The Involvement of Non Opioidergic Mechanism in the Antinociceptive and Antilocomotive Activity of Bacopa monnieri

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ABSTRACT
A hydroethanolic extract (HE-ext) of Bacopa monnieri (BM) was studied for antinociceptive effect in the animal models of acetic-acid-induced writhing test and antilocomotive effect in mice. Standard centrally-acting analgesic, morphine (MP), and peripherally-acting one, diclofenac (Diclo), were also tested along with the extract for comparison. The extract exhibited significant antinociceptive effect (p < 0.001) in this test, not antagonized by the opioid receptor antagonist, naloxone (NLX) in a fashion similar to diclofenac. This excluded the involvement of opioids in the mediation of antinociceptive response of Bacopa monnieri. Moreover, the BM HE-ext exhibited highly significant antilocomotive (p < 0.0001) that was also unaffected by naloxone. These results indicate that Bacopa monnieri possesses antinociceptive and antilocomotive effect that may be mediated through non-opioidergic mechanism.

Keywords: Bacopa monnieri, Hydroethanolic extract, Antinociceptive activity, Acetic-acid-induced writhing test, Antilocomotive effect

MATERIALS AND METHODS

Bacopa monnieri

Bacopa monnieri was collected from Ramli stream near Quaid-e-Azam University Islamabad, Pakistan and authenticated by Dr. Muhammad Ibrar, Professor of Botany University of Peshawar. A reference specimen (029006/Bot. University of Peshawar) was obtained.

Preparation of Bacopa monnieri extract

Aerial parts were separated from roots, dried under shade and coarsely ground. The coarsely-ground material was extracted with 70% ethanol and was concentrated on rotary evaporator at 60 °C, and then to semisolid form (% yield: 37.25).
**Chemicals and Drugs**

Ethanol was obtained from Khazana Sugar Mills Mardan through proper channel. Diclofenac sodium was gratefully donated by Zinta Pharmaceutical Pvt, Peshawar, Pakistan. Morphine was secured through proper channel (PDH Lahore, Pakistan). Opioid antagonist, naloxone was purchased from Sigma, USA.

For experiments, all drugs and extracts were dissolved in water for injection.

**Animals**

Balb-C mice bred in the animal house of the Department of Pharmacy, University of Peshawar, were used in this study. Animals were housed in groups of eight in cages with sawdust bedding. Experiments were carried out during the light phase between 9.00 am and 3.00 pm strictly in accordance with procedures laid down under the Animal Scientific Procedure Act (1986). Both anti-nociceptive and locomotive studies were carried out on mice of either sex weighing 18-22 g. Control animals received equal volume of normal saline (0.9% NaCl). Animals were marked for their proper identification.

**Procedures**

Acetic-acid-induced writhing test

Balb-C mice of either sex (n=8) weighing 18-22 g were used. Animals were withdrawn from food and water 2 hours before the start of experiment. Writhing behavior was tested, in which 1% acetic acid (AA) was administered intraperitoneally (i.p.) and number of abdominal constrictions occurring over the period of 20 minutes were counted just after 1% AA (10 mL/kg) was administered subcutaneously. All drugs were administered in the volume of 0.1 mL/20 g i.p. and 0.1 mL/20 g s.c.

**Statistical analysis**

Results were analyzed by one-way analysis of variance (ANOVA) with post hoc tests for multiple comparisons and Student's t test. Effects were considered significant at p < 0.05.

**RESULTS**

**Antinociceptive effect of morphine, diclofenac and hydroethanolic extract of Bacopa monnieri in acetic-acid-induced writhing test**

As shown in the Fig 1, hydroethanolic extract (80, 160 mg/kg) were administered orally (PO) 1 hour before Bacopa monnieri (80, 160 mg/Kg Body weight), administering 1% AA. For antagonism, naloxone (0.5 mg/kg body weight) was administered subcutaneously (s.c.) 5 minutes before AA administration. All drugs were administered in the volume of 0.1 mL/20 g i.p and 0.1 mL/20 g PO. Percent analgesia was calculated with the help of following formula:

\[
\%\ Protection = (1 - \frac{\text{Mean no. of abdominal constrictions of treated drug}}{\text{Mean no. of abdominal constrictions of control}}) \times 100
\]

Fig 1. Antinociceptive effect of diclofenac, morphine and hydroethanolic extract of Bacopa monnieri calculated as percent protection in acetic acid induced writhing test in mice. Each column represents mean ± S.E.M. (n=8). **p < 0.01, ***p < 0.001. Difference between treatment groups and saline control was analyzed by one way analysis of variance with Dunnett's post-hoc test.

Fig 2. The effect of naloxone on morphine and diclofenac induced antinociception calculated as percent protection in acetic acid induced writhing test in mice. Each column represents the mean ± S.E.M. (n=8). **p < 0.01, ***p < 0.001, values showed significant antagonism by naloxone as compared to morphine treated groups when analyzed by Student's t test.
Antinociceptive/Antilocomotive B monnieri

The nociceptive response in the acetic-acid-induced writhing test results from the liberation of histamine, kinins, Prostaglandins, serotonin and substance P. The nociceptive activity of acetic acid may be due to cytokine release, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells [12]. It has been reported that intraperitoneal administration of acetic acid causes an increase in the concentration of glutamate and aspartate in the cerebrospinal fluid [13].

The production of prostaglandins [14,15] results through the action of the constitutive enzyme cyclooxygenase-1 (COX-1) and its isoform COX-2, which produce pain [15,16]. Induction of this mechanism through COX enzymes and stimulation of these sensory pathways in the mouse peritoneum incites a viscerosomatic reflex and the abdominal constrictions observed in response to an algogenic agent such as acetic acid [15,16]. Acetic-acid-induced writhing assay is sensitive procedure to evaluate peripherally and centrally mediated anti-inflammatory and antinociceptive activity in mice.

**Fig 3.** Effect of naloxone on BM HE-extract induced antinociception calculated as percent protection in acetic acid induced writhing test in mice. Each column represents mean ± S.E.M. (n = 8). Student’s t-test revealed no significant difference between two comparison groups (p > 0.05).

**Fig 4.** Effect of morphine and hydroethanolic extract of Bacopa monnieri after acute administration on locomotor activity in mice. Each column denotes mean line crossings ± S.E.M. (n = 8). ***p < 0.0001, values were significantly different as compared to control (ANOVA with Dunnett’s post hoc test).

**Fig 5.** Effect of naloxone pre-treatment on morphine and BM HE-ext induced locomotor activity in mice. Each column denotes mean line crossings ± S.E.M. (n = 8). Student’s t-test revealed significant difference between two comparison groups (***p < 0.01).

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose (mg/Kg)</th>
<th>SAL</th>
<th>SAL+NLX</th>
<th>BM HE-ext</th>
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**Discussion**

As depicted in Fig 2, pretreatment with naloxone (0.5 mg/Kg, s.c.) reversed the antinociceptive response of morphine (3 mg/Kg body weight) significantly (***p < 0.01). However, the antinociceptive effect of diclofenac (12.5 mg/Kg, i.p.) was unaffected with naloxone (0.5 mg/Kg, s.c.) pretreatment.

As shown in the Fig 3, naloxone did not antagonize the antinociceptive effect of hydroethanolic of Bacopa monnieri administered PO at the dose level of 80, 160 mg/Kg body weight.

As depicted in the Fig 4, acute administration of morphine (10 mg/Kg, i.p.) or hydroethanolic extract (80 mg/Kg, i.p.) significantly reduced locomotor activity when compared to control (***p < 0.0001).

As shown in the Fig 5, in contrast to morphine (10 mg/Kg B.w.), the antilocomotive effect of hydroethanolic extract of Bacopa monnieri (80 mg/Kg) was not antagonized with naloxone (0.25 mg/Kg, s.c.) pretreatment.

**Antagonism of Bacopa monnieri morphine- and diclofenac-induced antinociception with naloxone**

As shown in the Fig 3, naloxone did not antagonize the antinociceptive effect of hydroethanolic of Bacopa monnieri administered PO at the dose level of 80, 160 mg/Kg body weight.

**Antagonism of Bacopa monnieri hydroethanolic extract induced antinociception with naloxone.**

As shown in the Fig 3, naloxone did not antagonize the antinociceptive effect of hydroethanolic of Bacopa monnieri administered PO at the dose level of 80, 160 mg/Kg body weight.

**Effect of acute administration of morphine and hydroethanolic extract of Bacopa monnieri on locomotor activity in mice**

As depicted in the Fig 4, acute administration of morphine (10 mg/Kg, i.p.) or hydroethanolic extract (80 mg/Kg, i.p.) significantly reduced locomotor activity when compared to control (***p < 0.0001).

**Effect of naloxone pretreatment on morphine and hydroethanolic extract of Bacopa monnieri induced locomotor activity in mice**

As shown in the Fig 5, in contrast to morphine (10 mg/Kg B.w.), the antilocomotive effect of hydroethanolic extract of Bacopa monnieri (80 mg/Kg) was not antagonized with naloxone (0.25 mg/Kg, s.c.) pretreatment.
18 centrally acting analgesics [17–22] and sensory afferents in the peritoneum carry α2–adrenoceptors, β2-adrenoceptors and opioid receptors on their terminals [23]. When activated by appropriate agonists, these receptors depress the generation of pain impulses, in some instances there being an interaction between α- and opioid receptors in the mouse peritoneum [10,11,23,24].

In this study, morphine, diclofenac and hydroethanolic extract of *Bacopa monnieri* produced significant antinociceptive effect in acetic-acid-induced writhing method. In order to investigate further the mechanism of antinociceptive effect, the extract of *Bacopa monnieri*, and morphine were examined in the presence of non-selective opioid receptor antagonist, naloxone. In contrast to morphine, the antinociceptive effects of HE-water and diclofenac were not antagonized with naloxone. The fact that hydroethanolic extract of *Bacopa monnieri* inhibits chemical-induced nociception and that nociception is not antagonized with naloxone suggests that the extract does not possess opioid-mediated antinociceptive activity. This finding is in contrast to as reported by vohora et al. [25].

Opioids have been known to possess sedative effect and that is believed to due their action at opioid receptors within the central nervous system [28]. Naloxone has been known to antagonize the sedative effect of opioid by acting on opioid receptors [29]. Our study has also revealed that hydroethanolic extract of *Bacopa monnieri* was able to promote a motor depressant effect in mice. Thus, administered acutely at the dose level of 80 mg/Kg body weight, the extract exerted significant decrease in locomotor activity, indicating sedative properties of the extract. Furthermore, the anti-locomotor effect of the extract was not antagonized with naloxone, excluding the involvement of opioid receptors in the mediation of antilocomotor activity of the extract. However, naloxone pretreatment antagonized the antilocomotive activity of morphine at the dose of 10 mg/kg.

In conclusion, this study has demonstrated that hydroethanolic extract of *Bacopa monnieri* possesses antinociceptive effect and inhibited the locomotor activity involving a non opioidergic mechanism as the both activities were not affected by opioid receptor antagonist, naloxone.

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REFERENCES


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