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 grinded. 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).
The effect of naloxone on morphine and diclofenac induced antinociception was studied. For antagonism, naloxone (0.5 mg/kg) was administered subcutaneously 5 minutes before AA administration. All drugs were administered in the volume of 0.1 mL/20 g i.p and s.c. and 0.2 mL/10 g PO. Percent analgesia was calculated with the help of following formula:

\[ \text{% Protection} = 1 - \frac{1}{E} \times 100 \]

where \( E \) is the number of abdominal constriction counts of treated drug / Mean no. of abdominal constriction counts of control.

**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 5 minutes before AA administration. All drugs were administered in the volume of 0.1 mL/20 g i.p and s.c. and 0.2 mL/10 g PO. Percent analgesia was calculated with the help of following formula:

\[ \text{% Protection} = 1 - \frac{1}{E} \times 100 \]

where \( E \) is the number of abdominal constriction counts of treated drug / Mean no. of abdominal constriction counts of control.

**Discussion**

The hydroethanolic extract of *Bacopa monnieri* showed significant antinociception as compared to morphine treated groups when analyzed by Student's t test.

**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 \( p < 0.05 \).

**Conclusion**

The hydroethanolic extract of *Bacopa monnieri* was found to possess significant antinociceptive activity.

**Acknowledgment**

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**References**

[1] Abbas, O., et al., Protection = (1 - No. of abdominal constrictions of treated drug / Mean no. of abdominal constrictions of control) 100

**Figures**

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 Bacopa monnieri

**Effect of naloxone pretreatment on morphine and hydroethanolic 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).

**Discussion**

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 isof orm 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 visco-somatic reflex and the abdominal contractions 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

**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 Dunnet’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).
REFERENCES


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