ORIGINAL ARTICLE

Encouraging effect of *Brahmi Ghrita* in amnesia

Kapil Deo Yadav, K. R. C. Reddy, Vikas Kumar[1]
Departments of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, [1]Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Context: *Brahmi Ghrita* (BG) contains *Brahmi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*), *Kushtha* (*Saussurea lappa*), *Shankhapushpi* (*Convolvulus pluricaulis*) *and Puran Ghrita*, prepared as per sneha paka process. **Aim:** The aim of this study was to assess the learning and memory activity of BG in Amnesic rats. **Materials and Methods:** The Learning and memory activity of BG (400 and 800 mg/kg, per oral) was evaluated in scopolamine (1 mg/kg, sub cutaneous) challenged rats, using elevated maze plus, passive avoidance test and active avoidance test. **Results:** BG treated scopolamine challenged rat demonstrated a significant decrease in transfer latency in modified elevated plus maze test and increase in step through latency in passive avoidance test compared to control rats in a dose dependent manner. BG treated rats took significant less number of total trials, shock trial and total time for jumping into safe compartment. **Conclusion:** BG antagonises the action of scopolamine.

Key words: *Brahmi Ghrita*, modified elevated plus maze test, scopolamine

INTRODUCTION

Memory is the process by which organisms are able to record their experiences and use this information to adapt their responses to the environment.[1] Impairment of memory is an organic brain disorder defined as 'loss of intellectual ability of sufficient severity to interfere either with occupational functioning, usual social activities or relationship of a person in the absence of gross clouding of consciousness or motor involvement'.[2] Decreased cholinergic firing in the brain,[3] rise in oxidative stress,[4] hypercholesterolemia[5] and neuro inflammatory reactions[6] have been demonstrated to play an etiological role in memory decline. One of the study in India showed that the median survival time determined to be 3.3 years for patients with dementia.[7] Scopolamine, a non-selective muscarinic cholinergic antagonist, is a well-known centrally acting cholinergic probe, which causes impairment in learning.[8] In addition, scopolamine also causes an increase in cognitive impairment in healthy elderly subjects compared to young adults.[9] In *Ayurveda*, *Brahmi Ghrita* (BG) is an important formulation used for the treatment of memory disorders. It contained *Brahmi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*) *Shankhapushpi* (*Convolvulus pluricaulis*), *Kushtha* (*Saussurea lappa*) and *Puran Ghrita* (old clarified butter). *Brahmi* have Antioxidant[10] and Hepatoprotective[11] action, *A. Calamus* possess beneficial memory enhancing property on memory impairment, learning performance, behaviour modifying and enhances the clarity of perception. *C. pluricaulis* is synonyms of *Evolvulus alsinoides*. It is used as nootropic or brain tonic in traditional systems of medicines, potential memory enhancing agent used in treating dementia[12] nurses the mind as well as central nervous system (CNS).[13] *Brahmi* has an anti-fertility effect[14] and *Kushtha* have a special property of improving sperm purification[15] and also have anti-inflammatory activity.[16] The individual herbs was reported for improving memory, in this study. We examined the combined effect of BG on scopolamine challenged rats.

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MATERIALS AND METHODS

Animals

Charles Foster rats of either sex weighing between 160 g and 180 g were used for experimental study. The animals were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in polypropylene cages at an ambient temperature of 25°C ± 1°C and 45-55% relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum* unless stated otherwise. They were acclimatized to laboratory conditions for at least 1 week before using them for the experiments. Principles of laboratory animal care (National Institute of Health publication number #85-23, revised in 1985) guidelines

Address for correspondence: Dr. K. R. C Reddy, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. E-mail: drkrcreddybhu@yahoo.co.in
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were always followed and prior approval of Institutional Animal Ethical Committee (No. Dean/10-11/150) of Banaras Hindu University was obtained before commencing.

Plant Material and Preparation of *Brahmi* Formulation

BG was prepared as described in one of our earlier studies.[17] Briefly, it was prepared by adding paste of *B. monneri* (40% w/w), *A. calamus* (20% w/w), *C. pluricaulis* (20% w/w) and *S. lappa* (20% w/w) in freshly prepared 3 l juice of *B. monneri* in stainless steel vessel having 750 ml clarified butter. Above mixture was heated for 9 h and filtered after acquiring completion test. In this way, BG was prepared.

Drug Treatment

For the present study, totally twenty four animals were used and these animals were divided in to four groups including the control group that is six animals in each groups. In the control group, (first groups) no drugs were given only diet and water was provided. In the second group only single dose of scopolamine that is (1 mg/kg body weight) was administered intra peritoneal after training. In the third group, BG in a dose of 400 mg/kg body weight was administered once a day along with scopolamine in a dose of 1 mg/kg body weight. In the fourth group, BG in a dose of 800 mg/kg body weight was administered once a day along with scopolamine in a dose of 1 mg/kg body weight. BG was administered orally at 400 and 800 mg/kg body weight to rats once in a day.

Anti-amnestic Study

Scopolamine induced amnesia

Scopolamine hydrobromide (1 mg/kg, i.p.) was administered immediately after the learning trial on day 1 to all eighteen animals. Then, these animals were divided in to three groups (II, III, IV), and BG was given in III and IV groups in the dose of 400 and 800 mg/kg body weight. The behaviour experimental procedures mentioned below was used in the study.

Transfer latency on elevated plus maze

This test was used to assess the retention of learning and memory.[18] The plus maze consisted of two opposite open arms, 50 cm × 10 cm, crossed with two enclosed arms of the same dimensions with walls 40 cm high. The arms were connected with a central square (10 cm × 10 cm) to give the apparatus a plus sign appearance. The maze was kept in a dimly light room elevated 50 cm above floor level. On day 1, a rat was placed on the far end of one of the open arms, facing away from the centre and the time taken by the animal to enter one of the closed arms (transfer latency on day 1) was recorded with the help of a stop watch. The rat was left in the enclosed arm for 10-15 s and returned to its home cage. On day 2, same procedure was repeated and similarly after an interval of 1 week, on day 9, the transfer latency was again recorded.

Passive avoidance test

This test uses normal behaviour of rats[19] and was developed by Kings and Glasser (1970). The step through passive avoidance behaviour was evaluated by using the light-dark apparatus, which had two walls of wood and the remaining two walls of transparent plexiglass. It was divided into two equal compartments (30 cm × 25 cm × 30 cm) by a plexiglass with a 10 cm × 10 cm. opening in the centre. A guillotine door between the two compartments controlled the opening. The light compartment was painted white and a 15 W lamp illuminated it.

The interior of the dark chamber was painted black and had a ceiling. Each compartment had a copper grid floor. To ensure electrical separation, there was a 1.5 cm gap between the two floors in the light-dark box, at the opening between the two chambers.

In all four groups excluding control group (details of grouping was mentioned in drug treatment section) drug was given for 1 week before starting the experiment. On day 1, a rat was placed in the white box and time taken to enter into the dark box was noted. As soon as, the rat entered the dark box, the guillotine door was closed and foot electric shock (0.5 mA, 3 s) was delivered. The rat was then placed to its home cage. On the following day (24 h retention interval), each rat was again placed in the white box and was given a 5 min inhibition period. Latency to step through to the dark chamber was recorded. Electric shock was not delivered on 2nd day. If the animal remained in the white box for a 5 min test period, the maximum score of 300 s will be assigned. On day 9 (after a gap of 1 week), latency to step through was again recorded to test the retention of the passive avoidance learning.

Active avoidance test

Active avoidance learning acquisition and its retention was tested by the method of Spignoli *et al*., 1986.[20] The apparatus used, was the conventional shuttle avoidance box (Techno, India), which consisted of two grid-floor compartments (29 cm × 29 cm × 25 cm each) separated by a plexiglass transparent partition with a single opening (14 cm × 17 cm), and buzzer.

The rats were placed individually on the right compartment of a shuttle box and allowed to adapt for 15 s. Thereafter, the rats were exposed to a 15 s acoustic buzzer stimulus (conditioned stimulus, (CS) followed by both the acoustic stimulus and electric shock (unconditioned stimulus, 1.5 mA, 50 Hz) through the grid floor of the right for 30 s. Jumping to the un-electrified adjacent (safe) left

compartment during CS was designated as conditioned response (CR1), while jumping to the safe left chamber during the initial 15 s adaptation period was designated as anticipatory CR2. The number of trials required by the animal to reach the criterion of two consecutive correct responses represents the learning rate. A 60 min inter-trial interval period was maintained. For statistical analysis, rats not reaching criterion within eight trials was arbitrarily assigned a score of 9.

All the rats were subjected to this training schedule and were retested 24 h later and at day 9 (after a gap of 1 week) for retention of the learned task. Besides CR1, CR2 and trial scores, total time taken and the total number of shocks received to reach criterion was also recorded.

Statistical Analysis

The data, expressed as Mean ± SD, were subjected to Kruskal-Wallis one way analysis of variance (ANOVA). Inter group comparisons were made by Mann-Whitney-U-test (two tailed) for only those responses, which yielded significant treatment effects in the ANOVA test. $P < 0.05$ was considered statistically significant.

RESULTS

Elevated Plus Maze Test

In elevated plus maze model, rats treated with scopolamine showed a significant increase in transfer latency on 2nd and 9th day when compared with the control group rats. Treatment with BG significantly reversed the amnesia induced by scopolamine. Results have been summarized in Table 1.

Passive Avoidance Test

Rats treated with scopolamine showed a decrease in step through latency on 2nd and 9th day in comparison to control group rats. Treatment with BG significantly reversed the amnesia induced by scopolamine. Results have been summarized in Table 2.

Active Avoidance Test

In active avoidance model, rats treated with BG took significant less number of total trials, shock trial and total time for jumping into safe compartment in 2nd and 9th day as compared to scopolamine challenged rats. Treatment with BG significantly reversed the amnesia induced by scopolamine. Results have been summarized in Table 3.

DISCUSSION

Transfer latency on modified elevated plus maze might be shortening if the animal has previous experience of entering the open arm and shortened transfer latency could be related to memory. Scopolamine, acetylcholine receptor antagonist, is reported to impair cognitive performances[21] especially spatial learning and memory.[22] It exerts amnesic effect equally in various behavioural models of memory including Morris water maze,[23] etc., and considered as a reliable tool to study anti-amnesic effects. It is muscarinic cholinergic antagonist, and capable of inducing transient memory impairment in normal subjects[24] due to this rats treated with scopolamine showed an increase in transfer latency on elevated maze plus, increase step through latency in passive avoidance and increased total trial, shock trial and total time in active avoidance test indicate that scopolamine produces amnesia in animals that is it acts as anti-amnestic agent. BG treated rats shows increased transfer latency in elevated maze and other modules used in the present study indicate that it reverse the effect of scopolamine in rats, i.e., increases the memory in scopolamine challenged rats. In this formulation *B. monneri* present in

Table 1: Effect of BG on transfer latency in the elevated plus maze test against scopolamine

Treatment	Dose (mg/kg)	Transfer latency (s)		
		Day 1	Day 2	Day 9
Control		45.50±6.38	42.00±3.29	41.67±5.32
SCP	1	41.83±6.62	52.00±7.48**	48.17±7.68**
SCP+BG	1+400	42.83±4.45	35.50±4.09*,#,$	29.83±4.88***,###,$$$
SCP+BG	1+800	43.33±7.47	32.67±5.85*,#,$	25.83±4.36***,###,$$$

BG – *Brahmi ghrita*; SCP – Scopolamine, *n* – Six animals in each group; Values are mean±SD, *P<0.05, **P<0.01, ***P<0.001 compared to control, #P<0.05, ##P<0.01, ###P<0.001 compared to day 1, $$P$<0.05, $$$$P$<0.001 compared to scopolamine

Table 2: Effect of BG on step through latency in passive avoidance test against scopolamine

Treatment	Dose (mg/kg)	Step through latency (s)		
		Day 1	Day 2	Day 9
Control		29.83±5.53	32.67±3.88	33.67±5.05
SCP	1	39.83±5.12	16.17±3.49**	21.83±3.31*
SCP+BG	1+400	35.33±4.76	51.67±8.45###,$$$	70.17±6.99###
SCP+BG	1+800	33.33±5.16	55.67±5.05***,###,$$$	61.00±5.22***,##,$$$

BG – *Brahmi ghrita*; SCP – Scopolamine; *n* – Six animals in each group; Values are mean±SD, *P<0.05, **P<0.01, ***P<0.001 compared to control, #P<0.05, ##P<0.01, ###P<0.001 compared to day1, $$P$<0.05, $$$$P$<0.001 compared to scopolamine

Table 3: Effect of BG in learning, acquisition, and retention in active avoidance test against scopolamine

Treatment	Dose (mg/kg)	Total trial	Shocked trial	CR1	CR2	Total time (s)
Acquisition						
Control		3.33±0.52	1.17±0.75	1.67±0.52	0.33±0.82	86.00±7.54
Scopolamine	1	4.17±0.75	2.00±0.89	1.33±0.52	0.67±0.52	95.17±8.45
Scopolamine+BG	1+400	2.83±0.41	0.83±0.41	1.83±0.41	0.17±0.41	81.00±8.20
Scopolamine+BG	1+800	2.67±0.52	0.67±0.52	1.83±0.41	1.67±3.61	88.5±6.57
Retention after 24 h						
Scopolamine	1	3.67±0.82*	1.5±0.84	1.5±0.84	0.5±0.84	110.00±8.44*
Scopolamine+BG	1+400	2.17±0.41***,#,$$	0.17±0.41	1.5±0.55	0.50±0.55	60.50±5.54***,###,$$$
Scopolamine+BG	1+800	2.17±0.41**,$$	0.2±0.41	0.83±0.75	1.17±0.75	46.7±4.59***,$$$
Retention on after 1 week						
Control		3.33±0.52	1.00±0.00	1.67±0.52	0.5±0.55	84.5±5.65
Scopolamine	1.00	4.00±0.63*	1.67±0.82	1.33±0.82	0.83±0.75	117.67±0.52***
Scopolamine+BG	1+400	2.17±0.41***,#,$$$	0.17±0.41	1.67±0.52	0.33±0.52	59.17±7.14***,###,$$$
Scopolamine+BG	1+800	2.17±0.41**,$$$	0.17±0.41	1.67±0.52	0.33±0.52	43.33±6.49***,$$$

BG – *Brahmi ghrita*; CR – Conditioned response; SCP – Scopolamine; *n* – Six animals in each group; Values are mean±SD; *P<0.05, **P<0.01, ***P<0.001 compared to control, #P<0.05, ##P<0.01, ###P<0.001 compared to day 1, $$P$<0.05, $$$$P$<0.001 compared to scopolamine

a large percentage (approx. 40% w/w), hence properties of combined formulation was mainly dependent on properties of *B. Monneri*. *B. monneri* is reported to reverse neurotoxin and colchicines-induced depletion of acetylcholine and suppression of cholinesterase activity and muscarinic receptor binding in the frontal cortex and hippocampus.[25] It is also documented to inhibit the acetylcholine esterase activity dose dependently.[26] *Brahmi* reversed the scopolamine induced amnesia significantly mainly by improving calmodulin and by partially attenuating protein kinase C and cyclic AMP responce-element binding protein (pCREB).[27,28] Beside *Brahmi* other ingredients like *A. calamus* also inhibits the acetyl cholinesterase due to this its rhizomes of are used in loss of memory given in combination with other drugs,[29] extracts of *Shankhapushpi* significantly improved learning and memory in rats and significantly reversed the amnesia induced by scopolamine[30] and ghee is also prescribed for anxiety, depression, dementia, insanity, epilepsy, and other disorders of consciousness. Ghee is older than 1 year especially good for healing the mind. Distribution of drug in blood is chiefly influenced by its lipid solubility, ionization, differences in the regional blood flow, etc., A water soluble drug is usually distributed in the extracellular spaces and it may not readily diffuse in to cerebro spinal fluid (CSF) and other body cavities while the lipid soluble drugs are rapidly distributed throughout the intra and extra cellular spaces. The drugs that are rapidly absorbed from the gut due to their lipid solubility are known to readily diffuse into the CSF and the brain. Those drugs which are medicated with *Ghrita* and also dispense in the form of Ghee are rapidly absorbed and distributed in the target areas of the body like the nervous system in this case. The main reason behind this is the molecular structure of the blood brain barrier. The membrane separating the CNS tissue and the circulating blood is lipophilic in nature. Thus, it selectively allows the passage of lipids and lipid soluble drugs across it. Therefore, any drug given in the form of ghee will not only be digested and absorbed fast, but will also be able to reach some of the most distant areas of body like the CNS.[31] All drugs used in BG; however, influence cholinergic function by increasing high affinity choline uptake these drugs facilitate acetylcholine production and turnover with varying actions at both muscarinic and nicotinic receptors.[32] The combination of these ingredients used in BG in a specific quantity and manner of blending creates a powerful synergy for memory benefits. Significant reversal of scopolamine (amnestic agent) induced amnesia by BG, indicates an underlying cholinergic mechanism as scopolamine impair spatial cognition.[33]

CONCLUSION

Scopolamine is producing amnesia by cholinergic mechanism and BG reversed the effect of scopolamine in all animal models. The effect of BG might be antagonising action of scopolamine that is by affecting cholinergic system or antioxidant effect by reducing oxidative stress in animals in brain.

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