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Macrosolen cochinchinensis (Lour.): Anti-nociceptive and antioxidant activity

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ABSTRACT

Objective: To investigate the anti-nociceptive activities of *Macrosolen cochinchinensis* (*M. cochinchinensis*) (Lour.) leaves extract at 100 and 200 mg/kg doses in mice and *in vitro* antioxidant potential. **Methods:** Acetic acid induced writhing, formalin test and tail immersion method were used to investigate anti-nociceptive activities of methanol extract of *M. cochinchinensis* in mice. The *in vitro* antioxidant potential was determined by DPPH free radical scavenging activity and reducing power capacity. **Results:** Methanol extract of *M. cochinchinensis* at 200 mg/kg significantly ($P<0.01$) reduced the acetic acid-induced abdominal contractions (54.82%) comparable to standard drug, diclofenac sodium (10 mg/kg). But the lower dose (100 mg/kg) produced mild analgesia (22.66%). It also significantly decreased latency to discomfort by formalin test and increased the reaction time of mice from (1.80 ± 0.31) s to (6.03 ± 0.08) s in tail immersion method where as reference drug nalbuphine (10 mg/kg) increased to (11.12 ± 0.13) at 90 min. Maximum scavenging activity 39.47% was found at the concentration of 500 μg/mL. **Conclusions:** These findings demonstrate that the leaves extract of *M. cochinchinensis* have excellent anti-nociceptive activities and moderate antioxidant properties that may possibly explain the use of the plant in traditional medicine.

1. Introduction

In recent years there has been an increased use of opioids in the management of non-malignant chronic pain causing a new and growing problem with addiction and misuse of opioids[1] and opiates lack potent analgesic efficacy in neuropathic pain[2]. NSAIDs are widely indicated for the treatment of acute or chronic conditions of pain and inflammation although relate to direct and indirect irritation of the gastrointestinal tract. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.

Macrosolen cochinchinensis (*M. cochinchinensis*) (Loranthaceae) is a much branched, quite glabrous, epiphytic parasitic shrub with swollen nodes that attaches itself to the host tree by modified roots[3,4] and in Bangladesh it is commonly known as Pargacha or Chota Banda[4].

The leaf paste is taken as a folk remedy for jaundice[5]. Tea from leaves is used for headache while the stems juice is commonly employed to expel the after-birth. Moreover, fruits are useful for symptomatic treatment of cough[6,7].

It has been scientifically proven that *M. cochinchinensis* possesses relaxation effect on vascular smooth muscle[7] and some antiviral and cytotoxic activities[6]. A number of natural components such as flavonoids, steroids and high concentrations of condensed tannins have been reported previously by primary phytochemical screening[6].

The aim of the present study was to investigate anti-nociceptive and antioxidant activities of *M. cochinchinensis* leaves extract by using different experimental methods. However, no earlier studies have been conducted experimentally to characterize the analgesic and antioxidant effect of this plant.

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2. Materials and methods

2.1. Plant material

Fresh leaves of *M. cochinchinensis* were collected from a local village (Gopalpur) of Rangpur, Bangladesh and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

2.2. Preparation of crude extract

The leaves were sun dried for a period of one week and ground into a fine powder using a milling machine. The ground leaves (300 g) were soaked in sufficient amount of methanol for ten days at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1.

The solvent was evaporated under reduced pressure at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

2.3. Experimental animals

Swiss Albino mice weighing 25-30 g of both sexes were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of (26±2) ℃.

Animals were allowed free access to drinking water and pellet diet, collected from ICDDRB Dhaka. Mice were acclimatized for 7 d in the laboratory environment prior to the study.

All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals (Pub No. 85-23 revised 1985).

2.4. Drugs and chemicals

Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh), nalbuphine (Square Pharmaceuticals Ltd., Pabna, Bangladesh), formaldehyde (MERCK, Mumbai, India), acetic acid (MERCK, Mumbai, India), 1, 1-Diphenyl-2-picrylhydrazyl (Loba Chemicals, Mumbai, India), ascorbic acid (Loba Chemicals, Mumbai, India) and normal saline solution (0.9% NaCl) were used. All other reagents were analytical grade.

2.5. Assay for anti-nociceptive activity

2.5.1. Acetic acid induced writhing test

Mice were divided into four groups of either sex containing five of each. For writhing test, 0.6% (v/v) acetic acid solution (10 mL/kg body weight) was injected intraperitoneally to each mice and the number of writhing and stretching was counted over 20 min[8,9]. Group I served as control received distilled water, Group II received diclofenac sodium (10 mg/kg) as a standard, Group III and Group IV treated with methanol extract of M. *cochinchinensis* (100 and 200 mg/kg) orally 30 min before acetic acid injection.

2.5.2. Formalin induced nociception

20 μL of 2.5% Formalin in saline was injected subcutaneously to a hind paw of the mice after 30 min administration of the diclofenac sodium 10 mg/kg, methanol extract of *M. cochinchinensis* 100 mg/kg and methanol extract of *M. cochinchinensis* 200 mg/kg p.o to the Group II, III and IV respectively. Group I as control received only formalin (20 μL of 2.5%) during the experiment.

The time spent licking and biting responses of the injected paw was taken as an indicator of pain response and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection[10].

2.5.3. Tail immersion method

Mice were divided into four groups of five animals each. Group 1 received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control and group II and III received 100 and 200 mg/kg of methanol extract of *M. cochinchinensis* orally, respectively. Group IV received the standard drug nalbuphine (10 mg/kg b.w.) subcutaneously. The animal withdrawing his tail from hot water within 5 s were selected for the study.

The lower 3 cm portion of the tail of mice was dipped in a water bath maintaining at temperature of (55.0 ± 0.5) ℃. The time in second (s) for tail withdrawal from the water was taken as the reaction time and recoded by a stopwatch at before (0) and 30, 60 and 90 min after the administration of test samples.

A maximum immersion time of 15 s was maintained to prevent thermal injury to the animals[11].

2.6 Assay for antioxidant activity

2.6.1. DPPH free-radical scavenging activity

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) scavenging activity was carried out by the method of Braca *et al*[12]. The absorbance of different concentrations (500, 300, 100, 50 and 10 μg/mL) of methanol extract of *M. cochinchinensis* were taken at 517 nm against a blank, and the % inhibition activity was calculated from [(A0 − A1)/A0] × 100, where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration. Control sample was prepared containing the same volume without any extract and reference drug. Methanol was served as blank. The inhibition curves were prepared and the half maximal inhibitory concentration (IC_{50}) values were calculated using linear regression analysis.

2.6.2. Reducing power capacity

Reducing power of the extract was evaluated by Oyaizu method[13]. Different concentrations of methanol extract of *M. cochinchinensis* (125, 250, 500, 1000 μg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1% w/v). The mixture was incubated at 50 ℃ for 20 min. After incubation, 2.5 mL of 10% trichlorocacetic acid solution was added to each tube and the mixture was centrifuged at 3000 rpm for 10 minutes. Five millilitre of the upper layer solution was mixed with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1% w/v) and the absorbance was measured at 700 nm.

The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard. Phosphate buffer (PH 6.6) was used as blank solution.

2.7. Statistical analysis

Experiments results were analyzed by one-way ANOVA followed by Bonferroni test using SPSS Data Editor for Windows, Version 16.0 (SPSS Inc., U.S.A.). Values are represented as mean ± SEM.

3. Results

3.1. Anti-nociceptive activity

3.1.1. Effect of M. cochinchinensis in acetic acid induced writhing

Treatment with methanol extract of *M. cochinchinensis* 100 and 200 mg/kg, *p.o.* significantly decrease the number of writhing after acetic acid induction in mice (Table 1).

Maximum analgesic activity (54.82%) was found at 200 mg/kg. Diclofenac sodium (10 mg/kg) shown 59.76% protection against acetic acid induced writhing in mice.

Table 1
Effect of *M. cochinchinensis* leaves extract on acetic acid induced writhing response in mice (mean±SEM, *n*=5).

Treatment	Dose (mg/kg)	Number of writhes	% Inhibition
Control	–	49.20±2.59	–
Diclofenac sodium (DS)	10	19.80±1.10[a]	59.76
MEMC	100	38.05±1.08[b]	22.66
MEMC	200	22.20±1.10[a]	54.82

MEMC- Methanol extract of *M. cochinchinensis*; [a]*P*<0.01, [b]*P*<0.05 *vs* control.

3.1.2. Effect of M. cochinchinensis in formalin test

The effect of methanol extract of *M. cochinchinensis* in formalin test is shown in Table 2. At both doses, there was dose dependent decrease of paw licking time in early phase but dose of 200 mg/kg significantly (*P*<0.05) reduced latency to discomfort in late phase compared to the late phase of the test control. In contrast, the reference antinociceptive drug diclofenac sodium (10 mg/kg) significantly reduced the licking activity against both phases of formalin-induced nociception.

Table 2
Antinociceptive profile of *M. cochinchinensis* leaves extract assessed by the formalin test in mice (mean±SEM, *n*=5).

Treatment	Dose (mg/kg)	Latency to discomfort (min)	
		Early phase	Late phase
Control	–	1.87±0.18	3.02±0.15
Diclofenac sodium (DS)	10	1.09±0.19[b]	1.02±0.43[a]
MEMC	100	1.70±0.21	2.17±0.33
MEMC	200	0.98±0.18	1.14±0.05[b]

MEMC- Methanol extract of *M. cochinchinensis*; [b]*P*<0.05, [a]*P*<0.01 *vs* control.

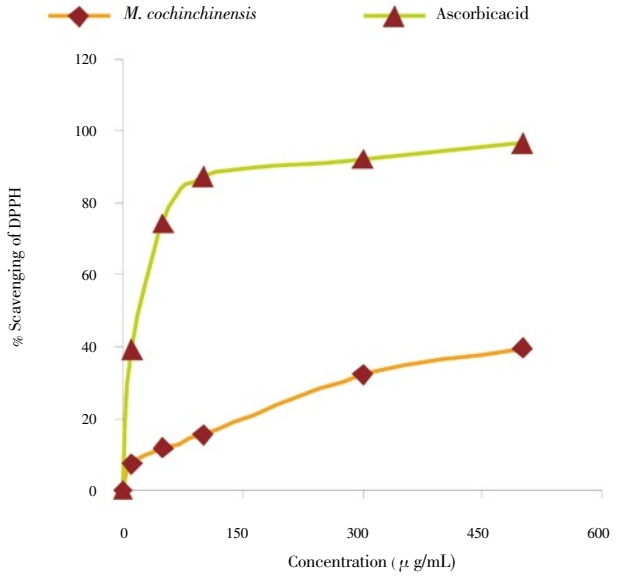

Figure 1. DPPH radical scavenging activity of MEMC. Values are the average of triplicate experiments and represented as mean ± SEM.

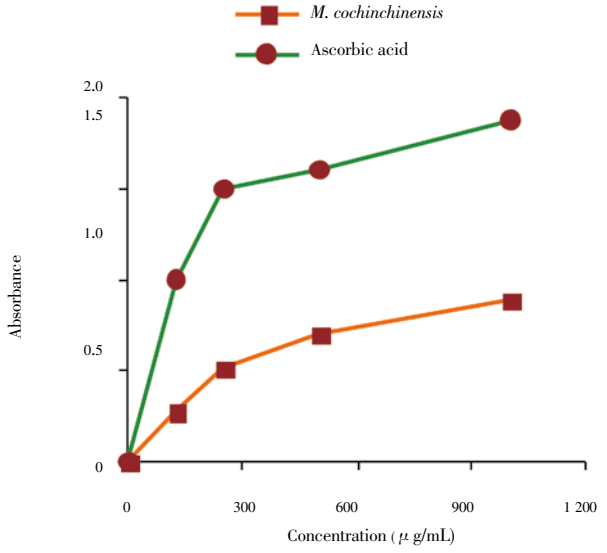

Figure 2. Reducing power of the *M. cochinchinensis*. Values are the average of triplicate experiments and represented as mean ± SEM.

Table 3
Effect of *M. cochinchinensis* leaves extract on tail immersion test in mice (mean ± SEM, $n = 5$).

Treatment	Dose (mg/kg)	Post treatment reaction time (s)			
		0 min	30 min	60 min	90 min
Control	–	2.21±0.01	2.26±0.12	2.91±0.03	2.39±0.02
Nalbuphine	10	2.27±0.04	6.07±0.08[a]	9.12±0.10[a]	11.12±0.13[a]
MEMC	100	1.80±0.31	3.31±0.31[b]	3.90±0.31	2.85±0.30[b]
MEMC	200	2.53±0.50	3.67±0.13[a]	4.92±0.66[b]	6.03±0.08[a]

[b]$P < 0.05$, [a]$P < 0.01$ *vs* control.

3.1.3. Effect of M. cochinchinensis in tail immersion method

In the tail immersion method the extract showed dose dependent activity against conduction of heat induced analgesia in mice (Table 3). Values were found to be significant ($P<0.01$) when compared with control group. Reaction time was increased at 200 mg/kg up to 90 min and analgesic activity was 52.49% which was comparable to diclofenac sodium (78.50%), whereas it was declined at dose of 100 mg/kg. Reaction time was gradually increased upto 90 min and analgesia were 16.14% and 52.49% at 100 and 200 mg/kg doses respectively, which was comparable to nalbuphine (78.50%) at 10 mg/kg (Figure 3).

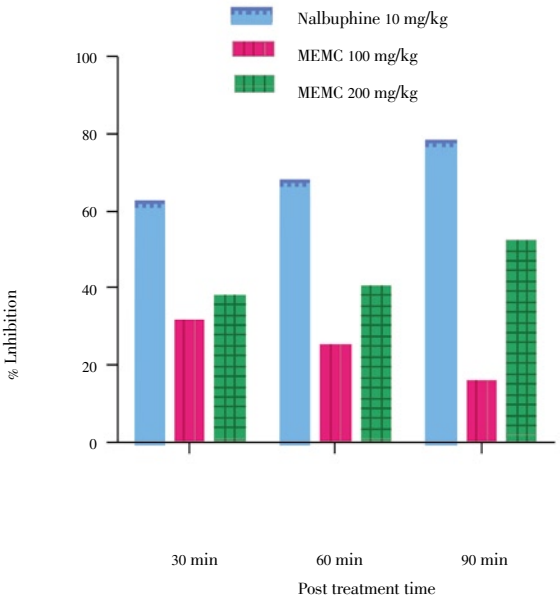

Figure 3. Comparative % inhibition of pain of MEMC and nalbuphine on tail immersion test.

3.2. Antioxidant activity

The DPPH radical scavenging activity of *M. cochinchinensis* is shown in (Figure 1). This activity was found to increase moderately with increasing concentration of the extracts.

Maximum scavenging activity (39.47%) was found at 500 μg/mL. The IC_{50} value of methanol extract of *M. cochinchinensis* was 599.64 μg/mL while the IC_{50} value of ascorbic acid was 48.38 μg/mL. In reducing power assay, the extract showed dose dependent reducing activity as was with the reference ascorbic acid (Figure 2).

4. Discussion

The experimental data presented here suggests that the methanol extract of *M. cochinchinensis* possess anti-nociceptive and antioxidant activities. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics[14].

In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings[15,16].

Since methanol extract in this study 100 and 200 mg/kg, *p.o.* significantly inhibited nociception in mice by 22.66% and 54.82% respectively compared to control while dose at 200 mg/kg showed more statistically significant ($P<0.01$) antinociceptive activity than other one in acetic acid induced pain, it may be predicted as the analgesic effect of extract.

The extract was then tested against other model of experimental pain. In formalin test, the nociceptive response has two phases. The acute phase (0–5 min) and chronic phase (15–30 min) representing the neurogenic and inflammatory pain responses, respectively[17]. The control group has the highest licking time and the extract reduces remarkably at both phases.

Pain inhibitions are 9.09%, 47.59% at acute phase and 28.15%, 62.25% at chronic phase by 100 and 200 mg/kg, respectively. The standard drug diclofenac sodium (10 mg/kg) also produced significant inhibition of pain in both phases.

The thermal stimulation in tail immersion test is also considered to be selective to screen out centrally acting analgesic activity[18]. It was demonstrated that oral administration of methonal extract of *M. cochinchinensis* (100 and 200 mg/kg) significantly prolonged the latency time to the heat stimulus compared to control. This effect began early at 30 min after administration of methonal extract of *M. cochinchinensis* and persisted until the following 90 min.

Opioids inhibit both peripheral and central mechanism of pain but central neuropathic pain appears to respond less well to opioids than peripheral neuropathic pain[19,20].

Reactive oxygen species (ROS) including free radicals and non free radicals along with various forms of active oxygen are involved in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, aging, hepatic damage, neurodegenerative, cardiovascular complications *etc*[21]. The antioxidant activity of the extract might also act as synergist to reduce pain.

The effect of the extract on this pain model indicates that it might be centrally acting and may comply with the folk use for headache[5,6]. The extract also showed moderate DPPH scavenging activity and reducing capacity compared to reference ascorbic acid (Figure 1 and Figure 2). The present study on leaves extract of *M. cochinchinensis* revealed that the plant has significant anti-nociceptive activity against both chemical and physical heat induced pain in mice and exhibits moderate antioxidant properties but for demonstration the mechanisms of this crude extract and their biological active component (s) more investigations are needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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