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# Evaluation of Nootropic activity of Two Marketed Drugs of *Bacopa monnieri* In Scopolamine Induced Amnesic Models

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## ABSTRACT

Evaluation of Nootropic activity of Two Marketed Drugs of *Bacopa Monnieri* In Scopolamine Induced Amnesic Models. The Learning and memory parameters were evaluated by using Morris water maze and Passive avoidance model in Swiss albino mice. The two marketed products of *Bacapa monnieri* was administered at a dose of 100 mg/kg body weight orally for 14 days to the respective groups. Piracetam (400mg/kg,i.p.) was used as a standard nootropic agent. It was observed that two products of *B.monniere* at the above-mentioned dose after 14 days of administration in the respective groups significantly reversed scopolamine (40mg/kg i.p.) induced amnesia, as evidence by a decrease in the acquisition and retension phases were in the and step down latency in the passive avoidance task. In this study brand 2 shown significant response when compare to brand-1, may be due to reduction in the brain ChE activity in mice may prove to be a useful memory restorative agent in the treatment of dementia seen in Alzheimer's disease.

## 1. INTRODUCTION

Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as cognition. Memory is a fundamental mental process and without it we are capable of nothing. It is a faculty by which sensations, impressions, and ideas are stored and recalled. Learning and memory is one of the most intensively studied subjects in the field of neuroscience. Dementia is a syndrome caused by disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation and comprehension, calculation, learning capacity, language and judgement. Aging demographic transition is proceeding rapidly especially in India, China, and Latin America, where dementia is rapidly becoming the major public health problem. Approximately 10% of the adults older than 65 years, and 50% of the adults older than 90 years have dementia.[1]

Medicinal herbs and plant extracts are now generally considered as effective medicines to be respected, appreciated and they play a major role in modern pharmacy. World Health Organization estimated that about 80% of the world's population relies on herbs for their primary health care needs[2]. Although, herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored. Numbers of plants are claimed to have medicinal uses and many researches are going on in this view.

Learning is utilized by many animals living in complex and changing habitats, enabling them to adapt their behavior to suit contemporary, local environmental conditions. The ability to learn and remember allows animals to draw on previous experience when faced with challenging decisions so that they can make the appropriate response. Spatial learning and memory is one area of behavioral research that has generated a large amount of interest recently because of its broad applicability, making it ideal for comparative studies[3]. The main aim of this type of behavioral research is to understand how animals navigate around their local habitat using learning and memory to aid in the relocation of food sources, shelter, and mates and to avoid potential dangers such as predators. These studies used a comparative approach to explore the differences in learning abilities and strategies of closely related species found in different environments to elucidate how natural selection shapes spatial learning abilities.

A study is reported on the effects of Brahmi (*Bacopa monnieri*) on memory. The results show a

significant effect of the Brahmi on a test for the retention of new information. Follow-up tests showed that the rate of learning was unaffected, suggesting that Brahmi decreases the rate of forgetting of newly acquired information. Tasks assessing attention, verbal and visual short-term memory and the retrieval of pre-experimental knowledge were unaffected. Questionnaire measures of everyday memory function and anxiety levels were also unaffected[4].

Due to more adverse effects of allopathic drugs people are more heading towards ayurvedic medicines. In order to avoid adverse drug reactions allopathic and ayurvedic drug combinations are used5. The present study plans to systematically evaluate *Bacopa monnieri* having various medicinal properties which is widely used in Alzheimer's disease. This study is designed to compare the two marketed products of *Bacopa monnieri* for its learning and memory deficit in mice with scopolamine (Hyoscine) induced amnesia and to know the efficient drugs in the market and to provide information about the best choice and to avoid economic waste for the patients.

## 2. MATERIALS AND METHODS

Healthy albino mice 20-35gm are used for the experiment were procured from the animal house of Shadan college of pharmacy, Peeran Cheru, Hyderabad. Animals were housed under standard conditions of temperature and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water. The Institutional animal ethics committee (IAEC) had approved the experimental protocol from Shadan Institute of medical sciences. Reg.No:1864/PO/Re/S/16/CPCSEA and care was taken as per guidelines of CPCSEA, Department of Animal Welfare Government of India.

Scopolamine, Brahmi (Brand-01 of Himalaya), Brahmi (Brand-02 of Amway neutralite), Piracetam were obtained from Spectrum Labs, Hyderabad. All other chemicals and reagents used were of analytical grade.

**Preparation of stock solution for dosing:**

**Preparation of *Bacapa monnieri* (Brand 01 i.e. Himalaya):** Two tablets of *Bacapa monnieri* each having a weight of 250mg.crushed in the mortar with the help of pistle and dissolved it in 50ml of water.

**Preparation of *Bacapa monnieri* (Brand 02 i.e. Amway Nutralite):** Two tablets each having a weight of 200gms.crushed in the mortar with the help of pestle and dissolved it in 20ml of water.

**Preparation of Scopolamine:** One ampoule of which contains 1ml of scopolamine (Concentrated solution) and make up the volume up to 150 ml with distilled water.

**Preparation of Piracetam:** One ml of Piracetam (Concentrated solution) and make up the volume up to 5ml with distilled water.

**Assessment of nootropic activity:** Most of the currently used paradigms for learning and memory can be conveniently discussed under behavioral tasks: 1.Behavior on mazes (ex: Morris Water Maze) 2.Passive Shock Avoidance. Mazes are the traditional tool in assessing learning and memory performance in laboratory animals**.**

**Methodology:** Chronic administration of piracetam causes significant memory impairment.

***In Vivo* Models:** Morris water maze method.

**Experimental Design:** Animals were trained to swim to a visible platform in a circular pool (60 cm in diameter and 20 cm in height) located in a test room. In principle, mice can escape from swimming by climbing onto the platform and over time the mice apparently learn the spatial location of the platform from any starting position at the circumference of the pool. The pool was divided into four equal quadrants and filled with water to a height of 40 cm. During the acquisition phase, a movable circular platform (9 cm diameter) was placed in one of the quadrants of the pool approximately 2 cm above the water level, and during the retention phase, a similar platform was placed in the pool 2 cm below the water level. The water was made opaque by adding a nontoxic dye and four locations were equally spaced around the edge of the pool (N, S, E, and W) and used as starting points for the acquisition phase.

**Animal Grouping:** Thirty Male Swiss albino mice were randomized into 5 groups (G1 –G5), each with 6 animals.

Animals in G1 are treated with only vehicle. (Normal Saline)

Animals in G2 are treated with scopolamine (0.4mg/kg) (I.P.) only on 6$^{th}$ day.

Animals in G3 are treated with *Bacapa monnieri* (brand-01) (100mg/kg p.o) and scopolamine, (0.4mg/kg i.p.) for 15 days.

Animals in G4 are treated with *Bacapa monnieri* (brand-02) (100mg/kg p.o.) and scopolamine, (0.4mg/kg i.p.) for 15 days.

Animals in G5 are treated with standard drug Piracetam (400mg/kg)(I.P.) and scopolamine (0.4mg/kg) (I.P.).

The dosing was done for a period of 14days and on day 14 amnesia was induced by scopolamine 0.4mg/kg IP to Group-02 to Group-05.The group-02 received just one dose of scopolamine on Day-14 itself 45mins after administration of scopolamine trial was conducted Step Down Latency was recorded and retention was observed for 24hours.

**Maze Acquisition Phase (training):** Animals received a training session consisting of four trials with a gap of 5 minutes between the two trials on Day 11-14. Four different starting positions were used during all four trials. A trial was started by releasing the animal into the maze facing the wall of the pool and the latency to find the escape platform was record to a maximum of 90 seconds. If the mice did not escape onto the platform within 90 seconds, it was guided to the platform and was allowed to remain there for 20 seconds. The time taken by the animal to reach the platform was considered as the initial acquisition latency.

**Maze Retention Phase (testing for retention of learned task):** Following training, the time taken to find the hidden platform (retention latency, RL) was assessed on Day 15 (24 hours after the last training session). In brief, the animals were released into the pool randomly at one of the edges facing the wall of the pool and the time taken to reach the platform was recorded. The change in RL from Daewy 11 to Day 15 was used to evaluate the learned skill or memory.

**Passive shock avoidance paradigm:**

**Experimental Design:** Passive avoidance behavior based on negative reinforcement was used to examine the long- term memory. The apparatus consisted of a box (27 X 27 X 27cm) having three walls of wood and one wall of Plexiglass, featuring a grid floor (3mm stainless steel rods set 8 mm apart), with a wooden platform (10 X 7 X 1.7 cm) in the centre of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20V AC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shock was delivered for 15 sec and the step-down latency (SDL) was recorded.SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with all its paws on the grid floor.

Animals showing SDL in the range (2-15sec) during the first test was used for the second session and the retention test. The second session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks was delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except that the electric shock was not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec.

**Animal Grouping:** Thirty Male albino mice were randomized into 5 groups (G1 –G5), each with 6 animals.

Animals in G1 are treated with only vehicle. (Normal saline)

Animals in G2 are treated with scopolamine (0.4mg/kg) (I.P.) only on 6$^{th}$ day.

Animals in G3 are treated with *Bacapa monnieri* (brand-01) (100mg/kg p.o) and scopolamine, (0.4mg/kg i.p) for 12 days.

Animals in G4 are treated with *Bacapa monnieri* (brand-02) (100mg/kg p.o.) and scopolamine (0.4mg/kg i.p) for 12 days.

Animals in G5 are treated with standard drug Piracetam (400mg/kg) (I.P.) and scopolamine (0.4mg/kg) (I.P).

The dosing was done for a period 14days and on day 14 amnesia was induced by scopolamine 0.4mg/kg IP to Group-02 to Group-05.The group-02 received just one dose of scopolamine on Day-14 itself 45mins after administration of scopolamine trial was conducted Step Down Latency was recorded and retention was observed for 24hours.

## 3. RESULTS AND DISCUSSION

**Morris water maze test:**

**Table.1.Effect of *Bacapa monnieri of* Brand-1 and Brand-2 on Morris water maze test in scopolamine induced amnesia**

| Groups | Treatment | Acquisition phase in sec | Retention phase in sec |
|---|---|---|---|
| I | Normal Saline | 5.51±2.43 | 7.81 ±3.22 |
| II | Control : Scopolamine hydrobromide,1mg/kg i.p | 11.87 ±1.73 | 10.25± 5.22 |
| III | Brand-1(Himalaya) *B.monnieri* 100 mg/kg,p.o.+ scopolamine(1), i.p | 9.37±4.11 | 6.31 ±1.32 |
| IV | Brand-2 (Nutralite) *B.monnieri* 100 mg/kg, p.o. + scopolamine (i.p) | 4.25± 3.55 | 5.62 ±3.21 |
| V | Standard (Piracetam) 400mg/kg i.p. | 4.81 ±2.22 | 6.87 ±4.22 |

**All the values are expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's post-test compared with control**

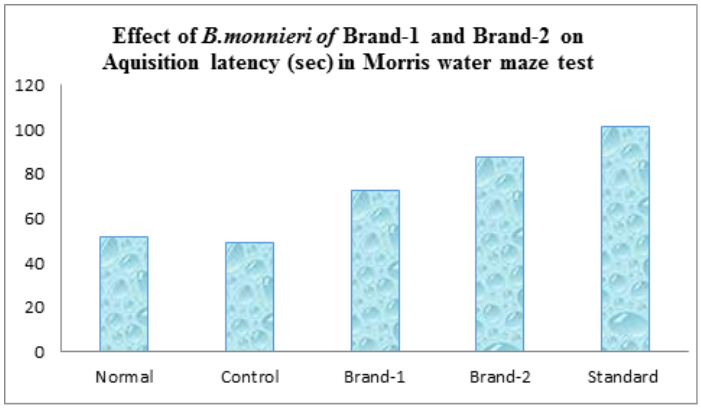


**Figure.1.Effect of *Bacapa monnieri* of Brand-1 and Brand-2 on Acquisition latency (sec) in Morris water maze test in scopolamine induced amnesia**

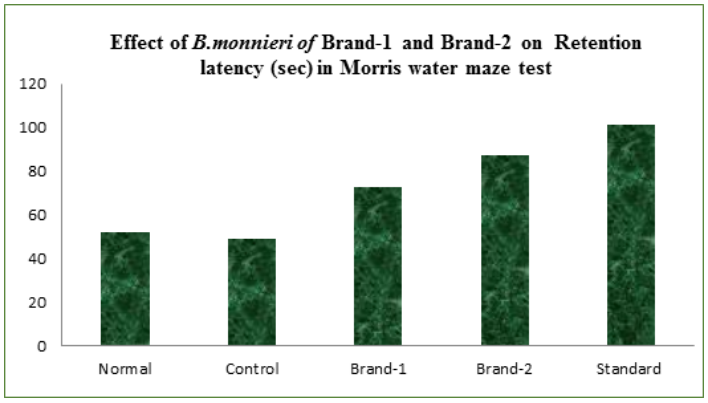


**Figure.2.Effect of *Bacapa monnieri* of Brand-1 and Brand-2 on Retention latency (sec) in Morris water maze test in scopolamine induced amnesia**

Oral administration of scopolamine results in the deterioration of learning and memory skills in albino mice, which is supported by the literature reports cited in the present study. Animals treated only with scopolamine showed learning and memory deficits in the Morris water maze task compared to normal controls ($p < 0.01$). However Brand-1(Himalaya) and Brand- 2 (Nutralite) 100 mg/kg and piracetam (400 mg/kg; $p < 0.01$) offered treated groups significant protection against scopolamine-induced learning and memory deficits. Interestingly, the Brand-2 (Nutralite) (100 mg/kg) and piracetam (400 mg/kg; $p < 0.01$) was found to be therapeutically more potent than Brand-1(Himalaya) 100mg/kg ($P<0.05$) against scopolamine-induced toxicity the results of the study are given in Table-1 and depicted in Figure 1 and 2

**Passive shock avoidance:**

**Table.2.Effect of *Bacapa monnieri of Brand*-1 and Brand-2 on Step down latency (Passive avoidance paradigm) in scopolamine induced amnesia**

| Groups | Treatment | SDL(sec) 14th day ± SEM on acquisition day | SDL (sec)15th day ± SEM on retention day |
|---|---|---|---|
| I | Normal Saline | 44.67±1.453 | 52.00 ±1.983 |
| II | Control : Scopolamine hydrobromide,1mg/kg i.p | 36.67±0.44** | 49.00±1.21** |
| III | Brand-1 (Himalaya)*B.Monniera* 100 mg/kg ,p.o.+ scopolamine(1),i.p | 53.10±1.38* | 72.50±0.25*** |
| IV | Brand-2 (Nutralite) *B.Monniera* 100 mg/kg, p.o. + scopolamine(i.p) | 85.13±33.27*** | 87.47±1.16*** |
| V | Standard (Piracetam) 400mg/kg i.p. | 96.17±3.45*** | 101.2±1.55*** |

**All the values are expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's post-test compared with control**

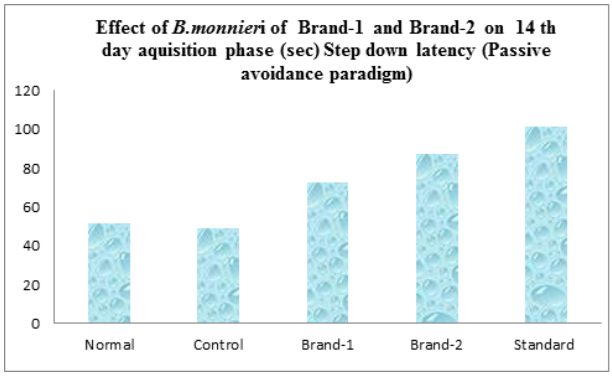


**Figure.3.Effect of *Bacapa monnieri* of Brand-1 and Brand-2 on 14th day acquisition phase (sec) Step down latency (Passive shock avoidance) in scopolamine induced amnesia**

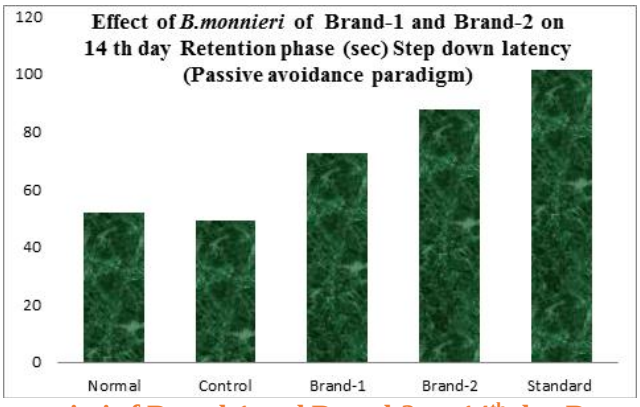


**Figure.4.Effect of *Bacapa monnieri* of Brand-1 and Brand-2 on 14th day Retention phase (sec) Step down latency (Passive shock avoidance) in scopolamine induced amnesia**

Effect of *Bacapa monnieri* on learning and retention was tested using passive avoidance paradigm apparatus. When compared to vehicle control group scopolamine induced animals exhibited decreased SDL. Piracetam treated group showed significant ($P<0.001$) increase in the SDL. On 14th day *Bacapa monnieri* brand-1 and 2 (100 mg/kg, p.o.) treated groups and Piracetam showed increase in the SDL as compared to scopolamine induced animals on day 14. However, Brand-2 (Nutralite) 100mg/kg dose there was significant increase in SDL. On day 15, Piracetam and Brand-1 and 2 *Bacapa monnieri* 100mg/kg, p.o.) treated groups showed significant increase ($p<0.001$) in SDL. Brand-2 (Nutralite) at the dose of 100mg/kg, p.o. had significant effect ($P<0.001$) as of standard drug Piracetam 400mg/kg, i.p. The results are tabulated in the Table 2 and depicted in Figure 3 and 4.

**Discussion:** In most therapeutic areas, multiple drug options are increasingly becoming available, but there is often a lack of evidence from head-to-head clinical trials that allows for direct comparison of the efficacy and/or safety of one drug *vs.* another. Herbal medicines have been used throughout written history, and probably even longer. Archaeological evidence suggests the use of herbal medicines for various conditions as early as 60,000 years ago. More recently, the use of specific extracts of herbal medicines was popular in the United States and Canada from the nineteenth century until the 1930s before slowly falling out of favor with the advent of modern pharmaceuticals. Resurgence was noted in the 1970s when studies from several European countries (most notably Germany) began applying scientific principles to testing the use of herbal medicines in clinical settings. Preclinical and clinical research on this topic has been increasing at a staggering rate in recent years.

Ayurvedic and other herbal medications have gained increased acceptance as they are found to be safer than the synthetic counterparts. Ayurveda or the Indian system of Medicine viz. Sushruta Samhita, Charak Samhita, and Atharva Veda describe plants which have a prabhava (specific action) on the intellect and memory as Medhya Rasayana (Medhya - intellect or retention, Rasayana - procedure or preparation).

Traditionally, Mandukaparni, Yastimadhu, Guduchi, and Shankhapushpi have been mentioned to have a memory enhancing action. Others like Brahmi, Vacha, and Jatamamsi although inadequately emphasized have been known for their efficacy. Vedic scholars of ancient India have been known to consume Medhya Rasayana that helped them memorize lengthy scriptures 3,000 years ago.

There is also evidence for potential attenuation of dementia, Parkinson's disease, and epilepsy. Current evidence suggests BM acts via the following

mechanisms—anti-oxidant neuroprotection (via redox and enzyme induction), acetylcholinesterase inhibition and/or choline acetyltransferase activation, β-amyloid reduction, increased cerebral blood flow, and neurotransmitter modulation (acetylcholine [ACh], 5-hydroxytryptamine [5-HT], dopamine [DA]). BM appears to exhibit low toxicity in model organisms and humans; however, long-term studies of toxicity in humans have yet to be conducted. Nootropic agents or cognition enhancers are few synthetic medicines e.g. tacrine, donepezil and the natural product based rivastigmine for treatment of cognitive dysfunction and memory loss associated with dementia. Reported to have adverse effects including gastrointestinal disturbances and problems associated with bioavailability, which necessitates the interest in finding better from natural resource. For many years, the amnesia action produced in animals by the administration of intra peritoneal, particularly scopolamine has been a widely used model for the characterization of potential cognition enhancing drugs. Scopolamine induced amnesic rodent model is one of the well-established animal model for memory dysfunction. Scopolamine-induced amnesia was proposed to be due to blockage of cholinergic neurotransmission, this substance is used to model the cognitive deficits that could be observed in dementia. Systemic administration Scopolamine induces central cholinergic blockade, produced a reversible and well described impairment in both maintaining attention and processing of information and the acquisition of new knowledge in rodents and in humans.

In the present study' Animals treated only with scopolamine showed learning and memory deficits in the Morris water maze task compared to normal controls, however Brand-1(Himalaya) and Brand- 2 (Nutralite) 100 mg/kg and piracetam (400 mg/kg) offered treated groups significant protection against scopolamine-induced learning and memory deficits. Interestingly, the Brand-2 (Nutralite) (100 mg/kg) and piracetam (400 mg/kg) was found to be therapeutically more potent than Brand-1(Himalaya) 100mg/kg against scopolamine-induced toxicity the results of the study are given in Table-1. Effect of *Bacapa monnieri* on learning and retention was tested using passive avoidance paradigm apparatus. When compared to vehicle control group scopolamine induced animals exhibited decreased SDL. Piracetam treated group showed significant increase in the SDL. On 14$^{th}$ day *Bacapa monnieri* brand-1 and 2 (100 mg/kg, p.o.) treated groups and Piracetam showed increase in the SDL as compared to scopolamine induced animals on day 14. However, Brand-2 (Nutralite) 100mg/kg dose there was significant increase in SDL. On day 15, Piracetam and Brand-1 and 2 *Bacapa monnieri* 100mg/kg, p.o.) treated groups showed significant increase in SDL. Brand-2 (Nutralite) at the dose of 100mg/kg, p.o. had significant effect as of standard drug Piracetam 400mg/kg, i.p. Piracetam, the established nootropic agent was used in the present study as standard because; it improves memory by facilitation of synaptic transmission (increase choline uptake in cholinergic nerve endings, thereby facilitating cholinergic transmission) in brain.

## 4. CONCLUSION

No two molecules are exactly the same. Even minor differences in molecular structure can sometimes result in important differences in pharmacological activity Alzheimer's disease is a neurodegenerative disorder currently without an effective treatment. Impairment of memory is the initial and most significant symptom of AD. AD is associated with a decline in cognitive abilities. The most common cause of dementia in the elderly is probably AD. Despite the severity and high prevalence of this disease. The central cholinergic system plays an important role in learning and memory. In this study brand 2 shown significant responses when compare to brand-1, may be due to reduced the brain ChE activity in mice may proved to be a useful memory restorative agent in the treatment of dementia seen in Alzheimer's disease. *Bacapa monnieri* contains majorly Steroids and antioxidant prosperity which may responsible for the anti-amnesic effect

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