

## ORIGINAL ARTICLE

## 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

*Bacopa monnieri* (family: Scrophulariaceae) [1] also known as *Bacopa monniera*, water hyssop, *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]. *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 cardiogenic, 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.

### 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 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).

## 57 Chemicals and Drugs

58 Ethanol was obtained from Khazana Sugar Mills  
59 Mardan through proper channel. Diclofenac sodium was  
60 gratefully donated by Zinta Pharmaceutical Pvt,  
61 Peshawar, Pakistan. Morphine was secured through  
62 proper channel (PDH Lahore, Pakistan). Opioid  
63 antagonist, naloxone was purchased from Sigma, USA.  
64 For experiments, all drugs and extracts were dissolved  
65 in water for injection.

## 66 Animals

67 Balb-C mice bred in the animal house of the  
68 Department of Pharmacy, University of Peshawar, were  
69 used in this study. Animals were housed in groups of  
70 eight in cages with sawdust bedding. Experiments were  
71 carried out during the light phase between 9.00 am and  
72 3.00 pm strictly in accordance with procedures laid  
73 down under the Animal Scientific Procedure Act  
74 (1986). Both anti-nociceptive and locomotive studies  
75 were carried out on mice of either sex weighing 18-22  
76 g. Control animals received equal volume of normal  
77 saline (0.9% NaCl). Animals were marked for their  
78 proper identification.

## 79 Procedures

### 80 Acetic-acid-induced writhing test

81 Balb-C mice of either sex (n=8) weighing 18-22 g  
82 were used. Animals were withdrawn from food and  
83 water 2 hours before the start of experiment. Writhing  
84 behavior was tested, in which 1% acetic acid (AA) was  
85 administered intraperitoneally (i.p.) and number of  
86 abdominal constrictions occurring over the period of 20  
87 minutes were counted just after 1% AA (10 mL/kg)  
88 administration [10,11]. Morphine (3 mg/kg) or  
89 diclofenac (12.5 mg/Kg) or normal saline (SAL) were  
90 administered i.p. 30 minutes before 1% AA  
91 administration. However, hydroethanolic extract (80,  
92 160 mg/kg) were administered orally (PO) 1 hour before  
93 administering 1% AA. For antagonism, naloxone (0.5

94 mg/kg body weight) was administered subcutaneously  
95 (s.c.) 5 minutes before AA administration. All drugs  
96 were administered in the volume of 0.1 mL/20 g i.p and  
97 s.c. and 0.2 mL/10 g PO. Percent analgesia was  
98 calculated with the help of following formula:

$$99 \quad \% \text{ Protection} = (1 - \text{Mean no. of abdominal} \\ 100 \text{ constrictions of treated drug} / \text{Mean no. of abdominal} \\ 101 \text{ constrictions of control}) / 100$$

### 102 Locomotor activity

103 Balb-C mice of either sex (n=8) weighing  $22 \pm 2$  g  
104 were used. Animals were acclimatized under red light  
105 (40 Watt red bulb) one hour before the start of  
106 experiment in laboratory with food and water available  
107 *ad libitum*. The locomotor activity arena measured 50 x  
108 40 cm and the floor was divided by lines into 4 equal-  
109 sized rectangular zones. Doses of BM HE-ext (80  
110 mg/kg), or morphine (10 mg/kg), or saline were  
111 administered intraperitoneally and animals were placed  
112 in the recording apparatus 30 minutes later. Group mean  
113 line crossing counts were subsequently recorded  
114 between 1- 30 mins. For antagonism, naloxone (0.25  
115 mg/kg) was administered s.c. 25 minutes after drug  
116 administration. All drugs were administered in the  
117 volume of 0.1 mL/10 g i.p. and 0.1 mL/20 g s.c.

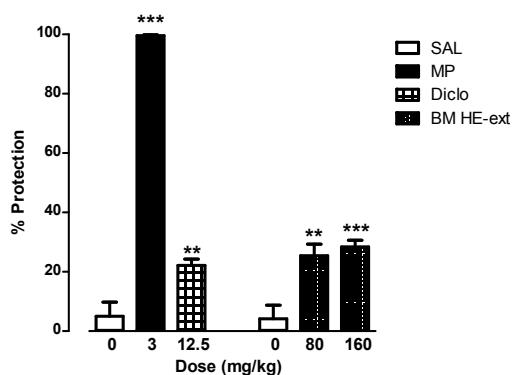
### 118 Statistical analysis

119 Results were analyzed by one-way analysis of  
120 variance (ANOVA) with post hoc tests for multiple  
121 comparisons and Student's t test. Effects were  
122 considered significant at  $p < 0.05$ .

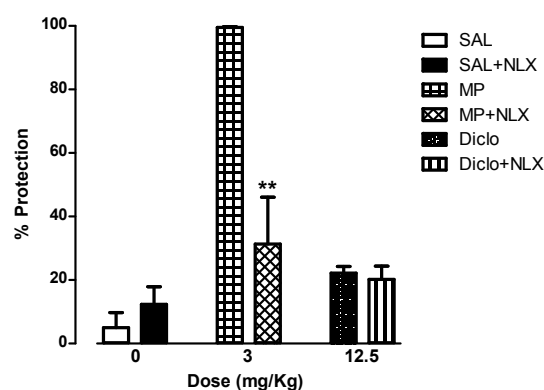
## RESULTS

### 124 Antinociceptive effect of morphine, diclofenac and 125 hydroethanolic extract of *Bacopa monnieri* in 126 acetic-acid-induced writhing test

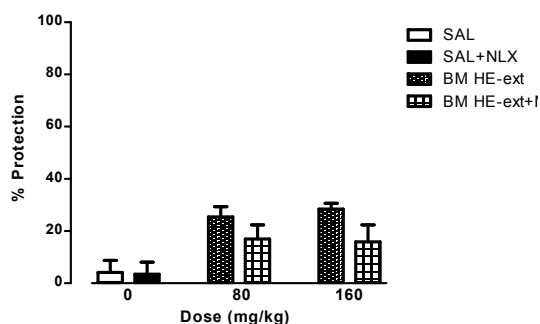
127 As shown in the Fig 1, hydroethanolic extract of  
128 *Bacopa monnieri* (80, 160 mg/Kg Body weight),  
129 morphine (3 mg/Kg body weight) and diclofenac (12.5



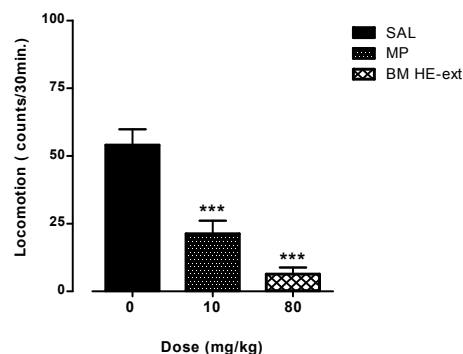
**Fig 1.** Antinociceptive effect of diclofenac, morphine and hydroethanolic extract of *Bacopa monnieri* calculated as percent protection in acetic acid induced writhing test in mice. Each column represents mean  $\pm$  S.E.M. (n=8). \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , Difference between treatment 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  $\pm$  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.



**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  $\pm$  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  $\pm$  S.E.M. (n=8). \*\*\* $p < 0.0001$ , values were significantly different as compared to control (ANOVA with Dunnett's post hoc test).

130 mg/Kg body weight) exhibited antinociceptive effect by 163  
131 decreasing the number of acetic-acid-induced 164  
132 abdominal constrictions in mice that was statistically 165  
133 significant.

#### 134 Antagonism of *Bacopa monnieri* morphine- and 135 diclofenac-induced antinociception with naloxone

136 As depicted in Fig 2, pretreatment with naloxone 170  
137 (0.5 mg/kg, s.c.) reversed the antinociceptive response 171  
138 of morphine (3 mg/Kg body weight) significantly (\*\* $p < 0.01$ ). However, the antinociceptive effect of 172  
139 diclofenac (12.5 mg/Kg, i.p.) was unaffected with 173  
140 naloxone (0.5 mg/Kg, s.c) pretreatment. 174

#### 142 Antagonism of *Bacopa monnieri* hydroethanolic 143 extract induced antinociception with naloxone.

144 As shown in the Fig 3, naloxone did not antagonize 175  
145 the antinociceptive effect of hydroethanolic of *Bacopa* 176  
146 *monnieri* administered PO at the dose level of 80, 160  
147 mg/Kg body weight. 177

#### 148 Effect of acute administration of morphine and 149 hydroethanolic extract of *Bacopa monnieri* on 150 locomotor activity in mice

151 As depicted in the Fig 4, acute administration of 178  
152 morphine (10 mg/Kg, i.p.) or hydroethanolic extract (80 179  
153 mg/Kg, i.p.) significantly reduced locomotor activity 180  
154 when compared to control (\*\* $p < 0.0001$ ). 181

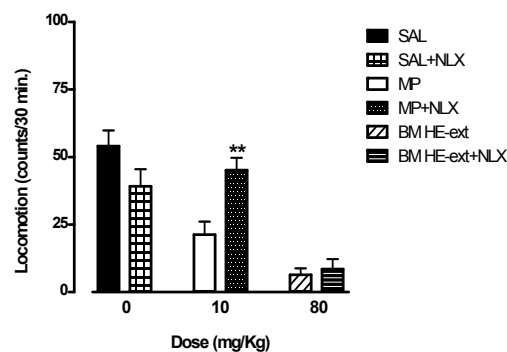
#### 155 Effect of naloxone pretreatment on morphine and 156 hydroethanolic extract of *Bacopa monnieri* induced 157 locomotor activity in mice

158 As shown in the Fig 5, in contrast to morphine (10 182  
159 mg/Kg B.w.), the antilocomotive effect of 183  
160 hydroethanolic extract of *Bacopa monnieri* (80 mg/Kg) 184  
161 was not antagonized with naloxone (0.25 mg/Kg, s.c.) 185  
162 pretreatment. 186

## DISCUSSION

The nociceptive response in the acetic-acid-induced 165  
166 writhing test results from the liberation of histamine, 167  
168 kinins, Prostaglandins, serotonin and substance P. The 169  
170 nociceptive activity of acetic acid may be due to 171  
172 cytokine release, such as TNF- $\alpha$ , interleukin-1 $\beta$  and 173  
174 interleukin-8, by resident peritoneal macrophages and 175  
176 mast cells [12]. It has been reported that intraperitoneal 177  
178 administration of acetic acid causes an increase in the 179  
180 concentration of glutamate and aspartate in the 181  
182 cerebrospinal fluid [13].

The production of prostaglandins [14,15] results 183  
184 through the action of the constitutive enzyme 185  
186 cyclooxygenase-1 (COX-1) and its isoform COX-2 187  
188 which produce pain [15,16]. Induction of this 189  
190 mechanism through COX enzymes and stimulation of 191  
192 these sensory pathways in the mouse peritoneum incites 193  
194 a viscerosomatic reflex and the abdominal constrictions 195  
196 observed in response to an algogenic agent such as 197  
198 acetic acid [15,16]. Acetic-acid-induced writhing assay 199  
200 is sensitive procedure to evaluate peripherally and



**Fig 5.** Effect of naloxone pre-treatment on morphine and BM HE-ext induced locomotor activity in mice. Each column denotes mean line crossings  $\pm$  S.E.M. (n=8). Student's t-test revealed significant difference between two comparison groups (\*\* $p < 0.01$ ).

centrally acting analgesics [17-22] and sensory afferents in the peritoneum carry  $\alpha_{1/2}$ -adrenoceptors,  $\beta$ -adrenoceptors and opioid receptors on their terminals [23]. When activated by appropriate agonists, these receptors depress the generation of pain impulses, in some instances there being an interaction between  $\alpha$ -adrenoceptors and opioid receptors in the mouse peritoneum [10,11,23,24].

In this study, morphine, diclofenac and hydroethanolic extract of *Bacopa monnieri* produced significant antinociceptive effect in acetic-acid-induced writhing method. In order to investigate further the mechanism of antinociceptive effect, the extract of *Bacopa monnieri*, and standards diclofenac and morphine were examined in the presence of non-selective opioid receptor antagonist, naloxone. In contrast to morphine, the antinociceptive effects of HE- ext and diclofenac were not antagonized with naloxone. The fact that hydroethanolic extract of *Bacopa monnieri* inhibits chemical-induced nociception and that nociception is not antagonized with naloxone suggests that the extract does not possess opioid-mediated antinociceptive activity. This finding is in contrast to as reported by vohora et al. 1997 [25].

Opioids have been known to possess sedative effect [26,27] and that is believed to due their action at opioid receptors within the central nervous system [28]. Naloxone has been known to antagonize the sedative effect of opioid by acting on opioid receptors [29]. Our study has also revealed that hydroethanolic extract of *Bacopa monnieri* was able to promote a motor depressant effect in mice. Thus, administered acutely at the dose level of 80 mg/Kg body weight, the extract exerted significant decrease in locomotor activity, indicating sedative properties of the extract. Furthermore, the anti-locomotor effect of the extract was not antagonized with naloxone, excluding the involvement of opioid receptors in the mediation of antilocomotor activity of the extract. However, naloxone pretreatment antagonized the antilocomotive activity of morphine at the dose of 10 mg/kg.

In conclusion, this study has demonstrated that hydroethanolic extract of *Bacopa monnieri* possesses antinociceptive effect and inhibited the locomotor activity involving a non opioidergic mechanism as the both activities were not affected by opioid receptor antagonist, naloxone.

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