Research Journal of Pharmacognosy (RJP) 3(4), 2016: 39-49
Received: June 2016
Accepted: Aug 2016

Original article

Neuroprotection and anxiety like behavior reduction of *Allium hirtifolium* and *Astragalus hamosus* in the Aβ-injected rat

Z. Bahaeddin[1], A. Yans[2], F. Khodagholi[3], S. Sahranavard[1*]

[1]*Traditional Medicine and Materia Medica Research Center and Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[2]*Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[3]*Neurobiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Abstract

Background and objectives: Traditional medicine is an important approach to achieve new therapeutic strategies in basic and clinical pharmacology. *Allium hirtifolium* Boiss. and *Astragalus hamosus* L. have been mentioned in Iranian Traditional Medicine references for a kind of dementia with features and symptoms similar to those of Alzheimer's disease (AD). In the present study, the neuroprotective effect of these herbs has been evaluated as new therapies in neurotoxicity model. **Methods:** Two separate groups of rats were fed with *A. hirtifolium* or *A. hamosus* extract (100 mg/kg/day) from 1 week before amyloid beta (Aβ) injection, for 16 consecutive days. One day after the last oral administration, behavioral test was done. The effect of these two extracts were assessed in anxiety-like behavior test using elevated plus maze. Furthermore, molecular pathways involved in apoptosis were assessed by Western blotting analysis. **Results:** The results showed that oral administration of both *A. hirtifolium* and *A. hamosus* decreased anxiety-like behavior and ameliorated the effect on apoptosis factors including Bax, Bcl-2 and caspase-3 in the rats with intra-hippocampal injection of Aβ. **Conclusion:** The results of this study revealed the potential neuroprotective properties of *A. hirtifolium* and *A. hamosus* as herbal remedies that could play a role in fostering healthy aging and be considered as useful candidates in decreasing AD related symptoms.

Keywords: *Allium hirtifolium*, Alzheimer's disease, anxiety, apoptosis, *Astragalus hamosus*

Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly, and is characterized by behavioral disturbances and psychological symptoms [1,2]. The prevalence of AD is one in nine people above 65 years in the United States. In addition to memory decline which is common in patients suffering from AD, anxiety symptoms are also a source of concern [1,2]. Behavioral disturbances associated with this disease could hurt families and caregivers [3,4]. The treatment of behavioral symptoms of AD is imperative in improving the condition of the patients and their caregivers [3,5]. Amyloid beta (Aβ) peptide which is the most common hallmark of AD pathogenesis [6] causes neuronal apoptosis [7] in the central nervous system. Apoptosis is the main reason for cognitive decline in AD [8]. Two of the main apoptotic

*Corresponding author: ssahranavard@sbmu.ac.ir, Tel/Fax: +9821-88776027

factors are changes in Bax/Bcl-2 ratio and cleavage of caspase-3 [9]. Aβ accumulation in the hippocampus causes apoptosis and anxiety-like behavior [10].

Medicinal herbs have the potential to be developed into optimum pharmaceuticals for complex situations such as AD because of their multi-function and multi-target characteristics [11,12]. In Iranian Traditional Medicine (ITM), numerous plants have been introduced for treatment of memory related disorders [13-15]. Among these plants based on the importance and accessibility, *Astragalus hamosus* L. and *Allium hirtifolium* Boiss. were chosen.

Persian shallot "*Mu-sir*" (traditional name) [16,17], with the scientific name of *Allium hirtifolium* Boiss., belongs to Amaryllidaceae family. *Allium* genus has more than 900 species in the world [18,19]. *Allium hirtifolium* grows as a wild plant in the Zagros mountains [18], and is one of the most frequently used spices in Iran [20]. Spices that exhibit antioxidant activity receive immense attention as food supplements to improve cognitive impairment against AD [21]. Phenolic compounds present in spicy plants possess bioactive properties which protecting cellular systems against oxidative stress [22]. *Allium hirtifolium* has been shown to have various pharmacological properties such as antioxidant, antimicrobial [23], anticancer [24], anti-inflammatory [25], antiatherosclerotic [26], antidiabetic [27], immunomodulatory [28], antinociceptive [29], and acetylcholinesterase inhibitiory properties [25].

Another plant used in this study was "*Iklil-ul-Malik*" (traditional name) [16,30] with the scientific name of *Astragalus hamosus* L., belongs to Fabaceae family. *Astragalus* genus is the largest genus of flowering plants, containing up to 3000 species, and Iran is one of the most important centers of diversity of this genus [31]. Recently it was shown that *A. hamosus* has protective effect on biological systems. In this regard, anti-inflammatory (pods) [32], analgesic [33], cytoprotective and antioxidant (aerial parts) [34] activities of *A. hamosus* have been reported. Hence, we investigated the effect of oral administration of *A. hirtifolium* and *A. hamosus* on changes in the main apoptotic factors (Bax, Bcl-2, caspase-3) in the hippocampus of Aβ-injected rats and anxiety-like behavior induced by Aβ.

Experimental

Plant material

Allium hirtifolium bulbs and *A. hamosus* fruits were purchased from a local market in Tehran. Their scientific names were authenticated and a specimen was deposited at the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences (332HMS, 333HMS, respectively). *Allium hirtifolium* bulbs were extracted twice with ethanol (80%) at room temperature. The extract was concentrated utilizing a rotary evaporator and dried using a vacuum drying oven [16,28]. The powdered *A. hamosus* fruits were extracted employing the decoction method based on the preparation method in the ITM. The mixture was filtered and the filtrate was freeze-dried [15,35]. The extracts were kept in a closed container and protected from light at 4-8 °C until use.

Animals

All animals were kept following approval from the animal care committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran and Guide for the Care and Use of Laboratory Animals of the National Institute of Health, Bethesda, United States of America [36]. Male Wistar rats (200–250 g) were obtained from the Pasteur Institute of Iran, Tehran. Rats were habituated to the laboratory for 7 days before the experiment. Animals were kept four per cage, at a temperature of 22±2 °C with free access to standard laboratory chow and water, under a 12h light–dark cycle (lights on at 7:00 a.m.).

Oral administration of plant extract

A. hirtifolium and *A. hamosus* extracts were dissolved in water separately and fresh solutions were prepared every day to feed the rats with a volume of 1 mL using gavage tube, one week

before surgery to 24 h before behavioral testing (in general, for 16 consecutive days). Gavage (100 mg/kg) was used daily between 8:00 to 9:00 AM.

Body weight gain

To determine the effect of *A. hirtifolium* or *A. hamosus* extracts on body weight gain in the rats, the weight was assessed twice before feeding the rats with the extracts and 7 days after oral administration of the mentioned extracts.

Preparation of Aβ

Preparation of Aβ powder (GenScript, Piscataway, USA) was carried out according to the method described previously to obtain the concentration of 50 ng/µL [37].

Surgery

The animals were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) and were placed in a stereotaxic apparatus (Stoelting, Wood Dale, USA). After cutting along the midline, the retracting of scalp was performed and the different parts including the bregma were dried and cleaned with the help of sterile cotton. Based on the Paxinos and Watson atlas of rat brain, the stereotaxic for dorsal hippocampus was determined [38] (anterior-posterior, -3.8 mm; lateral, ±2.2 mm from the central line, and ventral, -2.7 mm down from the surface of the skull). By means of a Hamilton syringe (1 µL/side), Aβ (50 ng/µL) injection was introduced into both side of the CA1 region of the hippocampus for 1 to 3 minutes. The needle was left in place to facilitate the diffusion of the injected materials and thereafter, the scalp was sutured.

Experimental groups

Rats were randomly assigned into six groups (eight rats per experimental group) as follows: (1) Vehicle group that only received phosphate buffered saline (PBS) (1 µL/ side) into the CA1 region and 1 mL of water every day using gavage tube for 16 days; (2) Aβ-group, which received bilateral intra-CA1 injection of Aβ (50 ng/µL PBS per side) and 1 mL of water by gavage; (3) *Allium hirtifolium* group that received PBS (1 µL/ side) bilaterally into the CA1 region and 100 mg/kg *A. hirtifolium* dissolved in 1 mL of water by gavage; (4) The *A. hirtifolium* and Aβ group, received both *A. hirtifolium* (100 mg/kg) orally and Aβ (50 ng/µL PBS) into the CA1 region; (5) *Astragalus hamosus* group that received PBS (1 µL/ side) bilaterally into the CA1 region and 100 mg/kg *A. hamosus* dissolved in 1 mL of water by gavage; (6) The *A. hamosus* and Aβ group, received both *A. hamosus* (100 mg/kg) orally and Aβ (50 ng/µL PBS) into the CA1 region.

Elevated plus maze (EPM) test

The EPM test was carried out on the ninth day after stereotaxic surgery. The EPM is a rodent model of anxiety paradigm that is employed as a screening test for putative anxiolytic or anxiogenic effects [39]. The wooden and sign (+) shaped maze, possessing four arms (two closed and two open) with a platform at the center (10 cm × 10 cm), was elevated 50 cm from the floor. The closed arms characteristics were 50 cm length, 10 cm width and 40 cm height, while the features of the open arms were 50 cm length and 10 cm width. The animals were individually placed in the center of the apparatus and were allowed to explore freely for 5 min. The percentage of time spent in the open arms [OAT%: (time in open arm/time in "open+closed" arm) ×100] and the percentage of number of entrances to open arms [OAE%: (number of open arm entries/number of "open+closed" arm entries) ×100] were counted as anxiety index. The total of closed and open arm entries were considered as an index for the locomotor activity [40].

Western blot test

Ten days after surgery (after the completion of maze test), the hippocampi were dissected and flash frozen in liquid nitrogen immediately. Thereafter, the tissue was stored at −80 °C. The hippocampal tissues were removed from the rats' brain, lysed in a buffer solution including Tris-HCl, SDS, NaCl, Sodium deoxycholate, EDTA,

Triton X-100 and cocktail protease inhibitor (Roche, Penzberg, Germany). Bradford test was used to determine the total concentrations of proteins using serum albumin as a standard. In this method, the proteins, which were loaded into the wells existed on the SDS-PAGE gel and were separated based on their molecular weights. The assay was continued by electroblotting the proteins on the polyvinylidene difluoride membranes (Millipore, USA) and covering the membrane with blocking solution. Addition of the primary antibodies against caspase-3, Bax, Bcl-2 and β-actin (Cell Signaling Technology, USA) paved the way for recognition of the level of these proteins by means of a secondary antibody (Cell Signaling Technology) which was conjugated with horseradish peroxidase enzyme. The substrate of electrochemiluminescent (Amersham Bioscience, USA) made the immunoreactive bands detectable on the autoradiography that was visualized on Kodak films. Subsequently, the software of Image J provided the opportunity to quantify the outcomes obtained from this experiment.

Statistical analysis

Data obtained from Western blot and other results were expressed as the Mean ± SEM (standard error of mean) and were processed using GraphPad Prism® 5.0. Comparison among the groups was carried out by means of ANOVA (one-way) using Tukey's post hoc test. $p<0.05$ was considered to be significant (n = 8).

Results and discussion

The effect of oral administration of *A. hirtifolium* and *A. hamosus* on body weight gain has been shown in table 1. During one week of oral administration of *A. hirtifolium* or *A. hamosus* (before stereotaxic surgery), body weight gain for the two groups of rats treated with the mentioned extracts did not show any significant changes, compared to the control group which received only 1 mL of water daily by gavage. The data showed feeding with *A. hirtifolium* and *A. hamosus* had no effect on body weight.

Table 1. Effects of oral administration of *Allium hirtifolium* and *Astragalus hamosus* on body weight gain

Groups	body weight gain (g) after 1 week
Control (water)	19.2±1.2
A. hirtifolium	16.3±2.2
A. hamosus	19.7±1.9

The values were measured 1 week after feeding. Data are mean ± SEM; the results did not show any significant difference between each group compared to the control group.

In order to evaluate anxiety-like behavior, EPM test was used on the ninth day after stereotaxic surgery. The EPM has been validated to assess the anxiolytic effects of the pharmacological agents [39]. In the current study, data analysis showed that the injection of Aβ into the hippocampus significantly decreased OAT% and OAE% in comparison with the vehicle group (both $p<0.001$). In addition, data analysis showed that oral administration of each extract of *A. hirtifolium* and *A. hamosus* significantly increased OAT% (both $p<0.05$) and OAE% ($p<0.001$ and $p<0.05$, respectively) in Aβ-injected rats compared to the Aβ-group (figures 1A and B). Moreover, locomotor activity did not show any significant changes among the different experimental groups (figure 1C). Our data exhibited that oral administration of *A. hirtifolium* and *A. hamosus* in sham animals (group 3 and 5) had no effect on anxiety like behavior and movement. According to the results of the study, the oral administration of the mentioned extracts decreased Aβ-induced anxiety-like behavior. In the present study, Western blot analysis of hippocampal lysates was carried out to detect the level of Bax, Bcl-2 and cleaved caspase-3 proteins. The high Bax/Bcl-2 ratio is affiliated with greater vulnerability to apoptotic activation [41]; in addition, caspase-3 cleavage is required as typical hallmark of apoptosis [42]. Six groups were run together on single blots and bands densities were normalized to β-actin (Figure 2A). As shown in figures 2B and C, the Bax/Bcl-2 ratio and the level of cleaved caspase-3 increased about 1.6- and 2.2-fold, respectively in the hippocampi of Aβ-injected group compared to the vehicle group.

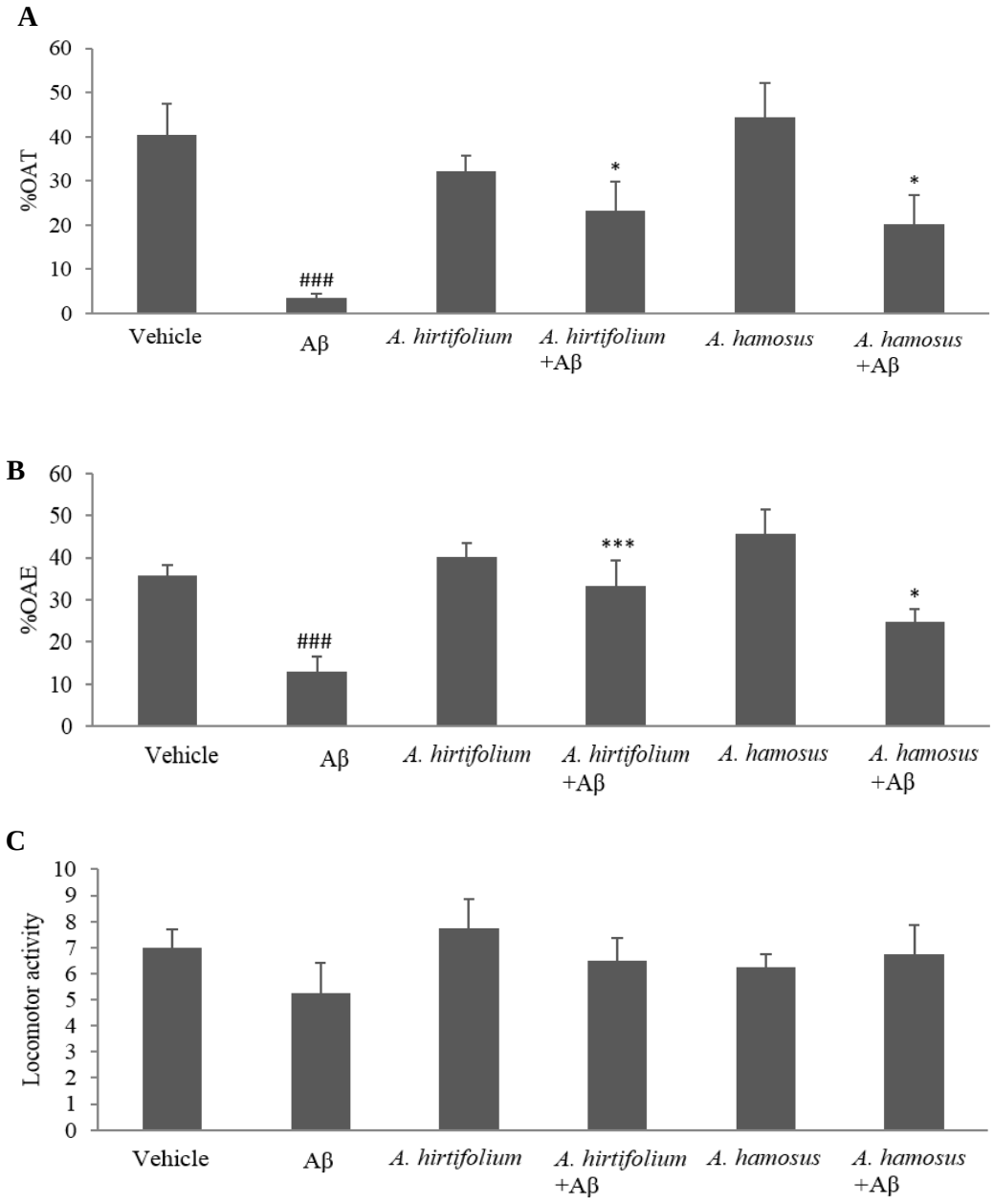

Figure 1. Effect of the oral administration with each of the extracts of *Allium hirtifolium* and *Astragalus hamosus* on anxiety-related behavior in the rats injected with Aβ (50 ng/μL). Each value represents the mean ± SEM. OAT% (A), OAE% (B) and locomotor activity (C) during 5 minutes exposure to EPM. Significant differences: ###$p<0.001$ different from the vehicle group. *$p<0.05$ and ***$p<0.001$ different from the Aβ-injected group.

Besides, the outcomes obtained from Western blotting assay showed that oral administration of *A. hirtifolium* in Aβ-injected rats diminished the Bax/Bcl-2 ratio and caspase-3 cleavage about 42% and 30% respectively compared with the Aβ-injected group. Furthermore, oral administration of *A. hamosus* decreased Bax/Bcl-2 ratio and caspase-3 cleavage about 47% and 49%, respectively, in comparison with Aβ-injected group, 10 days after surgery.

Studies have shown that Aβ is neurotoxic *in vitro* and *in vivo* [43] and its injection into hippocampus tissue can lead to apoptosis and anxiety-like behavior [7,10,44]. The results obtained from the present study revealed that the oral administration of the mentioned extracts reduced anxiety-like behavior in the rats which received Aβ. Moreover, the outcomes of the Western blotting test of Bax, Bcl-2 and caspase-3 showed that both *A. hirtifolium* and *A. hamosus* had an ameliorating effect on Aβ induced apoptosis.

Studies have shown that hippocampal apoptosis has an important effect on the creation of anxiety-related behavior [45,46]. The main components of the apoptosis in neurons are proteins of the Bcl-2 and caspase families [43]. Bax is a pro-apoptotic member of the Bcl-2 family and interacts with Bcl-2 [43]. One of the major caspases involved in neuronal apoptosis is caspase-3 which plays some key roles in apoptotic cell death [42]. Studies have suggested that selective caspase inhibition might be a possible therapeutic strategy in AD [43]. Researchers have shown an effective relationship between the function of mitochondria and anxiety disorders [47,48]. One of the major modulators of mitochondrial function is Bcl-2 proteins located in the inner mitochondrial membrane and reduction in the amount of this protein can be counted as the main factor for anxiety disorders [48].

In the current study, oral administration of *A. hirtifolium* and *A. hamosus* increased Bcl-2 and decreased proapoptotic factor in the hippocampi of rats, which consequently can be effective on anxiety-like behaviors reduction.

These beneficial protective effects of *A. hirtifolium* in neurotoxicity induced by Aβ injection could probably be due to the antioxidant capacity of its phenolic and organosulfur compounds [49-52]. Polyphenols have been considered as modulators and reducers of oxidative stress [53, 54]. Dietary polyphenols are capable of producing positive effects on mental health [55] and are particularly effective against anxiety [56]. The neuroprotective benefits exhibited by *A. hirtifolium* may be due to the polyphenol components which improve a number of pathological situations including neurodegenerative disorders [57]. In addition, organosulfur compounds content of this plant such as diallyl disulfide [27,58] and diallyl thiosulfinate (allicin) [24,28,59,60] contribute to this feature.

Astragalus plant species are rich sources of flavonoids [61,62]. Flavonoids such as isoquercitrin, astragalin, hyperoside and rhamnocitrin 4'-beta-D-galactopyranoside, were isolated from the aerial parts of *A. hamosus* [62]. Polyphenolic compounds like flavonoids in the plant may contribute to neuroprotective properties [55,56]. The species also contains selenium, an important micronutrient, which deficiency seems to have important relation with AD [63,64]. The neuroprotective effects may be caused by a complex mixture of phytochemicals and bioactive compounds in *A. hirtifolium* and *A. hamosus* and diverse compounds may complement or synergize to produce their beneficial actions [65].

In the present study, both *A. hirtifolium* and *A. hamosus* showed neuroprotective properties. Oral administration of *A. hamosus* resulted in more reduction of caspase-3 as a proapoptotic molecule than *A. hirtifolium*, but no significant difference in behavioral tests were observed. This could be as a result of the influence of *A. hamosus* on other molecular pathways involved in anxiety, which was not considered in this research and can be examined in future studies. As *A. hirtifolium* and *A. hamosus* proposed in

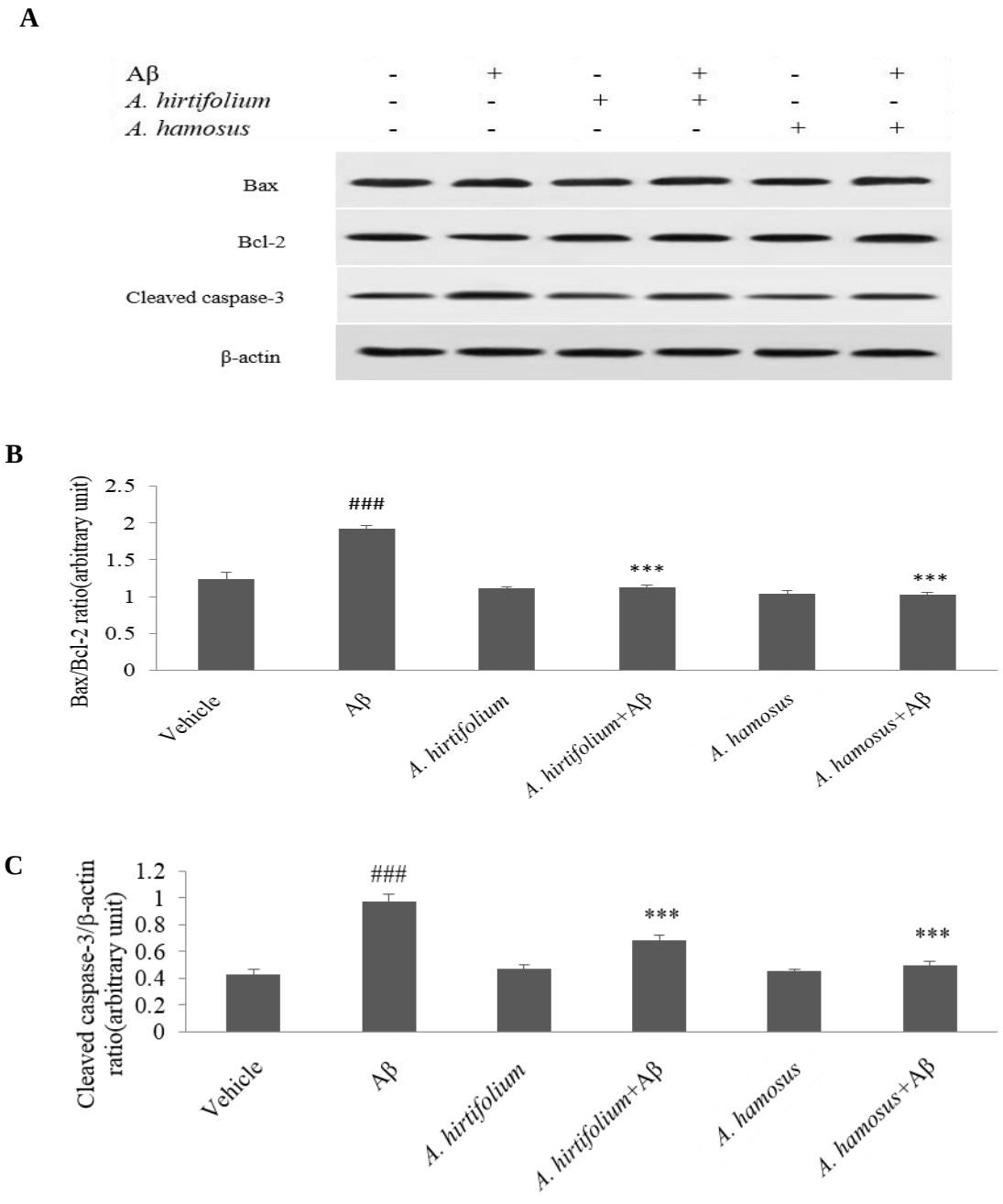

Figure 2. Western blot analysis to measure the effect of oral administration with each of the extracts of *Allium hirtifolium* and *Astragalus hamosus* on Bax, Bcl-2 and cleaved caspase-3 levels in the hippocampus of rats injected with Aβ (50 ng/μL) (A, B, C); one representative western blot is shown. The values represent the mean ± SEM. Significant differences: ###p<0.001 different from the vehicle group. ***p<0.001 different from the Aβ-injected group.

traditional medicine documents to have positive effect on nervous system, our data provide their neuroprotective effect on Aβ-injected rat model of Alzheimer disease.

Acknowledgements
The authors would like to thank Neurobiology Research Center and Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences for the laboratory supports. This work was part of the PhD thesis of traditional pharmacy (Zahra. Bahaeddin, No.143), granted by School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (No: 123 and 124)

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References
[1] Hart D, Craig D, Compton S, Critchlow S, Kerrigan B, McIlroy S, Passmore AP. A retrospective study of the behavioural and psychological symptoms of mid and late phase Alzheimer's disease. *Int J Geriatr Psych*. 2003; 18(11): 1037-1042.
[2] Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimers Dement*. 2015; 11(3): 1-83.
[3] Desai AK, Grossberg GT. Recognition and management of behavioral disturbances in dementia. *Prim Care Companion J Clin Psychiatry*. 2001; 3(3): 93-109.
[4] Sadowsky CH, Galvin JE. Guidelines for the management of cognitive and behavioral problems in dementia. *J Am Board Fam Med.* 2012; 25(3): 350-366.
[5] Kraus CA, Seignourel P, Balasubramanyam V, Snow AL, Wilson NL, Kunik ME, Schulz PE, Stanley MA. Cognitive-behavioral treatment for anxiety in patients with dementia: two case studies. *J Psychiatr Pract*. 2008; 14(3): 186-192.
[6] Goldsworthy MR, Vallence AM. The Role of ß-amyloid in Alzheimer's Disease-related neurodegeneration. *J Neurosci*. 2013; 33(32): 12910-12911.
[7] Miguel-Hidalgo JJ, Paul IA, Wanzo V, Banerjee PK. Memantine prevents cognitive impairment and reduces Bcl-2 and caspase 8 immunoreactivity in rats injected with amyloid β 1–40. *Eur J Pharmacol*. 2012; 692(1): 38-45.
[8] Kim SE, Ko IG, Kim BK, Shin MS, Cho S, Kim CJ, Baek SS, Lee EK, Jee YS. Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. *Exp Gerontol*. 2010; 45(5): 357-365.
[9] Shi L, Chen J, Yang J, Pan T, Zhang S, Wang Z. MiR-21 protected human glioblastoma U87MG cells from chemotherapeutic drug temozolomide induced apoptosis by decreasing Bax/Bcl-2 ratio and caspase-3 activity. *Brain Res*. 2010; 1352: 255-264.
[10] Zare N, Khalifeh S, Khodagholi F, Shahamati SZ, Motamedi F, Maghsoudi N. Geldanamycin reduces Aβ-associated anxiety and depression, concurrent with autophagy provocation. *J Mol Neurosci*. 2015; 57(3): 317-324.
[11] Anekonda TS, Reddy PH. Can herbs provide a new generation of drugs for treating Alzheimer's disease? *Brain Res Rev*. 2005; 50(2): 361-376.
[12] Kim HG, Oh MS. Herbal medicines for the prevention and treatment of Alzheimer's disease. *Curr Pharm Design*. 2012; 18(1): 57-75.
[13] Chashti HAK. *Exir-e-Azam*. 1st ed. Tehran: University of Medical Science, Institute for Islamic and Complementary Medicine, 2007.
[14] Rhazes. *Al-hawi.* 1st ed. Afsharipour S, (Trans.). Thehran: Academy of medical sciences publication, 2005.
[15] Avicenna. *The Canon of medicine*. 2nd ed. Sharafkandi A, Ed. Tehran: Soroush press, 1997.

[16] Aghili khorasani MH. *Makhzan-al-advia*. 1st ed. Shams Ardekani MR, Rahimi R, Farjadmand F, Eds. Tehran: Rahe kamal, 2009.

[17] Fritsch RM, Gurushidze M, Jedelska J, Keusgen M. More than apretty face-ornamental "drumstick onions" of *Allium* subg. melanocrommyum are also potential medicinal plants. *Planta Med.* 2007; 73(09): 26-59.

[18] Fritsch RM, Abbasi M. *A taxonomic review of Allium subg. melanocrommyum in Iran.* 1st ed. Gatersleben: IPK Gatersleben, 2013.

[19] Sobolewska D, Michalska K, Podolak I, Grabowska K. Steroidal saponins from the genus *Allium*. *Phytochem Rev*. 2016; 15(1): 1-35.

[20] Shahgholi H, Vazirimehr MRV, Hosein Talaei G, Rigi K. Effect bulb size and two specie mooseer to yield components bulb percent allicin in weather mashha. *J Bio & Env Sci*. 2014; 5(1):236-242.

[21] Kannappan R, Gupta SC, Kim JH, Reuter S, Aggarwal BB. Neuroprotection by spice-derived nutraceuticals: you are what you eat! *Mol Neurobiol*. 2011; 44(2): 142-159.

[22] Stankevičius M, Akuņeca I, Jākobsone I, Maruška A. Analysis of phenolic compounds and radical scavenging activities of spice plants extracts. *Maisto Chemija Ir Technologija*. 2010; 44(2): 85-91.

[23] Ghahremani-majd H, Dashti F, Dastan D, Mumivand H, Hadian J, Esna-Ashari M. Antioxidant and antimicrobial activities of Iranian mooseer (*Allium hirtifolium* Boiss) populations. *Hortic Environ Biote*. 2012; 53(2): 116-122.

[24] Azadi HG, Ghaffari SM, Riazi GH, Ahmadian S, Vahedi F. Antiproliferative activity of chloroformic extract of Persian Shallot, *Allium hirtifolium*, on tumor cell lines. *Cytotechnology*. 2008; 56(3): 179-185.

[25] Krejčová P, Kučerová P, Stafford GI, Jäger AK, Kubec R. Antiinflammatory and neurological activity of pyrithione and related sulfur-containing pyridine N-oxides from Persian shallot (*Allium stipitatum*). *J Ethnopharmacol*. 2014; 154(1): 176-182.

[26] Rafieian-kopaei M, Keshvari M, Asgary S, Salimi M, Heidarian E. Potential role of a nutraceutical spice (*Allium hirtifolium*) in reduction of atherosclerotic plaques. *J Herb Med Pharmacol*. 2014; 2(2): 23-28.

[27] Mahmoodi M, Hosseini J, Hosseini-Zijoud SM, Mirzaee M, Mirzajani E. The effect of Persian shallot (*Allium hirtifolium* Boiss.) extract on blood sugar and serum levels of some hormones in diabetic rats. *Pak J Pharm Sci*. 2013; 26(2): 397-402.

[28] Jafarian A, Ghannadi A, Elyasi A. The effects of *Allium hirtifolium* Boiss. on cell-mediated immune response in mice. *Iran J Pharm Res.* 2003; 2(1): 51-55.

[29] Mohammadi S, Zarei M, Mahmoodi M, Zarei MM, Nematian MA. *In vivo* antinociceptive effects of Persian shallot (*Allium hirtifolium*) in male rat. *Avicenna J Neuro Psycho Physiol*. 2015; 2(1): 1-5.

[30] Hooper D, McNair JB, Field H. *Useful plants and drugs of Iran and Iraq.* 1st ed. Chicago: Field Museum of Natural History, 1937.

[31] Ghahremaninejad F. Two new records of *Astragalus* species of the sections Anthylloidei DC. and Dissitiflori DC. from Iran. *Turk J Bot.* 2005; 29(5): 399-402.

[32] Hakim A, Tajuddin, Ghufran A, Nasreen J. Evaluation of anti-inflammatory activity of the pods of Iklil-ul-Malik (*Astragalus hamosus* Linn.). *Indian J Nat Prod Resour*. 2010; 1(1): 34-37.

[33] Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of *Astragalus hamosus* in animal models. *Iran J Pharm Res*. 2015; 14(1): 263-269.

[34] Kondeva-Burdina M, Krasteva I, Mitcheva M. Effects of rhamnocitrin 4-β-D-galactopyranoside, isolated from *Astragalus hamosus* on toxicity models in vitro. *Pharmacogn Mag*. 2014; 10(S3): 487-493.

[35] Handa SS, Khanuja SPS, Longo G, Rakesh DD. *Extraction technologies for medicinal and aromatic plants*. 3rd ed. Trieste: Earth, Environmental and Marine Sciences and Technologies, 2008.

[36] National Institutes of Health. *Guide for care and use of laboratory animals*. 6th ed. Bethesda: NIH Publication, 1985.

[37] Bahaeddin Z, Yans A, Khodagholi F, Hajimehdipoor H, Sahranavard S. Hazelnut and neuroprotection: Improved memory and hindered anxiety in response to intra-hippocampal Aβ injection. *Nutr Neurosci*. In press.

[38] Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 3rd ed. San Diego: Academic Press, 2007.

[39] Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*. 2007; 2(2): 322-328.

[40] Zarrindast MR, Khalifeh S, Rezayof A, Rostami P, Sereshki AA, Zahmatkesh M. Involvement of rat dopaminergic system of nucleus accumbens in nicotine-induced anxiogenic-like behaviors. *Brain Res*. 2012; 1460: 25-32.

[41] Jarskog LF, Selinger ES, Lieberman JA, Gilmore JH. Apoptotic proteins in the temporal cortex in schizophrenia: high Bax/Bcl-2 ratio without caspase-3 activation. *Am J Psychiat*. 2004; 161(1): 109-115.

[42] Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999; 6(2): 99-104.

[43] Yuan J, Yankner BA. Apoptosis in the nervous system. *Nature*. 2000; 407(6805): 802-809.

[44] Harkany T, O'Mahony S, Keijser J, Kelly JP, Kónya C, Borostyánkői ZA, Görcs TJ, Zarándi M, Penke B, Leonard BE, Luiten PG. β-Amyloid (1-42)-induced cholinergic lesions in rat nucleus basalis bidirectionally modulate serotonergic innervation of the basal forebrain and cerebral cortex. *Neurobiol Dis*. 2001; 8(4): 667-78.

[45] Revest J, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza P, Abrous DN. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Mol Psychiatr*. 2009; 14(10): 959-967.

[46] Hill AS, Sahay A, Hen R. Increasing adult hippocampal neurogenesis is sufficient to reduce anxiety and depression-like behaviors. *Neuropsychopharmacol*. 2015; 40(10): 2368-2378.

[47] Hroudová J, Fišar Z, Raboch J. *Mitochondrial functions in mood disorders*. In: Kocabaşoğlu N, Ed. *Mood disorders*. Rijeka: InTech, 2013.

[48] Einat H, Yuan P, Manji HK. Increased anxiety-like behaviors and mitochondrial dysfunction in mice with targeted mutation of the Bcl-2 gene: further support for the involvement of mitochondrial function in anxiety disorders. *Behav Brain Res*. 2005; 165(2): 172-180.

[49] Pirbalouti AG, Ahmadzadeh Y, Malekpoor F. Variation in antioxidant, and antibacterial activities and total phenolic content of the bulbs of mooseer (*Allium hirtifolium* Boiss.). *Acta Agric Slov*. 2015; 105(1): 15-22.

[50] Galato D, Ckless K, Susin MF, Giacomelli C, Ribeiro-do-Valle RM, Spinelli A. Antioxidant capacity of phenolic and related compounds: correlation among electrochemical, visible spectroscopy methods and structure-antioxidant activity. *Redox Rep*. 2001; 6(4): 243-250.

[51] Montine T, Neely M, Quinn J, Beal M, Markesbery W, Roberts L, Morrow JD. Serial review: causes and consequences of oxidative stress in Alzheimer's disease. *Free Radic Biol Med*. 2002; 33(5): 620-626.

[52] Kelsey NA, Wilkins HM, Linseman DA. Nutraceutical antioxidants as novel neuroprotective agents. *Molecules*. 2010; 15(11): 7792-7814.

[53] Espín JC, García-Conesa MT, Tomás-Barberán FA. Nutraceuticals: facts and fiction. *Phytochemistry*. 2007; 68(22): 2986-3008.

[54] Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J Diabetes Metab Disord*. 2013; 12(1): 1-9

[55] Gomez-Pinilla F, Nguyen TT. Natural mood foods: the actions of polyphenols against psychiatric and cognitive disorders. *Nutr Neurosci*. 2012; 15(3): 127-133.

[56] Dias GP, Cavegn N, Nix A, do Nascimento Bevilaqua MC, Stangl D, Zainuddin MSA, Nardi AE, Gardino PF, Thuret S. The role of dietary polyphenols on adult hippocampal neurogenesis: molecular mechanisms and behavioural effects on depression and anxiety. *Oxid Med Cell Longev*. 2012; Article ID 541971.

[57] Aquilano K, Baldelli S, Rotilio G, Ciriolo MR. Role of nitric oxide synthases in Parkinson's disease: a review on the antioxidant and anti-inflammatory activity of polyphenols. *Neurochem Res*. 2008; 33(12): 2416-2426.

[58] Kim JG, Koh SH, Lee YJ, Lee KY, Kim Y, Kim S, Lee MK, Kim SH. Differential effects of diallyl disulfide on neuronal cells depend on its concentration. *Toxicology*. 2005; 211(1): 86-96.

[59] Li XH, Li CY, Lu JM, Tian RB, Wei J. Allicin ameliorates cognitive deficits ageing-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways. *Neurosci Lett*. 2012; 514(1): 46-50.

[60] Li XH, Li CY, Xiang ZG, Zhong F, Chen ZY, Lu JM. Allicin can reduce neuronal death and ameliorate the spatial memory impairment in Alzheimer's disease models. *Neurosciences (Riyadh)*. 2010; 15(4): 237-243.

[61] Li X, Qu L, Dong Y, Han L, Liu E, Fang S, Zhang Y, Wang T. A Review of recent research progress on the *Astragalus* genus. *Molecules*. 2014; 19(11): 18850-18880.

[62] Krasteva I, Platikanov S, Nikolov S, Kaloga M. Flavonoids from *Astragalus hamosus*. *Nat Prod Res*. 2007; 21(5): 392-395.

[63] Cardoso BR, Ong TP, Jacob-Filho W, Jaluul O, Freitas MIdÁ, Cozzolino SMF. Nutritional status of selenium in Alzheimer's disease patients. *Brit J Nutr*. 2010; 103(06): 803-806.

[64] Shaw W, Anderson J. Comparative enzymology of the adenosine triphosphate sulphurylases from leaf tissue of selenium-accumulator and non-accumulator plants. *Biochem J*. 1974; 139(1): 37-42.

[65] Cao C, Wang L, Lin X, Mamcarz M, Zhang C, Bai G, Bai G, Nong J, Sussman S, Arendash G. Caffeine synergizes with another coffee component to increase plasma GCSF: linkage to cognitive benefits in Alzheimer's mice. *J Alzheimers Dis*. 2011; 25(2): 323-335.