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, Hydroethanotic 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).
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 i.p. and 0.1 mL/20 g s.c. 5 minutes later. Group mean abdominal constrictions occurring over the period of 20 minutes were counted just after 1% AA (10 mL/kg) administration. All drugs were administered intraperitoneally and animals were placed in the recording apparatus 30 minutes later. Group mean line crossing counts were subsequently recorded between 1- 30 mins. For antagonism, naloxone (0.25 mg/kg) was administered s.c. 25 minutes after drug administration. All drugs were administered in the volume of 0.1 mL/10 g i.p. and 0.1 mL/20 g s.c.

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

% Protection = (1 - Mean no. of abdominal constrictions of treated drug / Mean no. of abdominal constrictions of control) 100

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

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. Difference between treatment groups and saline control was analyzed by one way analysis of variance with Dunnett’s post-hoc test.
Antinociceptive/Antilocomotive B monnieri

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).

**DISCUSSION**

The nociceptive response in the acetic-acid-induced writhing test results from the liberation of histamine, 5-hydroxytryptamine, Prostaglandins, serotonin and substance P. The antinociceptive activity of acetic acid may be due to decrease in the production of prostanoids which produce pain ([16], results through the action of the constitutive enzyme cyclooxygenase-1 (COX-1) and its isofrom 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 visceral-somatic 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 acting analgesics.

**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.
REFERENCES


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