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EXPERIMENTAL STUDY

Learning and memory-enhancing effect of *Bacopa monniera* in neonatal rats

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Abstract: *Objective:* The aim of this study was to evaluate the learning and memory-enhancing effect of *Bacopa monniera* in neonatal rats.
Background: Learning is an acquisition and storage of information as a consequence of experience. Memory is a relatively permanent storage form of the learned information. In the process of 'learning', activation of neurons occurs in specific areas or specific memory systems of the brain concerned with the processing of the specific modality of sensory information. Rasayana plants are said to prevent ageing, re-establish youth, strengthen life, brain power and prevent diseases. *Bacopa monniera* (BM) is shown to be very useful in improving learning and memory.
Methods: In the present study neonatal rat pups (10 days old) were given different doses of BM extract orally for different periods of time. These rats were then subjected to spatial learning (T- Maze) and passive avoidance tests along with the age matched normal and gum acacia control rats. The data were compared with those of control rats.
Results: The results showed improvement in spatial learning performance and enhanced memory retention in neonatal rats treated with extract of BM.
Conclusion: We conclude that treatment with BM extract during growth spurt period of neonatal rats enhances learning and memory *(Tab. 3, Fig. 3, Ref. 45).* Full Text in free PDF *www.bmj.sk.*
Key words: *Bacopa monniera,* memory, passive avoidance, spatial learning.

Learning or acquisition, a highly specialized function of the brain, is a process of acquiring knowledge about the environment around the organism, while memory is the storage or retention of this learnt knowledge which can be retrieved later (1). In the process of 'learning' or 'acquisition', activation of neurons occurs in specific areas or specific memory systems of the brain concerned with the processing of the specific modality of sensory information (2).

Physiologically, memories are caused by changes in the capacity of synapses to transmit activity from one neuron to another in a neural circuit as a result of previous neural activity. These changes in turn establish new pathways to develop in the neural circuitry. The new pathways are called memory traces. They are important because once established, they can be activated by the thinking process to reproduce memories whenever required. The intellectual ability of an individual is dependent on memories to which one is adding constantly. The hippocampus and amygdala are concerned with the storage of recent memory and emotional behavior. The structural organization of these areas has been reported to be highly plastic, particularly in the hippocampus (3, 4).

Ayurvedic pharmacology classifies medicinal plants into different groups according to their actions. One of these is the 'Rasayana' group. The word 'Rasayana' literally means the path that 'Rasa' takes ('Rasa': plasma; Ayana: path). It is believed, in Ayurveda that the qualities of the 'Rasadhatu' influence the health of other dhatus (tissues) of the body. Hence any medicine that improves the quality of 'Rasa' should strengthen or promote the health of all tissues of the body. 'Rasayana' drugs act inside the human body by modulating the neuro-endocrino-immune system and have been found to be a rich source of antioxidants (5). Rasayana plants are said to possess the following properties: they prevent ageing, re-establish youth, strengthen life, brain power and prevent diseases (6, 7), all of which imply that they increase the resistance of the body against any onslaught.

In the ayurvedic system of medicine "Medhya drugs" are a group of medicines known to act on the nervous system. In the texts of Ayurveda, many medhya drugs have been claimed to improve mental ability. Some of the drugs, which act on the nervous system, include Brahmi (*Bacopa monniera*), Ashwagandha (*Withania somnifera*), Jyotishmati (*Celastrus panniculatus*),

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Shankapushpi (*Clitoria ternatea*), Jatamansi (*Nardostachys jatamansi*), Vacha (*Acorus calamus*) and Mandukaparni (*Centella asiatica*) (8). Among the above named plants, ‚Brahmi' or *Bacopa monniera* (BM) is shown to be very useful in improving learning and memory (9–11). BM, a member of the scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers (9, 12).

BM has been used in ayurvedic medicine and in traditional treatments for a number of disorders, particularly those involving anxiety, intellect and poor memory (13). 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.

BM given in combination with 'Ghrita' (animal fat), a well-known Ayurvedic medicine (brahmi ghrita) in cases of hysteria and epilepsy had shown improved results (14). It is also useful in insanity, neurasthenia, aphonia and hoarseness (15). Extract of BM was assessed on rat brain frontal cortical, striatal and hippocampal SOD, CAT and GSH-px activities, following administration for 7, 14 or 21 days which induced a dose-related increase in SOD, CAT and GSH-px activities (16, 17). It showed a dose-dependent free radical scavenging capacity and a protective effect on DNA cleavage and was confirmed by a significant protective effect on H2O2-induced cytotoxicity and DNA damage in human non-immortalized fibroblasts (18).

Significant antidepressant activity has been observed in brahmi extract using a rodent model of depression, comparable to imipramine after five days of oral administration (19). Additionally, anticholinesterase activity has been demonstrated (20). Extensive pharmacological studies, mostly conducted with standardized extracts, have shown that BM improves the acquisition, retention and retrieval of learned tasks (10, 21) along with protection against phenytoin-induced cognitive deficits (22). BM has also demonstrated a significant memory-promoting effect in animal models of Alzheimer's disease (21). Animal studies have found that BM attenuates scopolamine-induced dementia (20). The effects of chronic administration of an extract of BM on cognitive function in healthy human subjects have been reported (23).

BM is also considered to promote youthful vitality and longevity. In Ayurvedic medicine it is described as being cold, sweet, astringent, diuretic, laxative and as a tonic for the heart and nerves (24). In India, BM is used internally as a febrifuge, nervine and cardiac tonic, and a hot poultice of the plant is applied in acute bronchitis, cough and children's chest conditions (25). Bacoside A and B, the active components of BM were found to facilitate the capacity for mental retention in rats and were active in both positive and negative reinforcement experiments (26). An ethanolic BM extract had cytotoxic effect on Sarcoma-180 cells in vitro (27). The probable mode of action was inhibition of DNA replication in the cancer cells. An aqueous extract of BM improved carbon clearance in rats, indicating stimulation of the reticuloendothelial system (28). Brahmi Rasayan, an Ayurvedic preparation that has BM as its major active ingredient, had anti-inflammatory effects in large oral doses in various experimental models of inflammation (29).

Though there are reports showing BM improving learning and memory in rats, there are no studies with different dosages and longer duration. Thus this study was designed to study the effect of BM on learning and memory in neonatal rats in longer duration with different doses.

Materials and methods

Animals and experimental groups

10 days old Wistar rats of both sexes maintained in a 12 hours dark and 12 hours light cycle, provided with food and water ad libitum were used in the experiments. They were housed in polypropylene cages. The experimental protocol was subjected to scrutiny of institutional animal ethical committee for experimental clearance IAEC/KMC/02/2005-2006.

Rat pups were assigned 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 body weight dose groups (n = 8 for each dose). Each rat in the given dosage group was fed with the designated amount of standardized extract of BM daily for 2, 4 and 6 weeks. Along with these experimental groups, a normal control group and a vehicle (gum acacia) control group (n = 8 in both groups) were also 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.

Extraction: The extract was prepared by first extracting the botanically identified plant material with alcohol. The alcoholic extract was then re-extracted with water and the water soluble matter was taken. Final re-extract was concentrated and dried to make a powder. Phytochemical analysis revealed that the final extract contained approximately 10 % w/w of the active ingredients (Bacosides A and B) by HPLC & HPTLC.

Plant extract was administered orally along with 5 % gum acacia, using an oral feeding needle.

Behavioral tests

Following treatment, all the groups (NC, GAC and BM) of rats were subjected to behavioral tests. The behavioral tests included, 1) spatial learning (T-Maze) test and 2) passive avoidance test.

Spatial learning (T-maze) tests

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

The wooden T-maze apparatus consisted of a stem (35x12 cm), a choice area (15x12 cm) and two arms (35x12 cm). The start box (15x12 cm) was located at the beginning of the stem. The goal areas were at the ends of the two arms (each 15x12 cm) containing the 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 sidewall of the apparatus was about 40

cm. The apparatus was kept in a sound attenuated normally lit room.

Spontaneous alternation test (30). Two days prior to the starting of the test, the rats were deprived of food in order to motivate them for the food reward. Subsequently, the food was restricted so that the animal's body weight was maintained at 85 % of pre-test weight. This was followed by orientation, which was done to familiarize the rats with the T-maze. During orientation, the rats subjected for food restriction were placed in the start box for sixty seconds. The sliding door was then opened to allow the rat to explore the T-maze for thirty minutes, and to eat fifteen pellets (10 mg each) in each goal area. After thirty minutes the rat was returned to the start box. This procedure was carried out for two consecutive days for all rats of the group.

After the orientation, six trials were given daily for the following four days. In each trial, the rat was first placed in the start box. By opening the sliding door it was allowed to enter into the stem and allowed to choose any one of the arms. A rat was considered to have entered into a particular arm only when it entered that arm with all its limbs. Once the rat ate the pellet in the goal area of that arm, it was replaced back in the start box for the next trial. The inter trial interval was one minute.In each trial, the arm chosen by the rat was noted. At the end of four days i.e., twenty-four trials, the total number of alternations were also noted. The percentage bias was calculated for each rat using the following formula; Percentage bias = total number of choices of more frequently chosen side x 100 / total number of trials. Higher number of alternations and lower percentage of bias were considered as an index for improved learning ability.

Rewarded alternation test (30)

This test was started on the day after the completion of spontaneous alternation test. During this test, six trials per day were conducted for four days. Each trial had two runs namely, a forced run and a choice run. In the forced run, the animal was forced to one of the arms by blocking the other arm and was allowed to consume the pellet in the goal area. Once the animal ate the pellet 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. But both the arms were kept free for the rat to choose. Between each forced run and the choice run, a gap of one minute was given. Similarly there was a gap of one minute between the two trials again. The sequence of the forced arm was predetermined and was same for all the rats for a given day. On subsequent days it was alternatively changed. During the choice run, if the rat entered the arm opposite to the forced arm, then that response was considered as "correct response". If it entered the same arm to which it was forced during forced run, it was considered as "wrong response". Percentage of correct responses was calculated for each rat by using the following formula; percentage of correct responses = total number of correct responses x 100 / total number of trials. Increase in percentage of correct response was considered as an index of improved learning and memory.

Passive avoidance test (31)

The passive avoidance apparatus was fabricated locally. It had two compartments, a rectangular larger compartment with a 50x50 cm grid floor and wooden walls of 35 cm height. It had a roof, which could be opened or closed. In the centre, one of the walls had a 6x6 cm opening connecting the larger compartment to a dark smaller compartment. The smaller compartment had 15x15 cm electrifiable grid connected to a constant current stimulator, wooden walls of 15 cm height and a ceiling, which could be opened or closed. The connection between the two compartments could be closed with a sliding door. The larger compartment was illuminated with a 100 W bulb placed 150 cm above the centre.

The experiment included three parts, 1) exploration test, 2) an aversive stimulation and learning (passive avoidance acquisition), and 3) retention test.

During exploration test each rat was kept in the centre of the larger compartment facing away from the entrance to the dark smaller compartment. The door between the two 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 inter-trial interval of five minutes.After the last exploration trial, the rat was forced into the smaller compartment and the sliding door between the two compartments of the apparatus was closed. Three strong foot shocks (50 Hz, 1.5 mA, 1 sec duration) were given at approximately five-second intervals. The ceiling was then opened and the rat was returned to its home cage. Retention test was carried out after twenty-four hours of acquisition test. The rat was kept in the centre of the larger compartment facing away from the entrance to the smaller compartment. The sliding door between the two compartments was kept open. The rat was allowed to explore the apparatus for three minutes. After three minutes the rat was kept back in the home cage. With a gap of five minutes the trial was repeated for three times. In each trial, the time spent by the rat in the smaller compartment was noted.

Decrease in the time spent in the smaller compartment during retention test was considered as good memory retention performance.

Data analysis

Data was analyzed using analysis of variance (ANOVA) followed by Bonferroni's test (post hoc) using GraphPad Prism, version 2.01.

Results

2 weeks

Spatial learning (T-Maze test) (Tab. 1)

i. Spontaneous alternation test

During spontaneous alternation test the animals treated with 20 mg/kg of BM extract did not show any significant difference in their performance. However, animals treated with higher doses

Tab. 1. Results of spatial learning (T-maze) tests (2 weeks treatment).

Groups	n	T-maze test		
		Spontaneous alternation test		Rewarded alternation test
		Number of alternations	% bias	% of correct response
NC	8	10.88 ± 1.80	67.18 ± 9.03	69.85 ± 6.04
GAC	8	10.63 ± 1.40	64.58 ± 10.45	68.48 ± 5.84
BM 20 mg/kg	8	12.38 ± 2.06	59.36 ± 5.32	81.60 ± 4.17$^{\#\#}$
BM 40 mg/kg	8	15.13 ± 2.74**	52.60 ± 4.37**	90.66 ± 5.89***
BM 80 mg/kg	8	14.75 ± 2.37$^{\$\$}$	53.14 ± 6.15$^{\$\$}$	87.29 ± 6.26$^{\$\$\$}$

Each value represents Mean ± SD. NC vs BM 20 mg/kg: $^{\#\#}$ p<0.01; NC vs BM 40 mg/kg: ** p<0.01, *** p<0.001; NC vs BM 80 mg/kg: $^{\$\$}$ p<0.01, $^{\$\$\$}$ p<0.001 (One way ANOVA, Bonferroni's test). NC – normal control; GAC – gum acacia control, BM – *Bacopa monniera.*

Tab. 2. Results of spatial learning (T-maze) tests (4 weeks treatment).

Groups	n	T-maze test		
		Spontaneous alternation test		Rewarded alternation test
		Number of alternations	% bias	% of correct response
NC	8	12.88 ± 0.83	69.26 ± 4.42	67.38 ± 3.58
GAC	8	13.00 ± 1.06	68.21 ± 7.35	69.44 ± 2.12
BM 20 mg/kg	8	15.63 ± 1.18$^{\#\#}$	58.85 ± 7.18$^{\#\#}$	75.51 ± 5.20$^{\#\#}$
BM 40 mg/kg	8	18.50 ± 2.13***	51.57 ± 3.10***	87.49 ± 3.15***
BM 80 mg/kg	8	17.25 ± 1.28$^{\$\$\$}$	52.63 ± 2.17$^{\$\$\$}$	85.40 ± 4.98$^{\$\$\$}$

Each value represents Mean ± SD. NC vs BM 20 mg/kg: $^{\#\#}$ p<0.01; NC vs BM 40 mg/kg: *** p<0.001; NC vs BM 80 mg/kg: $^{\$\$\$}$ p<0.001 (One way ANOVA, Bonferroni's test). NC – normal control; GAC – gum acacia control, BM – *Bacopa monniera.*

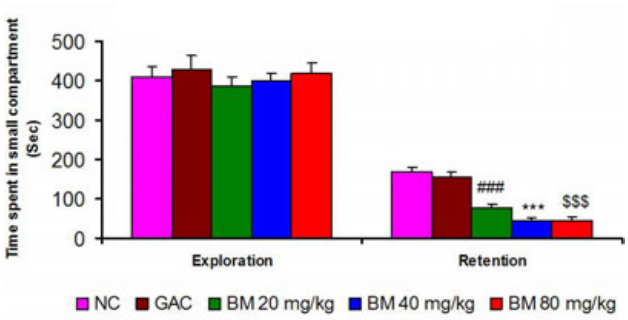

Fig. 1. Graph showing the time spent in small compartment in 2 weeks treatment group. Each bar represents Mean ± SD. NC vs BM 20 mg/kg: $^{\#\#\#}$ p<0.001; NC vs BM 40 mg/kg: *** p<0.001; NC vs BM 80 mg/kg: $^{\$\$\$}$ p<0.001 (NC – normal control; GAC – gum acacia control; n=8 in all groups).

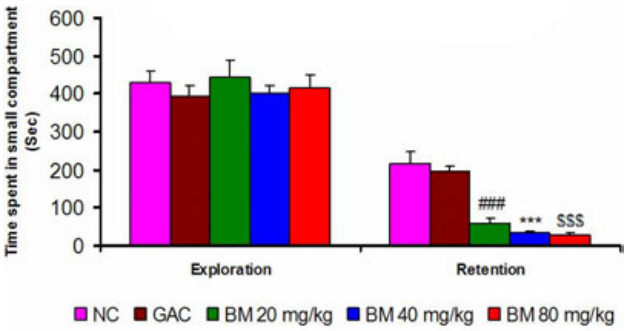

Fig. 2. Graph showing the time spent in small compartment in 4 weeks treatment group. Each bar represents Mean ± SD. NC vs BM 20 mg/kg: $^{\#\#\#}$ p<0.001; NC vs BM 40 mg/kg: *** p<0.001; NC vs BM 80 mg/kg: $^{\$\$\$}$ p<0.001 (NC – normal control; GAC – gum acacia control; n=8 in all groups).

of BM extract (40 and 80 mg/kg) showed significantly higher number of alternations when compared to NC group. Similarly, rats treated with higher doses (40 and 80 mg/kg) of BM showed significantly lesser percentage bias in comparison with NC rats.

ii. Rewarded alternation test

During rewarded alternation test, rats treated with BM extract (20, 40 and 80 mg/kg) showed a significant increase in the percentage of correct responses compared to NC rats.

Passive avoidance test

Total time spent in the small compartment (Fig. 1)

During exploration there was no significant difference between animals treated with the BM extract (20, 40 and 80 mg/kg) and NC animals. However, during retention test, it was seen that animals treated with BM extract spent significantly less time in the small compartment (166.30 ± 14.55 sec. in NC group vs. 79.00 ± 7.54 sec. in 20 mg/kg group, p<0.001, 43.75 ± 9.06 sec. in 40 mg/kg group, p<0.001 and 45.88 ± 8.42 sec. in 80 mg/kg group, p<0.001).

4 weeks

Spatial learning (T-Maze test) (Tab. 2)

i. Spontaneous alternation test

During spontaneous alternation test the animals treated with 20, 40 and 80 mg/kg of BM extract showed significantly higher number of alternations when compared to NC rats.

Similarly, rats treated with 20, 40 and 80 mg/kg of BM extract showed significantly lesser percentage bias in comparison with NC rats.

ii. Rewarded alternation test

During rewarded alternation test, rats treated with BM extract (20, 40 and 80 mg/kg) showed a significant increase in the percentage of correct response compared to NC group rats.

Passive avoidance test

Total time spent in the small compartment (Fig. 2)

During exploration there was no significant difference between animals treated with BM extract (20, 40 and 80 mg/kg) and NC animals. However, during retention test, it was observed

Tab. 3. Results of spatial learning (T-maze) tests (6 weeks treatment).

Groups	n	T-maze test		
		Spontaneous alternation test		Rewarded alternation test
		Number of alternations	% bias	% of correct response
NC	8	12.63 ± 1.30	68.14 ± 6.62	65.10 ± 4.41
GAC	8	12.00 ± 1.51	65.62 ± 6.58	67.18 ± 3.47
BM 20 mg/kg	8	16.38 ± 1.84$^{\#\#}$	57.29 ± 5.34$^{\#\#}$	85.41 ± 5.89$^{\#\#\#}$
BM 40 mg/kg	8	19.00 ± 1.69***	52.61 ± 3.10***	93.22 ± 4.42***
BM 80 mg/kg	8	18.13 ± 2.10$^{\$\$\$}$	53.65 ± 4.68$^{\$\$\$}$	91.66 ± 3.85$^{\$\$\$}$

Each value represents Mean ± SD. 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 (One way ANOVA, Bonferroni's test). NC – normal control; GAC – gum acacia control, BM – *Bacopa monniera.*

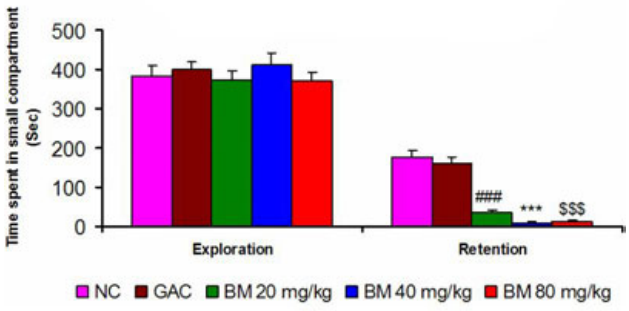

Fig. 3. Graph showing the time spent in small compartment in 6 weeks treatment group. Each bar represents Mean ± SD. NC vs BM 20 mg/kg: $^{\#\#\#}$ p<0.001; NC vs BM 40 mg/kg: *** p<0.001; NC vs BM 80 mg/kg: $^{\$\$\$}$ p<0.001 (NC – normal control; GAC – gum acacia control; n=8 in all groups).

that animals treated with BM extract spent significantly less time in the small compartment (217.1 ± 31.39 sec. in NC group vs. 57.63 ± 14.02 sec. in 20 mg/kg group, p<0.001, 33.63 ± 5.57 sec. in 40 mg/kg group, p<0.001 and 29.50 ± 6.78 sec. in 80 mg/kg group, p<0.001).

6 weeks

Spatial learning (T-Maze test) (Tab. 3)

i. Spontaneous alternation test

During spontaneous alternation test the animals treated with 20, 40 and 80 mg/kg of BM extract showed significantly higher number of alternations when compared to NC rats. Similarly, rats treated with 20, 40 and 80 mg/kg of BM extract showed significantly lesser percentage bias in comparison with NC rats.

ii. Rewarded alternation test

During rewarded alternation test, rats treated with BM extract (20, 40 and 80 mg/kg) showed a significant increase in the percentage of correct response compared to NC rats.

Passive avoidance test

Total time spent in the small compartment (Fig. 3)

During exploration there was no significant difference between animals treated with BM extract (20, 40 and 80 mg/kg) and NC animals. However, during retention test, it was observed that animals treated with BM extract spent significantly less time in the small compartment (178.0 ± 16.38 sec. in NC group vs. 34.25 ± 7.99 sec. in 20 mg/kg group, p<0.001, 10.50 ± 2.26 sec. in 40 mg/kg group, p<0.001 and 14.38 ± 1.59 sec. in 80 mg/kg group, p<0.001).

Discussion

To assess the spatial learning behavior of the animals, spontaneous and rewarded alternation tests were carried out in a T-maze. Many studies have examined how rats run different types of mazes, from T-mazes to radial arm mazes to water mazes (32–34). These maze studies are used to study spatial learning and memory in rats. Maze studies help uncover general principles about learning that can be applied to many species, including humans. Mazes are also used to determine whether different treatments or conditions affect learning and memory in rats. Rats are particularly gifted at running mazes. Their maze-running ability comes from their evolutionary history: rats are small burrowing rodents that have spent millenia digging and finding their way around underground tunnels. It's no wonder they have a knack with mazes.

The maze apparatuses (T-maze and radial maze) are useful for assessment of memory with little effort (33, 34). The T-maze tests have been used to assess the spatial learning behavior of rats by a number of scientists (33, 35-39).

In the present study animals treated with standardized extract of BM at 20 mg/kg for 2 weeks period did not show any significant improvement in spatial learning compared to NC rats. However rats treated with higher doses of BM extract (40 and 80 mg/kg) showed a higher number of alternations when compared to the NC rats. 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 rats treated with all doses of BM extract (20, 40 and 80 mg/kg) showed a significant increase in the percentage of correct responses when compared to NC group rats. In 4 and 6 weeks groups, increase in the number of alternations and decrease in percentage bias were seen in rats treated with 20, 40 and 80 mg/kg of BM extract during spontaneous alternation. During rewarded alternation rats of all the three BM treated groups showed an increase in percentage correct response when compared to NC group. However, in higher dose groups (BM 40 and 80 mg/kg) rats showed improved spatial performance. Such an improvement in learning performance by BM has been reported in literature using elevated plus maze model in rats (40) and Y-Maze test (41), but not by using T-maze tests. Accordingly our results are in consistence with other reports.

The term "passive avoidance" is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behavior. Passive avoid-

ance test is the test that assesses memory retention (42–44). According to Yamada et al (1983), passive avoidance response test is the most useful method in detection of impairments in learning and especially the memory retention ability (45). Passive avoidance tests are more revealing about a drug's effects when stay-time (time spent in the compartments) measures are used (46). In relation with rats there are three types of passive avoidance reactions; (i) Step-down, (ii) Step-through and (iii) Two-compartment. We followed two-compartment passive avoidance experiment as mentioned by Bures et al. (1983) to evaluate the type of learning in our study (31).

During passive avoidance test in the present study, there was no significant change in behavior during exploration. However, during retention test, animals of all the three dose groups (BM 20, 40 and 80 mg/kg) spent less time in the smaller compartment suggesting improved memory retention. This enhanced memory retention was observed in the animals treated with BM extract for 2, 4 and 6 weeks. These results clearly indicate an increased capacity of memory retention in BM treated rats.

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