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Nootropic activity of BacoMind™, an enriched phytochemical composition from *Bacopa monnieri*

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Abstract

The objective of this study was to evaluate the nootropic activity of BacoMind™, an enriched phytochemical composition from *Bacopa monnieri*, in different learning and memory paradigms *viz*., elevated plus maze, passive shock avoidance test and object recognition test. BacoMind™ was administered for 7 days at the dose of 40, 60 and 80 mg/kg to mice in elevated plus maze and passive shock avoidance test and 27, 40 and 54 mg/kg to rats in object recognition test. Scopolamine (0.3 mg/kg) was used to induce amnesia and piracetam (100 mg/kg) served as reference standard. In elevated plus maze test, BacoMind™ significantly ($p<0.01$) increased the inflexion ratio in scopolamine treated mice. In passive shock avoidance test, BacoMind™ significantly ($p<0.001$) reduced the latency to reach the shock free zone and number of mistakes in 15 min in both normal as well as scopolamine treated mice. In object recognition test, BacoMind™ significantly ($p<0.001$) increased the discrimination index in both normal as well as scopolamine treated rats. Thus, the findings of the present study revealed the nootropic activity of BacoMind™.

Keywords: BacoMind™, *Bacopa monnieri*, Nootropic activity, Bacosides, Elevated plus maze, Passive shock avoidance test, Object recognition test.

1. Introduction

Bacopa monnieri (*B. monnieri*), also referred to as *Bacopa monniera*, *Herpestis monniera*, water hyssop, and "Brahmi," has been in use since time immemorial as nerve tonic for improvement of memory. *B. monnieri* is a perennial creeping plant found throughout India in wet, damp and marshy areas [1, 2]. An infusion of the plant has been used in Indian folklore as a nerve tonic [3]. Traditionally, it was used as a brain tonic to enhance memory development, learning and concentration [4] and to provide relief to patients with anxiety or epileptic disorders [5].

The plant, plant extracts and isolated bacosides have been investigated for nootropic activity.

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Preliminary studies revealed that the treatment with *B. monnieri* plant [6, 7], its aqueous decoction [8] and the alcoholic extract [9] enhanced learning ability in rats. Alcoholic extract of *B. monnieri* has shown cognition facilitating effect in normal rats [10] and inhibited the amnesic effects of scopolamine, electroshock and immobilization stress [11]. In different behavioural response studies, alcoholic extract of *B. monnieri* facilitated the cognitive function and augmented the mental retention capacity [12]. Vohora *et al*., (2000) [13] have shown the potential protective effect of B. monnieri in phenytoin-induced cognitive deficit in mice by both acquisition and retention of memory without affecting its anticonvulsant activity. The butanolic extract of *B. monnieri* has shown memory enhancing effect in rats by increasing recognition index in differential exploration of familiar and new objects test [14].

Recently, Kishore and Singh (2005) [15] have reported that anterograde administration (before training) of alcoholic extract of *B. monnieri* in mice facilitated anterograde memory and attenuated anterograde experimental amnesia induced by scopolamine and sodium nitrite. The standardized extract of *B. monnieri* has also shown potent cognitive enhancing activity by attenuating the dementia effect of scopolamine in passive avoidance test [16]. A recent study reveals *B. monniera* extract is able to reduce amyloid levels in PSAPP mice which is a transgenic mice expressing the "Swedish" amyloid precursor protein and M146L presenilin-1 mutations [17].

The major chemical constituents shown to be responsible for the memory-facilitating action of *B. monnieri* are the steroidal saponins bacoside A and B, as these compounds have shown to exert facilitatory effects on mental retention in avoidance response in rats [18].

Based on the above, an enriched phytochemical composition, BacoMind™, was developed from *B. monnieri* extract for use as a cognition enhancing agent and it differs from the previously reported standardized extracts, in that it has been standardized to nine different bioactive constituents and with reference to *in vitro* bioassays. Hence, in order to establish the nootropic activity of this phytochemical composition, BacoMind™, the current study was undertaken in different learning and memory paradigms.

2. Materials and methods

2.1 Test substance

BacoMind™, an enriched phytochemical composition of *B. monnieri* extract was obtained from Natural Remedies Pvt. Ltd, Bangalore. BacoMind™ (patent pending) was standardized to the content of the following bioactive constituents *viz*., bacoside A_3 (>5.0% w/w), bacopaside I (>7.0% w/w), bacopaside II (>5.5% w/w), jujubogenin isomer of bacopasaponin C (>7.0% w/w), bacopasaponin C (>4.5% w/w), bacosine (>1.5% w/w), luteolin (>0.2% w/w), apigenin (>0.1% w/w) and β-sitosterol-D-glucoside (>0.3% w/w). It was further standardized using the following *in vitro* bioassays *viz*., lipoxygenase inhibition assay (IC_{50}< 600 µg/ml), ABTS radical scavenging assay (IC_{50}< 100 µg/ml), DPPH assay (IC_{50}< 200 µg/ml) and butyrylcholinesterase inhibition assay (IC_{50}< 3000 µg/ml).

2.2 Animals

Albino Wistar rats (150-175 g) and albino Swiss mice (20-22 g) of either sex were procured from National Toxicology Center, Pune and housed three animals per cage with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30-70 %. A 12:12 h light and dark cycle was followed. The animals had free access to feed and water *ad libitum*.

2.3 Drugs and Chemicals

Piracetam and scopolamine obtained from UCB India Ltd., Mumbai *as gratis* were used for this study.

2.4 Assessment of nootropic activity

2.4.1 Treatment schedule

In elevated plus maze and passive shock avoidance test, mice in groups of six were treated with vehicle or BacoMind™ (40, 60 and 80 mg/kg for 7 days, *p.o.*). Scopolamine (0.3 mg/kg, single dose, *i.p.*) was used to induce amnesia and piracetam (100 mg/kg, single dose, *i.p.*) served as reference standard. Another group was treated both with BacoMind™ (60 mg/kg, 7 days, *p.o.*) and scopolamine (0.3 mg/kg, single dose, *i.p.*).

In object recognition test, rats in groups of six were treated with vehicle or BacoMind™ (27, 40 and 54 mg/kg, 7 days, *p.o.*). Scopolamine (0.3 mg/kg, single dose, *i.p.*) was used to induce amnesia and piracetam (100 mg/kg, single dose, *i.p.*) served as reference standard. Another group was treated both with BacoMind™ (40 mg/kg, 7 days, *p.o.*) and scopolamine (0.3 mg/kg, single dose, *i.p.*).

2.4.2 Elevated plus maze test

The elevated plus maze consisted of two open arms (25 x 5 cm) crossed with two enclosed arms (25 x 5 x 20 cm). The arms were connected to a central platform (5 x 5 cm). The apparatus was elevated to a height of 25 cm in a dimly illuminated (25 W) room [19].

The mice were placed individually at the end of open arm of the elevated plus maze facing away from the center. The time taken by the mouse to move into the enclosed arm was noted as transfer latency (TL). On day 6, TL (L_1) was recorded before administration of BacoMind™. After determination of the TL, mice were allowed to explore the maze for 2 min and then transferred to their home cages. The TL (L_0) was again measured after 24 h *i.e.* 30 min after the administration of BacoMind™ on day 7. The TL was expressed as inflexion ratio.

$$\text{Inflexion ratio} = \frac{L_1 - L_0}{L_0}$$

Where L_0 is the TL after 24 h and L_1 is the initial TL.

2.4.3 Passive shock avoidance test

The apparatus consisted of an electric grid with a shock free zone (SFZ, 2 x 3 x 1 cm) in the center and the entire grid having a perplex enclosure. After 20 min of treatment on day 7, mice were placed individually on the electric grid and allowed to explore for one minute. A stimulus of 20 V with AC current of 5 mA was given and latency to reach SFZ was recorded for three consecutive times and considered as basal value. After 1 h of the first trial, each animal was placed on the electric grid again and the latency to reach SFZ and the mistakes (descents) the animal made in 15 min were recorded and considered as parameters for acquisition and retention respectively.

2.4.4 Object recognition test

Object recognition test apparatus consisted of white coloured box (60 x 60 x 30 cm). The apparatus was illuminated by a 60 W bulb suspended 50 cm above the box. The objects of different shapes to be discriminated were made of plywood but coloured black. The height of the objects was 8 cm [20].

The day before testing, the animals were allowed to explore the box for 2 min. On day 7 after 30 min of last dose, a session of two trials were given. An intertrial interval of 60 min was kept. In the first trial (T1), two identical objects were presented in the opposite corners of the apparatus and the amount of time taken by each animal to complete 20 sec of object exploration

was recorded. Exploration was considered, directing nose at a distance < 2 cm to the object or touching it with nose. During the second trial (T2), one of the objects presented in T1 was replaced by a new object and the animal was left individually in the apparatus for 5 min. The time spent for exploration of the familiar (F) and new (N) objects were recorded and Discrimination Index (D) was calculated as follows [21].

$$\text{Discrimination index (D)} = \frac{N - F}{N + F}$$

2.5 Statistical analysis

The values are expressed as Mean ± SEM. Statistical significance was analyzed employing one-way ANOVA followed by Dunnett's or Bonferoni test as the post-hoc method.

3. Results

3.1 Elevated plus maze test

BacoMind™ (40, 60 and 80 mg/kg) and piracetam showed a non-significant increase in the inflexion ratio whereas scopolamine showed a significant decrease in inflexion ratio as compared to the vehicle control. BacoMind™ (60 mg/kg) administered orally for 7 days protected the animals from scopolamine induced impairment in learning and memory and increased the inflexion ratio as compared to the scopolamine treated group (Table 1).

Table 1. Effect of BacoMind™ on transfer latency in elevated plus maze model expressed as inflexion ratio in albino Swiss mice

	Treatment groups	Inflexion ratio
I	Vehicle control (10 ml/kg)	0.76 ± 0.03
II	BacoMind™ (40 mg/kg)	0.85 ± 0.02
III	BacoMind™ (60 mg/kg)	0.81 ± 0.04
IV	BacoMind™ (80 mg/kg)	0.80 ± 0.02
V	Piracetam (100 mg/kg, single dose)	0.78 ± 0.01
VI	Scopolamine (0.3 mg/kg, single dose)	0.36 ± 0.02*
VII	BacoMind™ (60 mg/kg for 7 days) + Scopolamine (0.3 mg/kg, single dose)	0.68 ± 0.02#

Values are expressed as mean ± SEM; n = 6.
*p < 0.001 - vehicle control Vs scopolamine control.
#p < 0.01 - scopolamine control Vs scopolamine + BacoMind™.

Table 2. Effect of BacoMind™ on the latency to reach SFZ and the number of mistakes in 15 min in passive shock avoidance test model in albino Swiss mice

	Treatment groups	Latency to reach SFZ	Mistakes in 15 min
I	Vehicle control (10 ml/kg)	11.33 ± 1.33	19.50 ± 0.42
II	BacoMind™ (40 mg/kg)	7.16 ± 0.40*	13.60 ± 0.66*
III	BacoMind™ (60 mg/kg)	6.80 ± 0.40*	7.50 ± 0.42*
IV	BacoMind™ (80 mg/kg)	6.10 ± 0.30*	9.30 ± 0.49*
V	Piracetam (100 mg/kg, single dose)	4.00 ± 0.36*	7.50 ± 0.56*
VI	Scopolamine (0.3 mg/kg, single dose)	15.17 ± 0.79*	24.33 ± 1.76
VII	BacoMind™ (60 mg/kg for 7 days) + Scopolamine (0.3 mg/kg, single dose)	10.00 ± 0.51#	5.80 ± 0.74#

Values are expressed as mean ± SEM; n = 6, *p < 0.001 - vehicle control Vs BacoMind™/ piracetam/ scopolamine control, #p < 0.001 - scopolamine control Vs scopolamine + BacoMind™.

Table 3. Effect of BacoMind™ in object recognition test on the time to explore objects, expressed as discrimination index in albino Wistar rats

	Treatment groups	Discrimination index
I	Vehicle control (10 ml/kg)	0.162 ± 0.09
II	BacoMind™ (27 mg/kg)	0.400 ± 0.02*
III	BacoMind™ (40 mg/kg)	0.445 ± 0.017*
IV	BacoMind™ (54 mg/kg)	0.503 ± 0.01*
V	Piracetam (100 mg/kg, single dose)	0.428 ± 0.01*
VI	Scopolamine (0.3 mg/kg, single dose)	0.152 ± 0.02*
VII	BacoMind™ (40 mg/kg for 7 days) + Scopolamine (0.3 mg/kg, single dose)	0.385 ± 0.009#

Values are expressed as mean ± SEM; n = 6.
*$p < 0.001$ - vehicle control Vs BacoMind™ /piracetam /scopolamine control.
#$p < 0.001$ - scopolamine control Vs scopolamine + BacoMind™.

3.2 Passive shock avoidance test

BacoMind™ (40, 60 and 80 mg/kg) improved memory retention and showed significant decrease in latency to reach SFZ and number of mistakes in 15 min as compared to vehicle control. Piracetam also showed significant decrease in latency to reach SFZ and number of mistakes in 15 min as compared to vehicle control whereas scopolamine showed significant increase in latency to reach SFZ as compared to vehicle control. BacoMind™ when administered for 7 days not only facilitated the retention but also alleviated the scopolamine induced impairment of retention by significantly decreasing the latency to reach SFZ and number of mistakes in 15 min as compared to scopolamine treated group (Table 2).

3.3 Object recognition test

The results revealed a significant effect of BacoMind™ (27, 40 and 54 mg/kg) on the object recognition by significantly increasing the discrimination index as compared to the vehicle control. The reference standard piracetam also significantly increased the discrimination index while scopolamine significantly decreased the discrimination index. BacoMind™ (40 mg/kg) administered for 7 days improved memory by preventing scopolamine induced amnesia and significantly increased the discrimination index as compared to scopolamine treated group (Table 3).

4. Discussion

The remarkable ability of living creatures to optimize behavior based on past experiences is a result of the brain's ability to rapidly acquire new skills and consolidate into long-term memory that seems to be useful for future use. As stated more than 50 years ago by Donald Hebb in his 'dual trace model', when a task is being acquired, it is initially stored in short-term memory, and through consolidation, the same memory trace is transformed into long-term memory storage [22]. In general, learning is defined as the acquisition of information and skills, and subsequent retention of the information is called memory. Memory function is vulnerable to a variety of pathologic processes including neurodegenerative diseases like Alzheimer's disease, stroke, tumors, hypoxia, cardiac surgery, malnutrition, depression, anxiety, side effects of medication and normal ageing [23]. Memory loss is often the most disabling feature of many disorders, impairing the normal daily activities of the patients and profoundly affecting their families [24]. In the current scenario, it has become mandatory to find new therapy to prevent and treat memory impairment resulting from diseases of brain and associated with age.

Nootropic drugs are a class of psychotropic drugs that enhance learning, acquisition and reverse learning impairments in experimental animals, and are likely to be clinically effective in memory dysfunctions [25] and also improve memory in absence of cognitive deficit [26]. In the present study, nootropic activity of BacoMind™, an enriched phytochemical composition from *B. monnieri* extract, was evaluated in different learning and memory paradigms in rats and mice.

Many experimental models are currently available for the evaluation of agents that affect learning and memory process. Mazes are traditional tools in assessing learning and memory performance in laboratory animals. Originally designed to evaluate the antianxiety agents, elevated plus maze has also been recently extended to measure the spatial long-term memory in animals [27, 28]. Passive avoidance behavior is used to examine the long term memory based on negative reinforcement [29]. Object recognition test measures nonspatial memory with the characteristics of episodic memory [21].

In the present study, BacoMind™ improved significantly the learning and retention in normal rats in all the models tested except in elevated plus maze wherein a non significant improvement was noticed. Similarly, alchololic extract and bacosides of *B. monnieri* reported to improve acquisition, consolidation and retention in various animal models [9, 12]. The results indicate that BacoMind™ can be used to enhance memory in normal subjects. In addition, BacoMind™ also protected the animals from scopolamine induced impairment in learning and memory. Similarly, bacosides and extracts of *B. monnieri* inhibited the scopolamine [11, 15, 16] induced amnesia. Scopolamine, a centrally acting acetylcholine blocker, is known to cause amnesia similar to Alzheimer's disease by interfering with acetylcholine transmission in the central nervous system [30]. Protective effect of BacoMind™ on scopolamine induced amnesia indicates possible neuroprotective role of BacoMind™ and can be useful in neurodegenerative diseases. Episodic memory evaluated by object recognition test is sensitive to the effects of ageing and cholinergic dysfunction [20, 31]. In patients suffering from neurodegenerative diseases like Alzheimer's disease, episodic memory is also impaired earlier in the disease. The significant increase in discrimination index by BacoMind™ in object recognition test indicates possible improvement on episodic memory and can be useful in memory disorders, especially of episodic memory observed during ageing and at the initial stages of various chronic neurodegenerative diseases. Similarly, butanolic extract of *B. monnieri* showed memory enhancing effect in differential exploration of new and familiar objects [14].

The precise mechanism by which BacoMind™ elicites its nootropic effect is not known. But the mechanism of action of bacosides could be attributed to a combination of cholinergic modulation by acetylcholine release and muscarinic cholinergic binding [32], membrane dephosphorylation with a concomitant increase in protein and RNA turnover in specific brain areas [33], enhancement of protein kinase activity in the hippocampus [12], antioxidant effect [34, 35] and antistress effect [36].

In conclusion, based on the findings of the present study, BacoMind™ revealed nootropic activity by enhancing acquisition and retention of memory and can be useful in enhancing memory in normal and cognition impaired subjects.

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