Title: An Standardized Extract from Textured Soy Protein (Glycine Max L.) Has Ameliorating Effect on Memory Deficit and Learning Insufficiency in Scopolamine-Induced Amnesia

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To appear in: Basic and Clinical Neuroscience

Received date: 2020/03/14

Revised date: 2020/12/6

Accepted date: 2020/12/15
Please cite this article as:


DOI: http://dx.doi.org/10.32598/bcn.2021.2446.1
Abstract

Purpose of the study: Textured soy protein (TSP) and Ajil are two processed forms of soybean (Glycine max L.) which are widely consumed by Iranian for nutritional purpose. Recently, we have reported antioxidant and anticholinesterse effect of raw soybean (RS) which has been attributed to isoflavones such as genistein. In this work, we aimed to compare in vitro antioxidant and anticholinesterase effects of TSP, Ajil and RS to select the most effective one for learning capacity and spatial memory studies. Method: Genistein content was determined using high performance thin layer chromatography (HPTLC) while diphenylpicrylhydrazil (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) were used for antioxidant evaluation study and Ellman’s colorimetry method was used for anticholinesterase assay. TSP extract (TSPE) was administered to male rats (100, 200 and 400mg/kg, i.p for 7 days) before scopolamine (1mg/kg) injection. Learning capacity and spatial memory was evaluated by 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 MWM test at 200mg/kg. Conclusion: The memory improving effect of TSPE might be due to its antioxidant and anticholinesterase effect as well as neuroprotective effects of its isoflavones.

Key words: Alzheimer’s disease; Dementia; Textured soy protein; Morris water maze; Antioxidant; Scopolamine
Plain Language Summary

Due to the aging of the world's population and the financial and emotional costs associated with cognitive impairment in these patients, there is an emergency need for drugs that can prevent the onset of symptoms. Natural products are generally welcome, especially if they would be from nutrients and food preparations. In this study, the processed form of soybeans has been studied for improving the memory and learning power in scopolamine-induced amnesia. The results have shown that, processed form of soybean can improves symptoms of cognitive impairment and memory insufficiency compared to conventional drugs and would be a good candidate for clinical studies in patients with Alzheimer’s disease.
1. Introduction

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

Medicinal plants and their bioactive constituents due to their diverse biological activities such as aniconvulsant, cytotoxicity, …(Mahmoudvand et al., 2014; A. Mandegary, Arab-Nozari, Ramiar, & Sharififar, 2012; Sharififar et al., 2009) have been highly paying attention in drug development. Recently we have reported anticholineserase and antioxidant activity of raw soybean (Glycine max L.) (Sharififar, Moshafi, Shafazand, & Koohpayeh, 2012). This plant belongs to Leguminosae family and is one of the nutritional plants introduced from South East Asia. Although there is no history for the plant consumption in Persian medicine, however the plant has been welcomed by Iranians (Mohamadi, Sharififar, Koohpayeh, & Daneshpajouh, 2015; Sharifi far, F., 2011). Regardless of the nutritional value, soybean contains a large quantities of protein and phytochemicals such as phenolic acids, flavonoids, saponins and isoflavone phytostrogens such as genistein and daidzein. Isoflavones can improve memory skills and learning and decrease rates.
of chronic disease (Menze, Esmat, Tadros, Abdel-Naim, & Khalifa, 2015). Soybean protein has a high-quality balance of amino acids due to the presence of all the essential amino acids. The main ingredients of this protein are glycinin and β-conglycinin which comprise about 65–80% of the seed protein (Bader, 1981). Nonetheless, little of the soybean has been used directly and the two major forms of soy consumption in Iran are textured soy protein (TSP) known “soy meat” and “Ajil”. In TSP form, defatted soybean is used as a substitute for meat after quick cooking while in the Ajil form, the seeds are roasted with lemon juice and salt. Considering the antioxidant, anticholinesterase and improving effect of raw soybean (RS) on cognitive impairments (Khodamoradi, Asadi-Shekaari, Esmaeili-Mahani, Sharififar, & Sheibani, 2017; Ali 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, Ajil and RS and study the effectiveness of the most active form of soybean on spatial memory and learning in scopolamine-induced amnesia. In the other hand, genistein is the major isoflavone found in soybean and has beneficial effects on the brain function (Bagheri, Joghataei, Mohseni, & Roghani, 2011), so genistein content of soybean preparations has been determined too.

**Materials and methods**

**1.1. Plant materials**

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

1.2. Chemicals

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

1.2. 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 done on the soybean according to the previous methods (Sharififar, Mirtajadini, Azampour, & Zamani, 2011). The results confirmed the presence of saponins, alkaloids and terpenoids in the plant. Total content of the saponins, alkaloids and terpenoids were determined as explained by Adusei (Adusei, Otchere, Oteng, Mensah, & Tei-Mensah, 2019). For HPTLC, A CAMAG LINOMATS instrument was used for analysis. Different concentrations of genistein (5, 10, 20 and 30 µg/ml) were prepared in methanol. Silica gel 60F254, HPTLC plate (Merck, Germany) and the solvent system of chloroform: methanol (9:1) were used as stationary and mobile phase respectively. After developing in the solvent system, the plate was dried in the room temperature, was deposited in photo-documentation chamber after drying and was observed under 254 nm and 366 nm uv lamp (Alam, Yusufoglu, & Alam, 2013). Densitometry analysis was done using a CAMAG TLC Scanner 3 to quantify the bands using WINCATS software (Version 4 X).
1.3. In vitro antioxidant

Antioxidant effects of the extracts were evaluated using diphenylpicrilhydrazil (DPPH) method and ferre reducing antioxidant potential (FRAP) assay (Ramesh, Sharififar, Mehrabani, Pardakhty, & Farsinejad, 2018) and the percentage of DPPH radical inhibition and FRAP values (as equivalent mmol Fe^{2+}/g) were reported. Butylated hydroxytoluene (BHT) and Vit C were used as positive controls in DPPH and FRAP assays respectively.

1.4. In vitro anticholinesterase activity

Anticholinesterase effect of the extracts was studied on the basis of Ellman’s method with some modifications as explained previously (Sharififar et al., 2012). Regarding the results of antioxidant, anticholinesterase activity and the genistein content, TSPE was used for animal studies.

1.5. Pharmacological study

1.5.1. Experimental animals

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

A total number of 126 rats were randomly assigned into 9 groups for MWM and 9 groups for PAT tests each of 7 animals. All injections were done intraperitoneally (i.p) between 8 and 10 am, and the experiments were performed at the time of light between 8 am and 4 pm to prevent the effects of night-time rhythm on the experiments. Animals were acclimated to the laboratory condition 1
hour before the test. Experimental groups were as, 1: Control: No treatment, 2: normal saline (NS), 3: Solvent (10% DMSO in NS), 4: 0.3mg/kg physostigmine (Phys), 5: 200mg/kg piracetam (Pir), 6-8: 100, 200 and 400mg/kg TSPE, 9: scopolamine (Sco) 1mg/kg scopolamine. 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 minutes after the last dose of 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 of 2 and 3 were considered as negative controls while the groups of 4 and 5 were considered as positive controls.

1.5.2. Passive avoidance test (PAT)

In passive avoidance test (PAT) which was used for evaluation of long spatial memory, a plexiglass box was used which is consisted of two equal dark and light partition (20.20.20cm) separated by a remote door. The dark part is covered by a black roof, on the bottom of which there are steel bars at intervals of one centimeter and are connected to the shock device by a communication cable. This device is able to generate an electric current of half a milliampere for two seconds with a frequency of 50 Hz in these rods, which causes an electric shock to the animal's hands and feet. In learning stage, the animals were placed in the light partition and allowed to enter to the dark side after 10 seconds. Animals with delay more than 120 seconds were removed from the experiment. After 2 hours, in training phase, the animals were placed in the light partition again and the door was opened after 10 seconds for animal entrance to the dark side and the door was closed immediately. A shock was introduced into the animal’s foot through an electrical current (0.5 mA, 50 Hz, 2 sec.) and the animal was gave back to the cage immediately. The experiment was repeated after 5 minutes. When the animals stayed at least for 5 consecutive minutes in the light side, the experiment was stopped. After 24h, in retrieval phase, the animal was directed to the light side,
the door was opened after 10 seconds and initial avoidance and crossing latency to the dark part were recorded (Harandi et al., 2015).

1.5.3. *Morris water maze (MWM)*

This test is used for evaluation of short spatial memory. A black circular pond (60 * 160 cm) was filled with water (24± 2°C) and divided to four separated quadrants. Animals released to swim into water and the performance of animals was recorded using a computerized-camera system. In target quadrant (QT), a square platform (10cm diameter) was placed under the water and in learning stage, the animals were allowed to swim again through three blocks each of four consecutive 60 seconds trials with 30 minutes interval (each trial was started from a different quadrant). After finding the platform, animals could rest on it for 30 seconds and then were transferred to the cage for another 30 seconds until the next trial. The animals were guided to the platform if did not find it after 60 seconds. The time spent in the QT and the distance moved to find the platform were recorded. A single probe trial was done after two hours to find the plant effect on memory retrieval. The animals were freed from the quadrant opposed to QT into the water to swim in the absence of the platform for 60 seconds. The crossing number, time remaining in the QT and the distance traveled to discover the platform were documented. After 2 minutes, the platform was transferred above the water level and allowed the animal find that (D’Hooge & De Deyn, 2001).

1.6. *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 ANOVA was used. Tukey’s post hoc and LSD test were used to find the difference between them. Repeated measurement ANOVA
test was used to compare the results of the first, second and third blocks in Morris water maze test. Differences with p values <0.05 was considered as significant.

2. Results

2.1. Plant phytochemistry, standardization and HPTLC analysis

Phytochemical experiments confirmed the presence of flavonoid, alkaloids, saponins and terpenoids in all three soybean forms. The results of quantification of these secondary metabolites and the yield of extraction have been shown in Table 1. As shown in the Table, the extraction yield was 15.76%, 12.60% and 11.28% (w/w) for TSP, RS and Ajil respectively. The greatest amount of alkaloids was reported in RS (20.70± 2.67) g / 100g dried extract. There was no significant difference among soybean samples in respect to saponin and terpenoid content (p>0.05).

HPLTLC profile exhibited similar band of genistein with R_f value of 0.32 in the chromatogram of all three extracts. The related florescent bands were visible obviously at 254 nm. Genistein content of TSPE, RS and Ajil was determined as 10.37± 0.60, 8.26± 0.34 and 6.14± 0.35 (g/100 g dried extract) on the basis of its calibration curve (Table 2).

2.2. 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) in comparison to BHT as positive control (98.79%± 2.46) (The IC_{50} value of TSPE was determined 65.27± 3.10 µg/ ml (Table 2).
In FRAP test, the greatest FRAP values were reported for RS and TSPE (131.06± 1.64 and 127.23± 2.48 µmol/ml respectively) in comparison to Vit C as positive control (168.12 ± 3.21µmol/ml). The FRAP values of TSPE and RS were significantly different from Ajil (p<0.001) (Table 2).

2.3. Anticholinesterase activity

As shown in Table 2, all samples somehow inhibited AChE in a concentration-dependent manner. TSPE markedly inhibited AChE enzyme (72.64%±4.01), followed by RS (69.94%±2.31) and Ajil (43.28± 1.65) respectively. The IC$_{50}$ value was calculated and was reported (Table 2). The least IC$_{50}$ was due to TSPE (146.32± 8.23 µg/ml) in comparison to physostigmine (12.03±1.48µg/ml).

2.4. PAT test

The results of PAT test indicated that Sco significantly decreased initial latency (12.13± 2.29 seconds) and increased crossing latency (7.14 ±0.36) in comparison to control (122.17 ±2.68 seconds 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 seconds) (p<0.001). Avoidance to entrance was also significant at different doses of TSPE such that animals completely avoided to enter at TSPE 200mg/kg (crossing latency = 0.05±0.00) (Fig 1a-b).

2.5. MWM result

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

In probe test, Sco markedly caused a decrease in the time and distance spent as well as site crossing in TQ (30.39± 0.86 seconds, 25.62±1.31 cm and 2.67±0.21 respectively) while animals who received TSPE, Pir and Phys spent more time and distance in TQ (39.54± 1.96, 44.74± 3.80 and 49.61± 3.07 seconds time spent for TSPE 100, 200 and 400mg/kg respectively) and (50.96± 5.06, 42.65±2.71, 45.85±2.85 cm 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 that of Sco group too (p<0.001 and p<0.01) (Fig 3a,b,c). Comparison of swimming velocity of animals in TQ revealed no significant difference between experimental groups which indicates sensory motor factors didn’t play a role in spatial memory evaluation (data was not shown).

3. Discussion

In this work, genistein content has been compared in more common consumed forms of soybean in Iran by HPTLC methods. The highest content of genistein was found in TSPE and with a slight difference in RS which were consistent with the results of antioxidant and anticholinergic activity of the samples. In the other hand, phytochemical studies of three samples of soybean indicated that total alkaloid content of TSPE is significantly less than the other two samples (p<0.001) while the total saponin and total terpenoid content in all the three samples were not significantly different
(p>0.05). Difference in alkaloid content might be due to lipophilicity nature of them which increases their persistence in the oily part of the plant through soybean processing of TSPE.

In PAT test, TSPE increased passive avoidance latency in contrast to Sco which significantly reduced the latencies (p<0.001). In training phase of MWM, the time spent and distance moved for platform finding have 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 (a sign of a flaw in the memory), TSPE improved all of the learning indicators. TSPE activity was prominent at 200mg/kg and by increasing the dose, the activity was decreased. This effect can be attributed to hormetric 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 have been unknown and needs further studies, at least a part of this effect could be attributed to 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 on the role of cholinergic signaling alteration in cognitive impairments. Acetylcholine causes cortical response increasing to the 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 of 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 like lignins. Isoflavones of genistin and daidzin can improve cognitive functions through estrogenic effect (receptor β) (Bansal & Parle, 2010). Estrogen stimulates ACh release and markedly facilitates forebrain cholinergic
system function. Soybean isoflavones increase cholinergic transmission via AChE inhibition (Bennetau-Pelissero, Jamali, & Marighetto, 2011) and improve nonverbal memory and the other abilities in cognition disorders (Gleason et al., 2009). Neuroprotective effect of these isoflavones has been reported in animal model of Alzheimer’s disease (Bagheri et al., 2011). 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 which thereby induce neuroprotection (Kajta et al., 2013). On the other hand, antioxidant and neuroprotective effect of soybean has been reported against β amyloid plaques (Ali Mandegary et al., 2014; Sharififar et al., 2012). Our results showed that TSPE and RS which had higher content of genistein, also displayed stronger antioxidant and anticholinesterase effects than Ajil. In front of it, the heat used in the Ajil preparation might lead to the decomposition of its active ingredients and reduce the antioxidant and anticholinesterase activity. In conclusion, brain cholinergic neurons have vital effect on cognition impairment associated with neurodegenerative disorders and aging. Acetylcholinesterase implicates ACh regulation to appropriate levels, so disproportionate activity of AChE results defect of ACh activation 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 likely to be involved in oxidation progression in the brain normal aging process (Cobley, Fiorello, & Bailey, 2018) and it is likely that the positive effect of TSPE would be due to its antioxidant and anticholinesterase effect of the as well as neuroprotective effects of the plant isoflavones. However the precise mechanism is beyond of the present work and needs more studies. Toxicological studies and clinical trials are needed for more documentation.
Acknowledgement

The authors are thankful for financial support by Neuroscinece Research Center of Kerman University of Medical sciences.

Conflict of interest

The authors report no conflict of interest.
4. References:


Chemistry, 130(1). https://doi.org/10.1016/j.foodchem.2011.06.034
Practical Applications

Soybean is a common food in Southeast Asia and is believed to be useful for health promotion. However, two forms of the soybean, Ajil and textured soy protein (TSP) are common in Iranian people in ageing period and for memory enhancement.

In this work, we have compared the antioxidant and anticholinesterase activity of these two forms with raw soybean and then selected TSPE, the most active form of soybean, for pharmacological studies. The results of Morris water maze and Shuttle box tests indicated that TSPE potentially can reverse the dementia symptoms in scopolamine treated animal and is a good candidate for aging supplementation. The results of this work can help to have a supplement for health promotion especially in the elderly who face memory and learning problems. On the other hand, TSPE can be thought of as a source of novel drugs too.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>AChEIs</td>
<td>Acetylcholinesterase inhibitors</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxyl toluene</td>
</tr>
<tr>
<td>MWM</td>
<td>Morris water maze</td>
</tr>
<tr>
<td>NS</td>
<td>Normal saline</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>PAT</td>
<td>Passive avoidance test</td>
</tr>
<tr>
<td>Phys</td>
<td>Physostigmine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Pir</td>
<td>Piracetam</td>
</tr>
<tr>
<td>RS</td>
<td>Raw soybean</td>
</tr>
<tr>
<td>Sco</td>
<td>Scopolamine</td>
</tr>
<tr>
<td>TSP</td>
<td>Textured soy protein</td>
</tr>
<tr>
<td>TSPE</td>
<td>Textured soy protein extract</td>
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</table>
Table 1- The yield of extraction and total alkaloid content, total saponin content and total terpenoid content of three different samples of soybean

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Extraction yield (% w/w dried plant materials)</th>
<th>TAC (% w/w dried extract)</th>
<th>TSC (% w/w dried extract)</th>
<th>TTC (% w/w dried extract)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TSPE</td>
<td>15.76± 1.02</td>
<td>5.52± 0.59**</td>
<td>2.01± 0.25</td>
<td>12.36± 0.69</td>
<td>&lt;0.001</td>
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<tr>
<td>2</td>
<td>Ajil</td>
<td>12.60±0.92</td>
<td>15.1± 3.01*</td>
<td>2.11±0.04</td>
<td>12.09± 1.40</td>
<td>&lt;0.027</td>
</tr>
<tr>
<td>3</td>
<td>RS</td>
<td>11.28± 0.84</td>
<td>20.70± 2.67*</td>
<td>1.98± 0.31</td>
<td>11.67±1.09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

TSPE: textured soy protein extract; RS: raw soybean; TAC: total alkaloid content; TSC: total saponin content; TTC: total terpenoid content. *significantly different with p<0.05, **significantly different with p<0.001
Table 2- Comparison of extraction yield, genistein content, antioxidant and anticholinesterase effect of three different forms of soybean

<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Genistein content (% w/w)</th>
<th>DPPH inhibition (%)</th>
<th>P value</th>
<th>FRAP value (µmol/ml)</th>
<th>P value</th>
<th>AChE inhibition (%)</th>
<th>P value</th>
<th>IC50 value (µg/ml)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>TSPE</td>
<td>10.37±0.60</td>
<td>87.32±3.54</td>
<td>&lt;0.00</td>
<td>127.23±2.48**</td>
<td>&lt;0.00</td>
<td>72.64±4.01*</td>
<td>&lt;0.00</td>
<td>146.32±8.23</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>2</td>
<td>Ajil</td>
<td>8.26±0.34</td>
<td>49.67±2.36*</td>
<td>&lt;0.00</td>
<td>48.93±5.72**</td>
<td>&lt;0.00</td>
<td>43.28±1.65*</td>
<td>&lt;0.00</td>
<td>549.37±17.52*</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>3</td>
<td>RS</td>
<td>6.14±0.35</td>
<td>81.50±1.76**</td>
<td>&lt;0.00</td>
<td>131.6±1.64**</td>
<td>&lt;0.00</td>
<td>60.94±2.31**</td>
<td>&lt;0.00</td>
<td>203.57±11.08*</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>4</td>
<td>BHT</td>
<td>-</td>
<td>98.79±2.46**</td>
<td>.0001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;0.00</td>
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<tr>
<td>5</td>
<td>Vit C</td>
<td>-</td>
<td>-</td>
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<td>168.12±3.21**</td>
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<td>6</td>
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<td>99.38±2.67*</td>
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<td>12.03±1.48**</td>
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TSPE: Textured soy protein extract; RS: raw soybean; FRAP: Ferric reducing antioxidant potential, Phys: phystostigmine, BHT: butylated hydroxytoluene, **significantly different with p<0.001

Caption of figures

Figure 1- Effect of different concentrations of TSPE on initial latency (a) and crossing latency (b) in scopolamine-induced amnesic animals in comparison to piracetam and phystostigmine using passive avoidance test.
Groups each of 7 animals received piracetam (Pir, 200mg/kg), physostigmine (Phys, 0.3mg/kg) and TSPE (100, 200 and 400mg/kg) for 7 consecutive days. Animals treated with 1mg/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

Figure 2- Effect of different concentrations of TSPE on escape latency (a) and distance moved (b) in scopolamine-induced amnesic animals in comparison to piracetam and physostigmine in training phase of morris water maze test.

Groups each of 7 animals received piracetam (Pir, 200mg/kg), physostigmine (Phys, 0.3mg/kg) and TSPE (100, 200 and 400mg/kg) for 7 consecutive days. Animals treated with 1mg/kg scopolamine (i.p). 30 min after the last dose, distance moved and latency escape in target quadrant were evaluated through in 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

Figure 3- Effect of different concentrations of TSPE on time spent (a) and distance moved (b) and crossing number (c) in target quadrant in scopolamine-induced amneic animals in comparison to piracetam and physostigmine in requisition phase of morris water maze test
Groups each of 7 animals received piracetam (Pir, 200mg/kg), physostigmine (Phys, 0.3mg/kg) and TSPE (100, 200 and 400mg/kg) for 7 consecutive days. Animals treated with 1mg/kg scopolamine (i.p). 30 min after the last dose, distance moved and time spent and crossing number in target quadrant were evaluated through in 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, ** with p<0.01
Fig 3a,b,c