Original Article

Mung Bean-Derived Protein Protects Against Neurodegeneration and Memory Impairment in Animal Model of Menopause with Obesity

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Background: Menopause related neurodegeneration and memory impairment in obesity condition is increasing. Therefore, the neuroprotectant and cognitive enhancer against the mentioned disorder is required.

Objective: In the present study, we aimed to assess the effect of mung bean protein supplement on the neuroprotective and cognitive enhancing effects which was investigated in bilateral ovariectomized (OVX) rats with obesity.

Material and Methods: Female Wistar rats, weighing 180 to 200 g, were induced experimental menopause by bilateral ovariectomy and then they were induced obesity with high-fat diet. Ovariectomized (OVX) rats with obesity were fed with a high fat diet (HFD) containing 15% mung bean for 8 weeks. The assessments of spatial memory and biochemical profiles, oxidative stress status and apoptosis in the brain together with the serum lipid profiles were determined at the end of treatment.

Results: The results showed that mung bean protein treatment improved spatial memory in Morris water maze test. The acetylcholinesterase (AChE) activity and malondialdehyde (MDA) level in the hippocampus of OVX rats with obesity which received mung bean protein were decreased. The density of Bax+ cells decreased, but the density of Bcl+ cells in the hippocampus increased.

Conclusion: Therefore, mung bean protein is the potential neuroprotectant and cognitive enhancer. The possible underlying mechanism might occur partly via the improvement of cholinergic function, oxidative stress status, and apoptosis. However, the clinical trial study was still necessary to confirm this effect.

Keywords: Mung bean, Memory impairment, Neurodegeneration, Obesity, Menopause

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Menopause, a period of permanent cessation of menstruation resulting from the age-related ovarian follicle depletion, has been reported to produced numerous deleterious changes in various tissues including brain[(1)] giving rise to neurodegeneration and memory impairment[(2)]. It has been reported that obesity at this period also increased around three times[(3)]. Moreover, the ability to store protein in the woman at this period decreased leading to a malnutrition condition and loss of skeletal mass; protein supplement provides a health benefit for postmenopausal women[(4)]. Both protein malnutrition and obesity also enhance the progress of memory impairment[(5,6)] which in turn aggravates the poor quality of life. Therefore, the therapeutic strategy against neurodegeneration and memory impairment in menopause with obesity is effective, cheap and easy to approach is still required.

Recent studies demonstrate that some protein extracts such as silkworm-derived protein[(7)], soy-derived protein[(8)] and nut-derived proteins[(9)] also enhance brain function. However, plant based protein can provide benefit with less risk of increasing cholesterol and atherosclerosis[(10)] which in turn can produce negative effects on brain circulation. This information raises the possibility that plant based protein may improve brain function in menopause with obesity. Among various plants which are widely consumed in Asian countries, *Vigna radiata* L or mung bean is regarded as one important resource for plant protein. It contains protein content around 20.97 to 31.32%[(11)] and possesses a chemical score 76%[(12)]. Assessment of the biological activities has revealed that mung bean protein exhibits angiotensin converting enzyme inhibitory (ACEI) activity[(13)] and

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antioxidant activity[14]. In addition, it improves insulin and lipid metabolism and may be a benefit for obesity, metabolic syndrome and their associated disorders[15]. Moreover, mung bean is also rich in lysine and glutamic acid[12] which can exert the effect on the central nervous system by improving stress response and anxiety[16]. Based on aforementioned information, the authors hypothesized that mung bean-derived protein should protect against neurodegeneration and memory impairment in menopause with obesity. To test this hypothesis, the authors aimed to determine the effect of mung bean-derived protein on memory impairment and hippocampal neurodegeneration in animal model of menopause with obesity. The possible underlying mechanisms such as the alterations of cholinergic function, oxidative stress status, and apoptosis were also explored.

Materials and Methods

Preparation and analysis of mung bean protein

Vigna radiata (L.) or mung beans (Raitip: Thai cereals world (1957) Co., Ltd, Bangkok, Thailand) were purchased from a local store in Khon Kaen province. The seeds were soaked in distilled water for 3 hours at room temperature (25°C). The soaked bean was then washed with distilled water and then dried in a hot air dryer at 40°C for 12 hours. The dried mung beans were ground into flour using a blender. Mung bean flour was defatted twice with hexane at mung bean flour to hexane ratio of 1: 1 (w/v) under room temperature (approximately 25°C) for 24 hours. Defatted mung bean flour was filtered with filter paper (Whatman No. 1) followed by air drying overnight. Defatted mung bean flour was dispersed in water at defatted mung bean flour to water ratio of 1: 15 (w/v) and adjusted to pH 9.0 with 2M NaOH at 25°C. The sample was subjected to a 15 minutes-centrifugation at 1,000 g at 25°C at 2 hours following the processes just mentioned. The pellet was discarded whereas the supernatant was adjusted to pH 4.0 with 2M HCl at 4°C and stored overnight. Then, the sample was centrifuged at 5,000 g for 5 min. The recovered precipitated protein isolate was freeze-dried and preserved at -20°C for further use[17]. The amino acid profile of mung bean was analyzed by using gas chromatography-mass spectrometry by Central lab Thai, Khon Kaen province, Thailand. The main amino acids are glutamic acid, leucine and histidine. Other amino acids were also observed and were presented in Table 1.

Animals

Adult female Wistar rats, 8 weeks old (180 to 220 g), were obtained from National Animal Center, Salaya, Nakornpatom. The animals were housed in group of 5 per cage in standard metal cages at 22±2°C on 12: 12 h light: dark cycle. All animals were given freely access to food and water. The experiments were performed to minimize animal suffering and the experimental protocols were approved by the Institutional Animal Care and Use Committee Khon Kaen University, Thailand (AEMDKKU 001/2559).

Experimental groups and protocol

After 1 week of acclimatization, the animals were divided into 7 groups (n = 6) as follows:

Group 1 Control: all rats in this group received normal diet (CP mouse feed, Thailand) (13% fat, 55% carbohydrate, and 32% protein) and received no treatment.

Group 2 Sham + ND: all rats in this group were subjected to sham operation and received normal diet.

Group 3 Sham + HFD: all rats in this group were subjected to sham operation, and received high fat diet.

Group 4 OVX + ND: all rats in this group were subjected to bilateral ovariectomy, and fed with normal diet.

Group 5 OVX + HFD: all rats in this group were subjected to bilateral ovariectomy, and fed with high fat diet.

Group 6 OVX + HFD + isoflavone: all rats in this group were subjected to bilateral ovariectomy, fed with high fat diet and treated with isoflavone at dose of 15 mg/kg BW.

Group 7 OVX + HFD + mung bean protein: all rats in this group were subjected to bilateral ovariectomy, fed with high fat diet and 15% mung bean protein (this dose was selected based on our previous unpublished data which showed the highest potential in the biological activities associating with anti-obesity and memory enhancement).

All rats in group 4 to 7 were anesthetized with thiopental sodium at a dose of 40 mg/kg BW prior to the induction of experimental menopause by bilateral ovariectomy. Two weeks after surgery, rats in group 3 and 5 to 7 were induced obesity by subjecting to a 16-week high fat diet (Major ingredients consisted of standard chow, condensed milk and egg yolk at the concentrations of 27.58%, 43.93%, and 28.49%, respectively). Obese rats which showed the increase in body weight more than 25% of control group were recruited for further study. All recruited rats were randomly assigned for the interventions as following; sham + HFD, OVX + HFD, OVX + HFD + isoflavone and OVX + HFD + mung bean protein. The animals were assessed spatial memory at 1-week, 4-week and at the end of 8-week intervention period.

In addition, neuron density, the oxidative stress status, acetylcholinesterase (AChE), lipid profile and the density of Bax+ cells and Bcl-2+ cells in the hippocampus were also investigated at the end of the study.

Assessment of spatial memory

Spatial memory was assessed by using Morris Water Maze Test. In brief, a circular pool with a diameter of 147 cm and 60 cm in depth was filled with water (25±1°C) and divided into 4 equal quadrants (Northeast, Southeast, Southwest, and Northwest). The water surface was covered by a non-toxic milk powder. Rats were trained for 5 consecutive days to memorize the location of an immersed platformin one of the quadrants by using the relationship of its location and the platform location with the aid of the environmental cues. On the test day, each rat was placed into the quadrant which was opposite to the quadrant of the platform. The time which required for finding the platform

Table 1. Amino acid profiles of mung bean-derived protein

Amino acid	Amino acid content (mg/100 g of protein)
Alanine	1,115
Aspartic acid	4,120
Cysteine	174
Glutamic acid	12,237
Glycine	809
Histidine	5,193
Hydroxylysine	<5.00
Hydroxyproline	<5.00
Isoleucine	2,975
Leucine	6,512
Lysine	2,965
Methionine	342
Phenylalanine	2,972
Proline	1,427
Serine	843
Threonine	552
Tyrosine	4,414
Valine	2,486

and climbed onto the platform was recorded as escape latency. Then, all animals were exposed to water maze without an immersed platform at 24 hours later and the time which the animals spent swimming in the quadrant previously contained the platform was recorded as retention time[18,19].

Biochemical analysis

After the scarification, the hippocampus was isolated and prepared as hippocampal homogenate with 50 volume of 0.1 M phosphate buffer saline. Then, the assessments of acetylcholinesterase (AChE) activity, and oxidative stress status including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were performed.

The determination of AChE activity was performed by colorimetric method according to the method of Ellmann et al[20]. The mixture containing 20 µl of sample solution, 200 µl of 0.1 mM sodium phosphate buffer (pH 8.0) and 10 µl of 0.2 M DTNB (5, 5'-dithio-bis-(2-nitrobenzoic acid)) (Sigma-Aldrich, USA) was mixed with 10 µl of 15 mM acetylcholine thiochloride (ACTI) (Sigma-Aldrich, USA) and incubated at room temperature for 5 minutes. At the end of incubation period, the absorbance at 412 nm was measured by using microplate reader. The activity of AChE was calculated according to the equation below and expressed as nmol/min.mg protein.

AChE activity = (ΔA/1.36x104) x 1/(20/230) C

(ΔA = the difference of absorbance/minute, C = protein concentration of brain homogenate)

The assessment of MDA level was performed by using thiobarbituric acid reaction. In brief, 100 µl of sample solution, 100 µl of 8.1% sodium dodecyl sulphate (SDS) (Sigma-Aldrich, USA), and 375 µl of 20% acetic acid (Sigma-Aldrich, USA), 150 µl of distilled water (DW) were mixed together. Then, the mixture was mixed with 375 µl of 0.8% of thiobarbituric acid (TBA) (Sigma-Aldrich, USA), shaken and boiled at 95°C in the water bath for 60 minutes. Following this process, the mixture was cooling with tap water. An aliquot of 500 µl of water and 2.5 ml of the mixture of n-butanol and pyridine at the ratio of 15: 1 were added, mixed together and subjected to a 10 minute-centrifugation at a speed of 4,000 round per minute (rpm). The supernatant was harvested and determined an absorbance at 532 nm. MDA level was expressed as nmol/mg protein[21].

SOD activity was determined by using the nitro tetrazolium blue reduction method. According to this method, xanthine/xanthine oxidase system was served as a source of superoxide, the substrate of superoxide dismutase, and nitroblue tetrazolium was used as an indicator of superoxide. The mixture containing 57 mM phosphate buffer solution (KH_2PO_4) (Sigma-Aldrich, USA), 0.1 mM EDTA (Sigma-Aldrich, USA), 10 mM cytochrome C (Sigma-Aldrich, USA) solution, 50 µM of xanthine (Sigma-Aldrich, USA) solution at the volume of 200 µl and 20 µl of xanthine oxidase (0.90 mU/ml) (Sigma-Aldrich, USA) solution was mixed with 20 µl of tissue sample and centrifuged at 4,000 rpm for 10 minutes. After the centrifugation, the supernatant was harvested and determined an absorbance at 532 nm. SOD enzyme (Sigma-Aldrich, USA) activities at the concentrations of 0 to 25 units/ml were used as standard and the results were expressed as units/mg protein[19,22].

CAT activity was determined by mixingan aliquot of10 µl of brain homogenate with the reaction solution which contained 50 µl of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0) (BDH Chemicals Ltd, UK), 25 µl of H_2SO_4 (Sigma-Aldrich, USA), and 150 µl of 5 mM $KMnO_4$ (Sigma-Aldrich, USA). After 1 minute, an absorbance at 490 nm was measured and data were expressed as units/mg protein[19,23].

GPx activity in the hippocampus was also determined. In brief, the reaction mixture containing 10 µl of 1 mM dithiothreitol (DTT) (Sigma-Aldrich, USA) in 6.67 mM potassium phosphate buffer (pH 7), 100 µl of 1 mM sodium azide (Sigma-Aldrich, USA) in 6.67 mM potassium phosphate buffer (pH 7), 10 µl of 50 mM glutathione (Sigma-Aldrich, USA) solution and 100 µl of 30% hydrogen peroxide (BDH Chemicals Ltd, UK) was mixed with 20 µl of sample. Then, it was shaken for 5 minutes and the optical density at 412 nm was recorded against the blank using a spectro-photometer[24]. Data were expressed as units/mg protein.

Histology and immunohistological study

After the transcardial perfusion with fixative solution containing 4% paraformaldehyde (Sigma-Aldrich, USA) in 0.1 M phosphate buffer pH 7.4, brain was isolated and placed in 4% paraformaldehyde overnight. Then, it was fixed in 30% sucrose at 4°C for 72 hours. Following this process, brain was cut frozen on cryostat (Thermo Scientific™

HM 525 Cryostat) at 20 µm thick and the sections were mounted onto gelatin-coated slides. The sections were then stained with 0.2% cresyl violet (Sigma-Aldrich, USA) for 2 minutes and rinsed in distilled water, dehydrated in different grades of ethanol and subsequently cleared in xylene. Finally, slides were cover slipped using DPX Mounting medium (Merck, Germany). The sections were examined and photographed using a microscope (Axio Imager.A1, Carl Zeiss, Oberkochen, Germany) equipped with a digital camera system (Axio Cam MRc 5, Carl Zeiss) and the analysis of the hippocampus neuronal density was performed at a magnification of 400x. Cells were counted in 3 randomly selected fields and expressed as the mean number of Nissl positive cells/255 μm^2.

For immunohistochemical study, brain was cut using cryostat at 20 µm thick and all sections were collected into six well plates containing 0.1 M phosphate buffered saline (pH 7.4). Then, they were immersed in 0.01 M sodium citrate buffer (pH 6.0) and heated with microwave for 10 minutes. Following this step, the sections were left cool at the room temperature and washed with PBS 3 times, 5 minutes each. After the washing step, the sections were incubated in 0.3% hydrogen peroxide for 20 minutes, and washed with PBS as mentioned earlier. Next to this step, the sections were incubated in a mixture containing 0.3% Triton X-100 (Fluka Chemika, Buchs, Switzerland), 1% (w/v) bovine serum album (BSA) and 10% normal goat serum at room temperature for 20 minutes. At the end of incubation period, the sections were washing with PBS 3 times. After washing, they were incubated with primary antibody to Bcl-2 (1: 500, Abcam, Cambridge, MA, USA) or Bax (1: 500, Abcam, Cambridge, MA, USA) at 4°C overnight. After the incubation, they were washed with PBS again and incubated with the Dako REAL™ En Vision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse commercial kit (Dako, Glostrup, Denmark) at room temperature for 30 minutes. Prior to the exposure to a 5-minute incubation with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich, USA), all sections were also washed with PBS. Positive staining was recognized as a brown color. The control sections were stained only with secondary antibody and no immunoreactive neuron was detected. The sections were mounted on gelatin-coated slides and counterstained with cresyl violet and dehydrated with alcohols, cleared with xylene, mounted with DPX mountant and observed under a light microscope. Immunoreactive neurons were characterized by brown granules in the cytoplasm. Counts were performed in three adjacent fields and expressed as the mean number of positive cells/255 µm[25-27].

Statistical analysis

Data were presented as mean ± standard error of mean (SEM). The statistical analysis of the experiment was carried out using IBM SPSS Statistic (version 21). The analysis was performed using one-way analysis of variance (ANOVA), following by *post hoc* test. A probability levels less than 0.05 were accepted as significance.

Results

Effect on memory

Figure 1 showed the effect of various treatments on escape latency. At 1 week of treatment, no significant changes of escape latency were observed in any groups. It was found that both sham operation and HFD failed to produce the significant change of escape latency in rats which still have ovary intact both at 4 and 8 weeks of treatment which indicated that the surgery operation and HFD produced no effect on escape latency in ovary intact rat. When compared the effect of OVX and sham operation treatment, no significant change of this parameter was observed. However, OVX rats which received HFD significantly increased escape latency (p-value <0.001; compared to OVX + ND). This indicated that HFD failed to produce positive modulation effect on escape latency in ovary intact rats but it did increase escape latency both at 4 and 8 weeks of treatment. Interestingly, this elevation was mitigated by isoflavone and mung bean protein treatments both at 4 and 8 weeks of treatment (p-value <0.001 all; compared to OVX + HFD group).

The effect of various treatments was also assessed in this study and data were shown in Figure 1. At 1 week of treatment, all treatment failed to produce the significant changes of retention time. Again, both sham operation and HFD failed to produce the significant changes in retention time in ovary intact rats throughout the study period. It was found that OVX rats which received HFD also failed to produce a significant reduction in retention time. At 8 weeks of treatment, OVX rats which received either isoflavone or mung bean protein significantly increased retention time (p-value <0.01 all; compared to OVX + HFD group) at 8 weeks

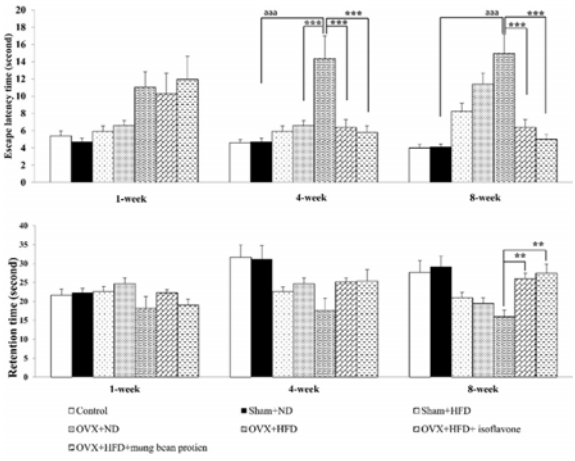

Figure 1. The effect of mung bean protein on the escape latency and on the retention time. Data are presented as mean ± SEM (n = 6/group). ^{aaa}p-value <0.001 compared to Sham + ND and **,***p-value <0.01, 0.001, respectively, compared to OVX + HFD.

of treatment.

Effect on biochemical parameters

Table 1 showed that sham operation failed to produce the significant change in AChE activity in hippocampus of normal rats. HFD also failed to show the change of aforementioned parameter in sham operation rats. In addition, OVX which received ND also failed to produce the significant change of AChE activity in the hippocampus (compared to control, sham + ND and sham + HFD). OVX rats which received HFD significantly increased AChE in the hippocampus (p-value <0.05 all; compared to control and compared to sham + ND group). Isoflavone failed to produce the significant mitigation effect on this parameter whereas mung bean protein significantly decreased AChE in the hippocampus of OVX rats which received HFD (p-value <0.01; compared to OVX + HFD group).

The alterations of oxidative stress markers in hippocampus of various groups were also assessed and data were shown in Table 2. When compared to control group, sham operation failed to produce the significant changes in MDA, SOD, CAT and GPx in the hippocampus. HFD also failed to produce the significant changes of aforementioned parameters in sham operation group. In addition, no significant changes of all parameters mentioned earlier were observed in OVX rats which received ND. HFD significantly increased MDA level (p-value <0.01 all; compared to control and compared to sham operation + ND) but decreased GPx activity in the hippocampus of OVX rats (p-value <0.001 all; compared to control; compared to sham operation + ND and compared to sham + HFD group). It was found that isoflavone failed to produce the significant modification effect on all oxidative stress markers while mung bean significantly mitigated the elevation of MDA level in the hippocampus of OVX + HFD (p-value <0.01; compared to OVX + HFD group). However, no changes in antioxidant enzymes were observed.

Effect on histology and immunohistological study

Effect of various treatments on the density of survival of neurons in CA1, CA2, CA3 and dentate gyrus (DG) were shown in Figure 2. Sham operation failed to produce the significant changes on the density of survival of neurons in all areas of the hippocampus mentioned earlier. OVX significantly decreased the density of survival neurons in all areas mentioned earlier (p-value <0.001 all; compared between OVX + ND and sham operation + ND group). Sham operation rats which received HFD showed the significant reduction in the density of survival neurons in CA1, CA2, CA3 and DG (p-value <0.001 all; compared between sham operation + ND and sham operation + HFD group). However, the positive modulation effect of HFD on this parameter in OVX rats was observed only in CA1 and CA3 (p-value <0.001 all; compared between OVX + ND and OVX + HFD group). Both isoflavone and mung bean protein significantly increased the density of survival neurons in all areas of the hippocampus investigated in this study (p-value

Table 2. The effect of mung bean protein on AChE activity, oxidative stress marker and antioxidant enzymes in hippocampus

Treatment groups	AChE (nmol/mg protein)	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	0.927±0.044	0.069±0.003	32.783±1.816	37.223±3.000	4.828±0.343
Sham+ND	0.906±0.091	0.064±0.006	33.841±3.177	37.026±4.583	4.813±0.317
Sham+HFD	1.019±0.145	0.107±0.010	22.295±1.982	37.379±2.979	7.551±0.340
OVX+ND	0.906±0.028	0.080±0.008	36.774±3.382	29.743±4.076	2.033±0.361
OVX+HFD	1.358±0.070^{a,b}	0.132±0.015^{aa,bb}	23.109±1.116	34.298±4.767	1.527±0.164^{aaa,bbb,ccc}
OVX+HFD+ isoflavone	0.901±0.252	0.081±0.024	32.388±5.138	35.991±9.093	4.426±0.995*
OVX+HFD+ mung bean protein	0.833±0.058*	0.063±0.007**	26.704±1.940	35.866±1.732	3.389± 0.247

Data are presented as mean ± SEM (n = 6/group). $^{a,aa,aaa}p$<0.05, 0.01, 0.001 respectively; compared to control rats that received normal diet (Control), $^{b,bb,bbb}p$-value <0.05, 0.01, 0.001 respectively; compared to Sham + ND, ^{ccc}p-value <0.001 compared to Sham+HFD, *,**p-value <0.05, 0.01 respectively; compared to OVX

<0.001 all; compared to OVX + HFD group).

Figure 3 showed the density of Bax+ cells in CA1, CA2, CA3 and DG of the hippocampus in various treatment groups. It was found that both OVX significantly increased the density of Bax+ cells in all areas mentioned earlier (*p*-value <0.001 all; compared between OVX + ND and sham operation + ND group). High fat diet produced the significant increase in density of Bax+ cells in all areas of the hippocampus investigated in this study (*p*-value <0.001 all; compared to sham operation + ND group) in ovary intact rats. However, OVX rats which received HFD showed the significant increase in density of Bax+ cells only in CA1 and CA3 (*p*-value <0.001 all; compared to OVX + ND group). Interestingly OVX rats fed with HFD and received either isoflavone or mung bean protein significantly decreased the density of Bax+ cells in all areas of the hippocampus investigated in this study (*p*-value <0.001 all; compared to OVX + HFD group).

The effect of various treatments on the density of Bcl-2+ cell in the hippocampus was also investigated and data were shown in Figure 4. Sham operation didn't produce the significant change in density of Bcl-2+ cell in all areas. OVX significantly decreased the density of Bcl-2+ cell in all areas of the hippocampus (*p*-value <0.001 all; compared to sham operation + ND group). In addition, HFD also significantly decreased the density of Bcl-2+ cell in all areas of the hippocampus (*p*-value <0.001 all; compared between sham operation + ND and sham operation + HFD and compared between OVX + ND and OVX + HFD group). Both isoflavone and mung bean protein could significantly increase this parameter in all hippocampal areas investigated in this study (*p*-value <0.001 all; compared to OVX + HFD group).

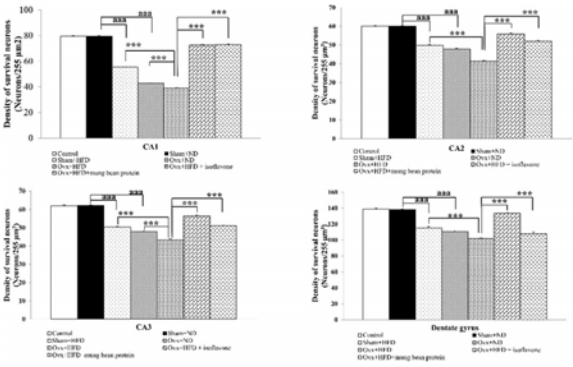

Figure 2. The effect of mung beanextract on neuron density in the CA1, CA2, CA3 and dentate gyrus, subregion of the hippocampus. Data are presented as mean ± SEM (n = 6/group). ^{aaa}p-value <0.001 compared to Sham + ND and $^{***}p$-value <0.001 compared to OVX + HFD.

Discussion

Our data have demonstrated that bilateral ovariectomy induces memory impairment whereas HFD failed to produce memory impairment both in ovary intact rats and OVX rats. However, both OVX and HFD decrease neuron density and apoptosis in the hippocampus. In addition, the current results also demonstrate that OVX or HFD alone fail to produce the significant changes in oxidative stress status and AChE in the hippocampus. Both isoflavone and mung bean protein can improve memory impairment, neuron density and apoptosis in hippocampus. Interestingly,

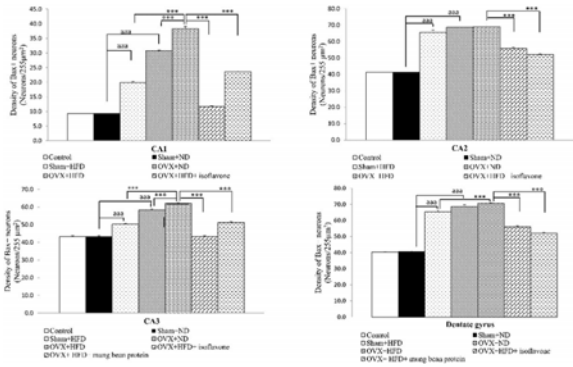

Figure 3. The effect mung bean protein on density of Bax+ cells in the CA1, CA2, CA3 and dentate gyrus, subregion of the hippocampus. Data are presented as mean ± SEM (n = 6/group). ^{aaa}p-value <0.001 compared to Sham + ND and $^{***}p$-value <0.001 compared to OVX + HFD.

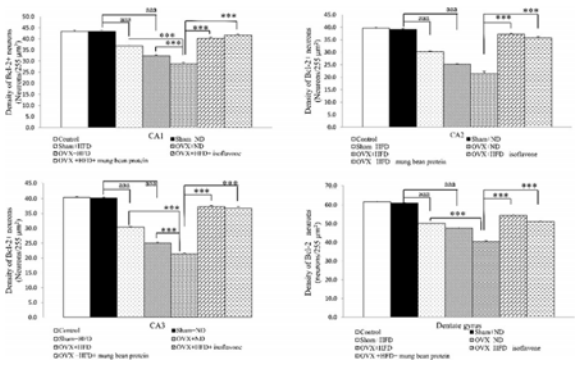

Figure 4. The effect of mung bean protein on density of Bcl-2+ cells in the CA1, CA2, CA3 and dentate gyrus, subregion of the hippocampus. Data are presented as mean ± SEM (n = 6/group). ^{aaa}p-value <0.001 compared to Sham + ND and $^{***}p$-value <0.001 compared to OVX + HFD.

mung bean protein suppresses AChE and oxidative stress whereas isoflavone fail to produce these effects in OVX rats fed with HFD.

Accumulative lines of evidence have revealed that OVX is typically associated with an estrogen reversible impairment of learning/memory behavior[28,29]. In addition, it has been shown that this impairment is associated with neurodegeneration[30,31]. The possible underlying mechanism partly involves with the increase in oxidative stress status[18,31]. Although our findings also demonstrate the increase in oxidative stress status in the hippocampus reflecting by the elevation of MDA level, no significant change is presented. The possible explanation may partly due to the difference in feeding system. In the previous studies, the vehicle was administered by intragastric needle which may induce stress more than our study which no stress is produced because the food is consumed by normal eating behavior. The present data point out that the mechanism for hippocampal neurodegeneration in OVX rats occurs via apoptosis by increasing apoptotic inducing protein but decreasing apoptotic protection protein. These findings are in agreement with the previous study which showed that OVX significantly increased apoptosis by increasing Bax+ but decreasing Bcl2+ cells in hippocampus. However, aforementioned changes can be attenuated with isoflavone[32].

The present study also demonstrates that not only OVX but HFD also induces apoptosis which in turn induces hippocampal neurodegeneration resulting in memory impairment. These results are in agreement with previous study which reveals that[33]. It was found that OVX rats which received HFD increase both AChE and MDA level together with the hippocampal neurodegeneration. The reduction of Bax+ cell is observed only in CA1 and CA3 whereas the elevation of Bcl-2+ cell is observed in all areas of the hippocampus investigated in the present study. These findings suggest that the possible underlying mechanisms of hippocampal neurodegeneration in OVX which received HFD are associated with the increase in apoptosis, oxidative stress status and the suppression of cholinergic function via the suppression of AChE in hippocampus. Both the reduction in cholinergic function[18,31] and the neurodegeneration can finally induce memory impairment. All of these changes can be attenuated by mung bean protein whereas isoflavone can significantly attenuate only apoptosis. The reduction of MDA and AChE also present in OVX rats which were fed with HFD and received isoflavone but no significant changes are observed. Since mung bean protein contains abundant amino acids possessing antioxidant activity such as histidine, tyrosine and phenylalanine[34] and AChE suppression activity such as phenylamine and tyrosine[35], the suppression of oxidative stress and AChE observed in OVX rats which fed with HFD and received mung bean protein may possibly occur as the result of amino acids mentioned earlier. In addition, some proteins such as tyrosine which can be oxidized to tyrosine oxidation product L-DOPA (L-3, 4-dihydroxy-phenylalanine) can induce apoptosis[36]. Moreover, nonessential amino acid such as proline also possesses anti-apoptosis effect[37]. Therefore, the reduction of apoptosis induced by mung bean protein may occur partly via the effect of amino acids just mentioned in the developed protein. Mung bean protein also contains abundant of glutamic acid which can activate learning and memory. Therefore, the memory enhancing effect of mung bean may also occur via the activation of glutamic acid via glutamic acid receptor[38] because it can pass the blood brain barrier (BBB)[39].

In conclusion, our study is the first study to demonstrate that mung bean protein can be the potential supplement for enhancing memory and protecting neurodegeneration in animal model of menopause. Since mung bean is not expensive, it can be served as the potential natural resource for developing neuroprotectant and cognitive enhancer which is cheap and easy to approach.

What is already known on this topic?

Vigna radiata L or mung bean is one of an important plant-based protein that widely consumed in Asia, which has protein content around 20.97 to 31.32%. Mung bean protein exhibits angiotensin converting enzyme inhibitory activity and antioxidant activity. Moreover, it can improve insulin and lipid metabolism. Additionally, lysine and glutamic acid which found abundant in mung bean can exert their effect on the central nervous system by improving stress response and anxiety.

What this study adds?

Mung bean protein can be the potential supplement for enhancing memory and protecting neurodegeneration in menopause.

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Potential conflicts of interest

The authors declare no conflict of interest

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