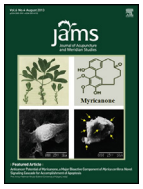
Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com



RESEARCH ARTICLE

Evaluation of *Bacopa monniera* for its Synergistic Activity with Rivastigmine in Reversing Aluminum-Induced Memory Loss and Learning Deficit in Rats

Agadi Hiremath Thippeswamy, Mohamed Rafiq*,
Gollapalle Lakshminarayana shastry Viswantha, Kethaganahalli J. Kavya,
Suryakanth D. Anturlikar, Pralhad S. Patki

Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Karnataka, India

Available online Mar 22, 2013

Received: Dec 4, 2012
Revised: Feb 1, 2013
Accepted: Feb 13, 2013

KEYWORDS
aluminum chloride;
Bacopa monniera;
elevated plus maze;
memory impairment;
Morris water maze;
rivastigmine

Abstract
The objective of this study was to evaluate the synergistic activity of *Bacopa monniera* with Rivastigmine against aluminum-chloride ($AlCl_3$)-induced cognitive impairment in rats. Adult male Wistar rats were divided into ten groups (n = 10) and subjected to their assigned treatments for 42 days. On the 20th day of the respective drug treatments, all the animals were trained in the Morris water maze (retention latency) and the elevated plus maze (transfer latency). After the initial training, the retention latency (RL) and the transfer latency (TL) were evaluated on the 21st and the 42nd days of the study. Chronic administration of $AlCl_3$ caused significant memory impairment associated with increased RL in the Morris water maze task and increased TL in the elevated plus maze test. Interestingly, animals treated with oral administration of *B. monniera* (100 and 200 mg/kg), Rivastigmine (5 mg/kg) or a combination of *B. monniera* (100 mg/kg) with Rivastigmine (5 mg/kg) showed significant protection against $AlCl_3$-induced memory impairment compared to animal treated with $AlCl_3$ *per se*. Additionally, the neuroprotective effect of *B. monniera* (100 and 200 mg/kg) was significantly improved when supplemented with Rivastigmine (5 mg/kg). These findings suggest that treatment with a combination of *B. monniera* with Rivastigmine may be highly beneficial compared to their *per-se* treatment.

* Corresponding author. Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore 562 123, Karnataka, India.
E-mail: dr.rafiq@himalayahealthcare.com

pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2013.02.004

1. Introduction

Developing effective and safe therapeutic treatment strategies for cognitive disorders, such as amnesia, attention deficit hyperactivity disorder, and Alzheimer's disease (AD), remains a challenge in the field of medicine [1]. The pathological hallmarks of cognitive disorders are memory loss and learning deficits. Environmental factors have been suggested as one of the possible contributory causes to the development of neurological disorders [2]. In this context, long-term consumption/exposure to aluminum is also thought to be a causative factor in the pathogenesis of AD [3]. Exposure to aluminum is not usually harmful and is not inherently toxic. However, a high concentration of aluminum is found in people with Parkinson's disease, senile dementia, amyotrophic lateral sclerosis, and senile dementia associated with AD [4].

Aluminum is a well-known neurotoxicant reported to accelerate oxidative damage to biomolecules. It crosses the blood—brain barrier through the high affinity transferrin receptors [5] and: (1) causes protein misfolding; (2) causes self-aggregation of highly phosphorylated cytoskeletal proteins, such as neurofilaments, microtubule-associated proteins, and amyloid-β; and (3) inhibits slow and fast axonal transports by damaging synaptic architecture, and induces neuroinflammation [6]. Additionally, aluminum is a potent cholinotoxin that causes apoptotic neuronal loss and also impairs hippocampal long-term potentiation. All these neurological perturbances ultimately result in learning and memory deficits in animals and humans. Further, aluminum has been reported to be found in both senile plaques and neurofibrillary tangle-bearing neurons in the brains of patients with AD [7].

In the context of treating cognition and related disorders, plant-based medicines have been used in folk medicine. Some medicinal plants have been scientifically proven to possess potent cognition-enhancing properties [8,9]. *Bacopa monniera* of the family Scrophulariaceae is a well known cognitive enhancer in the Indian system of traditional medicine [10]. It is also referred to as *Herpestis monniera* and is commonly called Brahmi [11]. *B. monniera* was traditionally used as a brain (nerve) tonic to enhance memory, learning, and concentration. It has been scientifically proven to possess anti-inflammatory [12], analgesic [13], antipyretic [14], sedative [15], antiepileptic [16], anxiolytic [14], antidepressant [17], and antioxidant properties [18].

Studies have reported that *B. monniera* has anticholinesterase activity (aids learning and memory skills), and adaptogenic and antidepressant activity in both acute and chronic stress situations [19]. It has also been shown to be beneficial in reversing scopolamine-induced oxidative stress and dementia in experimental animals [10,14,19]. In light of this, the present study was conducted to evaluate the possible synergistic activity of *B. monniera* with rivastigmine in reversing $AlCl_3$-induced learning deficits and memory loss in rats.

2. Materials and methods

2.1. Animals

Inbred adult male albino Wistar rats (180—200 g body weight) were used for the study. Animals were housed in standard isolation cages under standard environmental conditions with a temperature of 22 ± 2°C, relative humidity of 60 ± 5% and a 12 hour light—dark cycle. Rats were allowed free access to water and standard laboratory rat chow (Provimi Animal Nutrition India Pvt Ltd, Bangalore, India).

2.2. Ethics approval

All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of The Himalaya Drug Company and were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), India.

2.3. Chemicals and reagents

B. monniera (Bacopa) granules (The Himalaya Drug Company, Bangalore, India) and rivastigmine (Sun Pharmaceuticals Ltd, Mumbai, India) were used for the study; all other chemicals and reagents were of analytical grade (HiMedia Laboratories Pvt Ltd, Mumbai, India).

2.4. Experimental protocol

One hundred male Wistar albino rats were randomized into 10 groups (G1—G10), each with 10 animals. The animals in G1 were treated with vehicle and served as controls. Animals in G2 and G7 were treated with 5 mg/kg rivastigmine p.o., G3 and G8 with 100 mg/kg *B. monniera* p.o., G4 and G9 with 200 mg/kg *B. monniera* p.o., and G5 and G10 with a combination of 5 mg/kg rivastigmine and 100 mg/kg *B. monniera* p.o. Along with their assigned drug treatments, the animals in G7, G8, G9, and G10 were challenged with $AlCl_3$ (100 mg/kg p.o.) daily for 42 days to induce learning deficits and amnesia; animals in G6 served as positive controls and only received $AlCl_3$.

A flow chart of the experimental protocol is shown in Fig. 1.

2.5. Assessment of cognitive parameters by the Morris water maze test

Animals were trained to swim to a visible platform in a circular pool (180 cm in diameter and 60 cm in height) located in a test room. In principle, rats can escape from swimming by climbing onto the platform and over time the rats 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 [4,20].

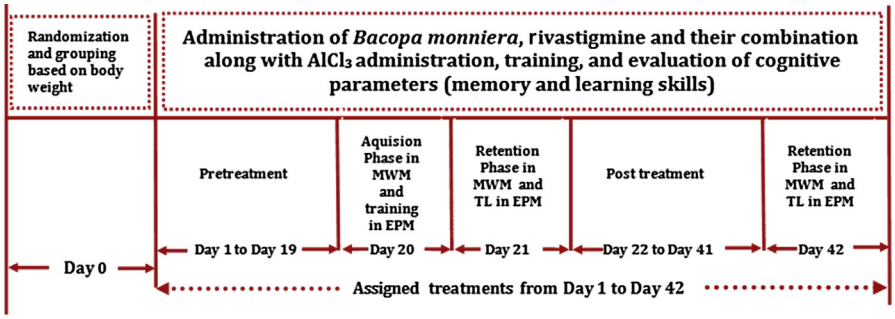


Figure 1 Flow chart of the experimental protocol. EPM = elevated plus maze test; MWM = Morris water maze test; TL = transfer latency.

2.5.1. 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 20. 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 rat 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 [4,20].

2.5.2. 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 21 (24 hours after the last training session) and Day 42. 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 Day 21 to Day 42 was used to evaluate the learned skill or memory.

2.6. Elevated plus maze test (transfer latency)

Animals of different groups received their assigned drug treatments (as mentioned previously) for 20 days. On Day 20, 1 hour after the assigned drug treatments, all animals were tested on the elevated plus maze (EPM) for transfer latency (TL). In brief, the animals were individually placed at the end of the open arm facing away from center of the maze and the time taken to enter the closed arm was recorded and termed the TL. On Day 20, all animals were allowed to explore the EPM for 90 seconds. The animals remaining in the open arm without entering into the closed arm within 90 seconds were gently pushed into one of the enclosed arms and TL was recorded as 90 seconds (first trial); these animals were once again allowed to explore the maze for another 10 seconds and returned to their home cages after completion of the first trial. Similarly, retention of memory was assessed as TL on Day 21 (24 hours after the first trial) and Day 42 [4, 20].

2.7. Statistical analysis

All the values were expressed as mean ± standard error of the mean. The results were analyzed statistically using ANOVA followed by Tukey's *post hoc* test. The minimum level of significance was fixed at $p < 0.05$.

3. Results

3.1. Morris water maze test

Chronic oral administration of $AlCl_3$ results in the deterioration of learning and memory skills in Wistar rats, which is supported by the literature reports cited in the present study. Animals treated only with $AlCl_3$ showed learning and memory deficits in the Morris water maze task compared to normal controls ($p < 0.01$). However, *B. monniera* (100 and 200 mg/kg; $p < 0.05$), rivastigmine (5 mg/kg; $p < 0.05$) and a combination of *B. monniera* (100 mg/kg) and rivastigmine (5 mg/kg; $p < 0.01$) offered treated groups significant protection against $AlCl_3$-induced learning and memory deficits. Interestingly, the combination of *B. monniera* (100 mg/kg) and rivastigmine (5 mg/kg) was found to be therapeutically more potent than either treatment alone against $AlCl_3$-induced toxicity.

The results of the study are given in Fig. 2.

3.2. EPM test

The EPM test is the most commonly used method for evaluating memory skills in experimental animals. In the EPM, memory was evaluated and expressed as TL. On Day 20, it was found that chronic administration of $AlCl_3$ significantly increased TL in animals treated only with $AlCl_3$ compared to normal controls ($p < 0.01$), which indicates that the memory deficits were due to $AlCl_3$ administration. In contrast to animals treated only with $AlCl_3$, administration of *B. monniera*, rivastigmine, and a combination of *B. monniera* (100 mg/kg) and rivastigmine (5 mg/kg) alleviated the $AlCl_3$-induced learning and memory deficits in Wistar rats. Exceptionally, the combination of *B. monniera* (100 mg/kg) and rivastigmine (5 mg/kg) was highly potent compared to either treatment alone (Fig. 3).

4. Discussion

Aluminum is one of the most abundantly found and commonly used metals in the food industry, especially for storage, packaging, and transportation [21]. Several studies

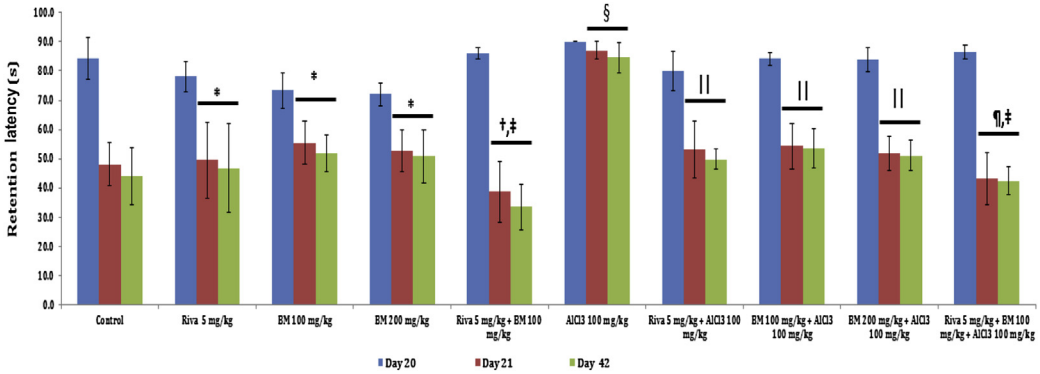


Figure 2 Evaluating the effect of *Bacopa monniera* (BM), rivastigmine (Riva), and their combination on memory retention in rats through the Morris water maze test. All values are expressed as mean ± standard error of the mean; means of various groups were statistically compared by ANOVA followed by Tukey's test using Graph Pad version 4.0. *$p < 0.05$ and †$p < 0.01$ compared to normal controls; ‡$p < 0.05$ compared to rivastigmine 5 mg/kg; §$p < 0.01$ compared to controls; ||$p < 0.05$, ¶$p < 0.01$ compared to $AlCl_3$; ‡$p < 0.05$ compare to rivastigmine 5 mg/kg.

have estimated that an adult will consume around 3–12 mg/day of aluminum either directly or indirectly [22]. Long-term consumption of aluminum leads to its accumulation in the brain, bone, muscle, kidney, and other organs, resulting in the development of neurodegenerative disorders [23,24], amyotrophic lateral sclerosis [25], encephalopathy due to chronic renal failure [25,26], osteomalacia [27], immunosuppression [28], and so on.

Aluminum is a potent pro-oxidant [29] and cholinotoxin [30], and is considered to be one of the environmental factors causing neurodegenerative disorders such as AD [31]. While aluminum is not a transition metal and does not directly initiate lipid peroxidation, it will potentiate the oxidative damage caused by iron under acidic and neutral conditions [32]. Also $AlCl_3$ causes effective alteration of the central nervous system, blood–brain barrier, and central cholinergic system. It may also decrease acetylcholine levels in the hippocampal area, which leads to the development of cognitive disorders such as AD, a decrease in spatial learning, and memory disorders [19]. The brain is covered with a thick lipid structure and has low antioxidant defense associated with high oxygen metabolism (it metabolizes about 20% of the total oxygen consumed by the body), making it highly vulnerable to oxidative damage [21].

Agents that enhance cholinergic transmission or decrease cholinesterase activity have proven useful in treating AD. In this regard, rivastigmine with its anticholinesterase activity has been shown to be beneficial in treating the symptoms of AD associated with learning deficits and dementia [33]. Similarly, *B. monniera* is also highly effective in alleviating learning and memory deficits. Furthermore, studies have reported that rivastigmine and *B. monniera* are also useful in preventing $AlCl_3$-induced neuronal damage [33–35]. Considering the beneficial effect of *B. monniera* and rivastigmine in treating the symptoms of AD, this study is an attempt to explore the possible drug–drug interaction of *B. monniera* and rivastigmine in reversing $AlCl_3$-induced learning deficits and dementia relevant to AD.

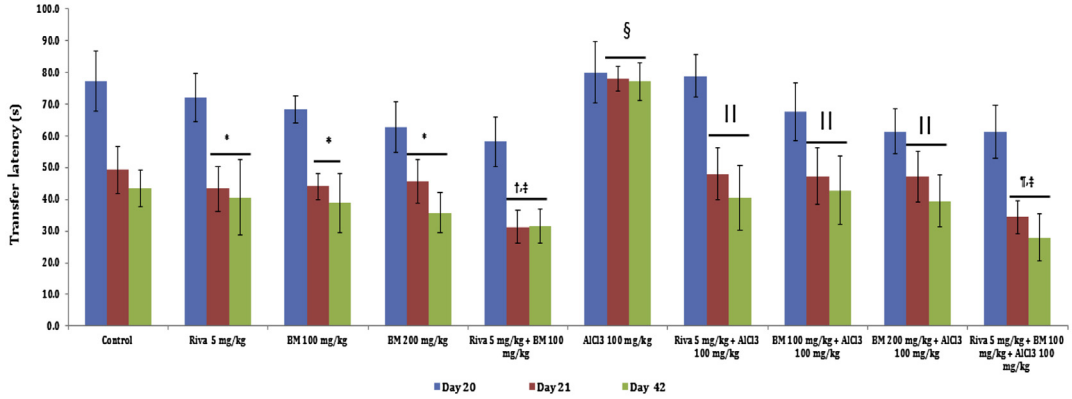


Figure 3 Evaluating the effect of *Bacopa monniera* (BM), rivastigmine (Riva), and their combination on memory retention in rats through the elevated plus maze test. All values are expressed as mean ± standard error of the mean; means of various groups were statistically compared by ANOVA followed by Tukey's test using Graph Pad version 4.0. *$p < 0.05$ and †$p < 0.01$ compared to normal controls; ‡$p < 0.05$ compared to rivastigmine 5 mg/kg; §$p < 0.01$ compared to controls; ||$p < 0.05$, ¶$p < 0.01$ compared to $AlCl_3$.

In the past, many researchers have reported that oral administration of $AlCl_3$ to experimental animals results in the development of learning deficits and dementia [36—38], and hence this is considered to be a suitable model for evaluating the beneficial effect of *B. monniera* and rivastigmine in experimental animals.

Consistent with the literature reports, chronic administration of $AlCl_3$ in this study caused functional disability in learning and memory skills, which was tested by the Morris water maze and EPM tasks. In short, spatial memory was evaluated in the Morris water maze test and showed decreased acquisition and retention latencies when the rats were tested on Day 21 and Day 42 of the treatment. However, groups treated with rivastigmine (5 mg/kg), *B. monniera* (100 and 200 mg/kg), and their combination showed better acquisition and retention latencies compared to groups treated only with $AlCl_3$, indicating significant protection against $AlCl_3$-induced deterioration in learning and memory skills. Similarly, in the EPM task, animals treated only with $AlCl_3$ showed diminished exploration (to locate the closed arm) and lack of retention (enhanced retention latency), indicating impaired learning and memory skills. In contrast, animals treated with rivastigmine (5 mg/kg), *B. monniera* (100 and 200 mg/kg), and their combination showed better learning and memory retention compared to the groups treated only with $AlCl_3$. Exceptionally, the combination of rivastigmine (5 mg/kg) with *B. monniera* (100 mg/kg) showed good results in both the Morris water maze and EPM tasks compared to either treatment alone. Furthermore, in earlier studies *B. monniera* has been shown to be effective in enhancing memory and learning skills [39] and the phytoconstituents, bacoside A and bacoside B, are thought to be responsible for its beneficial effect in treating memory and learning deficits. In exploring the underlying mechanism, a study conducted by Jyoti et al [35] on the beneficial effect of *B. monniera* against $AlCl_3$-induced neurotoxicity, reported that *B. monniera* through its potent antioxidant mechanism prevents $AlCl_3$-induced neurotoxicity in the brain, enhances neurogenesis and synaptic cholinergic neurotransmission in regions such as the cerebral cortex and hippocampus, and is thus useful as an antidementia drug during oxidative insults [19]. In examining the mechanism behind the synergistic effect of *B. monniera* and rivastigmine, it can be concluded that *B. monniera* (through its antioxidant mechanism) and rivastigmine (through its inhibitor anticholinesterase activity) act through dual mechanisms to prevent neuronal damage and/or enhance cholinergic neurotransmission, thereby showing better therapeutic effect compared to either treatment alone.

5. Conclusion

The findings of the present study suggest that *B. monniera* (100 and 200 mg/kg), rivastigmine (5 mg/kg), and a combination of *B. monniera* and rivastigmine could be highly beneficial in preventing the learning and memory impairment induced by chronic administration of $AlCl_3$. However, the combination of *B. monniera* (Bacopa) with rivastigmine was found to be more effective than either treatment alone in preventing $AlCl_3$-induced learning and memory deficits.

Conflict of interest

The authors declare that there are no conflict of interest.

Acknowledgments

The authors thank The Himalaya Drug Company, Bangalore for providing all necessary facilities to carry out this research work. The authors also acknowledge the work of Dr. Jayashree B. Keshav and team (Scientific Publications Division of the Himalaya Drug Company) for copy-editing and proofreading the article.

References

1. Joshi H, Parle M. Brahmirasayana improves learning and memory in mice. *eCAM*. 2006;3:79—85.
2. Campbell A. The potential role of aluminium in Alzheimer's disease. *Nephrol Dial Transplant*. 2002;17(suppl 2):17—20.
3. Rogers MAM, Simon DG. A preliminary study of dietary aluminium intake and risk of Alzheimer's disease. *Age Ageing*. 1999;28:205—209.
4. Prakash A, Kumar A. Effect of N-acetyl cysteine against aluminium-induced cognitive dysfunction and oxidative damage in rats. *Basic Clin Pharmacol Toxicol*. 2009;105:98—104.
5. Roskams AJ, Connor JR. Aluminum access to the brain: a role for transferrin and its receptor. *Proc Natl Acad Sci USA*. 1990; 87:9024—9027.
6. Campbell A, Becaria A, Lahiri DK, Sharman K, Bondy SC. Chronic exposure to aluminium in drinking water increases inflammatory parameters selectively in the brain. *J Neurosci Res*. 2004;75:565—572.
7. McLachlan DR, Bergeron C, Smith JE, Boomer D, Rifat SL. Risk for neuropathologically confirmed Alzheimer's disease and residual aluminum in municipal drinking water employing weighted residential histories. *Neurology*. 1996;46:401—405.
8. Manish KP. Neuroprotective properties of some Indian medicinal plants. *Inter J Pharma Biol Arch*. 2011;2:1374—1379.
9. Howes MJ, Houghton PJ. Ethnobotanical treatment strategies against Alzheimer's disease. *Curr Alzheimer Res*. 2012;9:67—85.
10. Vollala VR, Upadhyay S, Nayak S. Effect of *Bacopa monniera* Linn. (Brahmi) extract on learning and memory in rats: a behavioural study. *J Vet Behav*. 2010;5:69—74.
11. Kulkarni R, Girish KJ, Kumar A. Nootropic herbs (Medhya Rasayana) in Ayurveda: an update. *Pharmacogn Rev*. 2012;6: 147—153.
12. Viji V, Helen A. Inhibition of lipoxygenases and cyclooxygenase-2 enzymes by extracts isolated from *Bacopa monniera* (L.) Wettst. *J Ethnopharmacol*. 2008;118:305—311.
13. Rauf K, Subhan F, Abbas M, Badshah A, Ullah I, Ullah S. Effect of bacopasides on acquisition and expression of morphine tolerance. *Phytomedicine*. 2011;18:836—842.
14. Russo A, Borrelli F. Bacopa monneira, a reputed nootropic plant: an overview. *Phytomedicine*. 2005;12:305—317.
15. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of sedative and anticonvulsant activities of Unmadnashak Ghrita. *J Ethnopharmacol*. 2004;94:77—83.
16. Mathew J, Paul J, Nandhu MS, Paulose CS. Increased excitability and metabolism in pilocarpine induced epileptic rats: effect of *Bacopa monnieri*. *Fitoterapia*. 2010;81:546—551.
17. Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. *Phytomedicine*. 2002;9:207—211.

18. Bhattacharya SK, Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. *Phytother Res*. 2000;14:174—179.
19. Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK. A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba* anti-cholinesterase and cognitive enhancing activities. *Pharmacol Biochem Behav*. 2002;73:893—900.
20. Kumar A, Seghal N, Padi SV, Naidu PS. Differential effects of cyclooxygenase inhibitors on intracerebroventricular colchicine-induced dysfunction and oxidative stress in rats. *Eur J Pharmacol*. 2006;551:58—66.
21. Shati A, Elsaid G, Hafez E. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of *Crocus sativus* L. extraction and honey syrup. *Neuroscience*. 2011;175:66—74.
22. Yokel RA. Aluminum chelation principles and recent advances. *Coord Chem Rev*. 2002;228:97—113.
23. Suárez-Fernádez MB, Soldado B, Sanz-Medel AB, Vaga A, Novelli JAA, Fernández-Sánchez MT. Aluminum-induced degeneration of astrocytes occurs via apoptosis and results in neuronal death. *Brain Res*. 1999;835:125—136.
24. Garruto RM, Shankar SK, Yanagihara R, Salazar AM, Amyx HL, Gajdusek DC. Low-calcium, high-aluminum diet-induced motor neuron pathology in cynomolgus monkeys. *Acta Neuropathol*. 1989;78:210—219.
25. Van der Voet GB, Schijns O, de Wolff FA. Fluoride enhances the effect of aluminum chloride on interconnections between aggregates of hippocampal neurons. *Arch Physiol Biochem*. 1999; 101:15—21.
26. Van Landeghem GF, D'Haese PC, Lamberts LV, Barata JD, De Broe ME. Aluminum speciation in cerebrospinal fluid of acutely aluminium-intoxicated dialysis patients before and after desferrioxamine treatment; a step in the understanding of the element's neurotoxicity. *Nephrol Dial Transplant*. 1997;12:1692—1698.
27. Kausz AT, Antonsen JE, Hercz G, Pei Y, Weiss NS, Emerson S, et al. Screening plasma aluminum levels in relation to aluminum bone disease among asymptomatic dialysis patients. *Am J Kidney Disease*. 1999;34:688—693.
28. Golub MS, Takeuchi PT, Gershwin ME, Yoshida SH. Influence of dietary aluminum on cytokine production by mitogen stimulated spleen cells from Swiss Webster mice. *Immunopharmacol Immunotoxicol*. 1993;15:605—619.
29. Exley C. The pro-oxidant activity of aluminum. *Free Radic Biol Med*. 2004;36:380—387.
30. Gulya K, Rakonczay Z, Kása P. Cholinotoxic effects of aluminium in rat brain. *J Neurochem*. 1990;54:1020—1026.
31. Domingo JL. Aluminum and other metals in Alzheimer's disease: a review of potential therapy with chelating agents. *J Alzheimers Dis*. 2006;10:331—341.
32. Ohyashiki T, Karino T, Suzuki S, Matsui K. Effect of aluminum ion on Fe^{2+}-induced lipid peroxidation in phospholipid liposomes under acidic conditions. *J Biochem*. 1996;120:895—900.
33. Abdel-Aal RA, Assi AA, Kostandy BB. Rivastigmine reverses aluminium-induced behavioural changes in rats. *Eur J Pharmacol*. 2011;659:169—176.
34. Jyoti A, Sharma D. Neuroprotective role of *Bacopa monniera* extract against aluminium-induced oxidative stress in the hippocampus of rat brain. *Neurotoxicology*. 2006;27:451—457.
35. Jyoti A, Sethi P, Sharma D. *Bacopa monniera* prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. *J Ethnopharmacol*. 2007;111:56—62.
36. Kumar A, Prakash A, Dogra S. Neuroprotective effect of carvedilol against aluminium induced toxicity: possible behavioral and biochemical alterations in rats. *Pharmacol Rep*. 2011;63: 915—923.
37. Kumar A, Dogra S, Prakash A. Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: possible behavioral and biochemical alterations in rats. *Behav Brain Res*. 2009;205:384—390.
38. Miu AC, Benga O. Aluminium and Alzheimer's disease: a new look. *J Alzheimers Dis*. 2006;10:179—201.
39. Roodenryls S, Booth D, Bulzoni S, Phipps A, Micallef C, Smoker J. Chronic effects of Brahmi (*Bacopa monnieri*) on human memory. *Neuropsychopharmacology*. 2002;27: 279—281.