The Involvement of Non Opioidergic Mechanism in the Antinociceptive and Antilocomotive Activity of \textit{Bacopa monnieri}

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**ABSTRACT**

A hydroethanolic extract (HE-ext) of \textit{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 \textit{Bacopa monnieri}. Moreover, the BM HE-ext exhibited highly significant antilocomotive ($p < 0.0001$) that was also unaffected by naloxone. These results indicate that \textit{Bacopa monnieri} possesses antinociceptive and antilocomotive effect that may be mediated through non-opioidergic mechanism.

**Keywords:** \textit{Bacopa monnieri}, Hydroethanolic extract, Antinociceptive activity, Acetic-acid-induced writhing test, Antilocomotive effect

**MATERIALS AND METHODS**

\textit{Bacopa monnieri} (family: Scrophulariaceae) [1] also known as \textit{Bacopa monniera}, water hyssop, \textit{Herpestis monnieri} 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]. \textit{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, antiemetic, anti-inflammatory [6], antioxidant [7], anticancer, antipyretic, laxative, diuretic, antistress [8], and anxiolytic [9] properties. In this study, we have examined \textit{Bacopa monnieri} for antinociceptive and antilocomotive activity in animal models.

\textit{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 was submitted to the herbarium of the Botany Department, University of Peshawar and a voucher specimen (029006/Bot. University of Peshawar) was obtained.

\textit{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 a 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 in 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 40 cm and the floor was divided by lines into 4 equal-sized rectangular zones. Doses of BM HE-ext (80, 160 mg/Kg body weight), 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/10 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 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:

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

**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, values showed significant antagonism by naloxone as compared to morphine treated groups when analyzed by Student's t test.
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 viscerosomatic reflex and the abdominal constric tions 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 analgesic activity.

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 (***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.

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 extract 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.
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