Effect of Bramhi Ghrita, an polyherbal formulation on learning and memory paradigms in experimental animals

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ABSTRACT

Objective: To investigate the neuropsychopharmacological effect of a polyherbal formulation Bramhi Ghrita (BG) on learning and memory processes in rats by elevated plus maze, and in mice by Morris water maze model.

Material and Methods: BG contains Bacopa monneri (Bramhi), Evolvulus alsinoids, Acorus calamus, Saussurea lappa and cow’s ghee. Its effect (30, 50 and 100 mg/kg, p.o.) was tested on learning and memory processes. The activity of BG on memory acquisition and retention was studied using elevated plus maze model (EPM) in rats, and on spatial memory using Morris water maze model (MWM) in mice. The alcoholic extract of Bacopa monneri (40 mg/kg, p.o.) was also administered to one group of animals. The results were compared with the vehicle-treated group.

Results: Administration of Bramhi Ghrita (50 and 100 mg/kg, p.o.) showed significant reduction in transfer latency in EPM and escape latency in MWM as compared with the control group.

Conclusion: BG may act as a memory enhancer formulation and may also be useful as a supportive adjuvant in the treatment of impaired memory functions.

KEY WORDS: Bramhi ghrita, learning and memory, elevated plus maze, Morris water maze, Panchagavya

Introduction

Bramhi Ghrita is one of the polyherbal formulations mentioned in Ayurveda (an Indian system of medicine) containing Bacopa monneri (8 g), Acorus calamus (4 g), Evolvulus alsinoids (4 g), Saussurea lappa (4 g) and cow’s ghee (80 g). This formulation has been used traditionally as a memory enhancer and for its anticonvulsant activity. Bacopa monneri is a well-known nootropic plant reported for its tranquilizing, sedative, cognitive enhancer, hepatoprotective and antioxidant actions. Acorus calamus is known for its carminative, sedative and tranquilizing actions. Saussurea lappa has been reported to possess antiinflammatory and immunostimulant actions. Evolvulus alsinoids has been used traditionally as brain tonic, sedative, anthelmintic, antiepileptic and against leucodermia. Cow’s ghee (clarified butter fat) is believed to be useful as a memory enhancer, and antiinflammatory agent.

Learning is defined as the acquisition of information and skills, while subsequent retention of that information is called memory. One of the challenging tasks for neuroscientists is to elucidate the biochemical and molecular mechanisms underlying learning and memory. To assess the learning and memory paradigms in laboratory animals, mazes are used conventionally. There is a lack of scientific data regarding the effect of BG on learning and memory. The present study was, therefore, carried out for the authentication of traditional claims of BG as a memory enhancer using two animal models, namely elevated plus maze (EPM) and Morris water maze task (MWM).

Material and Methods

Animals

Male Swiss albino mice (25-30 g, body weight) and albino rats (150-200 g body weight) were used. The animals were housed in groups of five in standard laboratory conditions of temperature (23 ± 1°C), relative humidity (55 ± 5%), lighting (08:00-20:00 h) with food (Lipton India Ltd. pellets) and water freely available. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed between 08:00-16:00 h. The Institutional Animal Ethical Committee constituted for the purpose approved the protocol.
Bramhi Ghrita (BG)

The formulation BG was obtained as a gift sample for research from the Go-Vigyan Anusandhan Kendra, Deolapar Dist. Nagpur, India, which was prepared by an expert Ayurvedic practitioner. The formulation was used as received in the present study.

Plant extract

The aerial parts of *Bacopa monnieri* (BM) were collected from the local forest of Deolapar and dried under shade. The plant material was authenticated by the Department of Botany, Nagpur University, Nagpur. The plant material (750 g) was then subjected to defatting with petroleum ether (60-80) and extracted with ethanol (95% v/v) using Soxhlet extractor. The ethanol was removed from the extract by distillation under reduced pressure to obtain a dried residue. The dried extract (yield 6 g) was used to compare the activity of BG on different learning and memory paradigms.

Experimental

The formulation was fed up to 2 g/kg to the animals (rats and mice) and they were observed for 24 h for mortality if any.

1. Elevated plus maze

Rats were divided into 5 groups of 6 animals each as follows: Group I animals served as control and received distilled water (10 ml/kg, p.o.). Group II animals received alcoholic extract of BM 40 mg/kg orally for comparison. Groups III, IV and V were fed orally with BG (30, 50 and 100 mg/kg). The EPM apparatus used in the study consisted of two open and two closed arms facing each other. The maze was elevated at a height of 50 cm from the ground. The animals were placed individually 30 min after oral administration of either vehicle or test drug at the end of either of the open arms and the time taken by the animal to move from open to closed arm (Transfer latency) was noted on the first day. The time elapsed between the time that the animal was placed on the open arm and the time at which all 4 legs were inside the enclosed arms, was noted as transfer latency. The transfer latency was again recorded 24 h after the first exposure.

2. Morris water maze task

Mice were grouped as 6 animals each in 5 groups as follows: Group I animals served as control and received vehicle only. Group II animals received alcoholic extract of BM 40 mg/kg orally. Groups III, IV and V received BG in doses of 30, 50 and 100 mg/kg orally. The apparatus used is a circular water tank (100 cm in diameter) filled to a depth of 30 cm with water (25°C). Four points equally distributed along the perimeter of the tank served as starting locations. The tank was divided arbitrarily into four equal quadrants and a small platform (5 cm width) was located in the center of one of the quadrants. The platform remained in the same position during the training days. The mice were released into the water and allowed 90 s to find the platform. Animals received 4 trials for the first day and 8 trials per day with 5 min. inter-trial interval for 8 days until the performance was stable and the latency to find the platform was low (<10 sec). The test formulations were administered 30 min prior to the first trial daily. Time to find the hidden platform is considered as escape latency. The platform in the water maze was kept at the same position throughout the test to assess the effect of BG on spatial reference memory.

Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s test. The level of significance was set at $P<0.05$.

Results

The formulation BG was safe on oral administration up to the dose of 2000 mg/kg. No mortality was observed up to this dose.

1. Elevated plus maze

The rats showed a significant decrease in transfer latency in all groups (including control) on the second day. The analysis revealed significant differences in transfer latency in EPM performance between BG (100 mg/kg) and vehicle-treated animals on both the days ($P<0.05$). The results were comparable with the alcoholic extract of BM. (Figure 1).

2. Morris water maze

The time required to reach the hidden platform in the water maze is illustrated in Figure 2. There was a significant difference between BG and vehicle-treated animals on Days 2-5 and 8. Data for Days 1, 6 and 7 did not show a significant difference. The mice treated with BG (50 and 100 mg/kg) showed a significant decrease in escape latency as compared with the control group (Figure 2). The alcoholic extract of BM also showed a decrease which was not statistically significant when compared with control. The BG (100 mg/kg) treated animals reached the hidden platform in significantly lesser time than vehicle-treated animals.

Figure 1: Effect of Bramhi Ghrita on spatial memory in elevated plus maze in rats

* $P<0.05$ when compared to control (One-way ANOVA followed by Dunnett’s test)
Discussion

The elevated plus maze is used to measure the anxiety state in animals, however transfer latency i.e. the time elapsed between the movement of the animal from an open to an enclosed arm was markedly shortened if the animal had previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. Recent studies of several nootropics and amnestic agents on EPM made this model a widely accepted paradigm to study learning and memory processes in rodents.  

In EPM, acquisition (learning) can be considered as transfer latency on first day trials and the retention/consolidation (memory) is examined 24 h later. The animals treated with 100 mg/kg of BG showed a significant decrease in transfer latency as compared with the control group, which is an indication of the cognitive enhancer effect of BG in rodents.

The water maze task was introduced by Morris (1981) and colleagues as a spatial localization or navigation task. The task has been used extensively to study the neurobiological mechanisms that underlie spatial learning and memory, age-associated changes in spatial navigation and the ability of nootropic agents to influence specific cognitive processes.  

BG significantly decreased the escape latency as compared with control. The alcoholic extract of BM (40 mg/kg, p.o.) and BG (50 mg/kg, p.o.) showed decreased escape latency though the ef-

Figure 2: Effect of Bramhi Ghrita on spatial memory in Morris water maze task in mice

\( n = 6 \) in each group. *\( P<0.05 \) compared to control (One-way ANOVA followed by Dunnett’s test)
fect was not significant statistically. The plant *Bacopa monnieri* is a well-known nootropic plant and a proven memory enhancer agent. Bacosides are the main active nootropic principle present in the alcoholic extract of the plant. Apart from memory enhancer activity these bacosides have the potential to modulate the activities of heat stock protein (HSP70) expression, cytochrome P450 and superoxide dismutase in the rat brain. The bacosides of *Bacopa monnieri* thus act as an antistress buffer in the brain of rodents. As *Bacopa monnieri* is one of the constituents of Bramhi Ghrita (BG), BG may also serve as a protective agent, providing a buffer against rapid age-related decline in mental function. Certain factors need to be considered, like species differences, age of animals, duration of treatment and experimental protocol employed (e.g. cued vs. place learning) before definitive conclusions can be reached. More detailed and well-planned experimentation, which covers more parameters of learning, and memory processes, are necessary to evaluate the exact mechanism of the action of BG. From the present study it can be concluded that BG may act as a memory enhancer formulation and may also be useful as a supportive adjuvant in the treatment of impaired memory functions.

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**References**