

머루전초, 독활전초, 감초 혼합추출물의 Amyloid β Protein (25-35) 유발 신경 독성에 대한 억제효과

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Inhibitory Effect of an Ethanol Extract Mixture of *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix on Amyloid β Protein (25-35)-Induced Neurotoxicity

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ABSTRACT : The present study investigated an ethanol extract (SSB) of a mixture of three medicinal plants of *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix for possible neuroprotective effects on neurotoxicity induced by Amyloid β protein (A β) (25-35) in cultured rat cortical neurons and antidementia activity in mice. Exposure of cultured cortical neurons to 15 μ M A β (25-35) for 36 h induced neuronal apoptotic death. At 1-30 μ g/ml, SSB inhibited neuronal death, elevation of intracellular calcium concentration ([Ca²⁺]_i), and generation of reactive oxygen species (ROS) induced by A β (25-35) in cultured cortical neurons. Memory impairment and increase of acetylcholinesterase activity induced by intracerebroventricular injection of mice with 16 nmol A β (25-35) was inhibited by chronic treatment with SSB (25, 50 and 100 mg/kg, p.o., for 8 days). From these results, it is suggested that antidementia effect of SSB is due to its neuroprotective effect against A β (25-35)-induced neurotoxicity and that SSB may have a therapeutic role in preventing the progression of Alzheimer's disease.

Key Words : *Vitis amurensis*, *Aralia cordata*, *Glycyrrhizae* radix, Amyloid β Protein, Cultured Neurons, Memory Impairment

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder, and the most common type of dementia. It is characterized by pronounced loss of neurons, extracellular senile plaque, whose principle component is β -amyloid (A β), a 39-43 amino acid peptide derived from the cleavage of the amyloid precursor protein by β and γ -secretase (Ivins *et al.*, 1999). A β can directly induce neuronal cell death (Ueda *et al.*, 1997) and cause neurons vulnerable to excitotoxicity (Koh *et al.*, 1990) and oxidative insults (Chen *et al.*, 2007). It is suggested that the mechanisms underlying A β neurotoxicity are complex but may involve N-methyl-D-aspartate (NMDA) receptor, a glutamate receptor

subtype, modulation induced by glutamate release, sustained elevations of intracellular Ca²⁺ concentration ([Ca²⁺]_i), and oxidative stresses resulting from reactive oxygen species (ROS) generation (Ekinci *et al.*, 2000; Gray and Patel, 1995; Ueda *et al.*, 1997). A β has been reported to produce hydrogen peroxide, superoxide, and proinflammatory cytokines in neurons and glial cells (Gitter *et al.*, 1995). Therefore, antioxidants such as α -tocopherol and anti-inflammatory agents such as indomethacin reportedly slow the progression of AD (Gasparini *et al.*, 2004; Sano *et al.*, 1997).

Vitis, known as a kind of grapes, has various pharmacological effects including improvement against fatty liver, hypertension, cardiac diseases and arthritis (Wang *et*

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Received 2014 January 2 / 1st Revised 2014 January 24 / 2nd Revised 2014 February 7 / 3rd Revised 2014 February 17 / Accepted 2014 February 17
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al., 2004). *Vitis* contains large amount of polyphenolic compounds such as resveratrol possessing potent antioxidant properties (Rice-Evans *et al.*, 1995) and anti-inflammatory properties (Wang *et al.*, 2004). *Aralia* belongs to the families Araliaceae and has long been recognized as therapeutic herbs for antinociceptive, antidiabetic, antioxidant and anti-inflammatory activities in China, Japan and Korea (Bae, 2004). Diterpenes isolated from the root of this plant were known as active constituents for analgesic and anti-inflammatory agents (Han *et al.*, 1985). Recent studies have indicated that extracts from the aerial parts of *A. cordata* inhibit cyclo-oxygenase (COX)-1, COX-2, and COX-2-dependent prostaglandin E2 (PGE2) generation, as well as hyperalgesia during peripheral inflammation (Dang *et al.*, 2005; Lee *et al.*, 2006; Park *et al.*, 2005). *Glycyrrhizae* radix, the root of *Glycyrrhizae uralensis* (Lycophodiaceae), has been widely used in Chinese medicine and food. Current European and Chinese texts list *Glycyrrhizae* radix as an expectorant in the treatment of bronchitis, catarrh, and coughs, as an anti-inflammatory agent in gastric ulcers, arthritis, and rheumatism, and as an adrenocorticotrophic agent in adrenocorticoid insufficiency (Huang, 1999). Moreover, *Glycyrrhizae* radix has been reported to have antioxidant effects (Tang *et al.*, 2004).

There has been much effort to develop beneficial agents from medicinal plants with free radical scavenging and anti-inflammatory properties to achieve neuroprotection in our laboratory (Jung *et al.*, 2012; Kim *et al.*, 2011). In our previous studies, aerial parts, leaves and stems, of *Vitis amurensis* and *Aralia cordata* were demonstrated to show neuroprotective effect against A β (25-35)-induced neurotoxicity in *in vivo* and *in vitro* (Cho *et al.*, 2009; Jeong *et al.*, 2010). *Glycyrrhizae* radix also showed neuroprotection in cultured neurons, but not in *in vivo* (Lee *et al.*, 2012). Therefore, we hypothesized that mixture of three plants might show more effective and synergistic neuroprotective effect with the reduction of possible individual toxicity due to large quantity.

The present study investigated the neuroprotective property of an ethanol extract of mixture of *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix, which was named as SSB, against A β (25-35)-induced memory deficits in mice and neuronal damage in cultured rat cortical neurons.

MATERIALS AND METHODS

1. Plant Materials and Extraction

The leaves and stems of *V. amurensis* and the aerial parts of *A. cordata* were collected at Keryong Mountain in Daejeon, Korea, from May to July and *Glycyrrhizae* radix (Korean) was purchased from Daegu Oriental Pharm Co. at Daegu, Korea. These plants were identified by Dr. KiHwan Bae, Chungnam National University. Each 200 g of the three plants was mixed, extracted with ethanol (3 L \times 24 h \times 3) at room temperature, filtered, and concentrated under reduced pressure using a rotary evaporator to yield an ethanol extract (SSB, 64 g) (Choung *et al.*, 2013), which was then stored at -20°C until required.

2. Experimental Animals

Pregnant Sprague-Dawley (SD) rats for primary neuronal culture and male ICR mice for the passive avoidance test were supplied by Daehan BioLink Co., Ltd. (Chungbuk, Korea) and housed in an environmentally controlled room at $22 \pm 2^{\circ}\text{C}$, with a relative humidity of $55 \pm 5\%$, a 12-h light/dark cycle, and food and water ad libitum. The procedures involving experimental animals complied with the animal care guide lines of the National Institutes of Health and the animal ethics committee of Chungbuk National University.

3. Induction of Neurotoxicity in Primary Cultures of Rat Cerebral Cortical Neurons

Primary cortical neuron cultures were prepared using embryonic day 15 to 16 SD rat fetuses, as previously described (Ban *et al.*, 2006). Neurotoxicity experiments were performed on neurons after 3-4 days in culture. Cultured neurons were treated with $15 \mu\text{M}$ A β (25-35) (Bachem, Bubendorf, Switzerland) in serum-free DMEM (Sigma, St. Louis, MO, USA) at 37°C for 36 h (unless otherwise indicated) to produce neurotoxicity. An A β (25-35) stock solution of 2 mM was prepared in sterile distilled water, stored at -20°C , and incubated for more than 2 days at 37°C to aggregate before use. SSB was dissolved in dimethyl-sulfoxide (DMSO) at concentrations of 50 mg/ml and further diluted in experimental buffers. The final concentration of DMSO was less than 0.1%, which did not affect cell viability. For each experiment,

SSB was applied 20 min prior to treatment with 15 μ M A β (25-35). It was also present in the medium during A β (25-35) incubation.

4. Measurements of A β (25-35)-induced Neuronal Death and Intracellular Biochemical Changes

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) assay and Hoechst 33342 (Molecular Probes, Eugene, OR, USA) staining were performed to measure neuronal death 36 h after exposure of cultured neurons to 15 μ M A β (25-35), as previously described (Ban *et al.*, 2006). Changes in $[Ca^{2+}]_i$ were measured with Fluo-4 AM (Molecular Probes), a Ca^{2+} -sensitive fluorescent dye, using a laser scanning confocal microscope (LSM 510, Carl Zeiss, Oberkochen, Germany) with 488-nm excitation argon laser and 515-nm longpass emission filters. The microfluorescence of 2',7'-dichlorofluorescein, the fluorescent product of 2',7'-dichlorofluorescein diacetate (H_2DCF -DA; Molecular Probes), and a laser scanning confocal microscope (MRC 1024ES, Biorad, Maylands, UK) with 488-nm excitation and 510-nm emission filters were used to monitor the generation of ROS in neurons treated with 15 μ M A β (25-35) for 24 h.

5. Measurement of Memory Impairment and Brain Cholinesterase Activity

Intracerebroventricular (i.c.v.) injection of the aggregated A β (25-35) (16 nmol) was performed to induce memory impairment in mice, as previously described (Kim *et al.*, 2009). SSB (25, 50 and 100 mg/kg) suspended in distilled water was orally administered to 5-week-old ICR mice 30 min before the injection of A β (25-35) and further administered daily for 7 days. Passive avoidance apparatus (Gemini Avoidance System, SanDiego, CA, USA) was used to measure memory acquisition according to the method previously described (Kim *et al.*, 2009). Mice were trained on step-through passive avoidance task 30 min after administration of SSB on day 7 of i.c.v. injection of A β (25-35). Retention trial was given 24 h after the acquisition trial. Cholinesterase activity of mice whole brain isolated immediately after the retention trial of passive avoidance test was determined using the spectrophotometric method (Ellman *et al.*, 1961).

6. Statistical Analysis

Data were expressed as mean \pm SEM and statistical significance was assessed by one-way analysis of variance (ANOVA) and Tukey's tests. *P* values of <0.05 was considered significant.

RESULTS

1. SSB Inhibited A β (25-35)-Induced Neuronal Cell Death

Cultured cortical neurons exposed to 15 μ M A β (25-35) for 36 h showed $60.5 \pm 2.5\%$ absorbance of that of the untreated controls in the MTT assay (Fig. 1), indicating that A β (25-35) caused neuronal cell death. Pretreatment of cortical neurons with 10 and 30 μ g/ml SSB reduced the neuronal death induced by A β (25-35) (absorbance, $74.4 \pm 2.8\%$ and $82.0 \pm 3.0\%$ of control, respectively; Fig. 1).

An additional experiment was performed with Hoechst 33342 staining to detect condensed or fragmented DNA, which is indicative of A β (25-35)-induced neuronal apoptotic death. Treatment of neurons with 15 μ M A β (25-35) produced apoptosis of $41.6 \pm 2.7\%$ of the total population of cultured cortical neurons, as compared with $13.4 \pm 2.1\%$ of apoptotic neurons in control cultures. The addition of SSB (1, 10 and 30 μ g/ml) significantly decreased the A β (25-35)-induced apoptotic cell death, showing 22.2 ± 3.2 , 21.0 ± 2.1 and $16.2 \pm 2.5\%$, respectively (Fig. 2).

2. SSB Inhibited A β (25-35)-Induced $[Ca^{2+}]_i$ Elevation

Increase in $[Ca^{2+}]_i$ has been associated with A β -induced cell death. In cultured neurons, $[Ca^{2+}]_i$ rapidly increased in response to treatment with 15 μ M A β (25-35) and then showed a slow and gradual decrease over 10 min (Fig. 3). In contrast, pretreatment with SSB (30 μ g/ml) showed inhibition of the increase of $[Ca^{2+}]_i$ induced by 15 μ M A β (25-35).

3. SSB inhibited A β (25-35)-Induced ROS Generation

To explain the involvement of oxidative stress in A β neurotoxicity, the accumulation of ROS after the exposure of the neurons to A β (25-35) for 24 h was measured. In H_2DCF -DA-loaded cortical neurons, 15 μ M A β (25-35) increased the fluorescence intensity, indicating that ROS were generated. In neurons treated with 15 μ M A β (25-

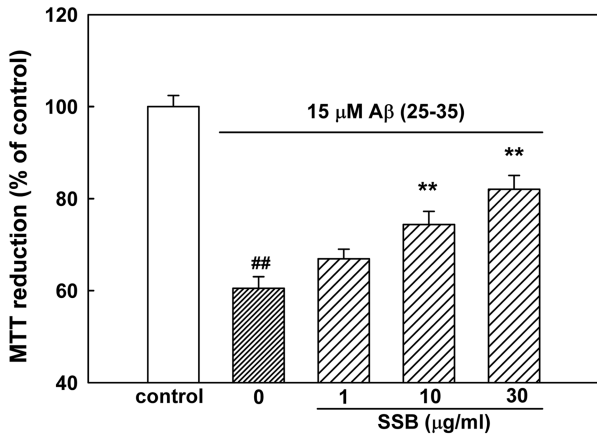


Fig. 1. Inhibitory effect of SSB on Aβ (25-35)-induced neuronal cell death in cultured cortical neurons. Neuronal cell death was measured using the MTT assay. The MTT absorbance from untreated cells was normalized to 100% as a control. Results are expressed as mean ± SEM of data obtained from 5 independent experiments. ##*p* < 0.01 vs. control; ***p* < 0.01 vs. 15 µM Aβ (25-35).

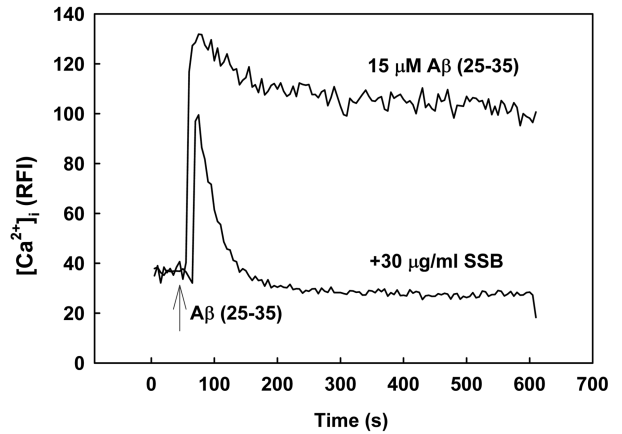


Fig. 3. Inhibitory effect of SSB on Aβ (25-35)-induced [Ca²⁺]_i elevation in cultured cortical neurons. [Ca²⁺]_i was monitored using Fluo-4 AM dye and a confocal laser scanning microscope. All images were processed to analyze changes in [Ca²⁺]_i at the single cell level. Results are expressed as the relative fluorescence intensity (RFI). Each trace shows a single cell that is representative of at least 3 independent experiments.

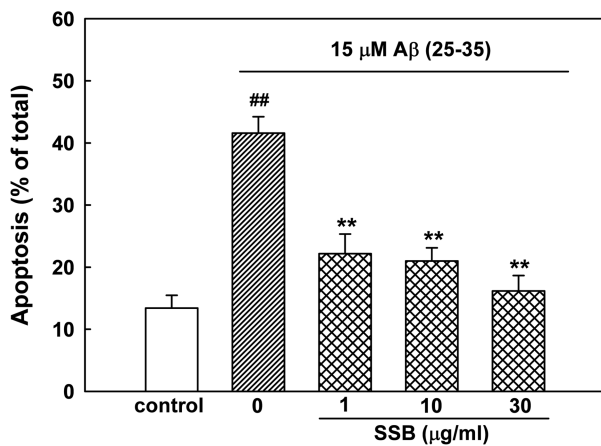


Fig. 2. Inhibitory effect of SSB on Aβ (25-35)-induced apoptosis of cultured cortical neurons. Apoptotic cells measured by Hoechst 33342 staining were counted in 5 to 6 fields per well. The values represent the apoptotic cells as a percentage of the total number of cells expressed as mean ± SEM of data obtained from 3 independent experiments. ##*p* < 0.01 vs. control; ***p* < 0.01 vs. 15 µM Aβ (25-35).

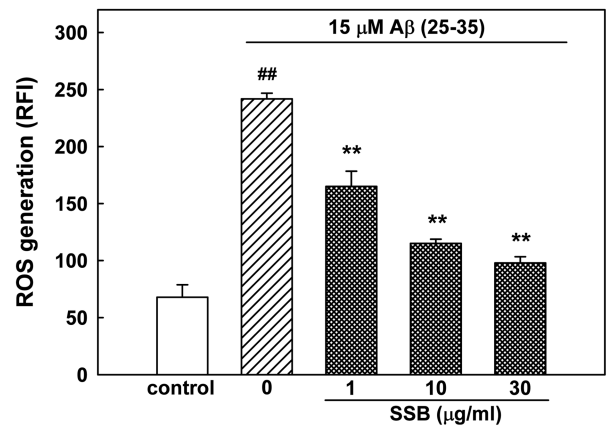


Fig. 4. Inhibitory effect of SSB on Aβ (25-35)-induced ROS generation in cultured cortical neurons. ROS was monitored using H₂DCF-DA dye and a confocal laser scanning microscope. Results are expressed as mean ± SEM of RFI obtained from 4 independent experiments. ##*P* < 0.01 vs. control; ***p* < 0.01 vs. 15 µM Aβ (25-35).

35), the relative fluorescence increased approximately 3.5-fold to 241.8 ± 5.0 compared with the value in control neurons (67.8 ± 10.9, Fig. 4). The Aβ (25-35)-induced increase in ROS generation was significantly inhibited by SSB (1, 10 and 30 µg/ml).

4. SSB Inhibited Aβ (25-35)-Induced Memory Impairment

In the initial acquisition trial of the passive avoidance

task, the step-through latency did not differ among the 5 groups (control, 16 nmol Aβ (25-35), Aβ (25-35) + 25 mg/kg SSB, Aβ (25-35) + 50 mg/kg SSB, and Aβ (25-35) + 100 mg/kg SSB; data not shown). The step-through latency of the Aβ (25-35)-treated group in the retention trial significantly decreased to 31.6 ± 9.9 s, compared with 213.6 ± 30.3 s in the control group, indicating that Aβ (25-35) impaired memory in mice. Chronically administered SSB markedly protected against

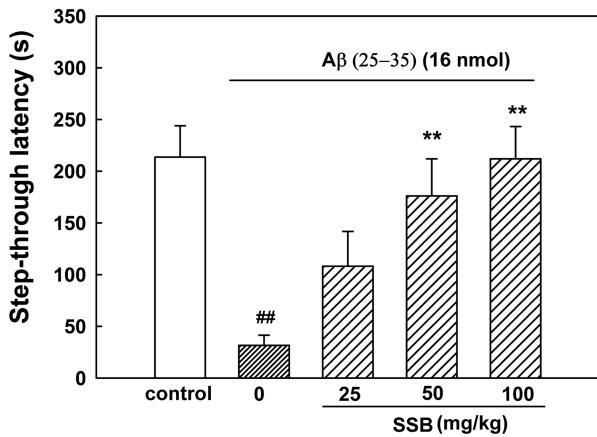


Fig. 5. Protective effect of SSB on A β (25-35)-induced memory impairment in mice. The learning and memory performance was assessed by the passive avoidance test. Values are expressed as mean \pm SEM of step-through latency (n = 8-12). ##*P* < 0.01 vs. sham-operated control; ***p* < 0.01 vs. 16 nmol A β (25-35).

Table 1. Effect of SSB on brain cholinesterase activity in mice.

Group	Dose	cholinesterase activity (μ mol/h mg protein ⁻¹)
Control	-	172.8 \pm 7.1
A β (25-35)	16 nmol/animal	222.2 \pm 9.9##
+ SSB	25 mg/kg	200.0 \pm 9.2
+ SSB	50 mg/kg	181.1 \pm 13.9*
+ SSB	100 mg/kg	163.7 \pm 12.6**

Results are expressed as mean \pm SEM of cholinesterase activity in brain (n = 8-12). ##*p* < 0.01 vs. sham-operated control; **p* < 0.05, ***p* < 0.01 vs. 16 nmol A β (25-35).

the memory impairment produced by A β (25-35). The step-through latency in groups administered SSB was 108.1 \pm 33.6, 176.0 \pm 35.9 and 212.0 \pm 31.2 s at doses of 25, 50 and 100 mg/kg, respectively (Fig. 5).

Cholinesterase activity in brain exposed to 16 nmol A β (25-35) was significantly increased. In the group consuming 50 mg/kg and 100 mg/kg SSB, the cholinesterase activity significantly decreased compared with the A β (25-35) group (Table 1).

DISCUSSION

A β (25-35), which is the core toxic fragment of full length A β (1-40), forms a β -sheet structure and induces neuronal cell death, neuritic atrophy, synaptic loss, and memory impairment (Maurice *et al.*, 1996; Pike *et al.*, 1995; Tohda *et al.*, 2004). It has been reported that A β

(25-35) causes neuronal cell death as shown in the present study, and it was blocked by MK-801, an NMDA receptor antagonist; verapamil, a L-type Ca²⁺ channel blocker; and and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, in cultured neurons (Cho *et al.*, 2009; Jeong *et al.*, 2010). These results suggest that NMDA glutamate receptor activation, Ca²⁺ influx through L-type voltage dependent Ca²⁺ channel (L-VDCC) and ROS generation are implicated in A β -induced neuronal apoptotic death. The present study provides evidence that A β (25-35)-induced injury to rat cortical neurons was prevented by SSB, an ethanol extract of a mixture of *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix. SSB was able to reduce the [Ca²⁺]_i increase, ROS generation, and apoptotic neuronal death in A β (25-35)-induced neurotoxicity. Additionally, SSB prevented memory loss induced by i.c.v. injection of A β (25-35) in mice.

Ca²⁺ influx via L-VDCC is considered as a primary insult after A β treatment in cultured neurons, because blockade of this channel or Ca²⁺ chelation prevents other consequences (Ueda *et al.*, 1997). In the present study, SSB inhibited a significant increase of [Ca²⁺]_i after the treatment with A β (25-35). Many reports have demonstrated the role of ROS formation in A β -induced neurotoxicity (Miranda *et al.*, 2000; Morais Cardoso *et al.*, 2002). It has been reported that vitamin-E, an antioxidant, blocked the A β -induced generation of ROS, but not Ca²⁺ influx, and reduction of extracellular Ca²⁺ inhibited the A β -induced increase in [Ca²⁺]_i as well as generation of ROS, indicating that ROS generation is a consequence of Ca²⁺ accumulation (Ekinci *et al.*, 2000). SSB also decreased the A β (25-35)-induced increase of ROS generation. These results indicate that SSB might prevent A β (25-35)-induced Ca²⁺ entry through VDCC- and / or NMDA-receptor-coupled channels to inhibit ROS generation and then neuronal death, although the mechanism by which SSB blocks the channels is not clear. *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix, the constituents of SSB, have been reported to possess antioxidant principles, resveratrol, oleanolic acid, and isoliquiritigenin (Du and Ko, 2006; Ha *et al.*, 2009a, b; Kumar *et al.*, 2007), respectively, suggesting that inhibition of A β (25-35)-induced neuronal death by SSB might be due to their ROS scavenging activity. Further

study should be conducted to elucidate the precise mechanism.

Many studies have demonstrated that A β is accompanied by multiple events culminating in apoptosis in *in vitro* neuronal experiments (Ekinici *et al.*, 2000; Yan *et al.*, 1999). In the present work, cultured cortical neurons exposed to A β (25-35) for 36 h showed increased chromatin condensation, a typical feature of apoptotic cell death, which was reduced by SSB. Activation of caspases after increased [Ca²⁺]_i signaling and ROS generation, or inflammatory responses in A β -stimulated neurons have been proposed to play pivotal roles in apoptosis. We have already demonstrated that an increase of caspase-3 activity in A β (25-35)-treated cultured cortical neurons is correlated with the increase of [Ca²⁺]_i, ROS generation and neural apoptotic death (Ban *et al.*, 2006; Lee *et al.*, 2005). In the previous studies, *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix inhibited caspase activity to reduce A β (25-35)-induced neuronal apoptosis (Cho *et al.*, 2009; Jeong *et al.*, 2010; Lee *et al.*, 2012). Therefore, SSB might work in the same way.

I.c.v. administration of A β (25-35) into mouse brain induces memory impairment in different behavioral paradigms, including spontaneous alternation, the water maze, and passive avoidance (Maurice *et al.*, 1996; Um *et al.*, 2006). A β (25-35) preferentially impairs spatial and non-spatial short-term memory, and these effects remain evident up to 6 months after even a single i.c.v. injection of the peptide (Stepanichev *et al.*, 2003). In the present work, memory impairment in the passive avoidance test was also confirmed in mice 7 days after the i.c.v. injection of A β (25-35). Chronic treatment with SSB effectively protected the mice against A β (25-35)-induced memory deficit. This result was essentially in agreement with its protective effect on A β (25-35)-induced neurotoxicity *in vitro*. A β accumulation associated with cognitive impairment in AD is accompanied by an increase in cholinesterase activity (Atack *et al.*, 1983). Equivalently, the increase of cholinesterase activity by A β (25-35) in the brain was inhibited by SSB in the present study.

Many reports have suggested that oxidative stress is responsible for the onset of the cognitive dysfunction as well as the progression of AD (Butterfield *et al.*, 2001;

Kontush, 2001). Elevated levels of A β induce oxidative stress, increasing the appearance of ROS such as superoxide and NO and subsequently producing ONOO⁻ by a rapid interaction, could mediate the damage seen in AD (Kontush, 2001; Smith *et al.*, 1997). A scavenger of ONOO⁻ protects against A β (25-35)-induced memory impairment (Alkam *et al.*, 2007), and anti-oxidants such as α -tocopherol protect against cytotoxicity *in vitro* as well as against learning and memory deficits induced by A β (Sano *et al.*, 1997). In the present study, 15 μ M A β (25-35) significantly increased the ROS level in cultured neurons, and this was inhibited by SSB. In addition, the constituents of SSB contain antioxidant components (Du and Ko, 2006; Ha *et al.*, 2009a,b; Kumar *et al.*, 2007). Therefore, it is possible that the favorable effect of SSB on A β (25-35)-induced cognitive deficits can be attributed to the inhibition of ROS generation.

In the previous studies (Cho *et al.*, 2009; Jeong *et al.*, 2010; Lee *et al.*, 2012), *Vitis amurensis*, *Aralia cordata*, and *Glycyrrhizae* radix were demonstrated to show neuroprotective effect against A β (25-35)-induced neurotoxicity in cultured neurons. Furthermore, *Vitis amurensis* (Jeong *et al.*, 2010) and *Aralia cordata* (Cho *et al.*, 2009) but not *Glycyrrhizae* radix, at the dosage of 50 and 100 mg/kg revealed protection against A β (25-35)-induced memory impairment in mice. Mixture of three plants, SSB, showed a maximal inhibition of A β (25-35)-induced memory impairment by a dosage of 100 mg/kg in the present study. These results indicate that three medicinal plants acted synergistically to produce neuroprotection, since maximal effect of SSB was shown at much lower concentration than the sum of maximal effect concentration of each plant.

In conclusion, it is evident that SSB could provide a marked protection against A β (25-35)-induced neuronal cell damage *in vitro* and A β (25-35)-induced memory deficit *in vivo*. These may explain the inhibitory action of SSB on the progression of AD.

ACKNOWLEDGEMENTS

This work was supported by the research grant of the Chungbuk National University in 2012.

REFERENCES

- Alkam T, Nitta A, Mizoguchi H, Itoh A and Nabeshima T. (2007). A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Abeta(25-35). *Behavioural Brain Research*. 180:139-145.
- Atack JR, Perry EK, Bonham JR, Perry RH, Tomlinson BE, Blessed G and Fairbairn A. (1983). Molecular forms of acetylcholinesterase in senile dementia of Alzheimer type: Selective loss of the intermediate(10S) form. *Neuroscience Letters*. 40:199-204.
- Bae K. (2000). The medicinal plants of Korea. Kyo-Hak Publishing Co. Seoul, Korea. p.362-363.
- Ban JY, Jeon SY, Bae K, Song KS and Seong YH. (2006). Catechin and epicatechin from *Smilaxis chinae* rhizome protect cultured rat cortical neurons against amyloid beta protein(25-35)-induced neurotoxicity through inhibition of cytosolic calcium elevation. *Life Sciences*. 79:2251-2259.
- Butterfield DA, Drake J, Pocernich C and Castegna A. (2001). Evidence of oxidative damage in Alzheimer's disease brain: Central role for amyloid beta-peptide. *Trends in Molecular Medicine*. 7:548-554.
- Chen CY, Jang JH, Park MH, Hwang SJ, Surh YJ and Park OJ. (2007). Attenuation of abeta-induced apoptosis of plant extract(Saengshik) mediated by the inhibition of mitochondrial dysfunction and antioxidative effect. *Annals of the New York Academy of Sciences*. 1095:399-411.
- Cho SO, Ban JY, Kim JY, Jeong HY, Lee IS, Song KS, Bae K and Seong YH. (2009). *Aralia cordata* protects against amyloid beta protein(25-35)-induced neurotoxicity in cultured neurons and has antidementia activities in mice. *Journal of Pharmacological Sciences*. 111:22-32.
- Choung MG, Hwang YS, Kim GP, Ahn KG, Shim HS, Hong SB, Choi JH, Yu CY, Chung IM, Kim SH and Lim JD. (2013). Antimelanogenic effect and whitening of anthocyanin rich fraction from seeds of *Liriope platyphylla*. *Korean Journal of Medicinal Crop Sciences*. 21:361-371.
- Dang NH, Zhang X, Zheng M, Son KH, Chang HW, Kim HP, Bae K and Kang SS. (2005). Inhibitory constituents against cyclooxygenases from *Aralia cordata* Thunb. *Archives of Pharmacol Research*. 28:28-33.
- Du Y and Ko KM. (2006). Oleonic acid protects against myocardial ischemia-reperfusion injury by enhancing mitochondrial antioxidant mechanism mediated by glutathione and alpha-tocopherol in rats. *Planta Medica*. 72:222-227.
- Ekinci FJ, Linsley MD and Shea TB. (2000). Beta-amyloid-induced calcium influx induces apoptosis in culture by oxidative stress rather than tau phosphorylation. *Brain Research*. 76:389-395.
- Gasparini L, Ongini E and Wenk G. (2004). Non-steroidal anti-inflammatory drugs(NSAIDs) in Alzheimer's disease: Old and new mechanisms of action. *Journal of Neurochemistry*. 91:521-536.
- Gitter BD, Cox LM, Rydel RE and May PC. (1995). Amyloid beta peptide potentiates cytokine secretion by interleukin-1 beta-activated human astrocytoma cells. *Proceedings of the National Academy of Sciences of the United States of America*. 92:10738-10741.
- Gray CW and Patel AJ. (1995). Neurodegeneration mediated by glutamate and beta-amyloid peptide: A comparison and possible interaction. *Brain Research*. 691:169-179.
- Ha DT, Chen QC, Hung TM, Youn UJ, Ngoc TM, Thuong PT, Kim HJ, Seong YH, Min BS and Bae K. (2009a). stillbenes and oligostilbenes from leaf and stem of *Vitis amurensis* and their cytotoxic activity. *Archives of Pharmacol Research*. 32:177-183.
- Ha DT, Kim H, Thuong PT, Ngoc TM, Lee I, Hung ND and Bae K. (2009b). Antioxidant and lipoxygenase inhibitory activity of oligostilbenes from the leaf and stem of *Vitis amurensis*. *Journal of Ethnopharmacology*. 125:304-309.
- Han B, Woo E, Park M and Han Y. (1985). Studies on the anti-inflammatory activity of *Aralia continentalis* (III). *Archives of Pharmacol Research*. 8:59-65.
- Huang KC. (1999). The pharmacology of Chinese herbs. CRC press LLC, Boca Raton, Florida, USA. p.364-368
- Ivins KJ, Ivins JK, Sharp JP and Cotman CW. (1999). Multiple pathways of apoptosis in PC12 cells. CrmA inhibits apoptosis induced by beta-amyloid. *The Journal of Biological Chemistry*. 274:2107-2112.
- Jeong HY, Kim JY, Lee HK, Ha do T, Song KS, Bae K and Seong YH. (2010). Leaf and stem of *Vitis amurensis* and its active components protect against amyloid beta protein(25-35)-induced neurotoxicity. *Archives of Pharmacol Research*. 33:1655-1664.
- Jung MH, Song KS and Seong YH. (2012). Inhibitory effect of *Chaenomeles sinensis* fruit on amyloid β protein(25-35)-induced neurotoxicity in cultured neurons and memory impairment in mice. *Korean Journal of Medicinal Crop Sciences*. 20:8-15.
- Kim JY, Kweon K, Lee HK, Kim SH, Yoo JD, Bae KH and Seong YH. (2011). Protective effect of an ethanol extract mixture of *Curcuma longae* radix, *Phellinus linteus*, and *Scutellariae* radix on oxidative neuronal damage. *Korean Journal of Medicinal Crop Sciences*. 19:31-37.
- Koh JY, Yang LL and Cotman CW. (1990). Beta-amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Research*. 533:315-320.
- Kontush A. (2001). Amyloid-beta: an antioxidant that becomes a pro-oxidant and critically contributes to Alzheimer's disease. *Free radical Biology & Medicine*. 31:1120-1131.
- Kumar S, Sharma A, Madan B, Singhal V and Ghosh B. (2007). Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. *Biochemical Pharmacology*. 73:1602-1612.
- Lee BY, Ban JY and Seong YH. (2005). Chronic stimulation of GABAA receptor with muscimol reduces amyloid beta protein(25-35)-induced neurotoxicity in cultured rat cortical cells. *Neuroscience Research*. 52:347-356.
- Lee HK, Yang EJ, Kim JY, Song KS and Seong YH. (2012). Inhibitory effects of *Glycyrrhizae* radix and its active component, isoliquiritigenin, on Abeta(25-35)-induced neurotoxicity in cultured rat cortical neurons. *Archives of Pharmacol Research*. 35:897-904.
- Lee IS, Jin W, Zhang X, Hung TM, Song KS, Seong YH and

- Bae K.** (2006). Cytotoxic and COX-2 inhibitory constituents from the aerial parts of *Aralia cordata*. Archives of Pharmacal Research. 29:548-555.
- Maurice T, Lockhart BP and Privat A.** (1996). Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. Brain Research. 706:181-193.
- Miranda S, Opazo C, Larrondo LF, Munoz FJ, Ruiz F, Leighton F and Inestrosa NC.** (2000). The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. Progress in Neurobiology. 62:633-648.
- Morais Cardoso S, Swerdlow RH and Oliveira CR.** (2002). Induction of cytochrome c-mediated apoptosis by amyloid beta 25-35 requires functional mitochondria. Brain Research. 931:117-125.
- Park HJ, Hong MS, Lee JS, Leem KH, Kim CJ, Kim JW and Lim S.** (2005). Effects of *Aralia continentalis* on hyperalgesia with peripheral inflammation. Phytotherapy Research. 19:511-513.
- Pike CJ, Walencewicz-Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG and Cotman CW.** (1995). Structure-activity analyses of beta-amyloid peptides: Contributions of the beta 25-35 region to aggregation and neurotoxicity. Journal of Neurochemistry. 64:253-265.
- Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM and Pridham JB.** (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research. 22:375-383.
- Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS and Thal LJ.** (1997). A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. The New England Journal of Medicine. 336:1216-1222.
- Smith MA, Richey Harris PL, Sayre LM, Beckman JS and Perry G.** (1997). Widespread peroxynitrite-mediated damage in Alzheimer's disease. The Journal of Neuroscience. 17:2653-2657.
- Stepanichev MY, Moiseeva YV, Lazareva NA, Onufriev MV and Gulyaeva NV.** (2003). Single intracerebroventricular administration of amyloid-beta(25-35) peptide induces impairment in short-term rather than long-term memory in rats. Brain Research Bulletin. 61:197-205.
- Tang SY, Whiteman M, Peng ZF, Jenner A, Yong EL and Halliwell B.** (2004). Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional chinese medicines. Free radical Biology & Medicine. 36:1575-1587.
- Tohda C, Matsumoto N, Zou K, Meselhy MR and Komatsu K.** (2004). Abeta(25-35)-induced memory impairment, axonal atrophy, and synaptic loss are ameliorated by M1, A metabolite of protopanaxadiol-type saponins. Neuropsychopharmacology. 29:860-868.
- Ueda K, Shinohara S, Yagami T, Asakura K and Kawasaki K.** (1997). Amyloid beta protein potentiates Ca_2^+ influx through L-type voltage-sensitive Ca_2^+ channels: A possible involvement of free radicals. Journal of Neurochemistry. 68:265-271.
- Um MY, Choi WH, Aan JY, Kim SR and Ha TY.** (2006). Protective effect of *Polygonum multiflorum* Thunb on amyloid beta-peptide 25-35 induced cognitive deficits in mice. Journal of Ethnopharmacology. 104:144-148.
- Wang Q, Yu S, Simonyi A, Rottinghaus G, Sun GY and Sun AY.** (2004). Resveratrol protects against neurotoxicity induced by kainic acid. Neurochemical Research. 29:2105-2112.
- Yan XZ, Qiao JT, Dou Y and Qiao ZD.** (1999). Beta-amyloid peptide fragment 31-35 induces apoptosis in cultured cortical neurons. Neuroscience. 92:177-184.