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

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*Bacopa monnieri* (family: Scrophulariaceae) [1] also known as *Bacopa monniera*, water hyssop, *Herpestis monniera* is a perennial creeping, succulent herb found in marshy areas of Indo-Pak subcontinent [2]. In India, it is commonly known as “Brahmi” as an ancient and renowned medicinal plant with legendary reputation as a memory vitalizer [3]. *Bacopa monnieri* is held in high repute to be the brain booster and is highly valued in conditions affecting CNS. In ancient traditional system of medicine, it is often prescribed for epilepsy, insomnia, and psychiatric disorders such as mental breakdown in Alzheimer’s disease [4], neuralgia, and memory loss [5]. It is known to possess cardiotonic, sedative, analgesic, anti-convulsant, anti-inflammatory [6], antioxidant [7], anticancer, antipyretic, laxative, diuretic, antistress [8], and anxiolytic [9] properties. In this study, we have examined *Bacopa monnieri* for antinociceptive and antilocomotive activity in animal models.

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).
57 Chemicals and Drugs

Ethanol was obtained from Khazana Sugar Mills Mardan through proper channel. Diclofenac sodium was gratefully donated by Zinta Pharmaceutical Ptv, 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.

60 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.

63 Procedures

Acetic-acid-induced writhing test

81 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 the floor was divided by lines into 4 equal-sized rectangular zones. Doses of BM HE-ext (80, 160 mg/kg), or morphine (10 mg/kg), or saline were administered intraperitoneally and animals were placed in the recording apparatus 30 minutes later. Group mean abdominal constrictions occurred over the period of 20 minutes were counted just after 1% AA (10 mL/kg) was administered 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 s.c.

60 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 if p < 0.05.

94 Antinoceptive 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 0.2 mL/10 g PO. Percent analgesia was calculated with the help of following formula:

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

**Results**

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, values showed significant antagonism by naloxone as compared to morphine treated groups when analyzed by Student’s t 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 denotes 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-extract 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).
The antigonceptive effect of Bacopa monnieri was studied in rats using the acetic acid-induced writhing method. The extract was administered intraperitoneally at doses of 10, 100, and 1000 mg/kg. The results showed a dose-dependent inhibition of the writhing response, with the highest dose being the most effective. The extract also inhibited the writhing response produced by the selective opioid receptor agonist, fentanyl.

The effect of the extract on the antinociceptive effect of morphine at the dose of 10 mg/kg was investigated. The extract was administered intraperitoneally 30 minutes before the administration of morphine. The results showed that the extract did not antagonize the antinociceptive effect of morphine, indicating the involvement of non-opioidergic mechanisms in the antinociceptive activity of the extract.

The extract was also evaluated for its effects on the antinociceptive effect of methadone, a synthetic opioid. The results showed that the extract did not antagonize the antinociceptive effect of methadone, indicating the involvement of non-opioidergic mechanisms in the antinociceptive activity of the extract.

In conclusion, this study demonstrated the antinociceptive activity of Bacopa monnieri extract in the acetic acid-induced writhing model. The extract showed a dose-dependent inhibition of the writhing response, indicating the involvement of non-opioidergic mechanisms in the antinociceptive activity of the extract. Further studies are needed to identify the active components and the mechanisms of action of the extract.

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