Effect of *Boswellia serrata* on Alzheimer's disease induced in rats

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Background/aim

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Increased oxidative stress has been shown to be a prominent and early feature in AD. Medicinal plants with antioxidant activities have been used traditionally in the treatment of several human diseases. The present study aimed to investigate the possible prophylactic and therapeutic effects of aqueous infusions of *Boswellia serrata* on AD induced in rats.

Materials and methods

Ninety adult male Sprague Dawley rats were enrolled in this study and were divided into 9 groups (ten each). Groups 1–5 for the protective study, 6–9 for the therapeutic study as follows: 1st group: negative control group in which rats were given daily oral dose of 1 ml tab water, 2nd group: induction of animal model mimicking AD by daily oral administration of aluminum chloride ($AlCl_3$) to rats in a dose of 17 mg/kg for 4 successive weeks; 3rd, 4th, and 5th groups: rats were orally given rivastigmine (0.3 mg/kg/day), *Boswellia serrata* (45 and 90 mg/kg /day respectively), for two weeks followed by combination of each treatment with $AlCl_3$ for another four successive weeks. Groups 6–9 for the therapeutic study: 6th group: AD induced group which acted as a model mimicking AD in humans received orally 1 ml of tab water only for 12 successive weeks and served as therapeutic untreated group. 7th, 8th and 9th groups: AD rats treated orally with rivastigmine (0.3 mg/kg/day), *Boswellia serrata* (45 and 90 mg/kg /day respectively) daily for 12 successive weeks. At baseline (before induction of AD), before treatment, then after each treatment, behavioral stress tests as activity cages, rotarod, and T-maze tests were done. At the end of all experiments rats' brains were dissected and divided sagitally into two portions, the first portion was homogenized for determination of acetylcholine (Ach) and acetycholinesterase (AchE) levels. The second portion was used for histopathologic examination.

Results

The present study indicated that *Boswellia serrata* when was used for treatment of $AlCl_3$ induced AD, its high dose only produced increased activity of rats in the activity cage, duration of rats revolving on the rotarod and reduction in the duration taken by rats to reach food in the T-maze test. Both doses produced elevation of Ach level and reduction of AchE activity in brain homogenates. These results were consistent with the histopathological findings in brain tissues where, the neurons appear more or less like normal ones.

Conclusion

This study revealed that the treatment of AD-induced rats with aqueous infusions of *B. serrata* significantly ameliorates the neurodegenerative characteristics of ADs in rats.

Keywords:

acetycholinesterase, acetylcholine, Alzheimer's disease, behavioral stress tests, *Boswellia serrata*, rats

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Introduction

Alzheimer's disease (AD), which represents one of the most financially draining diseases to society, is a neurodegenerative disorder characterized by progressive degeneration of the hippocampal and cortical neurons that leads to impairment of memory and cognitive ability. Impairment of short-term memory is usually the first clinical feature, whereas retrieval of distant memories is preserved relatively well into the course of the disease. When the condition progresses, additional cognitive abilities are impaired, such as the ability to calculate and use common objects and tools. The pathological hallmarks of AD are senile plaques, which are spherical

accumulations of β-amyloid protein accompanied by degenerating neuronal processes as well as neurofibrillary tangles composed of paired helical filaments and other proteins. This corresponds to the clinical features of marked impairment of memory and abstract reasoning, with preservation of vision and movement [1].

The selective deficiency of acetylcholine (Ach) in AD has given rise to the 'cholinergic hypothesis', which proposes that a deficiency of Ach is critical in the genesis of the symptoms of AD [2]. Therefore, a major approach to the treatment of AD involves attempts to augment the cholinergic function of the brain. This involves the use of inhibitors of acetyl cholinesterase such as tacrine, donepezil, rivastigmine, and galantamine [3]. Moreover, other hypotheses state that inflammation plays a key role in the pathogenesis of AD. In addition, excessive reactive oxygen species levels are implicated in the etiology of AD [4].

Medicinal plants have been traditionally used in the treatment of several human diseases, and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts. In particular, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects against various degenerative diseases, including cancer and neurological and cardiovascular diseases [5]. Thus, the antioxidant properties of plants have a full range of perspective applications in human healthcare [6].

Boswellia is a genus of trees known for their fragrant resin that has many pharmacological uses, particularly as anti-inflammatory agents. Boswellic acids, which are components of the resin, have shown promising results in the treatment for asthma and various inflammatory conditions [7]. Boswellia gum, extracted from the resin, is used in the prevention and treatment against colitis, ulcerative colitis, Crohn's disease, and ileitis. Moreover, *Boswellia serrata* shows satisfactory antioxidant activity in the cerebrovascular system [8].

The purpose of this study was to investigate the possible prophylactic and curative effects of aqueous infusions of *B. serrata* in an animal model mimicking AD induced by administration of $AlCl_3$. The prophylactic and therapeutic effects were evaluated using behavior stress tests and by measuring levels of Ach and acetylcholinesterase (AChE) in brain homogenates, and by histopathological examination of the brain tissue for all rats in all groups.

Materials and methods

Materials

$AlCl_3$(MW = 133.34) was purchased from Sigma-Aldrich Co. (Munich, Germany). Rivastigmine (0.3 mg) was purchased from Novartis Co. (Cairo, Egypt). The aerial parts of *B. serrata* were purchased from a local market in Cairo, Egypt, and were identified kindly by Dr Ibrahim El-Garf, Professor of Taxonomy, Faculty of Science, Cairo University.

Preparation of aqueous infusion of Boswellia serrata

A volume of 50 ml of boiling-hot distilled water was poured on 625 mg of the resin in a beaker. The mixture was allowed to stand for 30 min before it was filtered with a filter paper. An equivalent extract from 12.5 mg dried plant material per ml aqueous infusion was obtained. The infusion was always freshly prepared so as to prevent growth of fungi. The dose of *B. serrata* was calculated by conversion of the human anti-inflammatory dose (900–1000 mg/day) to the rat dose according to method described by Paget *et al*. [9].

Induction of Alzheimer's disease in rats

Induction of AD in the rats was carried out by administering $AlCl_3$ orally at a dose of 17 mg/kg body weight daily for 4 successive weeks, according to the procedure described by Krasovskii *et al*. [10].

Experimental design

The present study was carried out on 90 adult male Sprague–Dawley rats (weighing 150–200 g) obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were maintained on standard laboratory diet and water *ad libitum*. After an acclimation period of 1 week, the animals were housed in stainless steel cages in a temperature-controlled ($23 \pm 1^{\circ}C$) and artificially illuminated (12-h dark/light cycle) room free from any source of chemical contamination. All animals received human care, and the experiments performed on them were in accordance with the guidelines provided for animal experimentation, which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt. The animals used were divided into nine groups (10 rats each) as follows.

Group 1: Normal rats serving as the negative control group that were administered 1 ml tap water orally daily throughout the experiment.

Protective groups

Group 2: Animal models mimicking AD, which was induced by daily oral administration of $AlCl_3$ at a dose of 17 mg/kg for 4 successive weeks, serving as the protective positive control group.

Group 3: Rats orally given rivastigmine at a dose of 0.3 mg/kg/day [11] for 2 successive weeks, followed by a combination of rivastigmine with $AlCl_3$ for 4 successive weeks.

Group 4: Rats orally given an aqueous infusion of *B. serrata* at a dose of 45 mg/kg/day for 2 successive weeks, followed by a combination with *B. serrata* and $AlCl_3$ for 4 successive weeks.

Group 5: Rats orally given an aqueous infusion of *B. serrata* at a dose of 90 mg/kg/day for 2 successive weeks, followed by a combination of *B. serrata* with $AlCl_3$ for 4 successive weeks.

Therapeutic groups

Group 6: AD-induced rats administered $AlCl_3$ for 4 weeks and serving as the therapeutic positive control group.
Group 7: AD-induced rats treated daily for 12 successive weeks with rivastigmine at a dose of 0.3 mg/kg body weight [11].
Group 8: AD-induced rats treated daily with *B. serrata* extract for 12 successive weeks at a dose of 45 mg/kg body weight.
Group 9: AD-induced rats treated daily with *B. serrata* extract for 12 successive weeks at a dose of 90 mg/kg body weight.

Behavioral stress tests

In the activity cage and rotarod tests, the percentage change in behavior was calculated (considered 100%), for which square root transformation (%) was done (considered 1). These calculations were made so as to avoid normal biological variations in the activities of normal rats in all groups (provided that each group contains rats with approximately similar activity). Later on, the square root transformed (%) change of activity for each rat was compared with its baseline activity at every transitional step throughout the whole experiment.

Measurement of levels of activity using the activity cage test

Levels of activity were measured by detecting rat movements using a grid floor activity cage (Model No. 7430; Ugo Basile, Varese, Italy), according to the method described by Pavić *et al.* [12].

Measurement of motor coordination using the rotarod test

Motor coordination in this study was assessed using an accelerating rotarod (Model No. 7750; Ugo Basile), according to the procedure described by Vijitruth *et al.* [13].

Test for cognitive abilities using T-maze

Animals were introduced from the base of the T-maze and allowed to choose one of the goal arms abutting the other end of the stem. The trial was carried out twice in quick succession. At the second trial, the rodent tended to choose the arm not visited before, reflecting a memory of the first choice. This is called 'spontaneous alternation'. This tendency was reinforced by starving the animal for 24 h before the test and rewarding it with a preferred food item if it alternates. Both spontaneous and rewarded alternations are very sensitive to dysfunction of the hippocampus; however, other brain structures are also involved. Each trial was completed in less than 2 min [14].

Brain tissue sampling and preparation

At the end of the experimental period (after 3 months), the animals were kept fasting for 12 h and killed by decapitation. The whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried, and then weighed. Thereafter, each brain was sagitally divided into two portions. The first portion of each brain was homogenized immediately to give a 10% (w/v) homogenate in ice-cold medium containing 50 mmol/l Tris-Hcl (pH 7.4) and 300 mmol/l sucrose [15]. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (10%) was separated for biochemical analysis (Ach, AchE, and total protein estimation). The second portion of each brain was fixed in formalin buffer (10%) for histopathological examination.

Biochemical analysis

Brain Ach levels were determined by the colorimetric method using a choline/acetylcholine assay kit (BioVision Inc., California, USA), according to the method described by Oswald *et al.* [16]. Brain AchE levels were determined colorimetrically according to method of Den Blaauwen *et al.* [17], using kits from Biostc Co. (Los Angeles, USA). Moreover, brain total protein concentrations were estimated to express the concentration of different brain parameters per mg protein, according to the method of Lowry *et al.* [18], using kits from Biodiagnostic Co. (Cairo, Egypt).

Histopathological examination

The second portion of each brain was fixed in formalin buffer (10%) for 24 h. The brains were washed in tap water and then dehydrated using serial dilutions of alcohol (methyl, ethyl, and absolute ethyl). Specimens were cleared in xylene and embedded in paraffin in a hot air oven at 56°C for 24 h. Paraffin bees wax blocks were prepared for sectioning at 4 μm using a microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin stains [19] for histopathological examination using a light microscope.

Statistical analysis

In the present study, all results were expressed as mean ± SE of the mean. Data were analyzed by one-way analysis of variance using the statistical package for social sciences (SPSS) program, version 11 (SPSS Inc., Chicago, Illinois, USA). The least significant difference was calculated to compare significances between the groups. The square root transformation (%) was calculated according to the method described by Jones *et al.* [20]. Thereafter, comparisons between more than two groups were made using analysis of variance, followed by Dunn's multiple comparison test or the Tukey–Kramer multiple comparison test to analyze the results of the T-maze test. A difference was considered significant at a P value of less than 0.05.

Results

Protective groups

Activity cage

The results in Table 1 showed a significant decrease in activity of rats treated with $AlCl_3$ for 4 weeks (AD group) as compared with the baseline (before treatment with $AlCl_3$) of the same group. While, rats treated with rivastigmine and 90 mg/kg of *B. serrata* exhibited a

significant increase of rats activity after 4 weeks of administration of *B. serrata* in combination with $AlCl_3$. In contrast rats treated with 45 mg/kg of *B. serrata* showed insignificant effect in comparison with the AD group of rats. Moreover, rivastigmine-treated rats exhibited significant improvement in levels of activity compared with rats treated with 45 mg/kg of *B. serrata*.

Rotarod

The results in Table 2 showed an insignificant change in motor coordination, as tested by the rotarod, in the AD group of rats treated with either rivastigmine or *B. serrata* (dose of 45 or 90 mg/kg) alone or in combination with $AlCl_3$ when compared with the baseline values of the same groups or with the AD group of rats. As in all groups the duration of sustained balance of rats on the rotarod was not significantly affected by administration of $AlCl_3$ for 4 successive weeks in combination with the treating agent or alone.

T-maze

The results in Table 3 show significant increases in the time (s) taken by rats to reach the food in the T-maze behavior stress test for the groups of rats given $AlCl_3$ only for 4 successive weeks (AD group), whereas groups treated with rivastigmine or *B. serrata* (45 or 90 mg/kg) in combination with $AlCl_3$ for 4 successive weeks induced significant decreases in the time (s) taken by rats to the reach food in the T-maze test in comparison with the AD group of rats.

Moreover, insignificant changes in the time (s) taken by rats to reach the food was recorded in the groups treated with rivastigmine or *B. serrata* (45 or 90 mg/kg) alone in comparison with the baseline values (before treatment) of the same groups.

Biochemical parameters

The results in Table 4 show a significant decrease in ACh levels and a significant increase in AChE levels in brains of rats that received $AlCl_3$ only for 4 successive weeks in

Table 1 Protective effects of rivastigmine and *Boswellia serrata* on the levels of activity in Alzheimer's disease-induced rats in activity cages

	Time duration		
Group	Baseline (0 weeks)	2 weeks before treatment	4 weeks after treatment with $AlCl_3$
Control	100[a]	98.9 ± 2.3	97.2 ± 1.8
	1[b]	0.99 ± 0.06[d]	0.98 ± 0.05[d]
AD group $AlCl_3$ (17 mg/kg)	100[a]		51.7 ± 6.72
	1[b]		0.71 ± 0.04[c]
Rivastigmine (0.3 mg/kg)	100[a]	95.11 ± 1.57	93.44 ± 1.55
	1[b]	0.97 ± 0.008[d]	0.96 ± 0.08[d]
Boswellia serrata (45 mg/kg)	100[a]	93.1 ± 1.64	57.26 ± 9
	1[b]	0.96 ± 0.008[d]	0.74 ± 0.06[c,e,f]
Boswellia serrata (90 mg/kg)	100[a]	89.65 ± 3.54	88.26 ± 2.73
	1[b]	0.94 ± 0.02[d]	0.92 ± 0.01[d]

All data are expressed as means of movements ± SE.
AD, Alzheimer's disease.
[a]% change.
[b]Square root transformed % change.
[c]Significantly different from baseline of the same group at $P < 0.05$.
[d]Significantly different from AD group at $P < 0.05$.
[e]Significantly different from rivastigmine when each is used for 4 weeks in combination with $AlCl_3$ at $P < 0.05$.
[f]Significantly different from same group when given alone for 2 weeks at $P < 0.05$.

Table 2 Protective effects of rivastigmine and *Boswellia serrata* on the time spent on the rotarod by Alzheimer's disease-induced rats

	Time duration		
Group	Baseline (0 weeks)	2 weeks before treatment	4 weeks after treatment with $AlCl_3$
Control	100[a]	96.4 ± 3.1	97.3 ± 2.8
	1[b]	0.981 ± 0.17	0.986 ± 0.16
AD group $AlCl_3$ (17 mg/kg)	100[a]	–	83.07 ± 2.69
	1[b]	–	0.91 ± 0.01
Rivastigmine (0.3 mg/kg)	100[a]	95.02 ± 1.34	92.32 ± 1.4
	1[b]	0.97 ± 0.006	0.96 ± 0.007
Boswellia serrata (45 mg/kg)	100[a]	89.96 ± 2.75	85.7 ± 3.1
	1[b]	0.94 ± 0.01	0.92 ± 0.01
Boswellia serrata (90 mg/kg)	100[a]	90.47 ± 3.23	89.27 ± 6.2
	1[b]	0.95 ± 0.02	0.94 ± 0.03

All data are expressed in seconds as means ± SE.
AD, Alzheimer's disease.
[a]% change.
[b]Square root transformed % change.

Table 3 Protective effects of rivastigmine and *Boswellia serrata* on the time taken to find the food in the T-maze by Alzheimer's disease-induced rats

	Time duration		
Group	Baseline (0 weeks)	2 weeks before treatment	4 weeks after treatment with $AlCl_3$
Control	13.44 ± 0.91	14.1 ± 0.88	15.56 ± 1.3[b]
AD group $AlCl_3$ (17 mg/kg)	15.66 ± 1.07	–	115 ± 4.83[a]
Rivastigmine (0.3 mg/kg)	15.33 ± 1.63	13.16 ± 1.5	18.5 ± 1.4[b]
Boswellia serrata (45 mg/kg)	18.7 ± 3	22.42 ± 3.5	26 ± 4d[b]
Boswellia serrata (90 mg/kg)	11.7 ± 2	15 ± 2.3	21.2 ± 2[b]

All data are expressed in seconds as means ± SE.
AD, Alzheimer's disease.
[a]Significantly different baseline duration of the same group at $P<0.05$.
[b]Significantly different from $AlCl_3$ after 4 weeks induction at $P<0.05$.

Table 4 Protective effects of rivastigmine and *Boswellia serrata* on brain levels of acetylcholine and acetylcholinesterase in Alzheimer's disease-induced rats

Group	Acetylcholine (ACh) (μmol/mg protein)	Acetylcholinesterase (AChE) (unit/mg protein)
Control	5.54 ± 0.13	0.52 ± 0.008
AD group $AlCl_3$ (17 mg/kg)	0.83 ± 0.04[a]	0.79 ± 0.01[a]
Rivastigmine (0.3 mg/kg)	5.3 ± 0.12[b]	0.55 ± 0.02[b]
Boswellia serrata (45 mg/kg)	1.36 ± 0.31[a,c]	0.8 ± 0.02[a,c]
Boswellia serrata (90 mg/kg)	2.18 ± 0.2[a,b,c,d]	0.52 ± 0.02[b,d]

All data are expressed as means ± SE.
AD, Alzheimer's disease.
[a]Significantly different from control group at $P<0.05$.
[b]Significantly different from $AlCl_3$ group at $P<0.05$.
[c]Significantly different from rivastigmine group at $P<0.05$.
[d]Significantly different from *Boswellia serrata* 45 mg/kg at $P<0.05$.

comparison with the control group. Also rats that received *B. serrata* (45 or 90 mg/kg) in combination with $AlCl_3$ for 4 successive weeks exhibited a significant decrease in ACh levels. While rats treated with a low dose of *B. serrata* only exhibited a significant increase in AChE levels and those that received a high dose of *B. serrata* showed a significant decrease in AChE levels in comparison with the control group and AD groups, respectively. Moreover, rats treated with rivastigmine exhibited a significant increase in Ach levels, and significant decreases was in AChE levels when compared with those in the AD group of rats. Rats treated with 90 mg/kg of *B. serrata* reported a significant improvement.

Therapeutic effects

Activity cage

Boswellia serrata (45 and 90 mg/kg) treatment to AD-induced rats showed no significant difference compared to baseline activity of the same group, but both doses showed significant increase in activity compared to the same group before treatment, and to positive control group (Table 5).

Rotarod

The results in Table 6 showed a significant decrease in motor coordination activity, as tested by the rotarod test, in the AD group of rats that were given $AlCl_3$ for 4 successive weeks and left untreated for 12 successive weeks as well as in the group of AD rats treated with rivastigmine for 12 weeks, in comparison with the baseline values of the same groups. However, the group of AD rats treated with 90 mg/kg *B. serrata* for 12 successive weeks exhibited a significant increase in the duration of sustained balance on the rotarod in comparison with the baseline values of the same group as well as with the values of the AD groups. The duration of sustained balance on the rotarod for rats treated with the high dose of *Boswellia serrata* was more than the duration of sustained balance on the rotarod for rats treated with the low dose of *boswellia* and rivastigmine.

T-maze

The results in Table 7 showed a significant increase in the duration in seconds to reach the food in the T-maze by rats treated with $AlCl_3$ (AD group) for 4 successive weeks and left for 12 successive weeks without treatment as well as in the group of AD rats before rivastigmine treatment and before and after treatment with *B. serrata* (45 or 90 mg/kg), in comparison with the baseline values of the same groups. While the group of AD rats treated with rivastigmine for 12 successive weeks showed a significant decrease in the duration to reach the food in the T-maze test in comparison with the duration for same group before treatment as well as with the AD group left untreated for 12 successive weeks. The duration to reach food in the T-Maze by rats was directly proportionate to the dose of *Boswellia serrata*.

Biochemical parameters

The results in Table 8 showed a significant decrease in ACh levels in the AD group and in the group of rats treated with the lower dose of *B. serrata* (45 mg/kg). In contrast, a significant increase in AChE levels was served in the AD group in comparison with the control group. Rivastigmine and *B. serrata* at a dose of 90 mg/kg exhibited a significant improvement in the AD status as evidenced by an increase in the ACh levels in the brain homogenates, whereas the AD groups treated with rivastigmine as well as the groups treated with *B. serrata* showed significant decreases in AchE levels in brain homogenates in comparison with the AD group of rats. The high dose of *B. serrata* showed better effects compared with the low dose.

Table 5 Therapeutic effects of rivastigmine and *Boswellia serrata* on the levels of activity in Alzheimer's disease-induced rats in activity cages

	Time duration		
Group	Baseline (0 weeks)	$AlCl_3$ induction for 4 weeks	12 weeks (after stopping $AlCl_3$ induction or after treatment)
Control	100[a]	97.2 ± 1.8	97.9 ± 3.4
	1[b]	0.98 ± 0.05	0.99 ± 0.07[e,f]
AD group $AlCl_3$ (17 mg/kg)	100[a]	51.71 ± 6.72	22.4 ± 0.6
	1[b]	0.71 ± 0.04[c]	0.47 ± 0.006[c,d]
Rivastigmine (0.3 mg/kg)	100[a]	31.7 ± 5.15	232.72 ± 27.18
	1[b]	0.54 ± 0.04[c]	1.5 ± 0.09[c,d,e]
Boswellia serrata (45 mg/kg)	100[a]	47.97 ± 6.53	89.67 ± 16.61
	1[b]	0.67 ± 0.05[c]	0.91 ± 0.08[d,e,f]
Boswellia serrata (90 mg/kg)	100[a]	47.52 ± 3.59	217.7 ± 38.5
	1[b]	0.68 ± 0.02[c]	1.43 ± 0.12[c,d,e,g]

All results are expressed as means of movements ± SE.
AD, Alzheimer's disease.
[a]% change.
[b]Square root transformed %change.
[c]Significantly different from baseline of the same group at $P<0.05$.
[d]Significantly different from AD group of rats (4 weeks induction) in the same group at $P<0.05$.
[e]Significantly different from the $AlCl_3$ group 12 weeks after stopping $AlCl_3$ ($P<0.05$).
[f]Significantly different from rivastigmine group after 12 weeks of treatment at $P<0.05$.
[g]Significantly different from *Boswellia serrata* 45 mg/kg group after 12 weeks of treatment at $P<0.05$.

Table 6 Therapeutic effects of rivastigmine and *Boswellia serrata* on the time spent on the rotarod by Alzheimer's disease-induced rats

	Time duration		
Group	Baseline (0 weeks)	$AlCl_3$ induction for 4 weeks	12 weeks (after stopping $AlCl_3$ induction or after treatment)
Control	100[a]	97.3 ± 2.8	98.7 ± 2.5
	1[b]	0.986 ± 0.16	0.99 ± 0.09[e,f]
AD group $AlCl_3$, (17 mg/kg)	100[a]	83.07 ± 2.69	48.42 ± 11.32
	1[b]	0.91 ± 0.01	0.67 ± 0.07[c,d]
Rivastigmine (0.3 mg/kg)	100[a]	91.04 ± 3.67	70.45 ± 3.39
	1[b]	0.95 ± 0.01	0.83 ± 0.02[c,e]
Boswellia serrata (45 mg/kg)	100[a]	82.99 ± 2.4	84.63 ± 5.42
	1[b]	0.91 ± 0.01	0.91 ± 0.02[e,g]
Boswellia serrata (90 mg/kg)	100[a]	72.5 ± 4.7	176.02 ± 39.22
	1[b]	0.84 ± 0.02[c]	1.26 ± 0.13[c,e,f,g]

All data are expressed in seconds as means ± SE.
AD, Alzheimer's disease.
[a]% change.
[b]Square root transformed % change.
[c]Significantly different from base line of the same group ($P<0.05$).
[d]Significantly different from AD group of rates (4 weeks induction) in the same group at $P<0.05$.
[e]Significantly different from the $AlCl_3$ group after 12 weeks of stopping $AlCl_3$ ($P<0.05$).
[f]Significantly different from rivastigmine group after 12 weeks of treatment at $P<0.05$.
[g]Significantly different from *Boswellia serrata* 45 mg/kg after 12 weeks of treatment with *Boswellia serrata* alone ($P<0.05$).

Histopathological results for the protective and therapeutic groups

Examination of the brain tissue of negative control rats stained with hematoxylin and eosin revealed highly active nerve cells with huge nuclei with relatively pale-stained faint nuclear chromatin. The surrounding relatively inactive support cells had small nuclei with densely stained condensed chromatin and no visible nucleoli, indicative of normal cerebral tissue (Fig. 1a). While sections of rat brains (positive control groups) receiving only $AlCl_3$ (17 mg/kg) for 4 weeks showed necrosis of the brain, spongy appearance, plaques, and loss of normal structure, outlines, and nuclei of cells. Some nuclei appeared ring shaped and the recently dead ones appeared dark (Fig. 1b). Sections of brain of rat receiving $AlCl_3$ (17 mg/kg) for 4 successive weeks and left untreated for 12 weeks showed neurofibrillary tangles (which appear as long pink filaments in the cytoplasm), fatty changes, and necrosis of the brain (Fig. 1c).

On the other hand, brain sections of rats administered rivastigmine (0.3 mg/kg) and $AlCl_3$ (17 mg/kg) for 4 weeks as well as those of rats administered *B. serrata* at a dose of 45 or 90 mg/kg (when both were used for protection against AD) appeared more or less like normal sections but with some dark neurons (Fig. 2a–c).

Brain section of rats given rivastigmine (0.3 mg/kg) as well as those of rats administered *B. serrata* at a dose of 45 or

Table 7 Therapeutic effects rivastigmine and *Boswellia serrata* on the time taken to find the food in the T-maze by Alzheimer's disease-induced in rats

Group	Time duration		
	Baseline (0 weeks)	$AlCl_3$ induction for 4 weeks	12 weeks (after stopping $AlCl_3$ induction or after treatment)
Control	13.44 ± 0.91	15.56 ± 1.3	16.2 ± 1.2[c,d]
AD group ($AlCl_3$, 7 mg/kg)	15.66 ± 1.07	115 ± 4.83[a]	120 ± 0[a]
Rivastigmine (0.3 mg/kg)	18.33 ± 0.83	96.87 ± 7.57[a]	8.4 ± 0.73[b,c]
Boswellia serrata (45 mg/kg)	13.66 ± 0.07	46 ± 5.61[a]	120 ± 0[a,b,d,e]
Boswellia serrata (90 mg/kg)	30.66 ± 2.03	116.57 ± 3.42[a]	97.5 ± 6.71[a,b,c,d,e]

All data are expressed in seconds as means ± SE.
AD, Alzheimer's disease.
[a]Significantly different baseline duration of the same group at $P<0.05$.
[b]Significantly different from AD group of rates before treatment in the same group at $P<0.05$.
[c]Significantly different from $AlCl_3$ group after 12 weeks at $P<0.05$.
[d]Significantly different from rivastigmine group after 12 weeks at $P<0.05$.
[e]Significantly different from *Boswellia serrata* (45 mg/kg) group after 12 weeks at $P<0.05$.

Table 8 Therapeutic effects of rivastigmine and *Boswellia serrata* on brain levels of acetylcholine and acetylcholinesterase in Alzheimer's disease-induced rats

Group	Acetylcholine (ACh) (μmol/mg protein)	Acetylcholinesterase (AChE) (μmol/mg protein)
Control	6.54 ± 0.13	0.49 ± 0.02
AD group ($AlCl_3$, 17 mg/kg)	0.68 ± 0.05[a]	1.76 ± 0.04[a]
Rivastigmine (0.3 mg/kg)	6.17 ± 0.01[b]	0.37 ± 0.01[b]
Boswellia serrata (45 mg/kg)	2.21 ± 0.22[a,b,c]	0.92 ± 0.18[b,c]
Boswellia serrata (90 mg/kg)	5.68 ± 0.4[b,d]	0.96 ± 0.19[b,c]

All data are expressed as means ± SE.
AD, Alzheimer's disease.
[a]Significantly different from negative control group at $P<0.05$.
[b]Significantly different from $AlCl_3$ group at $P<0.05$.
[c]Significantly different from rivastigmine group at $P<0.05$.
[d]Significantly different from *Boswellia serrata* 45 mg/kg at $P<0.05$.

90 mg/kg for 12 weeks after induction of AD by $AlCl_3$ showed healthy neurons (Fig. 3a–c). In addition, Fig. 3b shows some vacuoles that contain condensed neurons or partially degenerated neurons, and Fig. 3c shows some dark neurons with a hyperchromatic nuclear chromatin.

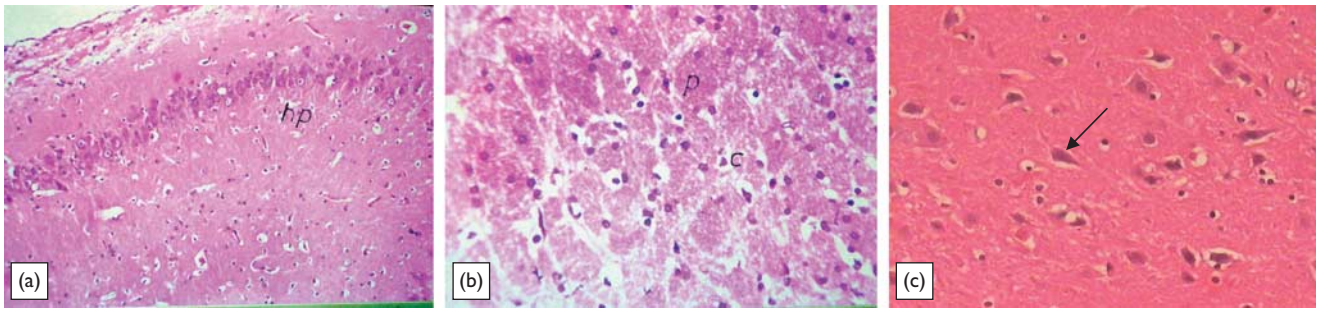
Discussion

AD is a neurodegenerative disorder characterized by progressive degeneration of the hippocampal and cortical neurons that leads to impairment of memory and cognitive ability. It is the most common cause of dementia [21], and its incidence increases with age [22]. Impairment of the short-term memory is usually the first clinical feature, and when the condition progresses, additional cognitive abilities are impaired, such as the ability to calculate and use common objects and tools [1]. Atherosclerotic diseases can also lead to AD [23]. Wimo and Prince [24] reported that there were 35.6 million individuals living with dementia worldwide in 2010, which will increase to 65.7 million by 2030 and to 115.4 million by 2050. Nearly two-thirds of these individuals live in low-income and middle-income countries, where the sharpest increases in numbers occur. Individuals with dementia and their families and friends are affected at personal, emotional, financial, and social levels. Costs in low-income and middle-income countries are rising at a rapid pace, compared with the costs of high-income countries. As a result of economic development, the per-person cost in the former countries will soon increase to levels seen in high-income countries as the increase in the number of individuals with dementia will be much sharper in those regions. Neurodegeneration in the hippocampus and neocortex are associated with spatial memory impairment [25]. Deficiency of Ach is critical in the genesis of the symptoms of AD [2]. Inflammation of the brain plays a key role in the pathogenesis of AD [26]. In addition, excessive accumulation of reactive oxygen species and oxidative stress accompanied by depletion of endogenous antioxidants levels are implicated in the etiology of AD [4]. It is believed that oxidative damage to critical molecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations [27].

It is well established that aluminum (Al) is a neurotoxic agent that induces the production of free radicals in the brain. Accumulation of free radicals may cause degenerative events of aging such as AD. In the present study, rats treated with $AlCl_3$ (AD group) showed a decrease in levels of activity in the activity cages and in the duration of rotation on the rotarod as well as an increase in the length of time taken by rats to reach the food in the T-maze test. The AD rats also showed a significant decrease in Ach levels as well as an increase in AchE activity. Histopathology of brain tissues revealed the presence of amyloid plaques in the hippocampus. $AlCl_3$ AD-induced rats showed significant increases in serum levels of MDA and NO and significant decreases in activities of SOD and TAC; this indicated that the mechanism by which $AlCl_3$ induced AD involves induction of oxidative stress [28]. Xie *et al.* [29] reported that aluminum potentiates the activity of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions to cause oxidative damage, leading to neurodegeneration.

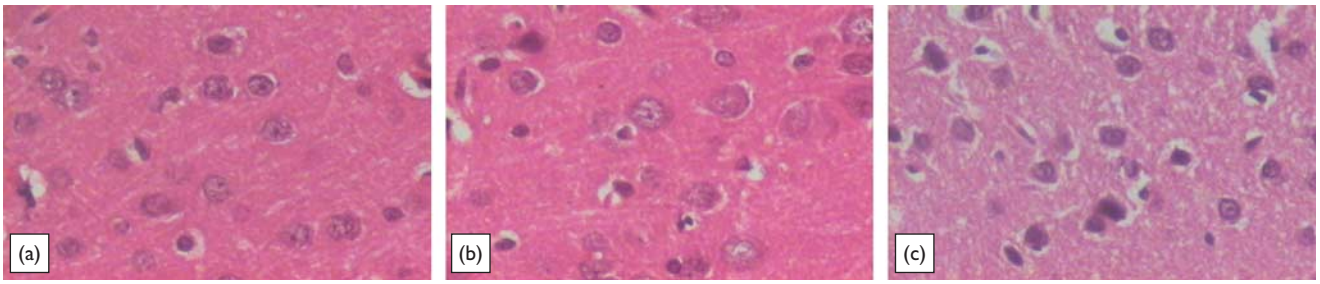
AChE inhibitors are the only agents approved by the Food and Drug Administration (FDA) for the treatment of

Figure 1



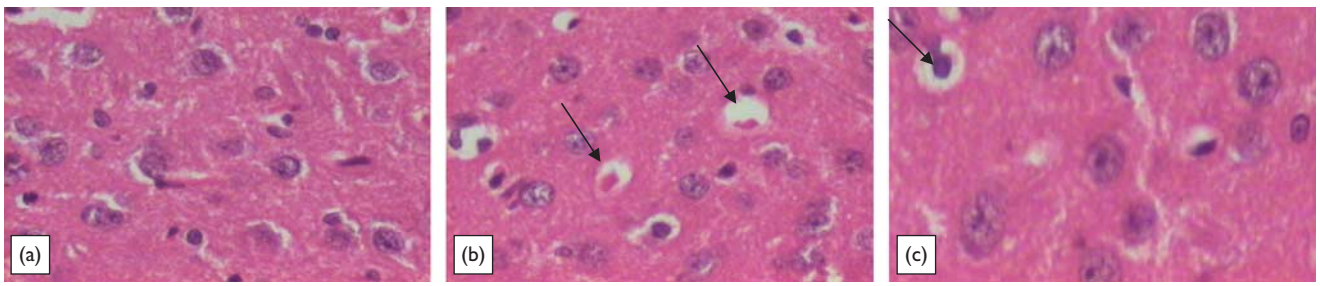
(a) Image of the brain section of a control rat (group 1) showing normal histological structure of the hippocampus (hp). (b) Image of the brain section of an Alzheimer's disease-induced rat (group 2) showing plaques (c) with plaques formation (p) in hippocampus (H&E, × 64). (c) Section of the brain of a rat given $AlCl_3$ (17 mg/kg) for 4 successive weeks and left untreated for 12 successive weeks, showing neurofibrillary tangles (arrow). The tangle appears as a long pink filament in the cytoplasm (H&E, × 100).

Figure 2



(a) A section of the brain of a rat treated with rivastigmine (0.3 mg/kg) only for 15 days, followed by a combination of rivastigmine (0.3 mg/kg) and $AlCl_3$ for 4 weeks, for protection against Alzheimer's disease (AD), showing neurons that appear more or less like normal ones. (b) Section of the brain of a rat treated with 45 mg/kg of *Boswellia serrata* only for 2 weeks, followed by a combination of 45 mg/kg of *B. serrata* and $AlCl_3$ for 4 weeks, for protection against AD, showing neurons that appear more or less like normal ones. Note some dark neurons. (c) Section of the brain of a rat treated with 90 mg/kg of *B. serrata* only for 2 weeks, followed by a combination of 90 mg/kg of *B. serrata* and $AlCl_3$ for 4 weeks, for protection against AD, showing neurons that appear more or less like normal ones. Note the dark neurons (H& E, × 400).

Figure 3



(a) Section of the brain of a rat treated with rivastigmine (0.3 mg/kg), for treatment of Alzheimer's disease (AD), for 12 weeks after induction of AD by $AlCl_3$, showing neurons that appear more or less like normal ones. (b) Section of the brain of a rat treated with 45 mg/kg of *Boswellia serrata* for 12 weeks after induction of AD by $AlCl_3$ showing healthy neurons. Note some vacuoles that contain condensed neurons or partially degenerated neurons (arrows). (c) Section of the brain of a rat receiving 90 mg/kg of *B. serrata*, for treatment of AD, for 12 weeks after induction of AD by $AlCl_3$, showing healthy neurons. Note some dark neurons with hyperchromatic nuclear chromatin (arrow) (H&E, × 400).

AD. All other agents prescribed for the treatment of AD are used on an off-label basis. Present research into new drugs focuses on agents that will prevent, slow down, and/or halt the progression of the disease. Hence, the importance for developing medicinal herb-derived and food plant-derived prophylactic agents directed at age-related disorders, especially neurological and psychiatric disorders, including memory dysfunction. In the present

investigation, we tried to study the protective and therapeutic effects of aqueous infusions of *B. serrata* (45 or 90 mg/kg) and of rivastigmine (as a reference drug) to determine their effects on the results of behavioral stress activities and on brain levels of Ach and AchE in $AlCl_3$-induced AD rats.

Rivastigmine was used as standardized drug as it is the only proven pharmacological therapy for the symptomatic treatment of AD [30]. Treatment of AD rats with rivastigmine as a protective or therapeutic agent led to an improvement in the oxidative stress status, as represented by a significant increase in the levels of activity in the activity cages and brain Ach levels as well as a significant decrease in the results of the T-maze and brain AchE levels when compared with the AD-induced groups of rats. Moreover, a significant increase in the activity on the rotarod was reported after 12 weeks of treatment. These results were confirmed by the histopathological findings of the brain tissues, wherein the amyloid plaques that are formed under the influence of $AlCl_3$ administration had disappeared in the samples from treated rats in comparison with those from the AD group. The efficacy of rivastigmine in the treatment of dementia has also been studied in patients with moderate-to-severe AD living in long-term care facilities. Rivastigmine treatment improves cognition, activities of daily living, and global function [31]. Rivastigmine binds to the AChE molecule in a pseudoirreversible manner; the acetyl moiety of AChE is dissociated rapidly but the carbamyl moiety remains attached for some time longer. Rivastigmine is metabolized by the synapse rather than by hepatic cytochrome enzymes [32]. The study by Andin *et al*. [33] provides the first evidence that the glutamatergic system is modulated after AChE inhibition by rivastigmine, a finding which is likely to be of importance for the clinical effects. Rivastigmine might act through the glutameric mechanism, decreasing the oxidative stress and restoring antioxidant defense [34,35]. In addition, selective AChE inhibitors also protect against the Aβ-induced oxidative stress [36]. Rivastigmine protects behavioral changes, restores antioxidant defense enzymes in the brain, and improves mitochondrial enzyme activity-induced neurotocixity [37].

Boswellia is a genus of trees known for their fragrant resin that has many pharmacological uses, particularly as anti-inflammatory agents. Boswellic acids, which are components of the resin, have shown promising results in the treatment for asthma and various inflammatory conditions [7]. Boswellia gum, extracted from the resins, is used in the prevention and treatment against colitis, ulcerative colitis, Crohn's disease, and ileitis. Moreover, *B. serrata* shows satisfactory antioxidant activity in the cerebrovascular system [8].

The results of the present study reveal that the protective and therapeutic groups of AD-induced rats treated with *B. serrata* (45 or 90 mg/kg) exhibited a significant improvement in the AD diseases induced in rats, as increase the activity in the activity cages and in brain Ach levels, as well as better performances on the rotaroad test in the therapeutic group only, in addition to significant decreases in the results of the T-maze test as well as in brain AchE levels, on comparing with AD-induced rats in a dose dependent manner. Histopathological analysis of the brain tissue from treated rats revealed that the brain cells appeared more or less similar to cells of the control group and that amyloid plaques had disappeared. However, the treatment with *B. serrata* at doses of 45 or 90 mg/kg proved more effective in the protective groups when compared with the therapeutic groups, with fewer vacuoles that contained condensed neurons or partially degenerated neurons and fewer dark neurons with hyperchromatic nuclear chromatin, respectively.

Boswellic acids of gum resin are the main constituents of all *B. serrata* extracts, which contain a range of triterpene acids such as β-boswellic acids, acetyl-β-boswellic acid, keto-boswellic acid and acetyl keto-boswellic acid, and α-boswellic acid [38]. Moreover, Mothana *et al.* [39] reported that methanol and hot water extracts of *B. serrata* showed good antioxidant potential at low concentrations. Therefore, the beneficial effects of *B. serrata* on $AlCl_3$-induced AD in this study could be attributed to its antioxidant activity, as it counteracts the neurotoxic effect of $AlCl_3$, which induces the production of free radicals in the brain.

Neuropathological examination of the AD brain showed extensive neuronal loss, accumulation of fibrillar proteins such as extracellular amyloid β (Aβ) plaques, and neurofibrillary tangles within neurons [40]. However, besides these pathological hallmarks, AD brains exhibit a clear evidence of chronic inflammation and oxidative damage [41,42]; these are also is thought to play a significant role in the onset and progression of AD. Support for this hypothesis came from epidemiological studies reporting that prolonged use of NSAIDs decreases the risk of developing AD and delays the onset of this disorder [43]. Several previous studies have reported the anti-inflammatory activity of the *B. serrata.* Administration of *B. serrata* to AD rats improved the pathogenesis of AD as demonstrated by an improvement in the behavioral stress tests (levels of activity and motor coordination) and cognitive abilities, increased brain Ach levels, and decreased AChE levels in the brains, which was further confirmed by an improvement in brain tissue characteristics on histopathological analysis. Kimmatkar *et al.* [44] reported that boswellic acids decreased levels of proinflammatory 5-lipoxygenase products such as 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B4 (LTB-4). In addition, Xia *et al.* [45] reported that boswellic acids of the gum resin of *B. serrata* have a chemical structure that is similar to other pentacyclic triterpenes and hence resemble anti-inflammatory drugs in their mode of action. Keto-boswellic acids (AKBA, acetyl-11-keto-β-boswellic acid, and KBA, 11-keto-β-boswellic acid) are orally active, direct, and nonredox and noncompetitive blockers of 5-lipoxygenase, which is the key enzyme in leukotriene biosynthesis. Sharma *et al.* [46] reported that boswellic acids significantly reduced the population of leukocytes in BSA-induced arthritis in rabbits as well as the infiltration of leukocytes into the knee joint. It was shown administration of

boswellin (methanol extract of the gum resin of *B. serrata*) to mice having inflammation and tumors led to an inhibitory effect [47].

A study done by Sharifabad and Esfandiary [48] on pregnant rats, revealed that prenatal maternal administration of *B. serrata* as an aqueous extract at a daily dose of 0.1 g/kg body weight during gestation (3weeks) improved learning and memory performances associated with an increase in the size of neuronal bodies in the Cornu Ammonis (CA3) of the hippocampus of their offsprings. The dendritic branching density was higher in experimental rats relative to that found in control rats, and this provides a neuroanatomical basis that may be relevant to the previously reported enhancement of learning and memory abilities in the offspring. The results of the above-mentioned study can support ours, as the administration of aqueous infusions of *B. serrata* to rats caused a reduction in the duration taken by rats to reach the food in the T-maze test; in other words, it improved the cognitive functions and memory in rats.

Moussaieff *et al.* [49] reported that incensole acetate, which is an acetylated boswellic acid fraction and a boswellia resin constituent, is a potent transient receptor potential vanilloid3 (TRPV3) agonist that causes anxiolytic-like and antidepressive-like behavioral effects in wild-type mice. Moreover, administration of incensole acetate showed that the performance of mice in both the elevated plus maze and Porsolt forced swimming tests was significantly TRPV3 dependent. TRPV3 mRNA has also been found in neurons throughout the brain. The results of the above-mentioned study can explain the improvement in the rat's memory observed in the present study on being given *B. serrata*. Increasing evidences implicate impairment of axonal integrity in mechanisms underlying neurodegenerative disorders. Therefore, the key factor that induces memory loss and impairment in AD patients could be neurite degeneration through microtubule proteins destabilization.

Karima *et al.* [50] reported the effect of β-boswellic acid (BBA), which is the major component of *B. serrata* gum, on neurite outgrowth and branching as well as on polymerization dynamics of tubulin *in vitro*, in which the morphometric parameters (axonal length and neurite branching) of which were examined microscopically after treating hippocampal cells with BBA. Their results revealed that BBA could significantly enhance neurite outgrowth, branching, and tubulin polymerization dynamics. The obtained results suggest that the enhancing effects of BBA on microtubule polymerization kinetics might be the reason for the increased axonal outgrowth and branching. In contrast, axonal stability could be a reflection of the stability of microtubules, which may consequently prevent axonal degeneration.

In conclusion, this study revealed that *B. serrata* has protective and therapeutic effects on AD-induced rats. It could ameliorate the neurodegenerative characteristics of AD. The effects of *B. serrata* at higher doses are better compared with those at lower doses. These results represented satisfactory therapeutic approaches for intervention against the progressive neurological damage associated with AD, with special reference to oxidative insults. Further clinical trials on humans are required to determine the efficacy of *B. serrata*, or one or more of its constituents, on neurodegenerative disorders.

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Conflicts of interest

There are no conflicts of interest.

References

1 Kimura R, Ohno M. Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. Neurobiol Dis 2009; 33:229–235.

2 Terry AV Jr, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003; 306: 821–827.

3 Daiello LA, Festa EK, Ott BR, Heindel WC. Cholinesterase inhibitors improve visual attention in drivers with Alzheimer's disease. Alzheimers Dement 2008; 4 **(4):** T498.

4 Zhu X, Perry G, Moreira PI, Aliev G, Cash AD, Hirai K, Smith MA. Mitochondrial abnormalities and oxidative imbalance in Alzheimer disease. J Alzheimers Dis 2006; 9:147–153.

5 Mentreddy SR. Medicinal plant species with potential antidiabetic properties. J Sci Food Agric 2007; 87:743–750.

6 Silva CG, Herdeiro RS, Mathias CJ, Panek AD, Silveira CS, Rodrigues VP, *et al.* Evaluation of antioxidant activity of Brazilian plants. Pharmacol Res 2005; 52:229–233.

7 Gupta I, Gupta V, Parihar A, Gupta S, Lüdtke R, Safayhi H, Ammon HP. Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. Eur J Med Res 1998; 3:511–514.

8 Assimopoulou AN, Zlatanos SN, Papageorgiou VP. Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food Chem 2005; 92:721–727.

9 Paget GE, Barnes JM. Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. In: Laurence DR, Bacarach AL, editors. *Evaluation of drug activities. Pharmacometrics*. 1 London: Academic Press; 1964. pp. 160–162.

10 Krasovskii GN, Vasukovich LY, Chariev OG. Experimental study of biological effects of lead and aluminum following oral administration. Environ Health Perspect 1979; 30:47–51.

11 Carageorgiou H, Sideris AC, Messari I, Liakou CI, Tsakiris S. The effects of rivastigmine plus selegiline on brain acetylcholinesterase, (Na^+, K^+)-, Mg^{2+}-ATPase activities, antioxidant status, and learning performance of aged rats. Neuropsychiatr Dis Treat 2008; 4:687–699.

12 Pavić R, Tvrdeić A, Tot OK, Heffer-Lauc M. Activity cage as a method to analyze functional recovery after sciatic nerve injury in mice. Somatosens Mot Res 2007; 24:213–219.

13 Vijitruth R, Liu M, Choi D-Y, Nguyen XV, Hunter RL, Bing G. Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J Neuroinflammation 2006; 3:6.

14 Deacon RMJ, Rawlins JNP. T-maze alternation in the rodent. Nat Protoc 2006; 1:7–12.

15 Tsakiris S, Schulpis KH, Marinou K, Behrakis P. Protective effect of l-cysteine and glutathione on the modulated suckling rat brain Na^+, K^+-ATPase and Mg^{2+}-ATPase activities induced by the *in vitro* galactosaemia. Pharmacol Res 2004; 49:475–479.

16 Oswald C, Smits SHJ, Höing M, Sohn-Bösser L, Dupont L, Le Rudulier D, *et al.* Crystal structures of the choline/acetylcholine substrate-binding protein ChoX from *Sinorhizobium meliloti* in the liganded and unliganded-closed states. J Biol Chem 2008; 283:32848–32859.

17 Den Blaauwen DH, Poppe WA, Tritschler W. Cholinesterase (EC 3.1.1.8) with butyrylthiocholine iodide as substrate: Age- and sex-dependent reference values with special reference values with special reference to hormonal effects and pregnancy. J Clin Chem Clin Biochem 1983; 21: 381–386.

18 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.

19 Banchroft JD, Steven A, Turner DR. *Theory and practice of histological technique*. 4th ed. New York: Churchill Livingstone; 1996.

20 Jones M, Onslow M, Packman A, Gebski V. Guidelines for statistical analysis of percentage of syllables stuttered data. J Speech Lang Hear Res 2006; 49:867–878.

21 Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006; 5:228–234.

22 Maccioni RB, Muñoz JP, Barbeito L. The molecular bases of Alzheimer's disease and other neurodegenerative disorders. Arch Med Res 2001; 32:367–381.

23 McKhann G, Drachman D, Folstein M. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984; 34:939–944.

24 Wimo A, Prince M. Alzheimer's Disease. International World Alzheimer Report. The Global Economic Impact of Dementia. *Alzheimer's Disease International (ADI)*; 2010.

25 Cain DP, Boon F. Detailed behavioral analysis reveals both task strategies and spatial memory impairments in rats given bilateral middle cerebral artery stroke. Brain Res 2003; 972 **(1–2):** 64–74.

26 Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, *et al.* Inflammation and Alzheimer's disease. Neurobiol Aging 2000; 21:383–421.

27 Baldeiras I, Santana I, Proença MT, Garrucho MH, Pascoal R, Rodrigues A, *et al.* Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. J Alzheimers Dis 2008; 15:117–128.

28 Mahdy K, Shaker O, Wafay H, Nassar Y, Hassan H, Hussein A. Effect of some medicinal plant extracts on the oxidative stress status in Alzheimer's disease induced in rats. Eur Rev Med Pharmacol Sci 2012; 16 **(Suppl 3):** 31–42.

29 Xie CX, Yokel RA. Aluminum facilitation of iron-mediated lipid peroxidation is dependent on substrate, pH, and aluminum and iron concentrations. Arch Biochem Biophys 1996; 327:222–226.

30 Mayeux R, Sano M. Treatment of Alzheimer's disease. N Engl J Med 1999; 341:1670–1679.

31 Onor M, Trevisiol M, Aguglia E. Rivastigmine in the treatment of Alzheimer's disease: an update. Clin Interv Aging 2007; 200:17–32.

32 Polinsky RJ. Clinical pharmacology of rivastigmine: a new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Clin Ther 1998; 20:634–647.

33 Andin J, Enz A, Gentsch C, Marcusson J. Rivastigmine as a modulator of the neuronal glutamate transporter rEAAC1 mRNA expression. Dement Geriatr Cogn Disord 2005; 19:18–23.

34 Porsteinsson AP, Grossberg GT, Mintzer J, Olin JT, Memantine MEM-MD-12 Study Group. Memantine treatment in patients with mild to moderate Alzheimer s disease already receiving a cholinesterase inhibitor: a randomized, double-blind, placebo-controlled trial. Curr Alzheimer Res 2008; 5:83–89.

35 Shah S, Reichman WE. Treatment of Alzheimer's disease across the spectrum of severity. Clin Interv Aging 2006; 1:131–142.

36 Xiao XQ, Wang R, Han YF, Tang XC. Protective effects of huperzine A on β-amyloid(25-35) induced oxidative injury in rat pheochromocytoma cells. Neurosci Lett 2000; 286:155–158.

37 Kumar P, Kumar A. Protective effect of rivastigmine against 3-nitropropionic acid-induced Huntington's disease like symptoms: possible behavioural, biochemical and cellular alterations. Eur J Pharmacol 2009; 615 **(1–3):** 91–101.

38 Graham AG. Synthesis of δ-Boswellic acid. Phytochemistry 1969; 8:208.

39 Mothana RA, Lindequist U, Gruenert R, Bednarski PJ. Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. BMC Complement Altern Med 2009; 9:7.

40 Clark CM. Clinical manifestations and diagnostic evaluation of patients with Alzheimer's disease. In: Clark CM, Trojanowski JQ, editors. *Neurodegenerative dementias: clinical features and pathological mechanisms*. New York: McGraw-Hill; 2000. pp. 95–114.

41 Praticò D, Trojanowski JQ. Inflammatory hypotheses: novel mechanisms of Alzheimer's neurodegeneration and new therapeutic targets? Neurobiol Aging 2000; 21:441–445.

42 Praticò D, Delanty N. Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease. Am J Med 2000; 109:577–585.

43 Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997; 48:626–632.

44 Kimmatkar N, Thawani V, Hingorani L, Khiyani R. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee – a randomized double blind placebo controlled trial. Phytomedicine 2003; 10:3–7.

45 Xia L, Chen D, Han R, Fang Q, Waxman S, Jing Y. Boswellic acid acetate induces apoptosis through caspase-mediated pathways in myeloid leukemia cells. Mol Cancer Ther 2005; 4:381–388.

46 Sharma ML, Bani S, Singh GB. Anti-arthritic activity of boswellic acids in bovine serum albumin (BSA)-induced arthritis. Int J Immunopharmacol 1989; 11:647–652.

47 Huang M-T, Badmaev V, Ding Y, Liu Y, Xie J-G, Ho C-T. Anti-tumor and anti-carcinogenic activities of triterpenoid, β-boswellic acid. BioFactors 2000; 13 **(1–4):** 225–230.

48 Sharifabad MH, Esfandiary E. A morphometric study on CA3 hippocampal field in young rats following maternal administration of *Boswellia serrata* resin during gestation. Iranian J Basic Med Sci 2007; 10:176–182.

49 Moussaieff A, Rimmerman N, Bregman T, Straiker A, Felder CC, Shoham S, *et al.* Incensole acetate, an incense component, elicits psychoactivity by activating TRPV3 channels in the brain. FASEB J 2008; 22:3024–3034.

50 Karima O, Riazi G, Yousefi R, Movahedi AAM. The enhancement effect of beta-boswellic acid on hippocampal neurites outgrowth and branching (an *in vitro* study). Neurol Sci 2010; 31:315–320.