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Research Paper

Ameliorating Effect of Standardized Extract From Textured Soy Protein (*Glycine max* L.) on Memory Deficit and Learning Insufficiency in Scopolamine-induced Amnesia

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ABSTRACT

Introduction: Textured soy protein (TSP) and nuts are two processed forms of soybean (*Glycine max* L.) that are widely consumed for nutritional purposes in Iran. Recently, we have reported the antioxidant and anticholinesterase effects of raw soybean (RS) attributed to isoflavones, such as genistein. In this work, we aimed to compare *in vitro* antioxidant and anticholinesterase effects of TSP, nuts, and RS to select the most effective one for learning capacity and spatial memory studies.

Methods: Genistein content was determined using high-performance thin layer chromatography (HPTLC), while diphenylpicrylhydrazil (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) were used to study antioxidant evaluation and Ellman's colorimetric method was used to measure anticholinesterase. TSP extract (TSPE) was administered to male rats (100 mg/kg, 200 mg/kg and 400 mg/kg, intraperitoneally [i.p] for 7 days) before scopolamine injection (1 mg/kg). Learning capacity and spatial memory were evaluated using passive avoidance test (PAT) and Morris water maze (MWM) methods compared to physostigmine and piracetam.

Results: The greatest antioxidant and anticholinesterase effect was observed for TSPE, which significantly prolonged initially latency in PTA ($P<0.05$) and improved all indicators in the MWM test at 200 mg/kg.

Conclusion: The memory-improving effect of TSPE may be due to its antioxidant and anticholinesterase effect as well as neuroprotective effects of its isoflavones.

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Highlights

- Different samples (nuts-raw soybeans-TSP) prepared from soybeans.
- All samples exhibited antioxidant and anti-cholinesterase effects in vitro studies.
- TSP showed the most biological activity and the greatest genistein content.
- TSP significantly improved memory and learning indicators at 200 mg/kg.
- These effects are attributed to its antioxidant and anticholinesterase activity.
- Plant isoflavones have neuroprotective effects.

Plain Language Summary

Alzheimer's disease (AD), is one of the problems of the elderly society, which has a lot of emotional and financial costs. AD is a type of progressive brain disease in which neurons are destroyed and memory is lost. This disease currently has no definitive treatment and the only way is to prevent the disease from spreading. Much research has been devoted to finding suitable and effective treatments for AD. Many food and herbal medicines have shown to be effective in controlling this disease. Soybean is a plant that is widely used as food and snacks in Iran in different ways. In this study, we prepared three preparation from soya beans which have been widely used by Iranian people including raw soya, nut (roasted form) and textured soy protein (TSP). The effect of these preparations have been studied on memory and learning in amnestic rats through different pharmacological studies. The results indicated that TSP due to antioxidant and anticholinesterase activity significantly can augment memory enhancing and learning ability Alzheimer's disease (AD), is one of the problems of the elderly society, which has a lot of emotional and financial costs. AD is a type of progressive brain disease in which neurons are destroyed and memory is lost. This disease currently has no definitive treatment and the only way is to prevent the disease from spreading. Much research has been devoted to finding suitable and effective treatments for AD. Many food and herbal medicines have shown to be effective in controlling this disease. Soybean is a plant that is widely used as food and snacks in Iran in different ways. In this study, we prepared three preparation from soya beans which have been widely used by Iranian people including raw soya, nut (roasted form) and textured soy protein (TSP). The effect of these preparations have been studied on memory and learning in amnestic rats through different pharmacological studies. The results indicated that TSP due to antioxidant and anticholinesterase activity significantly can augment memory enhancing and learning ability. TSP also contains some phytochemicals such as phytoestrogens which have shown neuroprotective activity in different studies.

1. Introduction

Memory and learning are two main functions of the brain, and failure in either is one of the most obvious features of dementia. Dementia is a progressive decline in cognition due to different brain diseases and problems. Globally, more than 25 million people worldwide suffer from dementia, especially Alzheimer's disease (AD), the most common form of the disease. This disease is age-related and is accompanied by neuronal loss in the basal forebrain (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). This issue suggests that cholinergic neurons in the hippocampus and neocortex are involved in learning and memory (Okada, Nishizawa, Kobayashi, Sakata, & Kobayashi, 2015). Although the accumulation of neurofibrillary tangles and amyloid plaques are the hallmark of AD, degeneration of central cholinergic neurons is correlated with disease progression and the manifestation of cognition problems (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016).

Medicinal plants and their bioactive constituents due to diverse biological activities, such as aniconvulsant and cytotoxicity, (Mahmoudvand et al., 2014; Mandegary, Arab-Nozari, Ramiar, & Sharififar, 2012; Sharififar et al., 2009) have been highly considered in drug development. Recently, we have reported anticholinesterase and antioxidant activity of raw soybean (RS) (*Glycine*

max L.) (Sharififar, Moshafi, Shafazand, & Koohpayeh, 2012). This plant belongs to the Leguminosae family and is one of the nutritional plants introduced from South East Asia. Although no history of plant consumption exists in Iranian medicine, the plant has been welcomed by Iranians (Mohamadi, Sharififar, Koohpayeh, & Daneshpajouh, 2015; Sharifi far, F., 2011). Regardless of nutritional value, soybean contains large quantities of protein and phytochemicals, such as phenolic acids, flavonoids, saponins, and isoflavone phytostrogens, such as genistein and daidzein. Isoflavones can improve memory and learning skills and decrease the rates of chronic disease (Menze, Esmat, Tadros, Abdel-Naim, & Khalifa, 2015). Soybean protein has a high-quality amino acid balance due to the presence of all essential amino acids. The main ingredients of this protein are glycinin and β-conglycinin, which comprise about 65% to 80% of seed protein (Bader, 1981). Nonetheless, a small amount of the soybean has directly been used and the two major forms of soy consumption in Iran are textured soy protein (TSP) known as "“soy meat" and "nuts". In TSP form, defatted soybean is used as a meat substitute after quick cooking, while in the nuts form, the seeds are roasted with lemon juice and salt.

Considering the effect of antioxidant, anticholinesterase, and improver of RS on cognitive impairments (Khodamoradi, Asadi-Shekaari, Esmaeili-Mahani, Sharififar, & Sheibani, 2017; Mandegary, Sharififar, Soodi, & Zarei, 2014; Sharififar et al., 2012), in the present work, we intended to compare *in vitro* antioxidant and anticholinesterase effects of TSP, nuts, and RS and to study the effectiveness of the most active form of soybean on spatial memory and learning in scopolamine-induced amnesia. On the other hand, genistein is the major isoflavone in soybean and has beneficial effects on brain function (Bagheri, Joghataei, Mohseni, & Roghani, 2011), therefore genistein content of soybean compounds (Acta Parasitologica and soybean preparations) have also been determined.

2. Materials and Methods

Plant materials

RS was authenticated by Dr. Mirtadzadini after gathering from the botanic garden of the Faculty of Pharmacy, Kerman University of Medical Sciences (KUMS), and a voucher specimen was inserted in the herbarium center of the Faculty of Pharmacy, Kerman University of Medical Sciences (KUMS) (KF1251). One part of RS was processed to prepare nuts via soaking plant seeds in a dilute mixture of water, lemon juice, and salt for 2h and roasting for about 30 min. TSP was prepared from the market (Sobhan, Iran). About 500g of each sample was milled, sieved (mesh 300), and extracted with 80% ethanol using the warm maceration method for 72h (for warm maceration, firstly the solvent is placed over a water bath at a temperature of about 40°C to warm. Heating induces plant cells to break apart and increases the solvent penetration into the plant cell).

Chemicals

Genistein was purchased from Roth (Germany), and physostigmine and piracetam were prepared from Darupakhsh (Medicinal Chemistry Co. Iran, Tehran). All other chemicals were from analytical grade and purchased from Merck, Germany.

Phytochemical screening, total content of flavonoids, alkaloids, saponins, and terpenoids, and plant standardization

A qualitative and quantitative analysis of major phytochemicals, such as alkaloids, flavonoids, saponins, tannins, and terpenoids was performed on the soybean according to previous methods. More attention has been paid for finding new anticholinesterase agents from medicinal plants (Sharififar, Mirtajadini, Azampour, & Zamani, 2011). The results confirmed the presence of saponins, alkaloids, and terpenoids in the plant. The total content of the saponins, alkaloids, and terpenoids was determined as explained by Adusei (Adusei, Otchere, Oteng, Mensah, & Tei-Mensah, 2019). For high-performance thin layer chromatography (HPTLC), A CAMAG LINOMATS instrument was used for analysis. Different concentrations of genistein (5 µg/mL, 10 µg/mL, 20 µg/mL and 30 µg/mL) were prepared in methanol. Silica gel 60F254, HPTLC plate (Merck, Germany), and the solvent system of chloroform-methanol (9:1 v/v) were used as stationary and mobile phases, respectively. After developing in the solvent system, the plate was dried at room temperature, deposited in the photo-documentation chamber after drying, and observed under 254 nm and 366 nm UV lamps (Alam, Yusufoglu, & Alam, 2013). Densitometry analysis was done using a CAMAG TLC Scanner 3 to quantify the bands using WIN CATS software (version 4 X).

In vitro antioxidant

Antioxidant effects of the extracts were evaluated using diphenylpicrilhydrazil (DPPH) method and ferric reducing antioxidant potential (FRAP) assay and

the percentage of DPPH radical inhibition and FRAP values (equivalent to mmol Fe+2/g) was reported (Rameshk, Sharififar, Mehrabani, Pardakhty, & Farsinejad, 2018). Butylated hydroxytoluene (BHT) and vitamin C were used as positive controls in DPPH and FRAP assays, respectively.

In vitro anticholinesterase activity

The anticholinesterase effect of the extracts was studied based on Ellman's method with some modifications explained previously (Sharififar et al., 2012). Regarding the results of antioxidant, anticholinesterase activity, and genistein content, textured soy protein extract (TSPE) was used for animal studies.

Pharmacological study

Experimental animals

Male Wistar rats (6-8 weeks with Mean±SD weight of 200±20g) were housed in individual cages in a controlled room on 12h and off 12h with free access to food and water. The experimental protocol of the work with animals was by the National Institutes of Health (NIH) guide and was approved by the Ethical Committee of the University Research Center (EC/KNRC/94-17).

A total of 126 rats were randomly assigned into 9 groups for MWM and 9 groups for passive avoidance test (PAT) each of 7 animals. All injections were done intraperitoneally (i.p) between 8 AM and 10 AM, and the experiments were performed during daylight between 8 AM and 4 PM to prevent the effects of nighttime rhythm on the experiments. Animals were acclimated to the laboratory condition 1 hour before the test. Experimental groups were 1: Control: No treatment; 2:Normal saline (NS); 3: Solvent (10% dimethyl sulfoxide [DMSO] in NS); 4: Physostigmine (Phys) 0.3 mg/kg; 5:Piracetam (Pir) 200 mg/kg; 6-8: TSPE 100 mg/kg, 200 mg/kg, and 400 mg/kg; 9:Scopolamine (Sco) 1 mg/kg. Animals received 1 mL/kg, i.p of drugs (7 consecutive days). Sco was used for amnesia induction and was injected (i.p) on the 7th day, 30 min after the last dose of the drug (Sco induces memory dysfunctions similar to those observed in dementia). Sco group just got 1mg/kg scopolamine and the experiment was performed 30 min later. The groups 2 and 3 were considered negative controls, while the groups 4 and 5 were considered positive controls.

Passive avoidance test (PAT)

In PAT, which was used to evaluate long spatial memory, a plexiglass box was used consisting of two equal dark and light partitions (20cm, 20cm, 20cm) separated by a remote door. The dark part is covered by a black roof, on the bottom of which steel bars are installed at one-centimeter intervals and connected to the shock device by a communication cable. This device can generate an electric current of 0.5 mA for two seconds with a frequency of 50Hz in these rods, which causes an electric shock to the animal's hands and feet. During the learning stage, animals were placed in the light partition and allowed to enter the dark side after 10s. Animals with a delay of more than 120s were removed from the experiment. After 2h, during the training phase, the animals were again placed in the light partition and the door was opened after 10s for the animals to enter the dark side and the door was immediately closed. An electrical shock (0.5mA, 50Hz, 2s) was delivered to the animal's foot, and the animal was immediately given back to the cage. The experiment was repeated after 5 min. When the animals remained on the light side for at least 5 consecutive minutes, the experiment was stopped. After 24h, during the retrieval phase, the animal was directed to the light side, the door was opened after 10s and initial avoidance and crossing latency to the dark part were recorded (Harandi et al., 2015).

Morris water maze (MWM)

This test is used to evaluate short spatial memory. A black circular pond (60×160cm) was filled with water (24±2°C) and divided into four separated quadrants. Animals were released to swim into the water and their performance was recorded using a computerized camera system. In the target quadrant (TQ), a square platform (10cm in diameter) was placed under the water and during the learning stage, the animals were again allowed to swim via three blocks each of four consecutive 60s trials with 30 min intervals (each trial was started from a different quadrant). After finding the platform, animals could rest on it for 30s and then were transferred to the cage for another 30s until the next trial. The animals were guided to the platform if did not find it after 60s. The time spent in the TQ and the distance moved to find the platform was recorded. A single probe trial was done after 2h to find the plant effect on memory retrieval. The animals were freed from the quadrant as opposed to TQ into the water to swim without the platform for 60s. The crossing number, time remaining in the TQ and the distance traveled to discover the platform was documented. After 2 min, the platform was transferred

above the water level and allowed the animal to find it it has become one of the most frequently used laboratory tools in behavioral neuroscience (D'Hooge & De Deyn, 2001). Many methodological variations of the MWM task have been and are being used by research groups in many different applications. However, researchers have become increasingly aware that MWM performance is influenced by factors such as apparatus or training procedure as well as by the characteristics of the experimental animals (sex, species/strain, age) nutritional state, exposure to stress or infection.

Statistical analysis

The results were shown as Mean±SEM. To compare the intergroup data and to detect the significance of the difference between the tested groups, one-way analysis of variance (ANOVA) was used. Tukey's post hoc and LSD tests were used to find the difference between them. A repeated measurement ANOVA test was used to compare the results of the first, second, and third blocks in the MWM test. Differences with $P<0.05$ were considered significant.

3. Results

Plant phytochemistry, standardization, and high-performance thin layer chromatography (HPTLC) analysis

Phytochemical experiments confirmed the presence of flavonoids, alkaloids, saponins, and terpenoids in all three soybean forms. Table 1 presents the results of the quantification of these secondary metabolites and the yield of extraction. As shown in Table 1, the extraction yield was 15.76%, 12.60%, and 11.28% (w/w) for TSP, RS, and nuts, respectively. The greatest amount of alkaloids were reported in RS (20.70±2.67) g/100g dried extract. No significant difference was observed among soybean samples in terms of saponin and terpenoid content ($P>0.05$).

HPTLC profile exhibited a similar band of genistein with Rf value of 0.32 in the chromatogram of all three extracts. The related fluorescent bands were visible at 254 nm. The genistein content of TSPE, RS, and nuts was determined 10.37±0.60, 8.26±0.34, and 6.14±0.35 (g/100g dried extract) based on its calibration curve (Table 2).

Antioxidant activity

All three extracts inhibited DPPH radical in a concentration-dependent manner. The greatest inhibition was shown by TSPE and RS (87.32%±3.54% and 81.50%±1.76%, respectively) compared to BHT as a positive control (98.79%±2.46%) (The 50% inhibitory concentration [IC50 value] of TSPE was determined 65.27±3.10 µg/mL) (Table 2).

In the FRAP test, the greatest FRAP values were reported for RS and TSPE (131.06±1.64 µmol/ mL and 127.23±2.48 µmol/ mL, respectively) compared to vitamin C as positive control(168.12±3.21µmol/mL). The FRAP values of TSPE and RS were significantly different from nuts ($P<0.001$) (Table 2).

Anticholinesterase activity

As shown in Table 2, all samples somehow inhibited acetylcholinesterase (AChE) in a concentration-dependent manner. TSPE markedly inhibited AChE enzyme (72.64%±4.01%), followed by RS (69.94%±2.31%) and nuts (43.28%±1.65%), respectively. The IC50 value was calculated and reported (Table 2). The least IC50 was due to TSPE (146.32±8.23 µg/mL) compared to physostigmine (12.03±1.48 µg/mL).

Passive avoidance test (PAT)

The results of the PAT test indicated that Sco significantly decreased the initial latency (12.13±2.29 s) and increased crossing latency (7.14±0.36) compared to the control (122.17±2.68s initial latency and 2.33±0.42 crossing latency) ($P<0.001$). The effect of Sco was not significantly different from NS and solvent groups ($P>0.05$). TSPE extract significantly prolonged initial latency especially at 200 mg/kg (300.00±0.00 s) ($P<0.001$). Entry avoidance was also significant at different doses of TSPE such that animals completely avoided entering at TSPE 200 mg/kg (crossing latency=0.05±0.00) (Figure 1a-b).

Morris water maze (MWM) result

In the acquisition phase of MWM, the Mean±SD of four trials in three different blocks has been shown. Animals treated with Sco significantly spent more time (52.19±2.68 s) and swam more distance (1533.00±83.75cm) compared to the control group ($P<0.001$). These results indicate that Sco causes a learning dysfunction. No significant difference was observed among Sco, solvent, and NS groups ($P>0.05$) (data not shown). As it implies, animals that received Pir, Phys, and TSPE (all three doses) could find the platform with significantly less time and distance, especially at TSPE 200 mg/kg (time spent of 20.54±2.38s and swimming moved of 446.48±66.21cm, respectively) ($P<0.001$ and $P<0.01$) (Figures 2a,b).

Table 1. Yield of extraction and total alkaloid content, total saponin content, and total terpenoid content of three different samples of soybean

Row	Sample	No. (%)/Mean±SD				P
		Extraction Yield, (w/w Dried Plant Materials)	TAC, (w/w Dried Extract)	TSC, (w/w Dried Extract)	TTC, (w/w Dried Extract)	
1	TSPE	15.76±1.02	5.52±0.59**	2.01±0.25	12.36±0.69	<0.001
2	Nut	12.60±0.92	15.1±3.01*	2.11±0.04	12.09±1.40	<0.027
3	RS	11.28±0.84	20.70±2.67*	1.98±0.31	11.67±1.09	<0.05

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TSPE: textured soy protein extract; RS: raw soybean; TAC: total alkaloid content; TSC: total saponin content: TTC: total terpenoid content.

* Significant difference P<0.05. ** Significant difference P<0.001.

In the probe test, Sco significantly decreased the time and distance spent as well as site crossing in TQ (30.39±0.86s, 25.62±1.31cm and 2.67±0.21, respectively) while animals that received TSPE, Pir, and Phys spent more time and distance in TQ (39.54±1.96s, 44.74±3.80s, and 49.61±3.07s time spent for TSPE 100, 200 and 400 mg/kg, respectively) and (50.96±5.06cm, 42.65±2.71cm, 45.85±2.85cm distance moved for TSPE 100, 200 and 400 mg/kg, respectively) which were significantly different from Sco group (P<0.001 and P<0.01). The crossing number to TQ in TSPE, Pir, and Phy groups was significantly greater than Sco group (P<0.001 and P<0.01) (Figures 3a, b, c). Comparing animals' swimming velocity in TQ revealed no significant difference between experimental groups indicating that sensory-motor factors did not play a role in the evaluation of spatial memory (data was not shown).

4. Discussion

In this work, genistein content has been compared with HPTLC methods in more commonly consumed forms of soybean in Iran. The highest content of genistein was found in TSPE and with a slight difference in RS which was consistent with the results of antioxidant and anticholinergic activity of the samples. On the other hand, phytochemical studies of three samples of soybean indicated that the total alkaloid content of TSPE is significantly less than the other two samples (P<0.001), while the total saponin and terpenoid con-

Table 2. Comparison of extraction yield, genistein content, antioxidant, and anticholinesterase effect of three different forms of soybean

Row	Sample	Mean±SD*			P	Mean±SD	P	Mean±SD*		P
		Genistein Content, (w/w)	DPPH Inhibition			FRAP Value (µmol/mL)		AChE Inhibition		
			Maximum Inhibition	IC50 Value (µg/mL)				Maximum Inhibition	IC50 Value (µg/mL)	
1	TSPE	10.37±0.60	87.32±3.54	65.27±3.10	<0.001	127.23±2.48	<0.001	72.64±4.01	146.32±8.23	<0.001
2	Nut	8.26±0.34	49.67±2.36	-	<0.001	48.93±5.72	<0.001	43.28±1.65	549.37±17.52	<0.001
3	RS	6.14±0.35	81.50±1.76	73.12±2.74	<0.001	131.6±1.64	<0.001	69.94±2.31	203.57±11.08	<0.001
4	BHT	-	98.79±2.46	2.94±0.61	<0.001	-		-	-	<0.001
5	Vit C	-	-	-		168.12±3.21	<0.001	-	-	
6	Phys	-	-	-		-		99.38±2.67	12.03±1.48	

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TSPE: textured soy protein extract; RS: raw soybean; FRAP: ferric reducing antioxidant potential; Phys: physostigmine; BHT: butylated hydroxytoluene.

* Significant difference P<0.001.

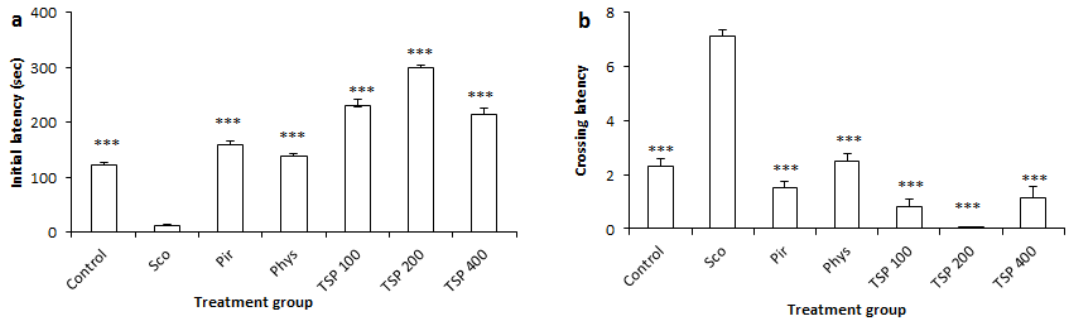

Figure 1. Effect of different concentrations of textured soy protein extract (TSPE)

On initial latency (a) and crossing latency (b) in scopolamine-induced amnesic animals compared to piracetam and physostigmine using passive avoidance test.

Groups of 7 animals each received Pir (200 mg/kg), Phys (0.3 mg/kg) and TSPE (100, 200 and 400 mg/kg) for 7 consecutive days. Animals treated with 1 mg/kg scopolamine (i.p). Initial latency and crossing latency were evaluated 30 min after the last dose in different experimental groups. The results were presented as Mean±SEM.

*** Significantly different from scopolamine group with P<0.001.

tent in all three Mean±SD samples were not significantly different (P>0.05). The difference in alkaloid content may be due to the lipophilicity nature of them which increases their persistence in the oily part of the plant via soybean processing of TSPE.

In the PAT test, TSPE increased passive avoidance latency compared to Sco, which significantly reduced the latencies (P<0.001). In the training phase of MWM, the time spent and distance moved to find the platform has been used as an indicator of spatial memory, especially when the swimming velocity is not affected. In the retrieval stage, if the consolidation of spatial memory takes place, the animal spends more time in TQ. In contrast to Sco which reduced the time and distance spent and entrance frequency into TQ (indicative of memory deficits), TSPE improved all learning indicators. TSPE activity was prominent at 200 mg/kg and the activity decreased as the dose increased. This effect can be attributed to the hormetic and paradoxical effect of the plants and phytochemicals which have demonstrated a biphasic effect in a wide range of biological activities. Although the accurate mechanism of hormesis in the plants has been unknown and needs further studies, at least part

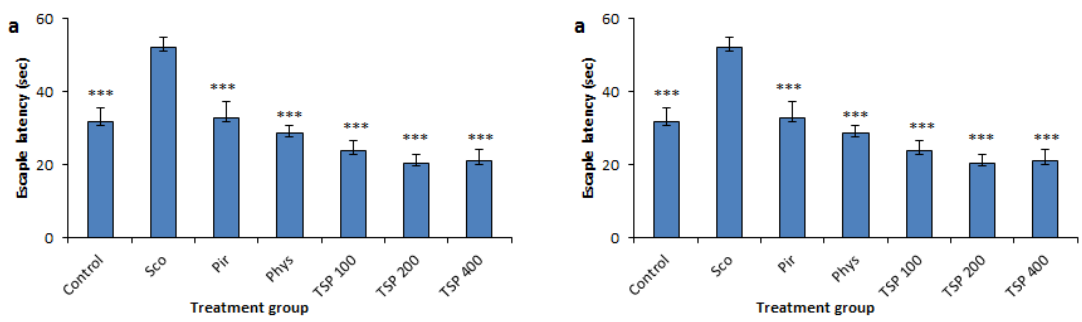

Figure 2. Effect of different concentrations of textured soy protein extract (TSPE)

On escape latency (a) and distance moved (b) in scopolamine-induced amnesic animals compared to Piracetam (Pir) and Physostigmine (Phys) in training phase of Morris Water Maze (MWM) Test.

Groups of 7 animals each received Pir (200 mg/kg), Phys (0.3 mg/kg) and TSPE (100, 200 and 400 mg/kg) for 7 consecutive days. Animals treated with 1mg/kg scopolamine (i.p). Thirty min after the last dose, distance moved and latency escape in the target quadrant was evaluated via three blocks in 4 consequent trials in different experimental groups. The results were presented as Mean±SEM.

*** Significantly different from the scopolamine group with P<0.001.

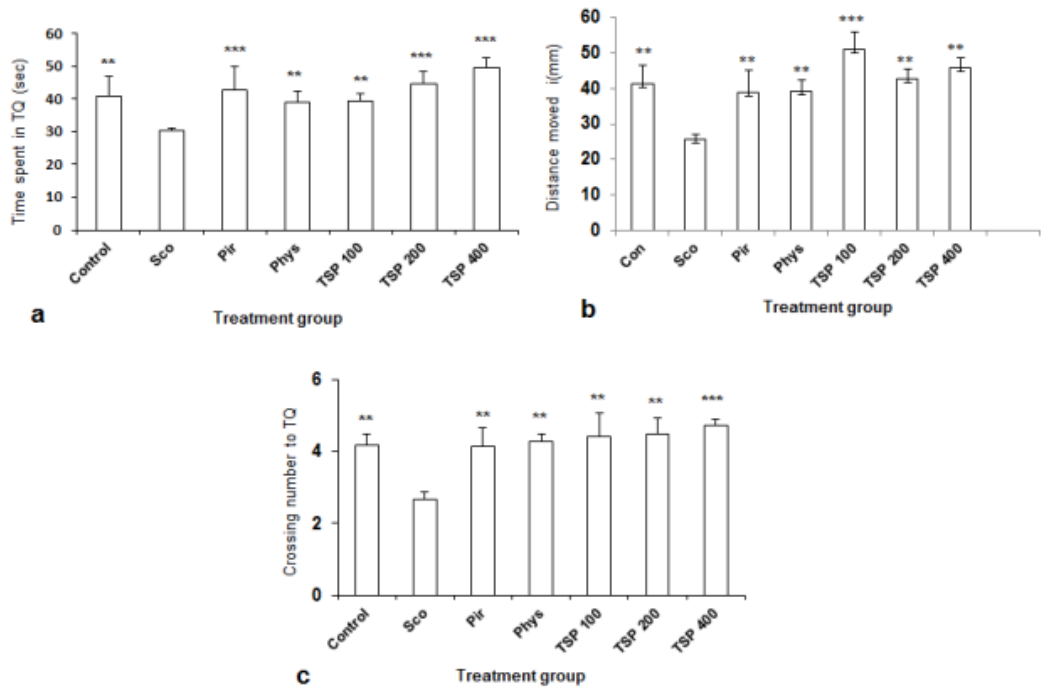

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Figure 3. Effect of different concentrations of textured soy protein extract (TSPE)

On time spent (a) and distance moved (b) and crossing number (c) in target quadrant in scopolamine-induced amneic animals compared to Piracetam (Pir) and Physostigmine (Phys) in requisition phase of Morris Water Maze (MWM) Test.

Groups of 7 animals each received Pir (200 mg/kg), Phys (0.3 mg/kg) and TSPE (100, 200 and 400 mg/kg) for 7 consecutive days. Animals treated with 1mg/kg scopolamine (i.p). Thirty min after the last dose, distance moved and time spent and crossing number in the target quadrant were evaluated via three blocks in 4 consequent trials in different experimental groups. The results were presented as Mean±SEM.

*** Significantly different from scopolamine group with P<0.001, ** P<0.01.

of this effect can be attributed to the complex composition of these natural sources. Sco is a non-selective and competitive inhibitor of muscarinic receptors and causes memory deficit (Flood & Cherkin, 1986). Recent studies emphasize the role of altered cholinergic signaling in cognitive impairments. Acetylcholine increases cortical response to external stimuli and leads to more concentration and attention (Higley & Picciotto, 2014). Our results in PAW and MWM revealed that TSPE improves memory and counteracts the negative impact of Sco on learning. It is believed that soybean is effective in preventing cardiovascular disease, cancer, and osteoporosis (Alekel et al., 2010; Korde et al., 2009; Liang et al., 2009). The plant contains flavonoids, phenolic acids, and more complex molecules, such as lignins. Isoflavones of genistin and daidzin can improve cognitive functions via estrogenic effect (receptor β) (Bansal & Parle, 2010). Estrogen stimulates ACh release and markedly facilitates the function of the forebrain cholinergic system. Soybean isoflavones increase cholinergic transmission via AChE inhibition (Bennetau-Pelissero, Jamali, & Marighetto, 2011) and improve nonverbal memory and other abilities in cognition disorders (Gleason et al., 2009) the findings from which are mixed. Isoflavone efficacy is dependent upon conversion of glycosides contained in soy foods and supplements to the biologically active aglycons. Of particular interest is the production of the metabolite, equol, which is dependent upon intestinal microflora and an integrous digestive system, both being altered by age and age-associated conditions. Unfortunately, few studies enrolled adults over the age of 70, and none included older men. We examined safety, feasibility and cognitive efficacy of soy isoflavone administration in older nondemented men and women (age: 62-89 years). The neuroprotective effect of these isoflavones has been reported in animal models of AD (Bagheri et al., 2011) heredity, and induced oxidative stress are among proposed risk factors. The increased frequency of the disease in women also suggests a role for estrogen in development of AD.

In a recent study, different supplements of soybean could reverse alprazolam-induced memory deficit (Bansal & Parle, 2010) and counteract the destructive influence of amyloid β in concomitant use with folic acid (Ma et al., 2009). In post-mortem AD patients, phytoestrogens exhibited brain protection (Jefremov et al., 2008) probably through estrogenic receptor pathways thereby inducing neuroprotection (Kajta et al., 2013). On the other hand, the antioxidant and neuroprotective effect of soybean has been reported against β amyloid plaques (Mandegary et al., 2014; Sharififar et al., 2012). Our results showed that TSPE and RS which had higher genistein content also showed stronger antioxidant and anticholinesterase effects than nuts. On the other hand, the heat used in the preparation of nuts may lead to the decomposition of its active ingredients and reduce the antioxidant and anticholinesterase activity. In conclusion, brain cholinergic neurons have a vital effect on cognition impairment associated with neurodegenerative disorders and aging. Acetylcholinesterase implicates ACh regulation at appropriate levels, hence the disproportionate activity of AChE results in ACh activation defects and cognition shortage (Pepeu & Giovannini, 2010). On the other hand, due to the higher susceptibility of the brain and CNS to oxidizing factors, these tissues are more involved in oxidation progression in the normal aging process of the brain (Cobley, Fiorello, & Bailey, 2018) and the positive effect of TSPE would likely be due to its antioxidant and anticholinesterase effect as well as neuroprotective effects of the plant isoflavones. However, the precise mechanism is beyond the present work and needs more studies. Toxicological studies and clinical trials are needed for more documentation.

Ethical Considerations

Compliance with ethical guidelines

All ethical guidelines have been observed in the research. The experimental protocol of the work with animals was by the National Institutes of Health (NIH) guide and was approved by the Ethical Committee of the University Research Center (EC/KNRC/94-17).

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Authors' contributions

Conceptualization and Supervision: Mandegary and Sheibani; Investigation, Writing–review & editing: Sharififar; Methodology: Nasehi Asadi; Identification and pharmacological experiment: Mirtadzadini and Hassanabadi.

Conflict of interest

The authors declared no conflict of interest.

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