Antiamnesic Effects of Ethyl Acetate Fraction from Chestnut (*Castanea crenata* var. *dulcis*) Inner Skin on A β _{25–35}-Induced Cognitive Deficits in Mice

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ABSTRACT To investigate neuronal cell protective effects of an ethyl acetate fraction from chestnut inner skin, *in vitro* assays, including 2',7'-dichlorofluorescein diacetate, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), and lactate dehydrogenase (LDH), were performed. Intracellular accumulation of reactive oxygen species resulting from hydrogen peroxide (H₂O₂) treatment of PC12 cells was significantly reduced when ethyl acetate fractions were present in the medium compared to PC12 cells treated with H₂O₂ only. In a cell viability assay using MTT, the ethyl acetate fraction protected against H₂O₂-induced neurotoxicity, and inhibited LDH release into the medium. In addition, the ethyl acetate fraction improved *in vivo* cognitive ability against amyloid β -peptide (A β)-induced neuronal deficit. High-performance liquid chromatography analyses showed that gallic acid, catechin, and epicatechin were predominant phenolics in the ethyl acetate fraction. Consequently, the results suggest that chestnut inner skin, including above phenolics, could ameliorate A β -induced learning and memory deficiency, and be utilized as effective substances for neurodegenerative disorders, notably Alzheimer's disease.

KEY WORDS: • antiamnesic effect • chestnut inner skin • neuronal protective effect • phenolics

INTRODUCTION

OXIDATIVE STRESS HAS BEEN LINKED to a variety of neurodegenerative disorders such as Alzheimer's disease (AD). The brains of AD patients are characterized by large deposits of amyloid β -peptide (A β). These aggregates are neurotoxic to the brain tissue and probably account for the cognitive deficiencies associated with AD.^{1,2} A β is known to increase oxidative stress in nerve cells.³ This oxidative stress is mediated by reactive oxygen species (ROS). When the metabolic balance between ROS and the antioxidant system is lost, the result is oxidative stress. ROS, including hydrogen peroxide (H₂O₂), superoxide anion, and hydroxyl radicals, are generated by multiple enzymatic and nonenzymatic pathways, leading to initiation of the oxidative stress cascade.⁴ ROS can attack cellular biomolecules. For example, increased oxidation of protein and DNA is reported in AD patients, and decreased levels of polyunsaturated fatty acids coupled with increased lipid peroxidation

are also found in the AD brain. The oxidative stress-induced damage disrupts cellular function and membrane integrity, thereby leading to apoptosis.^{5,6} Antioxidants are able to interfere with most, if not all, of these processes, including neurotoxicity.^{7,8}

Naturally occurring antioxidants have been reported to play a major role in ameliorating oxidative damage induced by free radicals. Recently, natural foods and food-derived components, such as antioxidative vitamins and phenolic phytochemicals, have received a great deal of attention because they are safe and not perceived as medicine; some of these are known to function as chemopreventive agents against oxidative damage.⁹ Chestnut inner skin is also rich in carbohydrates and is a good source of essential fatty acids and minerals.¹⁰ In addition, they have several vitamins and appreciable levels of fiber.¹¹ Some researchers also reported that chestnut inner skin contains gallic acid, epicatechin, and catechin as its main phenolics.¹² Although it has already been demonstrated that chestnut fruits and leaves contain phenolics and antioxidant activities,¹³ little is known about their protective effect on *in vivo* antiamnesic effects. Therefore, *in vitro* neuronal cell protective effects of the ethyl acetate fraction from chestnut inner skin against H₂O₂-induced cell death in PC12 cells were examined. In addition,

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its effects on A β -induced cognitive deficits in mice using *in vivo* learning and memory tests such as the Y-maze test were evaluated. According to the oxidative stress theory of AD, A β generates free radicals in neuronal cell membrane bilayers and then causes cellular dysfunction through lipid peroxidation.^{14–16} Therefore, cognitive dysfunction caused by neurotoxic A β has a direct correlation between oxidative stress-induced neurotoxicity and neurodegeneration. Consequently, the present study was undertaken to investigate the anti-amnesic effects of the ethyl acetate fraction from chestnut inner skin on A β -induced cognitive dysfunction in mice.

MATERIALS AND METHODS

Chemicals

2',7'-Dichlorofluorescein diacetate (DCF-DA), vitamin C, H₂O₂, dimethyl sulfoxide, HEPES, sodium bicarbonate, penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay kit, lactate dehydrogenase (LDH) assay kit, A β _{25–35}, and all other materials used were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Neuronal cell culture

PC12 cells (KCLB 21721; Korea Cell Line Bank, Seoul, Korea), which were derived from a transplantable rat pheochromocytoma, were propagated in an RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum, 25 mM HEPES, 25 mM sodium bicarbonate, 50 units/mL penicillin, and 100 μ g/mL streptomycin.

Animals and *in vivo* experimental design

Institute of cancer research mice (male, 4-week old) were obtained from Samtako (Osan, Korea). The mice were housed two per cage in a room maintained with a 12-h light–dark cycle, 55% humidity, and 23°C–25°C temperature. The ethyl acetate fraction of chestnut inner skin was mixed in drinking water at concentrations of 5, 10, and 20 mg/kg of body weight. All experimental procedures were approved by the guidelines established by the Animal Care and Use Committee of Gyeongsang National University. Subsequently, A β _{25–35} was administered via intracerebroventricular (ICV) injection. Control animals were injected with A β _{25–35} (Fig. 1).

FIG. 1. *In vivo* experimental design for amyloid β -peptide (A β)-induced amnesic mouse model.

A β was dissolved in 0.85% sodium chloride solution (w/v) and injected intracerebroventricularly into each mouse with a 25- μ L Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.5 mm. The injection volume was 5 μ L (dose 410 pmol/mouse).¹⁷

Plant materials and extraction

Chestnut inner skin was purchased from (Altari Co., Ltd. Jinju, Korea) in September 2010. The samples were washed with running tap water before being chopped into pieces. They were then oven-dried at 40°C for 2 days, and ground to a powder. The ethyl acetate fraction from the water extract of chestnut inner skin was obtained as follows. Powder of chestnut inner skin (100 g) was suspended and extracted with 500 mL of water at 70°C for 2 h. Water extracts were filtered through Whatman No. 2 filter paper (Whatman International Limited, Kent, England, United Kingdom), and evaporated to dryness. The dry materials were redissolved in 500 mL of distilled water. The solution was consecutively partitioned in a separatory funnel with the equivalent amount of ethyl acetate. The fraction was concentrated in a vacuum evaporator (N-N series; Eyela Co., Tokyo, Japan) at 40°C. This sample was stored at –20°C until use.

Measurement of intracellular oxidative stress

Levels of intracellular ROS were determined by DCF-DA assay.¹⁸ In brief, cells (10⁴ cells/well on 96-well) were treated for 48 h with the indicated concentrations of the ethyl acetate extract of chestnut inner skin or vitamin C. The cells were then treated with or without 200 μ M H₂O₂ for 2 h. At the end of the treatment, cells were incubated in the presence of 50 μ M DCF-DA in phosphate-buffered saline. Fluorescence was then quantified using a TECAN SER-NR 94572 fluorometer (San Jose, CA, USA) using 485-nm excitation and 530-nm emission filters.

Protection of neuronal cells against oxidative stress

MTT reduction assay was determined using the *in vitro* toxicology assay kit (TOX-1; Sigma). Neuronal PC12 cells were plated at a density of 10⁶ cells per well on 96-well plates in 100 μ L of RPMI. The cells were preincubated with the ethyl acetate extract of chestnut inner skin for 48 h before the 200 μ M H₂O₂ was added. The cells were treated with or without H₂O₂ for 2 h. The amount of MTT formazan product was determined by measuring absorbance using a microplate reader (680; Bio-Rad, Tokyo) at a test wavelength of 570 nm and a reference wavelength of 690 nm.¹⁹

Neuronal PC12 cells were precipitated by centrifugation at 250 g for 4 min at room temperature; 100 μ L of the supernatants was transferred into new wells, and LDH was determined using the *in vitro* toxicology assay kit (TOX-7; Sigma). Damage of the plasma membrane was evaluated by measuring the amount of the intracellular enzyme LDH released into the medium.¹⁹

Y-maze test

Recording spontaneous alternation behavior in the Y-maze test was used to assess the immediate working memory performance. The Y-maze test was performed 2 days after the A β injection. The maze was made of black-painted plastic, and each arm of the maze was 33-cm long, 15-cm high, and 10-cm wide and was positioned at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze for 8 min. The series of arm entries was recorded visually, and arm entry was considered to have been completed only when the hind paws of the mouse were placed completely in the arm of the maze. Alternation is defined as successive entries into the three arms in overlapping triplet sets. The percentage alternation was calculated as the ratio of actual to possible alternation (defined as the total number of arm entries minus two), multiplied by 100.²⁰

Determination of total phenolics

The total phenolics were determined by a spectrophotometric method.¹² The standard curve for total phenolics was made using the gallic acid standard solution (0–100 mg/L). The total phenolics in aqueous extract of propolis were expressed as milligrams of gallic acid equivalents per 1 g of sample.

Quantification by HPLC

Phenolics in ethyl acetate fraction from chestnut inner skin were measured at 280 nm by a photo diode array detector (U 3000 series; Dionex, Sunnyvale, CA). Separation was achieved with a Shiseido C₁₈ column (250 mm $\times$ 4.6 mm id, 5 μ m; Shiseido Co., Tokyo). The elution solvents were (A) 0.01 M potassium phosphate buffer adjusted to pH 3.0 by phosphoric acid and (B) methanol. The solvent gradient elution program used was as follows: initial 90% (A), hold for 9.5 min; linear gradient to 68% (A) in 3.5 min; linear gradient to 67% (A) in 17 min; linear gradient to 20% (A) in 1 min; linear gradient to 90% (A) 1 min, and hold for 10 min. The flow rate was 1.5 mL/min. Phenolics were identified by comparison of their retention time (RT) and UV spectra with those of known standards, and quantified by peak areas from the chromatograms. All analyses were run in triplicate, and mean values were calculated. Content of phenolics was expressed in mg/100 g extract.

Statistical analysis

All data were expressed as mean $\pm$ SD. The data were analyzed by Duncan's new multiple-range test of SAS (Cary, NC, USA). The statistical significance of differences among groups was calculated by a one-way analysis of variance.

RESULTS AND DISCUSSION

Measurement of intracellular oxidative stress

To examine intracellular accumulation of ROS, we used a DCF-DA probe that is freely permeable to the cell membrane.

In PC12 cells, the compound is hydrolyzed by the cellular esterase to DCF, which interacts with peroxide-forming fluorescent DCF. Exposure of PC12 cells to H₂O₂ for 2 h resulted in a 130.66% increase of ROS levels compared to control (Fig. 2). Intracellular ROS accumulation resulting from H₂O₂ treatment was significantly decreased when the ethyl acetate extract of chestnut inner skin was present in the medium compared to PC12 cells treated with H₂O₂ only. Vitamin C, a naturally occurring major nutrient, has antioxidant activity. At the level of 200 μ M vitamin C (101.89%) as a positive control, PC12 cells had significantly lower oxidative stress than PC12 cells treated with H₂O₂ only. Oxidative stress in AD may result from aging, energy deficiency, inflammation, or excessive production of A β . A β protein can induce cell death through a mechanism involving H₂O₂ production.¹⁶ In this respect, this result suggests that the ethyl acetate extract of chestnut inner skin with antioxidant activity may decrease the oxidative stress-induced risk by AD.

Neuronal cell protective effects on oxidative stress

H₂O₂ has been reported to induce apoptosis in cells of the central nervous system. In this study, the ethyl acetate fraction of chestnut inner skin was selected to investigate the neuroprotective effects against H₂O₂-induced damage because of strong antioxidant activity. Protective effects on H₂O₂-induced neuronal cells damage were confirmed by MTT assay. H₂O₂ caused a decrease in cell viability (32.87%), but pretreatment of PC12 cell with increasing concentrations of the ethyl acetate fraction of chestnut inner skin inhibited oxidative stress-induced cytotoxicity (Fig. 3A). Neuronal cell protective effects of the fraction at

FIG. 2. Protective effect of ethyl acetate fraction from chestnut inner skin against cellular oxidative stress. PC12 cells were pretreated for 48 h with various concentrations of the extract fraction. After 48 h, the cells were treated with 200 μ M hydrogen peroxide (H₂O₂) for 3 h. The increase of 2',7'-dichlorofluorescein diacetate (DCF-DA) fluorescence was measured by a fluorescence microplate reader. Results are presented as mean $\pm$ SD of three independent experiments in triplicate. ^{abcd}Different letters indicate significant differences at $P < .05$.

FIG. 3. Neuroprotective effect of ethyl acetate fraction from chestnut inner skin on H_2O_2 -induced cytotoxicity in PC12 cells. **(A)** Levels of cell viability measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. **(B)** Lactate dehydrogenase (LDH) activity in culture supernatants was measured with a colorimetric LDH assay kit. Basal and total LDH activities were determined in intact cells and cells solubilized with 0.2% Triton X-100, respectively, and LDH release was calculated as $[(\text{sample LDH} - \text{basal LDH}) / (\text{total LDH} - \text{basal LDH})] \times 100$ (%). Results are presented as mean $\pm$ SD of three independent experiments in triplicate. ^{abcde}Different letters indicate significant differences at $P < .05$.

100 $\mu\text{g/mL}$ on oxidative injury were similar to those of 200 μM vitamin C. This study demonstrated that PC12 cell cytotoxicity through oxidative stress was suppressed by pretreatment with the ethyl acetate fraction obtained from chestnut inner skin. MTT dye reduction assay is based on the catalytic activity of some metabolic enzymes in intact mitochondria. Mitochondria may be one of the most sensitive primary targets of oxidative injury in neuronal cells. These results suggest that PC12 cell protection by the ethyl acetate fraction of chestnut inner skin is partially due to the mechanisms with mitochondrial protection.

Protective effect on H_2O_2 -induced membrane damage

The neuronal membrane contains polyunsaturated fatty acids, which are vulnerable to oxidative stress induced by ROS such as H_2O_2 . Lipid peroxidation can alter the fluidity of the plasma membrane. LDH assay provided an estimate of the percentage of surviving PC12 cells. The LDH assay was used to further investigate the neuroprotective effect of the ethyl acetate fraction of chestnut inner skin. After the treatment of PC12 cells with 200 μM H_2O_2 for 3 h, the absorbance values at 490 nm increased. With oxidative treatment with H_2O_2 for 3 h, the amounts of LDH release of PC12 cells increased by 33.64% compared to that of the control without treatment. Compared with their effects on the corresponding H_2O_2 treatment group, the ethyl acetate fraction of chestnut inner skin was dose-dependently decreased. Especially, the fraction attenuated LDH release significantly to 26.70% of 200 μM H_2O_2 at 100 $\mu\text{g/mL}$. In addition, the fraction showed that the pattern of neuronal cell protection was similar to MTT assay. Above results showed that the fraction could protect PC12 cells against the lesion induced by H_2O_2 (Fig. 3B). Therefore, these data suggest that PC12 cell protection by the ethyl acetate fraction of chestnut inner skin is partially due to its protection of mitochondrial and cell membranes during H_2O_2 -induced neurotoxicity.

Effect of ethyl acetate fraction obtained from chestnut inner skin on Y-maze test

The Y-maze test was carried out 2 days after the $\text{A}\beta$ injection. The control group was injected with the nontoxic reverse fragment as $\text{A}\beta_{35-25}$. The $\text{A}\beta_{25-35}$ group was injected with 410 pmol of $\text{A}\beta_{25-35}$ per mouse. $\text{A}\beta$ was dissolved in a 0.85% (w/v) sodium chloride solution. In Figure 4A, the administration of $\text{A}\beta$ significantly impaired the spatial working memory (83.49%) compared with that of the control group (100%). Pretreatment with the sample increased spontaneous alternation behavior in the $\text{A}\beta$ -injected mice (5 mg/kg of ethyl acetate fraction: E5, 90.80%; 10 mg/kg of ethyl acetate fraction: E10, 98.26%; 20 mg/kg of ethyl acetate fraction: E20, 103.98%). In contrast, the number of arm entries did not change among all the experimental groups (Fig. 4B). ICV injection of $\text{A}\beta$ induced memory deficits.²¹ Therefore, this method of $\text{A}\beta$ exposure is a useful *in vivo* model for $\text{A}\beta$ toxicity and suggests that ethyl acetate fraction of chestnut inner skin exhibits protective effect against learning and memory deficits in AD mice.

Phenolic composition of ethyl acetate fraction from chestnut inner skin

Phenolics are secondary plant metabolites that are present in natural products. Many of the phenolics have been shown to contain high levels of antioxidant activities.²² To identify the major neuroprotective phenolics, the ethyl acetate fraction was subjected to further analysis by high-performance liquid chromatography (HPLC), and the fraction showed important phenolics (Table 1). By comparing the RTs and

FIG. 4. Effect of ethyl acetate fraction from chestnut inner skin against spontaneous alternation behavior in Aβ-induced amnesic mice. The spontaneous alternation behavior (A) and number of arm entries (B) were measured over 8 min. Control group was injected with saline (0.85%, w/v, 5 μL). Aβ group was injected with Aβ_{25–35} (410 pmol, 5 μL). Sample groups, C5, C10, and C20, were injected with Aβ after treatment with ethyl acetate fraction from chestnut inner skin at 5, 10, and 20 mg/kg of body weight per day, respectively. Values represented the mean ± SD (*n* = 8). **P* < .05 versus control group, #*P* < .05 versus Aβ group.

UV spectra of these compounds with those of standards, three phenolics (gallic acid, catechin, and epicatechin) were identified (Fig. 5). Furthermore, the HPLC results indicated that gallic acid (10.90 mg/100 g) was the predominant phenolic in the ethyl acetate fraction, followed by catechin and epicatechin (Table 1). Consequently, the physiological activities determined in ethyl acetate fraction from chestnut inner skin could be attributed to three phenolics, including gallic acid, catechin, and epicatechin. ROS is a key factor involved in the Aβ toxicity. Aβ insertion into the neuronal membrane bilayer generates oxygen-dependent free radicals, causing the lipid peroxidation and protein oxidation. The loss of membrane integrity leads to cellular dysfunction, such as loss of Ca²⁺ homeostasis, disruption of signal pathways, and activation of nuclear transcription factor and apoptotic pathway.²¹ The neuronal apoptosis is the ultimate

TABLE 1. PHENOLIC COMPOSITION OF ETHYLACETATE FRACTION FROM CHESTNUT INNER SKIN

	Phenolic content
Gallic acid	10.90 ± 0.22 ^a
Catechin	1.71 ± 0.35
Epicatechin	1.42 ± 0.08

^aValues represented the mean ± SD (*n* = 3), presented in mg/100 g dry weight.

consequence of these cellular dysfunctions.²³ Some phytochemicals are suggested to have efficacy in the management of oxidative stress-induced cellular dysfunctions such as AD. Many phytochemicals are also known to be potential chemopreventive agents.²⁴ Therefore, current results suggest that gallic acid, catechin, and epicatechin of the ethyl acetate fraction obtained from chestnut inner skin should mitigate Aβ-induced oxidative stress in neuronal cells, based on their abilities to protect neuronal cell membranes and mitochondrial dysfunction. Also, there are critical criteria for selecting herbal drugs for the treatment of amnesia, including high bioavailability, ability to cross the blood–brain barrier, and minimal toxicity.²⁴ Based on the studies of Manach *et al.*,²⁵ the bioavailability of different types of flavonoids is as follows (from most to least bioavailable): isoflavones, gallic acid, catechins, flavones, and quercetin glucosides. In conclusion, the ethyl acetate fraction obtained from chestnut inner skin inhibits neuronal cell death caused by Aβ-induced oxidative stress, and improves Aβ-induced learning and memory deficits.

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FIG. 5. HPLC chromatogram of ethyl acetate fraction from chestnut inner skin. Retention time (min): gallic acid, 3.937; epicatechin, 6.232; catechin, 10.167.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

- Selkoe DJ: Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001;81:741–766.
- Munoz DG, Feldman H: Cause of Alzheimer's disease. *CMAJ* 2000;162:65–72.
- Rottkamp A, Raina K, Zhu X, Gaier E, Bush I, Atwood S, Chevion M, Perry G, Smith A: Redox-active iron mediated amyloid- β toxicity. *Free Radic Biol Med* 2001;30:447–450.
- Smith DG, Cappai R, Barnham KJ: The redox chemistry of the Alzheimer's disease amyloid β peptide. *Biochim Biophys Acta* 2007;1768:1976–1990.
- Fiers W, Beyaert R, Declercq W, Vandenabeele P: More than one way to die: apoptosis, and necrosis and reactive oxygen damage. *Oncogene* 1999;18:7719–7730.
- Zana M, Janka Z, Kálmán J: Oxidative stress: a bridge between Down's syndrome and Alzheimer's disease. *Neurobiol Aging* 2007;28:648–676.
- Butterfield DA, Reed T, Newman SF, Sultana R: Role of amyloid β -peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radic Biol Med* 2007;43:658–677.
- Casley S, Land M, Sharpe A, Clark B, Duchem R, Canevari L: β -Amyloid fragment 25–35 causes mitochondrial dysfunction in primary cortical neurons. *Neurobiol Dis* 2002;10:258–267.
- Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY: Major phenolics in apple and their contribution to the total antioxidant capacity. *J Agr Food Chem* 2003;51:6516–6520.
- Borges O, Carvalho J, Correia P, Silva AP: Lipid and fatty acid profiles of *Castanea sativa* Mill. chestnuts of 17 native Portuguese cultivars. *J Food Compos Anal* 2007;20:80–89.
- Vaughan JG, Geissler CA: *The New Oxford Book of Food Plants*. Oxford University Press, Oxford, United Kingdom, 1997.
- Jeong CH, Choi GN, Kim JH, Kwak JH, Choi SG, Heo HJ: Characterization of antioxidant activities from chestnut inner skin extracts. *Food Sci Biotechnol* 2009;18:1218–1223.
- Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA: Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem* 2008;107:1106–1113.
- Butterfield DA, Drake J, Pocernich C, Castegna A: Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β -peptide. *Trends Mol Med* 2001;7:548–554.
- Mecocci P, Macgarvey U, Beal F: Oxidative damage to mitochondrial DNA is increases in Alzheimer's disease. *Ann Neurol* 1994;36:747–751.
- Behl C, Davis JB, Lesley R, Schubert D: Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* 1994;77:817–827.
- Yan JJ, Cho JY, Kim HS, Kim KL, Jang JS, Huh SO, Suh HW, Kim YH, Song DK: Protection against β -amyloid peptide toxicity *in vivo* with long-term administration of ferulic acid. *Br J Pharmacol* 2001;133:89–96.
- Heo HJ, Cho HY, Hong B, Kim HK, Kim EK, Kim BG, Shin DH: Protective effect of 4',5-dihydroxy-3',6,7-trimethoxyflavone from *Artemisia asiatica* against A β -induced oxidative stress in PC12 cells. *Amyloid* 2001;8:194–201.
- Heo HJ, Choi SJ, Choi SG, Shin DH, Lee JM, Lee CY: Effect of banana, orange, and apple on oxidative stress-induced neurotoxicity in PC12 cells. *J Food Sci* 2008;73:H28–H32.
- Heo HJ, Lee CY: Protective effects of quercetin and vitamin C against oxidative stress-induced neurodegeneration. *J Agr Food Chem* 2004;52:7514–7517.
- Fu AL, Dong AH, Sun MJ: Protective effect of N-acetyl-L-cysteine on amyloid β -peptide-induced learning and memory deficits in mice. *Brain Res* 2006;1109:201–206.
- Pietta PG: Flavonoids as antioxidants. *J Nat Prod* 2000;63:1035–1042.
- Yatin SM, Varadarajan S, Link CD, Butterfield DA: *In vitro* and *in vivo* oxidative stress associated with Alzheimer's amyloid beta-peptide (1–42). *Neurobiol Aging* 1999;20:325–330.
- Jang HD, Chang KS, Huang YS, Hsu CL, Lee SH, Su MS: Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. *Food Chem* 2007;103:749–756.
- Manach C, Williamson G, Morand C, Scalbert A, Remesy C: Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* 2005;81:230–242.