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Research Article

The Long-term Effect of *Bacopa monnieri* Extract on Spatial Learning and Memory in Rats

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Abstract

Memory is the process of gathering information from the world around us, processing it, storing it and later recalling that information. Many medicinal herbs used in Ayurveda to boost memory, sleep, immunity, learning and to relieve stress. Among these, *Bacopa monnieri* (BM), commonly called Brahmi, has been shown to be very useful in improving learning and memory. In our study, we are trying to establish the long-term consequences (either beneficial or adverse) of varying concentrations of Brahmi leaf extract powder (BM) in adult rats. Adult male Wistar rats of 10-12 months old used for this study. 10% and 20% BM diet is prepared using commercially available Brahmi (BM) powder. Rats were assigned to 3 month and 6 month treatment groups. Each rat in the given diet group was fed the designated BM diet (10% or 20%) daily for 3 months and 6 months duration and compared with the negative control. After the test duration, rat learning and memory is tested by T-maze and Passive avoidance tests. In the 3 month and 6-month treatment group, during the T-maze spontaneous alternation test, animals treated with the BM (10% and 20% showed significantly higher numbers of alternations and showed lesser percentage bias in comparison to NC rats. During the T-maze rewarded alternation test, rats treated with BM extract showed a significant increase in the percentage of correct responses when compared to the NC rats. In the passive avoidance tests, no significant change observed in behaviour during exploration trial. But, during the retention trial, rats treated with BM diet (10% and 20%) spent less time in the smaller compartment, suggesting improved memory retention. In both 3 month and 6 month diet groups, same pattern was observed. These results clearly prove that BM extract improved learning and memory in rats.

Keywords: *Bacopa monnieri* (BM); Brahmi; Spatial Learning; Passive Avoidance

Introduction

Memory is the ability of an individual to register, retain and recall the information at a later date when required. Poor memory or Amnesia and slow recall are common problems in today's stressful and competitive world. Several medicinal plants used in Ayurveda to boost memory, immunity, insomnia, anxiety. Important plants that act on the nervous system include: ''Brahmi'' (*Bacopa monnieri*), ''Vacha'' (Acorus calamus), ''Mandukaparni'' (*Centella asiatica*), ''Shankhapushpi'' (*Convolvulus pluricaulis*), ''Jyotishmati'' (*Celastrus paniculatus*), ''Jatamansi'' (*Nardostachys jatamansi*), and ''Ashwagandha'' (*Withania somnifera*) [1]. Among these, *Bacopa monnieri* (BM), commonly called Brahmi, has been shown to be very useful in improving learning and memory [2]. It has been used in ayurvedic medicine and the compounds within these herbs boosts neurotransmission in the brain cells and help to repair damaged neurons [3]. The plant has effective action on the central nervous system, where it improves learning, memory, cognition, and speech [4].

Various experiments have identified potent antioxidant activity in BM [5]. Significant antidepressant activity has been observed in BM extract using a rodent model of depression [6]. Additionally, anticholinesterase activity has been demonstrated [7]. A significant anti-ulcerogenic activity has also been reported for the fresh juice of the whole plant in an animal model of ethanol-induced gastric ulceration [8]. Studies also shown neurocognitive effect of Brahmi in Alzheimer's disease [9]. Antistress activity of Bacosides of Brahmi in rat brain was studied by Chaudhuri., *et al*. [10]. *Bacopa monnieri* also demonstrates stress decreasing activity in both acute and chronic stress situations [11]. The effects of chronic administration of an extract of BM on cognitive function in healthy human subjects have been reported [12]. Anti-ageing effect of *Bacopa monnieri* and Ashwagandha was studied by Patnaik., *et al*. [13] and showed positive results. Another study by Mallick., *et al*. [14], showed the anticancer potential of Brahmi against MCF-7 and MDAMB 231 cell line.

In another study by Vollala., *et al*. [15], 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.

The major chemical constituents shown to be responsible for the memory-facilitating action of BM are the steroidal saponins and bacosides A and B [16,17].

Some details about the composition and short-term impact of Brahmi extract is available. But its long term effect is unknown. Therefore, we are trying to establish the long-term consequences (either beneficial or adverse) of varying concentrations of Brahmi leaf extract powder (BM) in adult rats.

Materials and Methods

For the entire investigation, adult male wistar rats (10-12 months old) will be used. All of the rats will be kept in a climate-controlled room with a light-dark cycle of 12:12 hours and unrestricted access to food and water. All research will be conducted with conventional animal care after gaining approval from the Institutional animal ethics committee.

Preparation of Brahmi extract powder

Commercially available Brahmi extract powder is obtained from online store (Just Jaivik organic). 10% and 20% BM diet prepared. 10% (w/w) powder diet is prepared by mixing 10% BM powder and 90% control rat chow. 20% (w/w) powder diet is prepared by mixing 20% BM powder and 80% control rat chow. Both are mixed with water, food pellets prepared and sun-dried. This diet is given for 3 months and 6 months.

Rats were assigned to 3 month and 6 month treatment groups. Rats in each of these groups were divided into 10% and 20% diet groups (n = 6 for each diet group). Each rat in the given diet group was fed the designated BM diet daily for 3 months and 6 months duration. Along with these experimental groups, a normal control group (NC) was also included, which was daily given regular rat chow.

Behavioral tests

To assess the spatial learning memory of the Rat

After proper orientation, the rats were introduced to a new environment like T- maze (where T-maze have one starter box and two choice arms) and allowed to explore; the rodents will alternate their choice of the goal arm due to their natural tendency to explore the relatively novel environment. The rat tends to choose the arm that was not visited previously on the second trial run if two trials were immediately provided one after the other. This behaviour reflects the rat's "working memory" of the first choice, and their reaction on each trial alters dependent on what they did previously.

The term "spontaneous alternation" describes this. By starving the animal then rewarding it with a favourite meal if it completes the task correctly, this habit was reinforced. The alternation is particularly sensitive to identifying hippocampal impairment, whether it is rewarded or spontaneous.

T maze test as described by Rai., *et al*. [18].

T-maze apparatus

The T-maze device is made up of a starter area (6 x 4.5 inches), a stem area (14 x 4.5 inches), a choice area, and two arms (14 x 4.5 inches), each of which has a goal area (6 x 4.5 inches) with a well for pellets at the end. The height of the side walls is intended to be 16 inches. The stem is separated from the start area by a single sliding door. The T-maze will be kept in a soundproof room, and the experiment's trials were run essentially at the same time of day.

Spontaneous alternation test

The tests were done in three steps namely, food deprivation, orientation, and trials.

- **Food deprivation:** Rats were being deprived of food for 48 hours before the test to motivate them for a food reward. Subsequent food restrictions were continued throughout experiment and body weight was managed at 85% of the original weight.

- **Orientation:** The rat which has undergone food deprivation was placed in starter box for one minute. The door was kept open, and the rat was encouraged to explore the maze for thirty minutes daily for two successive days, to acclimatize them for the maze ambiance. During this orientation sessions, few pellets (10 mg approximately) were filled in well of each goal area.

- **Trials:** On the next four days, six trials were given daily. During each trial the rats were being placed in the starter box, a sliding door will be released, and the rat is grant entry into the stem portion and arm of the maze. The rat was deemed to have entered into a specific arm when it entered the arm with all its four limbs. After the rat had consumed the food pellet in the goal area, the rat was picked by the investigator and was placed back into the start area. During each trial, the arm chose by the rat, and the number of alteration was noted. The inter-trial interval were maintained constant for one minute. A special chart was prepared to mark the entry of rat into each arm during all the six trials and for all the four days, which in turn helped the investigator to calculate the mean number of alternation and mean percentage bias.

Percentage bias is calculated using the formula given below:

$$\text{Percentage bias} = \frac{\text{No. of choices of frequently chosen side}}{\text{No. of trials}} \times 100$$

Note

When the rats had more number of alternations and less percentage bias, it is understood that the working memory system in the rodent brain was better in reminding the previous choice and encourages them to explore the other arm, which is the index of good spatial learning and memory.

Rewarded alternation test as described by [19]

Rewarded alternation test was started immediately after the successful completion of spontaneous alternation test**.** This test consists of 6 trials/day for four successive days. Each trial of the rewarded alternation test had two steps *viz.,* forced run of the rat and choice run of the rat. During the forced run, the rodent was compelled to enter one arm by restricting the access to the alternative arm and allowing it to eat the pellet there. In the choice run, the food was removed from forced arm goal area, and the pellet is placed on the opposite arm. Both the arms of T-maze were kept open for the rat to run. To be considered a "proper response," the rat must now enter the arm that is opposite the forced arm. Every trial's forced arm is selected and kept the same for all of the rats on the same day. Later, on consecutive days, it was modified alternatively. The time between the force run, choice run, and next run was kept constant at one minute. Using the provided formula, "Percentage of Correct Response" was determined. Before each trial, the equipment was thoroughly cleaned and scrubbed with surgical spirit to remove any smell traces or cues that the preceding animal had mistakenly left behind.

$$\text{Percentage correct response} = \frac{\text{Total number of correct responses}}{\text{Total number of trials}} \times 100$$

Two compartments passive avoidance test as described by [18]

Avoidance tests include limiting an animal's natural behaviour to see if it can learn to avoid a painful or unpleasant occurrence after just one trial. A rat was exposed to a box with two compartments: a large, warm section lit by light, and a small, dark compartment with no light. Because rats are nocturnal creatures, they will naturally prefer the dark compartment and will try to hide there whenever possible. However, if the rat receives an electrical shock as punishment for going into a dark compartment, that stage of learning becomes extremely emotional and linked to pain and fear. Now the rat had efficiently learned that getting into this dark compartment of the box will punish it with the consequences of pain. This is called acquisition in the phase of learning. Retention is tested by comparing the time taken (latency) by the rat to enter the small cozy dark compartment (before and after the learning session). Increased latency to enter into the small cozy dark compart-

ment during retention test (after 48 hours) after unavoidable foot shock was considered as better retention performance.

Apparatus

The passive avoidance apparatus has a square shaped wooden box with a grid embedded floor of 19.5 × 19.5 inches and walls of 13.5 inches height. This box was brightened by 100 W bulb. The small dark compartment (6×6 inches) with the electrified floor grid was connected to a constant current stimulator. A (6×6 cm) opening in the partition between the two compartments was closed using a transparent plexiglass door.

Test procedure

The experiment will be carried out in three stages as follows.

Exploration-one day

On the day one, the Wistar rat was placed in the center of the box (large compartment) facing away from the sliding door of the small compartment. The plexi-door between the two compartments was raised and kept open. The animal was freely allowed to move around the entire apparatus (both compartments) for 5 minutes. The rats were then replaced to their cage.

Aversive stimulation

On the second day, the rat was again placed in the brightly illuminated large compartment and let on to explore the apparatus for 3 minutes with the plexi door kept open. The moment the rodent entered into the small dark compartment, the latency to enter into the dark, cozy compartment by the rodent was noted, and the plexi door was immediately shut closed and was delivered with three electric foot shocks (50 Hz, 1.5 mA, 1sec) through the floor grid using the current stimulator. The rat was kept back to its isolated new cage till the test was completed on all other rats. (The author believed this rat which got foot shock can always communicate to his cage mates about the shock and which might alter the behavior of other rats for the passive avoidance task).

Retention test

After the acquisition test has been completed for 48 hours, retention will begin. As a result, the rats were once more confined in the huge, brightly lit compartment and given three minutes to wander while the plexi door was left open. The rodents were returned to their original cage if the rat had entered the dark section once again. After a painful foot shock (during learning), a prolonged latency to enter a dark compartment during the retention phase (after 48 hours) was considered as a positive sign indicating significant retention performance.

Statistical analysis

Software GraphPad Prism will be used to do statistical analysis. We'll use one-way analyses of variance (one-way ANOVA) together with post hoc Newman-Keuls multiple comparison tests. Statistical significance will be determined by $p < 0.05$, and numerical data will be given as mean+/- SD.

Results

T-maze test: The results of these tests are showed in Tables 1 and 2. Animals treated with 10% and 20% BM extract showed a higher number of alternations when compared to the NC group. Similarly, rats treated with BM extract 10% and 20% showed lesser percentage bias in comparison with NC rats. During the rewarded alternation test, rats treated with of BM extract 10% and 20% showed a significant increase in the percentage of correct responses when compared to NC rats.

Group (n = 6/ group)	Spontaneous alternation test No of alternations % bias		Rewarded alternation test % of correct response
Negative control (NC)	15.1+/-1.5	65.0+/-4	75.2+/-5
BM 10%	18.12+/-1.7	54.75+/-3	95+/-4
BM 20%	17.0+/-1.2	56.0+/-3.5	92+/-3.5

Table 1: Results of T-maze (3 month treatment).

Note: Each value represents mean +/-SD, NCvsBM; $p < 0.05$.

In the 6-month treatment group, during the spontaneous alternation test, animals treated with the BM (10% and 20% 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, rats treated with BM extract showed a significant

increase in the percentage of correct responses when compared to the NC rats.

Group (n = 6/ group)	Spontaneous alternation test No of alternations % bias		Rewarded alternation test % of correct response
Negative control (NC)	16.25+/-2.3	60.1+/-3.2	70.15+/-3.9
BM 10% diet group	18.2+/-2.4	55+/-3.5	82.0+/-3.8
BM 20% diet group	18.6+/-2.5	52+/-2.4	92.65+/-5.7

Table 2: Results of T-maze (6 month treatment).

Note: Each value represents mean +/-SD, NC vs BM; $p < 0.05$.

Passive avoidance test results

Figures 1 and 2 shows the results of exploration and retention trials of Passive avoidance test.

In both 3 month and 6 month diet groups, there was no significant difference between animals treated with BM diet (10% and 20%) and NC in total time spent in small compartment during exploration trial. However, during the retention trial, it was seen that animals fed with BM diet spent significantly less time in the smaller compartment.

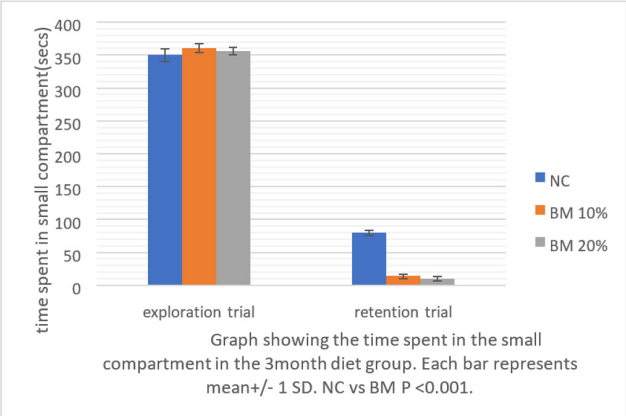

Figure 1

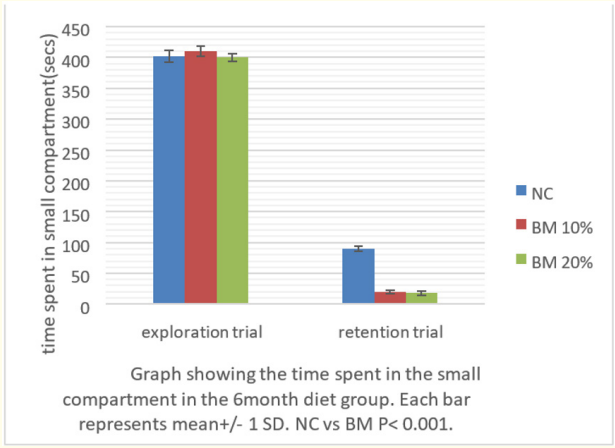

Figure 2

Discussion

The present study was designed to determine whether a BM diet administered at different strength and treatment duration would bring about any improvement in learning and memory in adult rats. In the 3month and 6-month treatment group, during the T-maze spontaneous alternation test, animals treated with the BM (10% and 20% showed significantly higher numbers of alternations and showed lesser percentage bias in comparison to NC rats. During the T-maze rewarded alternation test, rats treated with BM extract showed a significant increase in the percentage of correct responses when compared to the NC rats.

In the passive avoidance tests, no significant change observed in behaviour during exploration trial. But, during the retention trial, rats treated with BM diet (10% and 20%) spent less time in the smaller compartment, suggesting improved memory retention. In both 3 month and 6 month diet groups, same pattern was observed. These results clearly prove that BM extract improved learning and memory in rats.

Conclusion

We tried to establish the long-term consequences (either beneficial or adverse) of Brahmi diet in adult rats. Our study results clearly show that Brahmi diet improved the spatial learning and memory in rats when fed for longer period.

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