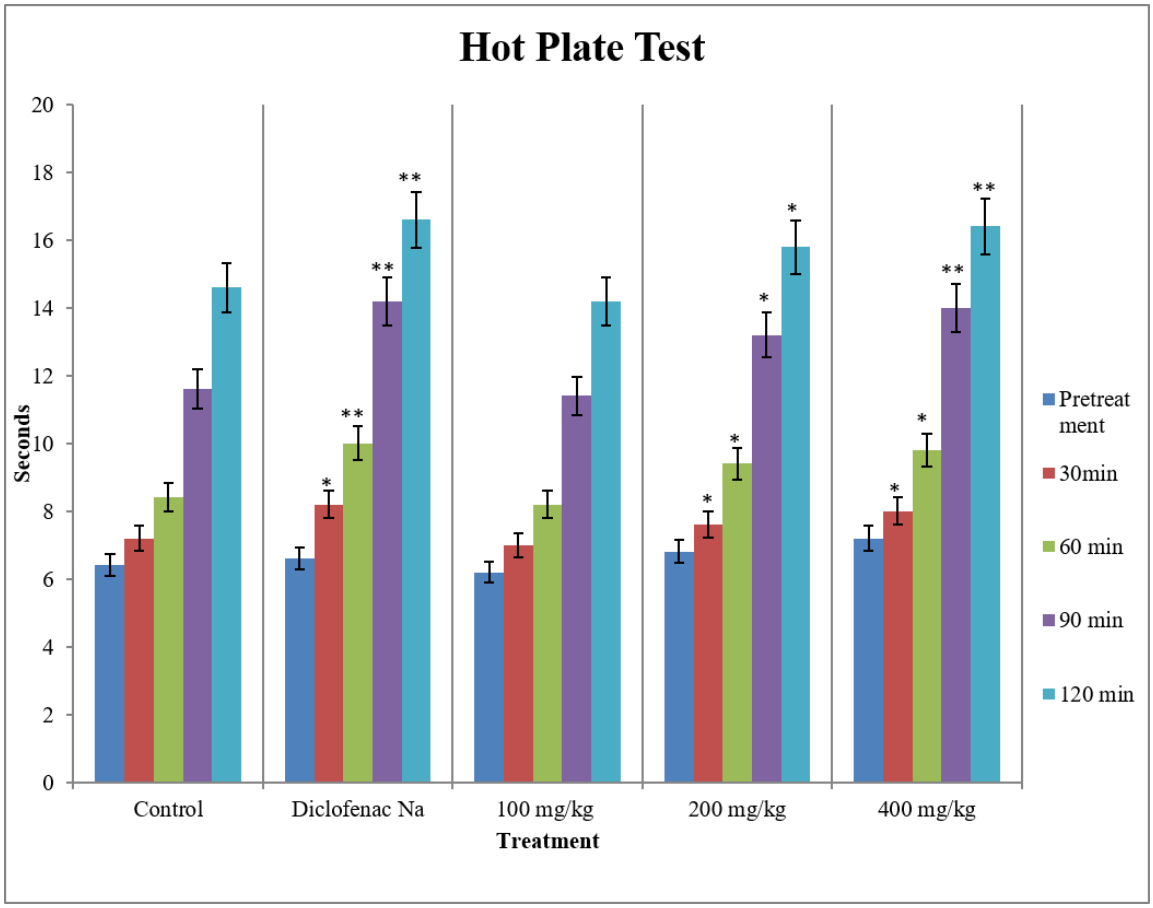
[MEHI = Methanolic Extract of *Heliotropium indicum* ; (+): Present; (-): Absent]

3.2. Acute toxicity

Mice used in the acute toxicity study were observed for the first four hours continuously then for 24 hours and for the next 14 days to see if there is any toxicity. The LD_{50} of the leave part of the plant was estimated to be above 4000 mg/kg as there were no visible signs of toxicity, gross behavioral changes, and mortality within 24 h as well as in the next 14 days.

3.3. Hot-plate test

In the hot plate test method, 200 mg/kg and 400 mg/kg doses of the extract produced a significant analgesic effect ($p < 0.05$ and $p < 0.01$) at 120 min when compared to the control group, and diclofenac sodium 10 mg/kg produced a significant analgesic effect at 60 and 90 min compared to the control group. In the contrast, 100 mg/kg of the extract produce no significant analgesic effect at an all-time interval compared to the control group as described in Table 2.



Values are presented as mean ± SEM (n = 5). ** $p < 0.01$ and *$p < 0.05$ compared with the control group (ANOVA followed by post hoc Dunnett's test).

Figure 1 Analgesic activity of *Heliotropium indicum* leaves extract and diclofenac Na in hot plate test

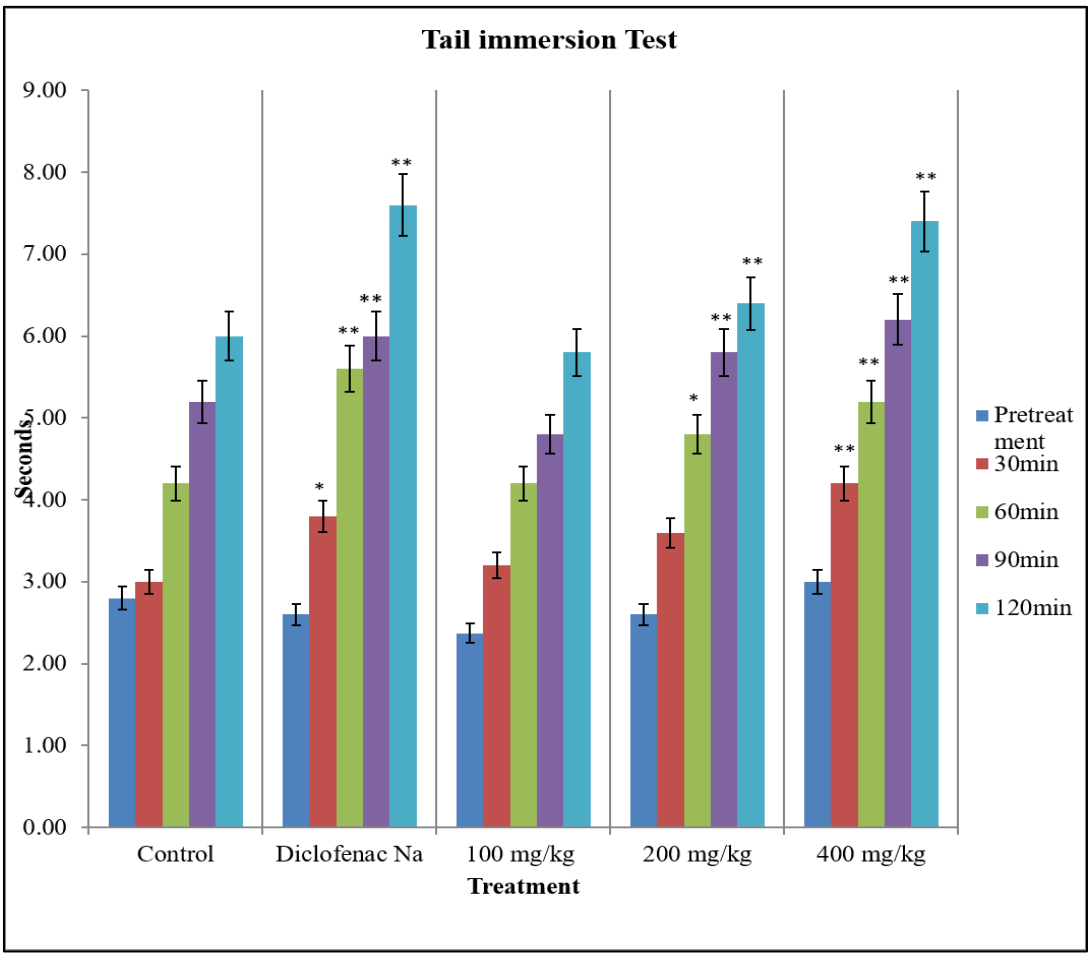
Table 2 Analgesic activity of leaf extract of *Heliotropium indicum* on hot plate test.

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	6.40 ± 0.51	07.20 ± 0.37	08.40 ± 0.40	11.60 ± 0.67	14.60 ± 0.51
Diclofenac Sodium	10	06.60 ± 0.24	08.20± 0.37*	10.00 ± 0.31**	14.20 ± 0.37**	18.00 ± 0.54**
MEHI	100	06.20 ± 0.37	07.00 ± 0.31	08.20 ± 0.58	11.40 ± 0.51	14.20 ± 0.37
MEHI	200	06.80 ± 0.37	07.60 ± 0.51*	09.40 ± 0.24*	13.20 ± 0.37*	16.80 ± 0.37*
MEHI	400	07.20 ± 0.37	08.00 ± 0.31*	10.40 ± 0.25*	14.00 ± 0.54**	17.20 ± 0.49**

Values are presented as mean ± SEM (n = 5). MEHI = Methanol extract of *Heliotropium indicum*;***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).

3.4. Tail immersion test

In the tail immersion test method, 200 mg/kg and 400 mg/kg doses of the extract produced a significant analgesic effect (p < 0.01) at 120 min when compared to the control group, and diclofenac sodium 10 mg/kg produced a significant analgesic effect at 60 and 90 min compared to the control group. In the contrast, 100 mg/kg of the extract produce no significant analgesic effect at an all-time interval compared to the control group as shown in Table 3.



Values are presented as mean ± SEM (n = 5). ** p < 0.01 compared with the control group (ANOVA followed by post hoc Dunnett's test).

Figure 2 Analgesic activity of *Heliotropium indicum* leaves extract and diclofenac Na in tail immersion test

Table 3 Effect of leaf extract of *Heliotropium indicum* extract on tail immersion test.

Treatment	Dose (mg/kg)	Response Time (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1ml/mouse	2.80 ± 0.37	3.00 ± 0.31	4.20 ± 0.37	5.20 ± 0.37	6.00 ± 0.31
Diclofenac Sodium	10	2.60 ± 0.24	3.80 ± 0.37*	5.60 ± 0.24**	6.40 ± 0.24**	7.60 ± 0.24**
MEHI	100	2.37 ± 0.25	3.20 ± 0.37	4.20 ± 0.37	4.80 ± 0.37	5.80 ± 0.37
MEHI	200	2.60 ± 0.24	3.60 ± 0.51	4.80 ± 0.37*	6.40 ± 0.25**	6.60 ± 0.24**
MEHI	400	3.00 ± 0.31	4.20 ± 0.37**	5.20 ± 0.37**	6.60 ± 0.24**	7.40 ± 0.24**

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

3.5. Acetic acid-induced writhing test

Table 4 showed the effect of the methanol extract of *Heliotropium indicum* Linn. on acetic acid-induced writhing test in mice. At dose of 100 mg/kg, 200 mg/kg and 400 mg/kg of body weight, the extract produced about 55.82%, 69.01% and 78.68% writhing inhibition in test animals, respectively. The results were statistically significant (P<0.05) and were comparable to the standard drug diclofenac sodium, which showed about 81.09% writhing inhibition (P < 0.05).

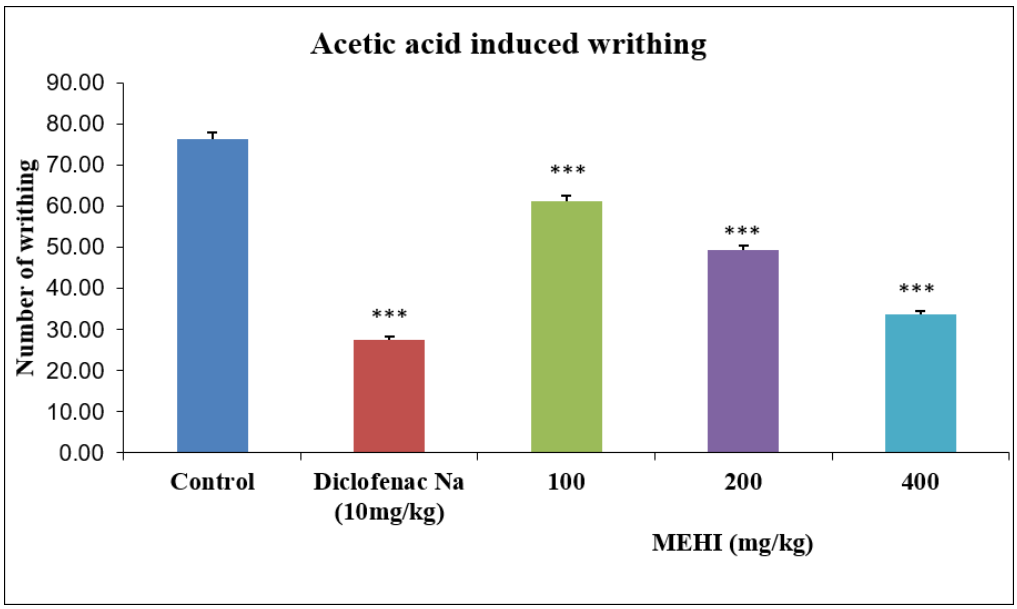


Figure 3 Analgesic activity of *Heliotropium indicum* leaves extract in acetic acid-induced writhing test. All values are presented as mean ± SEM (n = 5). * p < 0.05 compared with the control group (ANOVA followed by post hoc Dunnett's test)

Table 4 Effect of leaf extract of *Heliotropium indicum* extract on acetic acid-induced writhing test.

Treatment	Dose (mg/kg)	Mean ± SEM	% of Inhibition
Control	0.1 ml/mouse	76.30 ± 1.76	0.00
Diclofenac Sodium	10	27.40 ± 1.04***	81.09
MEHI	100	61.20 ± 1.36***	55.82
MEHI	200	49.30 ± 1.28***	69.01
MEHI	400	33.80 ± 0.81***	78.68

Values are expressed as Mean ±SEM (n=5); MEHI= Methanol extract of *Heliotropium indicum*; *** 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).

4. Discussion

Pain is the body's defense and protective mechanism to withdraw from a painful stimulus. The International Association for the Study of Pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [45]. Numbers of drugs are available in market to treat inflammation and pain, like non-steroidal anti-inflammatory drugs (NSADIs) and Opioids. Synthetic substitutes have no doubt taken over, but none of the anti-inflammatory and analgesic drugs available today can be considered ideal, for their toxic effects. There is extensive search going on for new drugs and molecules with fewer side effects and there is lot of scope for herbal medicine in this view.

To check for possible analgesic activity of the methanolic extract of *Heliotropium indicum*, hot plate and tail immersion tests were performed. The two tests are distinguished by their tendency to respond to the pain stimuli conducting through neuronal pathways [46]. Hot plate test at a constant temperature produces two kinds of responses: paw licking and jumping, which are both considered to be supra spinally integrated responses [47]. The test measures the complex response to an acute, non-inflammatory nociceptive stimulus [48]. This effect is largely dependent on central mechanism, playing an essential role in the endogenous opioid [49]. Tail immersion test, on the other hand, mediates spinal reflexes to nociceptive stimuli [50-51]. Substance P is released in excessive quantities due to the stimulation of non myelinated C fibers of mouse tail after the application of thermal heat. In case of acetic acid writhing test, prostacycline stimulates C fibers, whereas in thermal heat test C fibers are stimulated by thermal heat, serving as noxious stimuli. Narcotic analgesics are potential agonist of μ, κ and δ receptors. These receptors are specific for endogenous narcotics like endorphins, encephalin etc. After binding to these receptors, narcotic analgesics antagonize the action of substance P in the CNS by producing post-synaptic inhibitory action on interneuron, which processes the nociceptive information to be transmitted to the CNS. As our extract of *Heliotropium indicum* showed significant analgesic activity in thermal heat method, it can be assumed that the extract could act by a central anti-nociceptive mode like that of diclofenac. On the other hand, the significant analgesic activity of *Heliotropium indicum* extract in both hot plate-induced and tail immersion tests suggest a central action and could involve supraspinal and spinal mechanisms.

Analgesic activity of the methanolic extract of *Heliotropium indicum* was tested by acetic acid-induced writhing test in mice. The test represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [52]. Increased levels of PGE2 and PGF2α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [53-54]. The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 4). The chemical compounds present in the plant extract may be responsible for the obtained analgesic activity. Based on this result, the methanolic extract of *Heliotropium indicum* might possess analgesic activity in mice.

Heliotropium indicum is a medicinal plant whose beneficial effects have been shown by various researchers. Results from table 1 showed that *Heliotropium indicum* is rich in phytochemicals, including flavonoids, saponins, alkaloids, tannins, reducing sugars and terpenoids. The presence of these phytochemicals may be indicative of its numerous beneficial health effects that have been reported by several studies. Although pyrrolizidine alkaloids such as intermedine, supinine, lindelofin and trachelanthine isolated from the leaf methanolic extracts of *Heliotropium indicum* has been reported the analgesic effects [55].

5. Conclusion

Based on this result, the methanolic extract of *Heliotropium indicum* might possess analgesic activity in mice. The plant serves as a potential source of chemical compounds with promising biological activities. However, it is crucial to note that clinical trials for *Heliotropium indicum* are currently limited, posing a challenge in translating its potential benefits into routine clinical practice. Additional studies are necessary to further investigate and validate the safe traditional uses of *Heliotropium indicum*. Overall, the results give scientific support for the use of the plant in the management of pain.

Compliance with ethical standards

Acknowledgments

The authors are thankful to The Chairman, Department of Pharmacy, Stamford University Bangladesh, Dhaka, for providing laboratory facilities.

Disclosure of conflict of interest

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

Funding

This research work did not have any particular funding. All the studies had been self-funded by author and co-authors.

Authors' contributions

MAM and AK designed and coordinated all laboratory experiments, analyzed and interpreted results. All authors conducted all experiments. MAM did statistical analysis and drafted the manuscript. All authors read and approved the manuscript.

Statement of ethical approval

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/25) of Stamford University Bangladesh approved all experimental rules.

Statement of informed consent

The manuscript does not contain any individual person's data in any form. So, this information is not relevant.

References

[1] Calixto JB, Santos AR, Filho VC, Yunes RA. (1998). A review of the plants of the genus Phyllanthus, their chemistry, pharmacology, and therapeutic potential. Med Res Rev; 18(4):225-258.

[2] Sharma R, Singh B, Singh D, Chandrawat P. (2009). Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. J Med Plant Res; 3(13):1153-1175.

[3] Ghani A. (2003). Medicinal Plants of Bangladesh-Chemical constituents and uses. 2nd Ed. The Asiatic Society of Bangadesh: Dhaka;1-17.

[4] Dash KG, Abdullah SM. (2013). A Review on *Heliotropium Indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research; 4(4):1253- 1258.

[5] Kumar Pandi P, Ayyanar M, Ignacimuthu S. (2007). Medicinal plants used by Malasar tribes of Coimbatore district, Tamil Nadu. Indian Journal of Traditional Knowledge; 6(4):579-582.

[6] Das Kumar Ajit, Dutta KB, Sharma DG. (2008). Medicinal plants used by different tribes of Cachar district, Assam. Indian Journal of Traditional Knowledge; 7(3):446-454.

[7] Togola A, Diallo D, Dembélé S, Barsett H and Paulsen BS. (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. J Ethnobiol Ethnomedicine; 1(1): 7.

[8] Asprey GF and Thornton P. (1955). Medicinal plants of Jamaica, Part-III. West Indian Med J; 4(4): 69-82.

[9] Quisumbing E. (1951). Medicinal Plants of Phillipines. Tech. Bull Phillipines, Dept. Agr. Nat resources, Manila, 16, Rep: 1.

[10] Berhault J. (1974). Floore Illustree du Senegal. Govt. Senegal, Min Rural Development, Water and Forest Division, Dakar, Vol II: 110-114.

[11] Coee FG and Anderson GJ. (1996). Ethnobotany of the Garifuna of Eastern Nicaragua, Econ Bot; 50(1):7l-107.

[12] Duke JA. (1994). Amazonia Ethnobotanical Dictionary. CRC Press, Boca Raton FL. Peru: 181.

[13] Lin CC and Kan WS. (1990). Medicinal plants used for the treatment of hepatitis in Taiwan. Amer J Chinese Med; 18(112): 35-43.

[14] Wiart C. (2006). Medicinal Plants of the Asia-Pacific: Drugs for the Future? World Scientific Publishing Co. Pte. Ltd., Singapore.

[15] *Heliotropium indicum* L. Medicinal plants of Bangladesh. http://www.mpbd.info/plants/*heliotropium-indicum*.php. 15 August, 2015.

[16] Souza JSN. (2005). Pyrrolizidine alkaloids from *Heliotropium indicum*. J Braz. Chem. Soc; 16(6B):1410-1414.

[17] Willaman JJ and Schubert BG. (1961). Alkaloid bearing plants and their contained alkaloids, ARS, USDA, Tech Bull 1234, Supt Documents, Govt Print Off, Washington.

[18] Pandey VB, Singh JP, Rao YV and Acharya SB. (1982). Isolation and pharmacological action of heliotrine, the major alkaloid of *Heliotropium indicum* seeds. Planta Med; 45: 229-233.

[19] Ogunbinu AO, Flamini G, Cioni PL, Adebayo MA and Ogunwande IA. (2009). Constituents of *Cajanus cajan* (L.) Millsp., *Moringa oleifera* Lam., *Heliotropium indicum* L. and *Bidens pilosa* L. from Nigeria. Nat Prod Commun; 4(4): 573-578.

[20] Machan T, Korth J, Liawruangrath B, Liawruangrath S, Stephen G. (2006). Pyne Composition and antituberculosis activity of the volatile oil of *Heliotropium indicum* Linn. growing in Phitsanulok, Thailand, Flavour and Fragrance Journal;21(2):265–267.

[21] Dash GK, Abdullah MS. (2013). A review on *Heliotropium indicum L.* (Boraginaceae).Int. J. Pharm. Sci. Res.

[22] Rao PR; Nammi S, Raju ADV. (2002). Studies on the Antimicrobial Activity of *Heliotropium indicum* Linn. J. Nat. Remedies; 2:195–198.

[23] Sivajothi V, Shruthi SD, Jasmin Sajini R. (2015).Cytotoxic effect of *Heliotropium indicum* extracts on hela cell line. Int. J. Pharm. Pharm. Sci.

[24] Goyal N, Sharma SK. (2014). Bioactive phytoconstituents and plant extracts from genus *Heliotropium*. Int. J. Green Pharm: 8.

[25] Betanabhatla KS, Jasmin Sajni R, Karthik R, Raamamurthy J, Christina AJM, Sasikumar S. (2007). Anti-inflammatory and anti-nociceptive activities of *Heliotropium indicum* Linn. in experimental animal models. Pharmacologyonline**;** 3:438-445.

[26] Machan T, Korth J, Liawruangrath B, Liawruangrath S, Pyne SG.(2006). Composition and antituberculosis activity of the volatile oil of *Heliotropium indicum Linn*. growing in Phitsanulok, Thailand. Flavour Fragr. J.

[27] Reddy JS, Rao PR, Reddy MS. (2002).Wound healing effects of *Heliotropium indicum, Plumbago zeylanicum* and *Acalypha indica* in rats. J. Ethnopharmacol;79:249-251,

[28] Yeo D, Attioua B, Lehalle C, Kossi M, Jean David Ng, Djaman J, Lobstein A, Frossard N. (2011). Isolation of wound healing compounds from *Heliotropium indicum.* Journal of Applied Pharmaceutical Science;1:102-106.

[29] Sifat N, Zihad SMNK, Lovely F, Rouf R, Shajib GM, Al Alam MA, Shilpi JA, Uddin SJ. (2020). Supplementation of *Heliotropium indicum* Linn attenuates obesity and associated metabolic disorders in high-carbohydrate-high-fat diet-induced obese rats. J. Food Biochem:1–12,

[30] Kumar S, Vijaya VT, Kumar SS. (2015). Anticataract activity of ethanolic extract of *Heliotropium indicum* leaves on galactose induced in rats. Int. J. Pharmacol. Toxicol.

[31] Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM, Van Staden J. (2004). Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. J. Ethnopharmacol; 94:205-217.

[32] Ore A, Akinloye OA. (2019). Oxidative stress and antioxidant biomarkers in clinical and experimental models of non-alcoholic fatty liver disease. Med; 55:1–13.

[33] Hayes JD, Dinkova-Kostova AT, Tew KD. (2020). Oxidative Stress in Cancer. Cancer Cell;38:167–197.

[34] Cioffi F, Adam RHI, Broersen K. (2019). Molecular Mechanisms and Genetics of Oxidative Stress in Alzheimer's Disease. J. Alzheimer's Dis; 72:981–1017.

[35] Hemmati-Dinarvand M, Saedi S, Valilo M, Kalantary-Charvadeh A, Alizadeh Sani M, Kargar R, Safari H, Samadi N. (2019). Oxidative stress and Parkinson's disease: conflict of oxidant-antioxidant systems. Neurosci. Lett: 709.

[36] Zheng J, Winderickx J, Franssens V, Liu BA. (2018). Mitochondria-Associated Oxidative Stress Perspective on Huntington's Disease. Front. Mol. Neurosci; 11.

[37] Carrera-Juliá S, Moreno ML, Barrios C, de la Rubia Ortí JE, Drehmer E. (2020). Antioxidant Alternatives in the Treatment of Amyotrophic Lateral Sclerosis: A Comprehensive Review. Front. Physiol; 11:1–30.

[38] Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong X, Wen B, Zhang Z. (2018).The Pathogenesis of Diabetes Mellitus by Oxidative Stress and Inflammation: Its Inhibition by Berberine. Front. Pharmacol.

[39] Sun G, Lian T, Yang B, Gu Y, Li X. (2019). Ameliorative effect of Sargassum fusiforme polysaccharides on oxidative stress and inflammation in ethanol-induced gastric ulcer. Pharmacogn. Mag; 15:244-252.

[40] Ghani A. (2003) Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Edition, Asiatic Society of Bangladesh, Dhaka, 274.

[41] Mannan MA, Rony RR, Khan MFH. (2019). Evaluation of antinociceptive effects of methanolic extract of *Leucas aspera* leaves in mice.EC Pharmacology and Toxicology; 7.9: 1001-1012.

[42] Woolfee G and MacDonald AD. (1944). The evaluation of the analgesic action of pethidine hydrochloride. Journal of Pharmacology and Experimental Therapeutics; 80.3: 300-307.

[43] Cha DS, Eun JS, Jeon H. (2011). Antiinflammatory and antinociceptive properties of the leaves of *Eriobotrya japonica*. Journal of Ethnopharmacology134 (2):305-312.

[44] Zakaria ZA, Mustapha S, Sulaiman MR, Mat Jais AM, Somchit MN, Abdullah FC. (2007). The antinociceptive action of aqueous extract from *Muntingia calabura* leaves: the role of opioid receptors. Medical Principles and Practice; 16(2): 130-136.

[45] Ghodki SG, Borade SB, Pise H, Motghare V, Mehani RS, Wadgbalkar P. (2016). Evaluation of analgesic activity of *Aegle marmelossteam* bark in experimental animals. Int J Basic Clin Pharmacol;5:1081-1106.

[46] Zeashan H, Amresh G, Rao CV, Singh S. (2009). Antinociceptive activity of *Amaranthus spinosus* in experimental animals. J Ethnopharmacol, 122:492-496.

[47] Santos JS, Marinho RR, Ekundi-Valentim E. (2013). Beneficial effects of *Anadenanthera colubrina* (Vell.) Brenan extract on the inflammatory and nociceptive responses in rodent models. J Ethnopharmacol;148:218-222.

[48] Sulaiman MR, Hussain MK, Zakaria ZA. (2008). Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract, Fitoterapia; 79:557-561.

[49] Ramabadran K, Bansinath M. (1990). Endogenous opioid peptides and epilepsy. Int J Clin Pharmacol Ther Toxicol; 28:47-62.

[50] Taïwe GS, Bum EN, Talla E. (2011). Antipyretic and antinociceptive effects of *Nauclea latifolia* root decoction and possible mechanisms of action. Pharm Biol; 49 (1):15-25.

[51] Kabir S, Zahan R, Mosaddik A, Chowdhury AMS, Haque MR, Rashid MA. (2012). Evaluation of analgesic and anti-inflammatory activities of *Polyalthia simiarum*. Asian Pac J Trop Biomed:1-5.

[52] Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. (2003). Antiinflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J Ethnopharmacol; 84: 31-33.

[53] Derardt R, Jougney S, Delevalcee F, Falhout M. (1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol; 51: 17-24.

[54] Bose U, Gunasekaran K, Bala V, Rahman AA. (2010). Evaluation of phytochemical and pharmacological properties of *Dillenia indica* Linn. roots. J Pharmacol Toxicol;5:222-228.

[55] Adeniyi, Boluwaji M, Ikyenge, Barnabas A, Adah, Christiana A, Ibitoye, O,Ajisafe, Segun S, Oyewole,Omoniyi S. (2024). Isolation of Pyrrolizidine Alkaloid from *Heliotropium indicum* leaf for control of insects of stored grains, Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054);12:1.