Original Article

Brahmi rasayana Improves Learning and Memory in Mice

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Cure of cognitive disorders such as amnesia, attention deficit and Alzheimer's disease is still a nightmare in the field of medicine. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like Donepezil® are being used to improve memory, mood and behavior, but the resulting side effects associated with these agents have made their use limited. The present study was undertaken to assess the potential of *Brahmi rasayana* (BR) as a memory enhancer. BR (100 and 200 mg kg^{-1} p.o.) was administered for eight successive days to both young and aged mice. Elevated plus maze and passive-avoidance paradigm were employed to evaluate learning and memory parameters. Scopolamine (0.4 mg kg^{-1} i.p.) was used to induce amnesia in mice. The effect of BR on whole brain AChE activity was also assessed. Piracetam (200 mg kg^{-1} i.p.) was used as a standard nootropic agent. BR significantly improved learning and memory in young mice and reversed the amnesia induced by both scopolamine (0.4 mg kg^{-1} i.p.) and natural aging. BR significantly decreased whole brain acetyl cholinesterase activity. BR might prove to be a useful memory restorative agent in the treatment of dementia seen in elderly.

Keywords: acetylcholine – ayurveda – *Brahmi rasayana* – piracetam – scopolamine

Introduction

Alzheimer's disease is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes and ultimately death (1). The personality distortions interfere with the patient's professional life, social activities and relationships (2).

Nootropic agents such as piracetam (3), pramiracetam, aniracetam (4) and choline esterase inhibitors like Donepezil® are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use (5,6). Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders.

Ayurveda is the oldest medical science in the Indian sub-continent and has been practiced since the 12th Century BC. Its objective is to accomplish physical, mental, social and spiritual well-being by adopting preventive, health promoting and holistic approach towards life (7,8). *Vata*, *pitta* and *kapha* are the three psychobiological dimensions (energy) or biological rhythms regulating the entire functioning of the human body. *Vata*, is the energy that strengthens intellectual power, respiration and the activity of sensory organs. *Sadhak Pitta* regulates digestion of food and body temperature and is responsible for intelligence and memory. *Tarpak kafa* provides nutrition to the sense organs and is helpful in lubrications of the nervous tissue. According to ayurveda, Alzheimer's disease is an imbalance of *vata*, *pitta* and *kapha* (9). Medhya herbs such as *Convolvulus pluricaulis*, *Centella asiatica*, *Bacopa monnieri*, *Acorus calamus* and *Celastrus paniculatus* are beneficial in cognitive disorders (10).

Rasayanas are ayurvedic preparations that promote resistance against infections and other diseases by maintaining the equilibrium of *vata*, *pitta* and *kapha*. The *rasayanas* improve memory, intelligence and promote youthfulness, good lusture, complexion and efficiency (11). Various *rasayana* drugs like *Withania somnifera*, *Asparagus racemosus* and *Tinospora cordifolia* have proven their therapeutic worth (10).

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In the present study, the nootropic effects of a multi-herbal preparation, *Brahmi rasayana* (BR) were investigated by employing both exteroceptive and interoceptive models. The stimulus lies outside the body in the exteroceptive behavioral models, whereas it lies within the body in case of the interoceptive behavioral models. Elevated plus maze is a neutral exteroceptive model used to assess short-term memory, whereas passive-avoidance apparatus is a punishment-based exteroceptive model used to test long-term memory (12). Interoceptive behavioral models such as scopolamine and natural aging induced amnesia are widely cited as models simulating human dementia in general and Alzheimer's disease in particular.

Methods

BR comprises coarse powders of dried leaves of *B. monnieri*, flower buds of *Eugenia caryophyllus*, seeds of *Elettaria cardamomum*, inner bark of shoots of *Cinnamomum zeylanicum*, and fruits of *Piper longum* and *Piper nigrum* (13,14). BR prepared as per standard ayurvedic procedures (15) was procured from Dindayal ayurvedalaya, India, as a gift sample and the same were administered in doses of 100 and 200 mg kg^{-1} p.o. for eight successive days to the mice.

Drugs and Chemicals

Scopolamine hydrobromide (Sigma Aldrich, USA) and piracetam (Nootropil®; UCB India Pvt. Ltd, Vapi, Gujarat) were diluted in normal saline and injected intraperitoneally. Phenytoin (Dilantin® suspension; Parke Davis) was administered orally. Volume of administration was 1 ml per 100 g. All the drugs were administered in the morning session i.e. 8 a.m.–9 a.m. on each day.

Administration of BR

BR at different doses (50–500 mg kg^{-1}) was administered orally to the mice with the help of a specially designed oral needle connected to a polythene tube. BR was administered at the same time on each day (i.e. 8 a.m.–9 a.m.). During the first 4 h after the drug administration, the animals were observed for gross behavioral changes if any, for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality were observed. The doses selected for future studies were 100 and 200 mg kg^{-1} per day.

Mice

Swiss mice of either sex weighing ∼18 g (younger ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in the present study. Mice were procured from disease free animal house of CCS Haryana Agriculture University, Hisar (Haryana, India). They were acclimatized to the laboratory conditions for 5 days before behavioral studies. Mice had free access to food and water and were maintained under 12 h light/12 h dark cycles. All the readings were taken during the same time of the day i.e. between 8 a.m. and 11 a.m. The Institutional Animals Ethics Committee (IAEC) had approved the experimental protocol, and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Exteroceptive Behavioral Models

Elevated Plus Maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm), and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the mouse did not enter into one of the covered arms within 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for 10 s and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day (16,17).

Passive Shock Avoidance Paradigm

Passive-avoidance behavior based on negative reinforcement was recorded to examine long-term memory. The apparatus consisted of a box (27 × 27 × 27 cm^3) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 × 7 × 1.7 cm^3) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V 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, shocks were delivered for 15 s 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 its entire paw on the grid floor. Mice showing SDL in the range (2–15 s) during the first test were used for the second session and the retention test. The second session was carried out 90 min after the first test. When mice stepped down before 60 s, electric shocks were delivered for 15 s. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cutoff time of 300 s (18,19).

Experimental Design

Mice were divided into 24 groups and each group consisted of a minimum of five animals. Separate animals were used for each experiment.

Group I: It represented the control group for young mice ($n = 6$). Distilled water (DW), was administered orally for 8 days. TL was noted after 45 min of administration on the eighth day and again after 24 h, i.e. on the ninth day.

Groups II and XI: Piracetam, 200 mg kg^{-1} i.p. was injected to both young and aged mice, respectively. TL was noted after 45 min of injection and again after 24 h.

Group III: Scopolamine (0.4 mg kg^{-1} i.p.) was administered to young mice and TL was noted after 45 min of injection on the eighth day and again after 24 h, i.e. on the ninth day.

Groups IV and V: BR (100 and 200 mg kg^{-1}) was administered orally to young mice for 8 days. The last dose was given 45 min before subjecting the animals to elevated plus maze test. TL was noted on the eighth day and again after 24 h.

Group VI: BR (200 mg kg^{-1} p.o.) was administered to young mice for 8 days. After 45 min of administration of the last dose on the eighth day, scopolamine hydrobromide (0.4 mg kg^{-1} i.p.) was administered. TL was noted after 45 min of administration of scopolamine and again after 24 h, i.e. on the ninth day.

Group VII: Piracetam (200 mg kg^{-1} p.o.) was administered to young mice for 8 days. After 45 min of administration of the last dose on the eighth day, scopolamine hydrobromide (0.4 mg kg^{-1} i.p.) was administered. TL was noted after 45 min of administration of scopolamine and again after 24 h, i.e. on the ninth day.

Group VIII: Served as the control group for aged mice. DW was administered orally for 8 days. TL was noted after 45 min of administration on the eighth day and again after 24 h, i. e on the ninth day.

Groups IX and X: BR (100 and 100 mg kg^{-1}) was administered orally to aged mice for 8 days. The last dose was given 45 min before noting TL on the eighth day.

Group XII: Control group for young mice ($n = 6$). DW (1 ml per 100 g) was administered p.o. for 8 days. After 90 min of administration on the eighth day, SDL was recorded. Retention was examined after 24 h.

Groups XIII and XIV ($n = 5$ each): BR extract (100 and 200 mg kg^{-1}, respectively) was administered orally for 8 days to young mice. SDL was recorded after 90 min of administration on the eighth day and after 24 h.

Group XV: Scopolamine hydro bromide (0.4 mg kg^{-1}) was administered i.p. to young mice after training on the eighth day and SDL was recorded at 45 min after injection.

Group XVI: BR (200 mg kg^{-1} p.o.) was administered to young mice for 8 days. After 45 min of administration of the last dose on the eighth day, scopolamine hydrobromide (0.4 mg kg^{-1} i.p.) was administered. SDL was recorded after 90 min of administration on the eighth day and after 24 h.

Group XVII: Control group for aged mice ($n = 6$). DW (1 ml per 100g) was administered p.o. for 8 days to aged mice. After 90 min of administration on the eighth day, SDL was recorded. Retention was examined after 24 h.

Groups XVIII and XIX: BR (100 and 200 mg kg^{-1}, respectively) was administered orally for 8 days to aged mice. SDL was recorded after 90 min of administration on the eighth day and after 24 h.

Group XX: served as control and was treated with saline water, Group XXI was treated with phenytoin (12 mg kg^{-1} p.o.) and Group XXII was treated with piracetam (200 mg kg^{-1} p.o.). Group XXIII and Group XXIV were treated with BR (100 and 200 mg kg^{-1} p.o., respectively) for 8 days and acetyl cholinesterase (AChE) levels were determined.

Estimation of Brain AChE Activity

The time frame of cholinesterase activity estimation was similar to behavioral tests, i.e. 8 a.m.–11 a.m. on each day. On the ninth day animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using the Ellman method [20]. The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5,5′-dithionitrobenzoic acid (DTNB), and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of 3 min was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

$$R = \frac{\delta \text{ OD} \times \text{volume of assay (3 ml)}}{E \times \text{mg of protein}}$$

where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. δ OD is the change in absorbance per minute and E is the extinction coefficient, which is 13 600 M^{-1} cm^{-1}.

Statistical Analysis

All the results were expressed as mean ± standard error. The data were analyzed using ANOVA and Student's (unpaired) t-test. Kruskal–Wallis one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data. $P < 0.05$ was considered as significant.

Results and Discussion

Aged mice showed higher TL values on the first day and on the second day (after 24 h) as compared with young mice, indicating an impairment in learning and memory. Piracetam (200 mg kg^{-1} i.p.) pre-treatment for 8 days decreased TL of eighth day and ninth day as compared with distilled water treated group, indicating improvement in both learning and
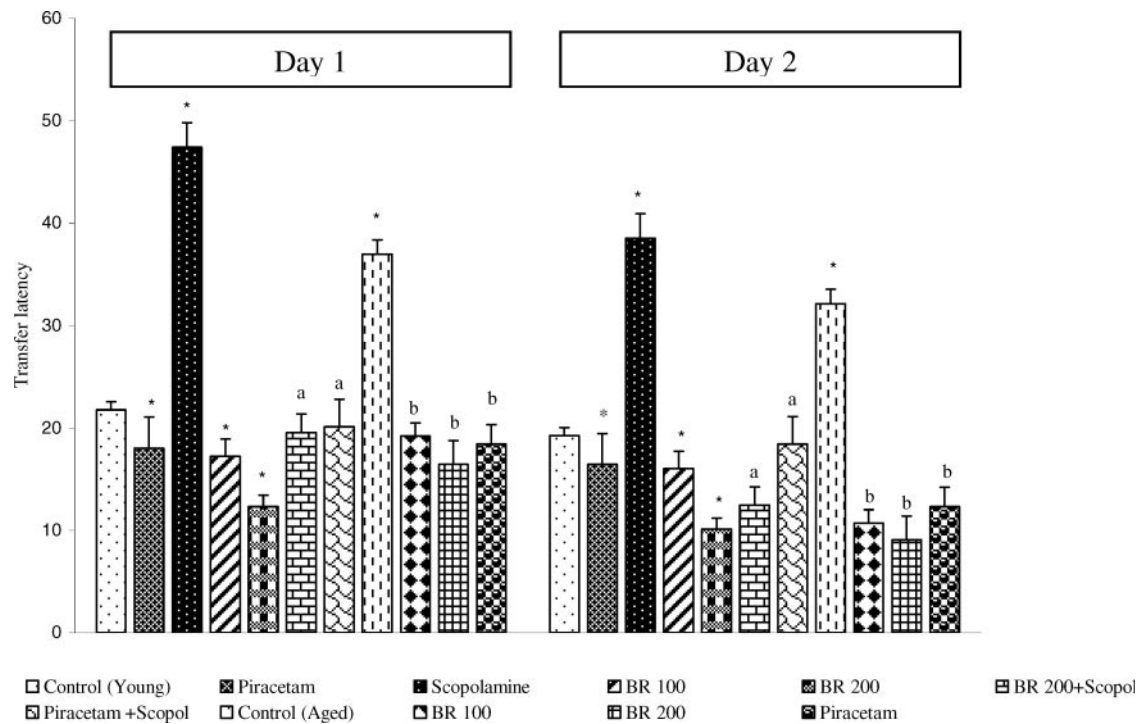
Figure 1. Effect of BR on transfer latencies of young and aged mice on elevated plus maze. Each group comprises a minimum of five mice. Values are expressed as mean ± SEM, ANOVA followed unpaired *t*-test, *$P < 0.05$ compared with control (young group), [a]$P < 0.05$ compared with scopolamine treated group, [b]$P < 0.05$ compared with control (aged group), first day TL: $F(11, 51) = 11.760$ ($P < 0.05$); second day TL: $F(11, 51) = 4.122$ ($P < 0.05$).

memory. Scopolamine (0.4 mg kg^{-1}) increased TL significantly [first day TL: $F(11, 51) = 11.760$ ($P < 0.05$); second day TL: $F(11, 51) = 4.122$, $P < 0.05$] in young mice on the first and the second day as compared with control, indicating impairment of both learning and memory (Fig. 1).

Higher Dose of BR Improved the Learning and Memory of Aged Mice

BR (100 mg kg^{-1} p.o.) decreased the TL on the eighth day and the ninth day in both young and aged mice ($P < 0.05$) when compared with their respective control groups. Higher dose of BR (200 mg kg^{-1} p.o.) improved learning and memory of aged mice rather than young mice as reflected by marked decrease in TL on the eighth day and the ninth day, when subjected to elevated plus maze tests (Fig. 1). BR pre-treatment for 8 days protected young as well as old mice ($P < 0.05$) against scopolamine-induced amnesia.

BR Reversed Amnesia

BR (100 and 200 mg kg^{-1} p.o.) treatment profoundly increased SDL as compared with the control group on the second day indicating improvement in memory of young mice. Scopolamine hydrobromide (0.4 mg kg^{-1} i.p.) decreased SDL on second day after training, indicating impairment of memory. BR (200 mg kg^{-1} p.o.) administered orally for 8 days significantly [$F(7, 32) = 59.312$, $P < 0.05$] reversed amnesia induced by scopolamine and natural ageing (Fig. 2).

The acetylcholinesterase activity of whole brain was markedly elevated ($P < 0.05$) after phenytoin (12 mg kg^{-1} p.o.) treatment. Piracetam (200 mg kg^{-1} p.o.) and BR (100 and 200 mg kg^{-1} p.o.) significantly ($H = 16.67$, $P < 0.05$) lowered AChE activity (Fig. 3).

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients often show non-cognitive symptoms, such as depression, apathy and psychosis that impair their day-to-day activities (21). The present study suggests that BR is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of learned task. Central cholinergic system plays an important role in learning and memory (22). In our study, phenytoin *per se* (12 mg kg^{-1} p.o.) significantly elevated brain AChE activity. Piracetam (250 mg kg^{-1} p.o.) and BR (100 and 200 mg kg^{-1} p.o.), on the other hand significantly ($P < 0.05$) lowered this activity indicating the counteracting actions of these drugs on the cholinergic system. BR also reversed the scopolamine-induced impairment in learning and memory, when assessed on passive-avoidance paradigm.

Pharmacological Properties of BR Components Include Anti-inflammatory, Antioxidant and Memory Improving Activities

The major components of BR include coarse powders of dried leaves of *B. monnieri*, flower buds of *E. caryophyllus*, seeds of *E. cardamomum*, inner bark of shoots of *C. zeylanicum*
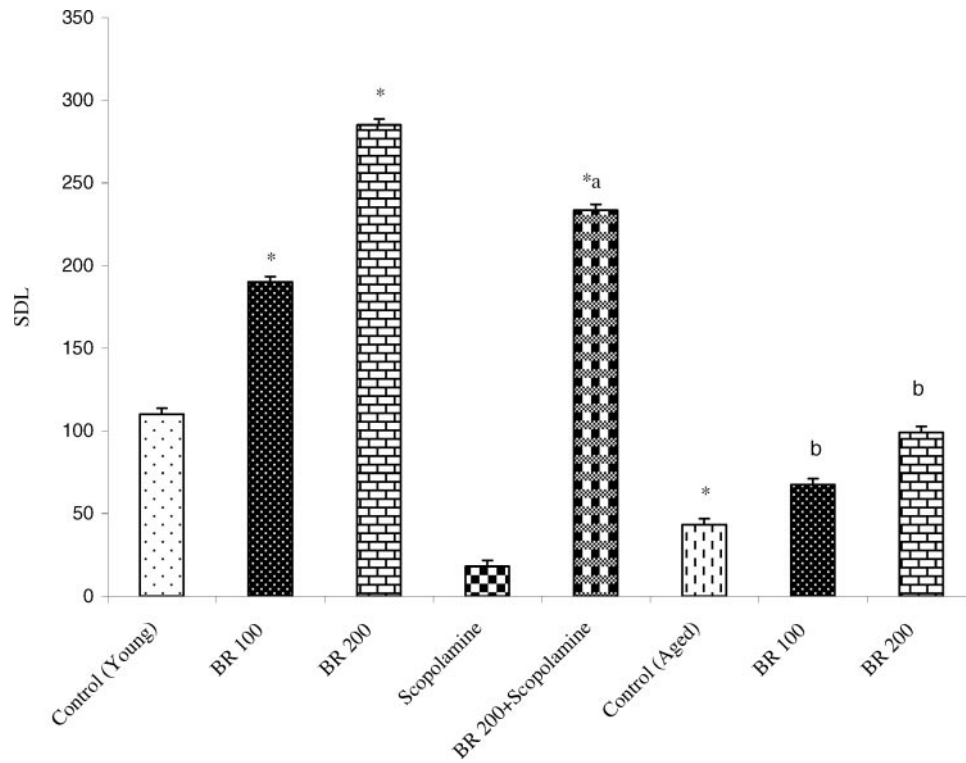
Figure 2. Effect of BR on SDL using passive-avoidance apparatus. Values are each mean ± SEM, ANOVA followed unpaired *t*-test, *$*P < 0.05$ compared with control (young mice), $^aP < 0.05$ compared with scopolamine treated group alone, $^bP < 0.05$ compared with control (aged mice alone), SDL: $F(7, 32) = 59.312$.
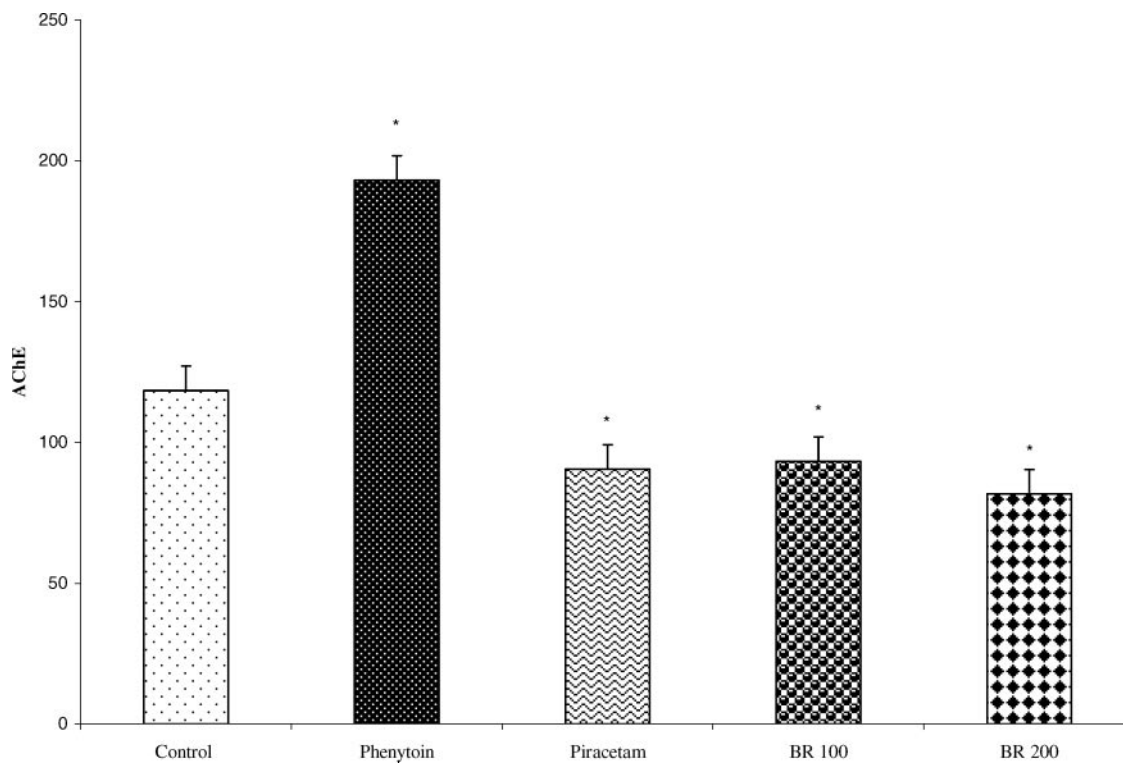

Figure 3. Effect of BR on AChE activity in aged mice. Values are mean ± SEM, AChE-whole brain AChE (µmol), *$*P < 0.05$ versus control (multiple range test), $H = 16.67$; df = 5; $I < 0.05$.

and fruits of *P. longum* and *P. nigrum*. These ingredients exhibit several pharmacological properties (23). Bacosides of *B. monnieri* are reported to potentiate human long-term memory (24) and exhibit anti-stress activity (23). Administration of *B. monnieri* extract to mentally retarded children has produced beneficial results (25). *E. cardamomum* was found to possess anti-inflammatory activity (26) and antioxidant activity (27). *E. caryophyllus* is reported to have anti-ulcer (28), anti-inflammatory (29) and antioxidant activity (30). *P. nigrum* and *P. longum* contain a major alkaloid, piperine, which possesses antioxidant (31), immunomodulatory (32), antitumor (33), antimutagenic (34), anticonvulsant (35), analgesic (36) and memory improving activities (37). Water-soluble polyphenol polymers from *C. zeylanicum* exhibited pro-healing (38), antioxidant activity (39) and reduced the risk factors associated with diabetes and cardiovascular diseases (40).

Therefore, the memory improving activity of BR may be attributed to its antioxidant, anti-inflammatory, neuroprotective, pro-cholinergic and anti-acetylcholinesterase properties of various components of the multi-herbal preparation and hence may be of enormous use in delaying the onset and reducing the severity of Alzheimer's disease. However, further investigations are warranted to explore the possible involvement of other neurotransmitters, such as glutamate, GABA and catecholamines (41), responsible for memory improving property of BR.

Acknowledgements

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