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Effect of *Bacopa monniera* Linn. (brahmi) extract on learning and memory in rats: A behavioral study

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KEYWORDS: *Bacopa monniera*; memory; passive avoidance; spatial learning

Abstract Extracts of *Bacopa monniera* (Brahmi, BM), a traditional ayurvedic medicine, have been reported to have memory-enhancing effects in animals. However, there are no studies in which different dosages or chronic use have been explored. The current study examined the effects of standardized extract of BM on behavioral changes of Wistar rats when administered the extract for various durations and in varying doses. We divided the animals into 2-, 4-, and 6-week treatment groups. Rats in each of these groups were divided into 20 mg/kg, 40 mg/kg, and 80 mg/kg dose groups (n = 8 for each dose). After the treatment period, the rats, along with age-matched normal and gum acacia control rats, were subjected to spatial learning (T-maze) and passive avoidance tests. The data were compared with those of age-matched control rats. The study was conducted at the Melaka Manipal Medical College, Manipal University, Manipal, Karnataka, India. The results showed improvement in spatial learning performance and enhanced memory retention in rats treated with BM extract. These results clearly indicate that oral administration of BM extract improved learning and memory in rats. © 2010 Elsevier Inc. All rights reserved.

Introduction

Memory is the ability of an individual to record sensory stimuli, events, and information; retain them over short or long periods of time; and recall the same at a later date when needed. Poor memory, lower retention, and slow recall are common problems in today’s stressful and competitive world. Several medicinal plants, designated as “Rasayan Drugs” in Ayurveda, are supposed to have specific influence on brain functions. Important plants that act on the nervous system include: “Brahmi” (*Bacopa monniera*), “Vacha” (*Acorus calamus*), “Mandukaparni” (*Centella asiatica*), “Shankhpushpi” (*Convulvulus pluricaulis*), “Jyotishmati” (*Celastrus paniculatus*), “Jatamansi” (*Nardostachys jatamansi*), and “Ashwagandha” (*Withania somnifera*) (Sharma, 1988). Among the above named plants, *Bacopa monniera* (BM) has been shown to be very useful in improving learning and memory (Dhawan and Singh, 1996; Warrier, 1996). *Bacopa monniera*, a member of the scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers (Bone, 1996; Warrier, 1996).

It has been used in ayurvedic medicine and in traditional treatments for a number of disorders, particularly those involving anxiety, intellect, and poor memory (Singh and Dhawan, 1997). The plant has prominent action on the central nervous system, where it improves understanding, memory, intellect, and speech, and corrects aberrations of emotions, mood, and personality in an individual.
Various experiments have identified potent antioxidant activity in BM (Tripathi et al., 1996; Bhattacharya et al., 2000). Significant antidepressant activity has been observed in BM extract using a rodent model of depression, comparable to imipramine after 5 days of oral administration (Sairam et al., 2002). Additionally, anticholinesterase activity has been demonstrated (Das et al., 2002). A significant anti-ulcer activity has also been reported for the fresh juice of the whole plant in an animal model of aspirin-induced gastric ulceration (Rao et al., 2000). Positive effects on learning skills, memory, and reaction times compared to controls have been reported (Singh and Dhawan, 1982; Dhawan and Singh, 1996), along with protection against phenytoin-induced cognitive deficits (Vohora et al., 2000). More recently, animal studies have found bacopa attenuates scopolamine-induced dementia (Das et al., 2002). Bacopa monniera also demonstrates stress-decreasing activity in both acute and chronic stress situations (Rai et al., 2003). The effects of chronic administration of an extract of BM on cognitive function in healthy human subjects have been reported (Stough et al., 2001). Bacopa monniera also exhibits a hepatoprotective effect on morphine toxicity (Sumathy et al., 2001). Anticancer (Elangovan et al., 1995), anti-ulcer (Sairam et al., 2002), calcium antagonist (Dar and Channa, 1999), vasodilatory (Channa et al., 2003), smooth muscle relaxant (Dar and Channa, 1997), anti-addictive (Sumathy et al., 2007), and mast cell-stabilizing (Samiuilla et al., 2001) properties of BM have also been demonstrated.

Though there are reports showing that BM improves learning and memory in rats, there are no studies in which different dosages or chronic use have been explored. Thus this experiment was designed to study the effect of BM on learning and memory in rats administered BM at various doses and for a variety of periods of time.

Materials and methods

Animals and experimental groups

Wistar albino rats of approximately of the same age and weighing about 150-200 g were used for the study. The breeding of animals was done in “the central animal house,” Manipal University, Manipal, India. The animals were fed Amrut laboratory animal feed, Amrut rat and mice pellet (Pranav Agro Industries, Ltd., Maharashtra, India). Four rats were housed in each polypropylene cage and maintained in a 12:12-hour cycle of dark and light. The experimental protocol was subjected to scrutiny by an institutional animal ethical committee for experimental clearance (IAEC/KMC/02/2005-2006).

Rats were assigned to 2-, 4-, and 6-week treatment groups. Rats in each of these groups were divided into 20 mg/kg, 40 mg/kg, and 80 mg/kg dose groups (n = 8 for each dose). Each rat in the given dosage group was fed the designated amount of standardized BM extract daily for 2, 4, and 6 weeks. Along with these experimental groups, an untreated normal control group (NC) and a gum acacia vehicle control group (GAC) (n = 8 in both groups) were maintained.

Extraction and administration of Bacopa monniera

Standardized plant extract of BM was supplied by the herbal manufacturer M/s. Natural Remedies Private Limited (Bangalore, India). The shelf-life of this extract is 2 years. The first step was extraction of the botanically identified plant material with alcohol. The alcoholic extract was then re-extracted with water, and the water-soluble matter was retained. The final re-extract was concentrated and dried to make a powder. Phytochemical analysis by high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) revealed that the final extract contained approximately 10% w/w (10% of the total mass of the extract) of the active ingredients (bacosides A and B). The plant extract was administered orally along with 5% gum acacia, using an oral feeding needle attached to a syringe. A volume of 0.75 mL-1 mL of BM extract was given, depending on the weight of the animal.

Behavioral tests

Following treatment, all groups (NC, GAC, and BM) were subjected to behavioral tests during the night (starting at 7 PM). The experiments were done at night since the animals were active during the night. The behavioral tests consisted of a spatial learning (T-maze) test and a passive avoidance test.

Spatial learning (T-maze) test

The purpose of this test was to assess the rats’ spatial learning ability. This test included spontaneous alternation and rewarded alternation tests.

The wooden T-maze apparatus consisted of a stem (35 × 12 cm), a choice area (15 × 12 cm), and 2 arms (35 × 12 cm). The start box (15 × 12 cm) was located at the beginning of the stem. The goal areas were at the ends of the 2 arms (15 × 12 cm each), each of which contained a food well. The stem and start box were separated by a sliding door. A cloth curtain separated the arm and goal areas. The height of the side wall of the apparatus was about 40 cm. The apparatus was kept in a sound-attenuated, normally lit room.

Spontaneous alternation test

This test was described by Dunnett et al. (1982). Two days prior to the start of the testing, the rats were completely deprived of food so as to enhance motivation for a food reward. Subsequently, food was restricted during trials so that the animal’s body weight was maintained at 85% of pretest weight. The rats then underwent orientation, which was done to familiarize the rats with the T-maze. During
orientation, the rats subjected to food restriction were placed in the start box for 60 seconds. The sliding door was then opened to allow the rat to explore the T-maze for 30 minutes and to eat 15 pellets (10 mg each) of consistent size in each goal area. After 30 minutes, the rat was returned to the start box. This procedure was carried out for 2 consecutive days for all rats in each group.

After the orientation period, 6 trials were conducted daily for the following 4 days. In each trial, the rat was first placed in the start box, the sliding door was opened, and the rat was allowed to enter the stem and choose either one of the arms. A rat was considered to have entered a particular arm only when it entered that arm with all of its limbs. Once the rat ate the pellets in the goal area of that arm, it was placed back in the start box for the next trial. The intertrial interval was 1 minute. There was no specific time limit for trials. In each trial, the arm chosen by the rat was noted.

After 4 days (i.e., 24 trials), the total number of alternations was also noted. The percentage bias was calculated for each rat using the following formula: percentage bias = total number of choices of most frequently chosen side \( \times 100 / \) total number of trials. A larger number of alternations and a smaller percentage bias were considered as indexes for improved learning ability.

**Rewarded alternation test**

This test was described by Dunnett et al. (1982). This test was started the day after the completion of the spontaneous alternation test. During this test, 6 trials per day were conducted for 4 consecutive days. Each trial had 2 runs: a forced run and a choice run. In the forced run, the animal was forced into one of the arms by blocking the other arm and was allowed to consume the pellets in the goal area. Once the animal ate the pellets in the goal area, it was placed back in the start box for a choice run. In the choice run, the goal area of the forced arm was kept empty and pellets were placed in the goal area of the opposite arm. Both arms were accessible to the rat. One minute passed between each forced and choice run. Similarly, there was a gap of 1 minute between each trial. The sequence of the forced arm was predetermined and was same for all rats for a given day. On subsequent days, it was alternated. During the choice run, if the rat entered the arm opposite to the forced arm, that response was considered as a “correct response.” If it chose the same arm it had been made to enter during the forced run, it was considered as giving the “wrong response.” The percentage of correct responses was calculated for each rat using the following formula: percentage of correct responses = total number of correct responses \( \times 100 / \) total number of trials. An increase in the percentage of correct responses was considered as an index of improved learning and memory.

**Passive avoidance test**

This test was modified from Bures et al. (1983). The passive avoidance apparatus was fabricated locally. It had 2 compartments, a larger rectangular compartment with a 50 \( \times \) 50-cm grid floor and 35-cm-high wooden walls. It had a top cover that could be opened or closed. In the center, one of the walls had a 6 \( \times \) 6-cm opening connecting the larger compartment to a smaller, dark compartment. The smaller compartment had a 15 \( \times \) 15-cm electrifiable grid connected to a constant current stimulator, 15-cm-high wooden walls, and a top cover that could be opened or closed. The connection between the 2 compartments could be closed with a sliding door. The larger compartment was illuminated with a 100-W bulb placed 150 cm above the center.

The experiment included 3 parts: (1) an exploration test; (2) an aversive stimulation and learning phase (passive avoidance acquisition); and (3) a retention test. During the exploration test, each rat was kept in the center of the larger compartment facing away from the entrance to the smaller, dark compartment. The door between the 2 compartments was kept open. The rat was allowed to explore the apparatus (both larger and smaller compartments) for 3 minutes. In each trial, the total time spent by the animal in the smaller compartment was noted. At the end of the trial, the rat was replaced in the home cage, where it remained during an intertrial interval of 5 minutes. This procedure was repeated 3 times for each rat. The intertrial interval was 5 minutes.

After the last exploration trial, the rat was forced into the smaller compartment, and the sliding door between the 2 compartments of the apparatus was closed. Three strong foot shocks (50 Hz, 1.5 mA, 1-second duration) were given at approximately 5-second intervals. The top cover was then opened, and the rat was returned to its home cage. The retention test was carried out 24 hours after the acquisition test. The rat was kept in the center of the larger compartment facing away from the entrance to the smaller compartment. The sliding door between the 2 compartments was kept open. The rat was allowed to explore the apparatus for 3 minutes, after which the rat was returned to the home cage. This procedure was repeated 3 times, with an inter-trial interval of 5 minutes. In each trial, the time spent by the rat in the smaller compartment was noted. A decrease in the time spent in the smaller compartment during the retention test was considered to indicate good memory retention performance.

The T-maze and passive avoidance equipment was cleaned between trials.

**Data analysis**

Data were analyzed using analysis of variance followed by Bonferroni’s test (post hoc) using GraphPad Prism, version 2.01 (GraphPad Prism Software Inc., USA, March 27, 1999).

**Results**

**Spatial learning (T-maze tests)**

The results of these tests are presented in Tables 1, 2, and 3. Animals treated with a standardized extract of BM
at 20 mg/kg for 2 weeks did not show any significant improvement in spatial learning compared to NC rats. However, animals treated with higher doses of BM extract (40 and 80 mg/kg) showed a higher number of alternations when compared to the NC group. Similarly, rats treated with higher doses of BM extract (40 and 80 mg/kg) showed lesser percentage bias in comparison with NC rats. During the rewarded alternation test, only rats treated with higher doses of BM extract (40 and 80 mg/kg) showed a significant increase in the percentage of correct responses when compared to NC rats.

In the 4-week treatment group, during the spontaneous alternation test, animals treated with the standardized extract of BM (40 and 80 mg/kg) showed a significantly higher number of alternations when compared to NC rats. Rats treated with the BM extract (20, 40 and 80 mg/kg) showed lesser percentage bias in comparison with NC rats. During the rewarded alternation test, only rats treated with BM extract showed a significant increase in the percentage of correct responses when compared to the NC group rats.

In the 6-week treatment group, during the spontaneous alternation test, animals treated with the standardized extract of BM (20, 40, 80 mg/kg) showed significantly higher numbers of alternations when compared to NC rats. Similarly, rats treated with the BM extract showed lesser percentage bias in comparison to NC rats. During the rewarded alternation test, only rats treated with BM extract showed a significant increase in the percentage of correct responses when compared to the NC rats.

### Passive avoidance test

Results of the passive avoidance exploration and retention performance tests are shown in Figures 1, 2, and 3. All of the BM treatment groups showed good memory retention.

In the 2-week treatment group (Fig. 1), during exploration, there was no significant difference between animals treated with BM extract (20, 40, and 80 mg/kg) and NC animals in the total time spent in the small compartment. However, during the retention test, it was seen that animals treated with high doses of BM extract (40 and 80 mg/kg) spent significantly less time in the smaller compartment (86.50 ± 9.39 in the NC group vs 21.25 ± 3.99 in BM 40 mg/kg group, P < 0.001 and 26.38 ± 5.60 in BM 80 mg/kg group, P < 0.001).

In the 4-week treatment group (Figure 2), during exploration, there was no significant difference between animals treated with BM extract (20, 40, and 80 mg/kg) and NC animals in total time spent in the small compartment. However, during the retention test, it was seen that animals treated with BM extract spent significantly less time in the smaller compartment (92.88 ± 11.97 in the NC group vs 75.50 ± 7.72 in the BM 20 mg/kg group, P < 0.05; 14.00 ± 2.39 in the BM 40 mg/kg group, P < 0.001; and 28.00 ± 5.42 in the BM 80 mg/kg group, P < 0.001).

In the 6-week treatment group (Figure 3), during exploration, there was no significant difference between animals treated with BM extract (20, 40, and 80 mg/kg) and NC animals in the total time spent in the small compartment. However, during the retention test, it was seen that animals

<table>
<thead>
<tr>
<th>Group (n = 8 in each group)</th>
<th>Spontaneous alternation test</th>
<th>Rewarded alternation test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of alternations</td>
<td>% bias</td>
</tr>
<tr>
<td>NC</td>
<td>10.13 ± 1.24</td>
<td>64.00 ± 4.07</td>
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<tr>
<td>GAC</td>
<td>11.00 ± 1.51</td>
<td>62.13 ± 3.48</td>
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<tr>
<td>BM 20 mg/kg</td>
<td>12.25 ± 1.66</td>
<td>59.25 ± 2.91</td>
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<tr>
<td>BM 40 mg/kg</td>
<td>15.13* ± 1.64</td>
<td>51.75* ± 3.01</td>
</tr>
<tr>
<td>BM 80 mg/kg</td>
<td>14.00 ± 1.30</td>
<td>53.88* ± 3.60</td>
</tr>
</tbody>
</table>

**BM, Bacopa monniera; GAC, gum acacia control; NC, normal control.**

Note: Each value represents mean ± standard deviation. NC vs BM 20 mg/kg: * P < 0.05, ** P < 0.01; NC vs BM 40 mg/kg: *** P < 0.001; NC vs BM 80 mg/kg: ** P < 0.01, *** P < 0.001.

Table 1 Results of spatial learning (T-maze) tests (2-week treatment)
treated with BM extract spent significantly less time in the smaller compartment (108.80 ± 15.12 in the NC group vs 25.38 ± 3.66 in BM 20 mg/kg group, P < 0.001; 10.88 ± 2.23 in BM 40 mg/kg group, P < 0.001 and 13.00 ± 2.00 in BM 80 mg/kg group, P < 0.001).

Discussion

Several research studies have identified natural compounds that serve as nootropic agents. Extracts and compounds have been selected and isolated from medicinal plants based on their value in traditional medicine systems. To date, pharmaceutical companies have been investing enormous resources in the identification of agents that could possibly alleviate debilitating disorders and slow the onset of mental retardation. There is a focus on phytochemicals, which seem to have these properties, though their full potential is yet to be determined and harnessed.

Bacopa Monniera is one such plant with wide medicinal properties that is being used as a treatment for memory-related disorders (Russo and Borrelli, 2005). The present study was designed to determine whether a standardized extract of BM administered at different dosages and treatment lengths would bring about any behavioral changes, especially in learning and memory in adult rats.

The results of T-maze tests in rats treated with lower doses of BM (20 mg/kg) for a 2-week period were not significantly different than those from the NC rats. However, rats treated for 2-weeks in the higher-dose groups (40 and 80 mg/kg) showed significant improvement in their learning behavior. When treated for a longer duration (4 and 6 weeks), rats showed significant improvement in their learning behavior in all (20, 40, and 80 mg/kg) dose groups. In the passive avoidance tests, there was no significant change in behavior during exploration. However, during the retention test, rats treated for 4 and 6 weeks at all 3 doses (20, 40, and 80 mg/kg) spent less time in the smaller compartment, suggesting improved memory retention. Animals treated with higher doses (40 and 80 mg/kg) for 2 weeks spent less time in the small compartment during the retention test. These results clearly indicate that oral administration of the BM extract improved learning and memory in rats.

In view of the importance of this plant, several groups of researchers have carried out systematic chemical examinations. Bose and Bose (1931) reported the isolation of the alkaloid “brahmine” from BM. Later, other alkaloids like nicotine and herpestine were also reported (Chopra et al., 1956). The isolation of D-mannitol and of a saponin (hersaponin) and potassium salt by Sastri et al. (1959) provided further details of the chemical components of BM. The major chemical constituents shown to be responsible for the memory-facilitating action of BM are the steroidal saponins.

**Table 3** Results of spatial learning (T-maze) tests (6-week treatment)

<table>
<thead>
<tr>
<th>Groups (n = 8 in each group)</th>
<th>Spontaneous alternation test</th>
<th>Rewarded alternation test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of alternations</td>
<td>% bias</td>
</tr>
<tr>
<td>NC</td>
<td>12.25 ± 2.12</td>
<td>65.25 ± 3.41</td>
</tr>
<tr>
<td>GAC</td>
<td>12.50 ± 1.60</td>
<td>64.13 ± 2.41</td>
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<tr>
<td>BM 20 mg/kg</td>
<td>16.50*** ± 2.33</td>
<td>57.50*** ± 3.58</td>
</tr>
<tr>
<td>BM 40 mg/kg</td>
<td>17.88*** ± 1.35</td>
<td>51.50*** ± 2.39</td>
</tr>
<tr>
<td>BM 80 mg/kg</td>
<td>16.88*** ± 2.53</td>
<td>53.88*** ± 3.09</td>
</tr>
</tbody>
</table>

BM, Bacopa monniera; GAC, gum acacia control; NC, normal control.

Note: Each value represents mean ± standard deviation. NC vs BM 20 mg/kg: ** P < 0.01, *** P < 0.001; NC vs BM 40 mg/kg: *** P < 0.001; NC vs BM 80 mg/kg: *** P < 0.001.

![Figure 1](image1.png)

**Figure 1** Graph showing the time spent in the small compartment in the 2-week treatment group. Each bar represents mean ± SD. NC vs BM 40 mg/kg: ***P < 0.001; NC vs BM 80 mg/kg: ***P < 0.001. (BM, Bacopa monniera; GAC, gum acacia control; NC, normal control; n = 8 in all groups.)

![Figure 2](image2.png)

**Figure 2** Graph showing the time spent in the small compartment in the 4-week treatment group. Each bar represents mean ± SD. NC vs BM 20 mg/kg: *P < 0.05; NC vs BM 40 mg/kg: ***P < 0.001; NC vs BM 80 mg/kg: ***P < 0.001. (BM, Bacopa monniera; GAC, gum acacia control; NC, normal control; n = 8 in all groups.)
and the restoration of synaptic activity, and ultimately in nerve impulse transmission.

References


