ORIGINAL ARTICLE

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

MUZAFFAR ABBAS, FAZAL SUBHAN, KHALID RAUF, IKRAM-UL-HAQ, and SYED NADEEM-UL-HASSAN MOHANI

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 (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 [15]. It is known to possess cardiotonic, sedative, analgesic, anticonvulsant, 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.

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 Islamabad, Pak subcontinent) was submitted to the herbarium of the Botany Department, University of Peshawar and a voucher 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 7 mice 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 number of abdominal constrictions occurring over the period of 20 minutes were counted just after 1% AA (10 mL/kg) was administered intraperitoneally (i.p.) and number of abdominal constrictions was analyzed by one way analysis of variance 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 of 80 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 saline were administered intraperitoneally (i.p) and number of abdominal constrictions of treated drug / Mean no. of abdominal constrictions of control) 100

**Locomotor activity**

Balb-C mice of either sex (n=8) weighing 22 ± 2 g were used. Animals were acclimatized under red light (40 Watt red bulb) one hour before the start of experiment in laboratory with food and water available ad libitum. The locomotor activity arena measured 50 x 80 cm and the floor was divided by lines into 4 equal-sized rectangular zones. Doses of BM HE-ext (80 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 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/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.

![Fig 1](image1.png)  
**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](image2.png)  
**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 represents mean ± S.E.M. (n=8). Student’s t-test revealed no significant difference between two comparison groups (p > 0.05).

As shown in the Fig 3, 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 when administered with naloxone (0.5 mg/Kg, s.c) pretreatment.

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

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 when compared to control (ANOVA with Dunnett’s post hoc test).

**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, leukotrienes, prostaglandins, serotonin and substance P. The antinociceptive 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 acting antinociceptive agents.

**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).
REFERENCES


**CURRENT AUTHOR ADDRESSES**

Muzaffar Abbas, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan, E-mail: mabbas14@yahoo.com, Mob. No. +923435224679, Fax: +92-91-5841460 (Corresponding Author)

Dr. Fazal Subhan, Department of Pharmacy, University of Peshawar, Peshawar, Pakistan, E-mail: fazal_subhan@upesh.edu.pk

Khalid Rauf, Department of Pharmacy, University of Peshawar, Peshawar, Pakistan, E-mail: khalidrauf@upesh.edu.pk

Ikram-ul-Haq, Department of Pharmacy, University of Peshawar, Peshawar, Pakistan, E-mail: ikram_pharmacist@yahoo.com

Syed Nadeem-ul-Hassan Mohani, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan, E-mail: nadeem.fls@suit.edu.pk