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ANTINOCICEPTIVE ACTIVITY OF METHANOL EXTRACT OF *COMMELINA BENGHALENSIS* LINN. WHOLE PLANT.

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ABSTRACT

Objectives: The present study was to investigate anti-nociceptive action of the methanol extract of *Commelina benghalensis*. **Methods:** The animals were submitted to acetic acid-induced writhing test and Formalin induced licking test to assess antinociceptive activities, respectively. Two doses 400 and 200 mg/kg were administered for testing. **Results:** The methanol extract of *C. benghalensis* at both doses, exhibited a significant ($p < 0.05$ - < 0.01) dose-dependent antinociceptive activity in acetic acid writhing test and Formalin test. In acetic acid-induced writhing test, oral administration of CB (200 and 400 mg/kg) also decreased the writhing significantly while compared to control. The dose 400 mg/kg showed maximum percentage of pain inhibition 52.24% and 64.29% for respectively. Diclofenac sodium (10 mg/kg) was used as reference antinociceptive drugs. **Conclusions:** The leaf extract has potential antinociceptive activity. The present study supports the use of *C. benghalensis* in different depressive states.

KEYWORDS: *C. benghalensis*, anti-nociceptive, Acetic acid writhing test, Formalin.

INTRODUCTION

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is a warning signal and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Excessive pain may be unbearable and cause other effect-sinking sensation, apprehension, sweating, nausea, palpitation and raise or fall in BP, tachypnea. Analgesics relieve pain as a symptom, without affecting its cause.[1] Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their side effect profile. Opiate analgesic such as morphine has strong addictive potential and other side effects including respiratory depression, drowsiness, decreased gastrointentinal motility, nausea and several alterations of endocrine and autonomic nervous system while NSAIDs are well known for their ability to produce gastrointestinal bleeding, ulceration etc.[2,3] Therefore, search for new analgesic drugs with promising pharmacological actions has become an urgent need.

Commelina benghalensis (family Commelinaceae) is a perennial herb native to tropical Asia and Africa, used in the Indian subcontinent as a folk medicine for the treatment of leprosy, headache, fever, constipation, jaundice and snake bite.[4,5] The plant is also used for mouth thrush, inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children[6], insanity and exophthalmia. *C. benghalensis* is used medicinally as a diuretic, febrifuge and anti-inflammatory.[7][8] It is used as an animal fodder, eaten by humans as a vegetable in Pakistan, also used there medicinally, but with different purported effects, including as a laxative and to cure inflammations of the skin as well as leprosy.[9] The plant is also reported to have antitumor, anticancer and antioxidant activity.[10,11] Previous phytochemical investigations of the Commelina genus were reported on *C. undulata* R.Br., *C. benghalensis* L. and *C. communis* L. from which several types of compounds such as alkaloids, steroids, terpenoids, iridoids, flavonoids, lignans, aliphatic alcohols, polyols, and phenolic acids were obtained.[12-14]. Moreover, the whole plant of *C. benghalensis* was reported to contain alkaloid, volatile oil, wax[15], vitamin-C and higher levels of both lutein and β-carotene.[16].
The purpose of this experiment was to test the antinociceptive activity of *C. benghalensis* on mice using Acetic acid writhing test and Formalin test.

MATERIALS AND METHODS

Experimental animals

Swiss albino mice, weighing about 25–30 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were provided with standard laboratory food and distilled water ad libitum and maintained at natural day-night cycle having proper ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The animals were acclimatized to laboratory condition for 10 days prior to experimentation.

Plant material and preparation of extract

C. benghalensis was collected from Sadarghat area of Chittagong, Bangladesh and authenticated by Dr. Shaikh Bokhtear Uddin (Professor, Department of Botany, and University of Chittagong, Bangladesh). The collected plant was washed thoroughly with water and air dried for a week at 35 to 40°C and pulverized in an electric grinder. For methanol extract, 250 g powder of whole plant was boiled in 1 liter of methanol for 30 min. Subsequently, the mixture was filtered using Whatman filter paper. The filtrate was concentrated over the vapor of the water bath and dried under vacuum.

Antinociceptive activity

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. Group I served as control received normal saline 10ml/kg), Group II received Diclofenac sodium 10 mg/kg as a standard, Group III and Group IV treated with MECB extract (200 and 400 mg/kg) orally 30 min before acetic acid injection.[17]

Formalin induced licking test

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, MECB extract 200 mg/kg and 400 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.[17]

Statistical analysis

The data was analyzed by one-way ANOVA followed by Dunnet's test to estimate significant differences between the test and control groups with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $p < 0.05$ - 0.01 were considered as statistically significant.

RESULTS

Analgesic Activity

Acetic acid test

Treatment with methanol extract of *C. benghalensis* 200 and 400 mg/kg, p.o. significantly decreases the number of writhing after acetic acid induction in mice (Table 1 and Figure 2). Maximum analgesic activity (52.24%) was found at 400 mg/kg. Diclofenac sodium (10 mg/kg) shown 69.66% protection against acetic acid induced writhing in mice.

Table 1: Effect of *C. benghalensis* extract on acetic acid induced writhing response in mice.

Treatment	Writhing	% inhibition
Control(1% tween)	59.33±1.84	-
Diclofenac-Na (10mg/kg)	18±0.82**	69.66
MECB (200mg/kg)	35.67±1.84*	39.88
MECB (400mg/kg)	28.33±1.03**	52.24

MECB=Methanol extract of *C. benghalensis;* *P<0.05, **P<0.01 as control.

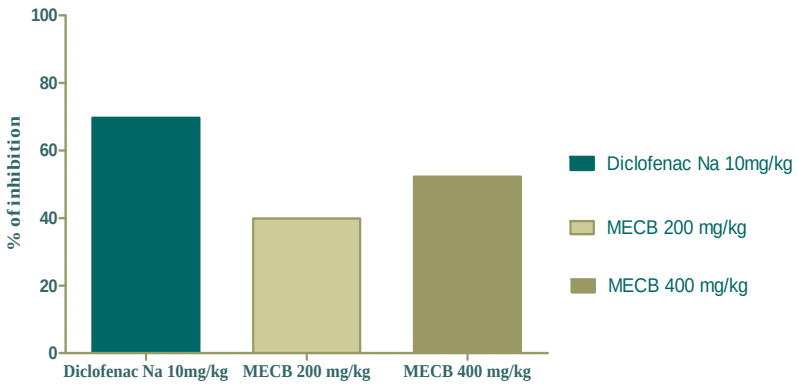

Figure 1: Effect of *C. benghalensis* whole plant extract on acetic acid induced writhing response in mice.

Formalin test

The effect of methanol extract of *C. benghalensis* in formalin test is shown in Table 2 and Figure 2. At both doses, there was dose dependent decrease of paw licking time in early phase but dose of 400 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 Analgesic drug diclofenac sodium (10 mg/kg) significantly reduced the licking activity against both phases of formalin-induced nociception.

Table: 2 Analgesic profile of *C. benghalensis* whole plant extract assessed by the formalin test in mice.

Treatment	Early Phase (1st 5 mins)	% inhibition	Late Phase (Last 15 mins)	% inhibition
Control(1% tween)	57.31±1.06		41.74±1.46	
Diclofenac-Na (10mg/kg)	14.95±0.60**	73.91	13.26±0.94**	68.22
MECB(200mg/kg)	37.47±0.66*	34.61	21.54±2.04**	49.11
MECB(400mg/kg)	27.70±0.90**	51.66	14.90±0.92**	64.29

MECB=Methanol extract of *C. benghalensis*; *P<0.05, **P<0.01 as control.

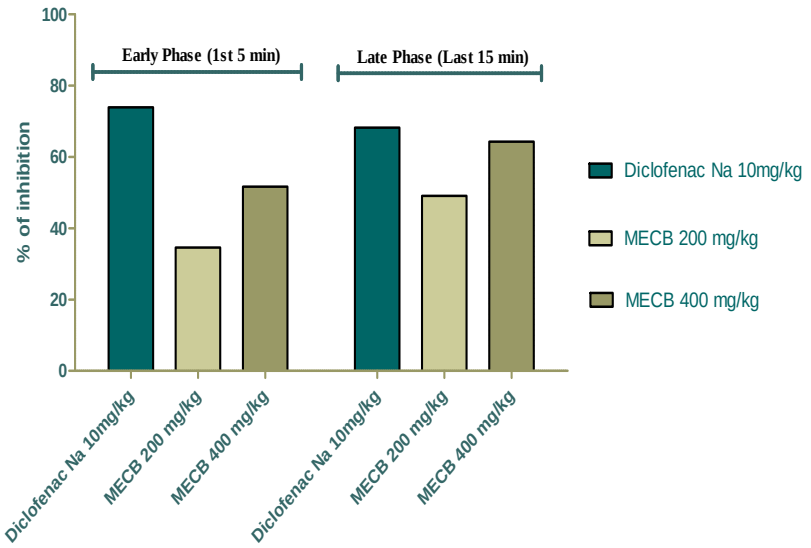

Figure 2: Effect of the methanolic extract of *C. benghalensis* on hind paw licking in the formalin test in mice.

DISCUSSIONS

Acetic acid induced writhing test, which is the visceral pain model, was employed to evaluate the peripheral analgesic activity of the plant material. The abdominal constriction response induced by acetic acid is a sensitive procedure to determine analgesia at peripheral level. This

response is thought to involve local peritoneal receptors.[18] Acetic acid is known to trigger the production of noxious substances such as prostaglandins specifically PGE2 and PGF2 as well as lipoxygenase products.[19] These prostaglandins and lipoxygenase products cause inflammation and pain by increasing capillary permeability.[20] Acetic acid may also cause release of other algesic mediators such as bradykinin, histamine and 5-hydroxytryptamine.[21] The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.[22]

It was observed from the study that in both analgesic activity assay models the plant extract demonstrated analgesic effects. This means that the extract may possess both peripheral and central analgesic effects. The whole plant extract of *C. benghalensis* exhibited significant dose dependent inhibition of acetic acid–induced writhing in mice in comparison to that of the control (saline). Acetic acid induces inflammatory pain by impelling capillary permeability[23], and releasing substances that excite pain nerve endings.[24] The peripheral analgesic effect is generally mediated by the NSAIDs through inhibition of cyclooxygenase and/or lipoxygenase (and other inflammatory mediators) or inhibition of pain responses mediated by noiceptors peripherally.[25] Therefore, it is possible that methanol extract of *C. benghalensis* may be showing analgesic effect through these mechanisms although the exact mechanism of action is yet to be discovered. Again in hot plate test the extract also showed prominent antinociceptive effect against the standard drug Diclofenac Na. Moreover, activation of μ2 opioid subtype receptor leads to spinal analgesia.[26] Therefore, by considering the test report, it may be assumed that the antinociceptive activity of *C. benghalensis* extract is likely to be mediated centrally although the exact mechanism is yet to be discovered. Previous studies on different plant extracts showed analgesic effect in animal models and their effects have been attributed to the presence of alkaloids, glycosides, flavonoids and saponins.[27][28] And previous studies proved that, this plant has alkaloids, glycosides, flavonoids and saponins in its leave.

CONCLUSION

The study showed that methanol extract of *Commelina benghalensis* possesses significant analgesic activity which was validated by various pain models in this study. The results substantiate the ethnomedicinal use of *C. benghalensis* to palliate pain disorder. The findings of present studies warrant further studies for isolation and identification of the responsible bioactive component(s) and to elucidate the mechanism(s) lying with these effects.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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