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Acer okamotoanum protects SH-SY5Y neuronal cells against hydrogen peroxide-induced oxidative stress

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Abstract Oxidative stress by over-production of reactive oxygen species (ROS) in brain is widely known as a cause of neurodegenerative disease. We investigated protective effects of *Acer okamotoanum* against oxidative stress by hydrogen peroxide (H_2O_2) in SH-SY5Y neuronal cells. *Acer okamotoanum* reduced ROS production and lactate dehydrogenase release in H_2O_2-induced SH-SY5Y cells, resulting in elevation of cell viability. To elucidate protective mechanisms, we measured inflammation and apoptosis-associated protein expressions. Treatment with *A. okamotoanum* dose-dependently decreased pro-inflammatory proteins such as inducible nitric oxide synthase and cyclooxygenase-2. Treatment with *A. okamotoanum* showed down-regulation of pro-apoptosis genes such as cleaved caspase-3, cleaved caspase-9, and Bax, and up-regulation of anti-apoptosis protein including Bcl-2, in H_2O_2-induced SH-SY5Y cells. We demonstrated potential anti-inflammatory and anti-apoptotic effect of *A. okamotoanum* in H_2O_2-induced SH-SY5Y cells. These results suggest that *A. okamotoanum* may possess neuroprotective potential, but further study is necessary to elucidate its pharmacological effects in neurodegenerative diseases.

Keywords *Acer okamotoanum* · Apoptosis · Hydrogen peroxide · Inflammation · Oxidative stress

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Introduction

Oxidative stress produced by excessive reactive oxygen species (ROS) is thought to be an important cause of various neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (Finkel and Holbrook, 2000; Gorman et al., 1996). ROS, including superoxide anions, hydroxyl radicals and hydrogen peroxide (H_2O_2), are generated in normal conditions, but imbalances, due to over production and accumulation of ROS, attack biological components such as proteins, lipids, and nucleic acids (Finkel and Holbrook, 2000). These effects may contribute to the pathology caused by mitochondrial dysfunction, inflammation, and apoptosis (Perticone et al., 1997; Somayajulu et al., 2005). ROS production activates pro-inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), to release inflammatory cytokines including interleukin (IL)-1β, IL-6, and IL-8 (Barnes and Karin, 1997). In addition, ROS-induced oxidative stress elevates the expression of pro-apoptotic proteins such as cleaved caspase-3, cleaved caspase-9 and Bcl-2-associated X protein (Bax), decreasing the expressions of anti-apoptotic proteins such as B cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra-large (Bcl-xl), resulting in neuronal cell death (Thayyullathil et al., 2008). Protection against oxidative stress by modulating inflammation and apoptosis is thought to be an important therapeutic strategy for neurodegenerative diseases.

Numerous studies have been attempted to establish neuroprotective effects of antioxidant from natural sources such as curcumin, green tea and *Ginkgo biloba* (Koo et al., 2004; Mandel et al., 2008; Shi et al., 2010). In addition, antioxidants from natural sources have attracted much attention due to their low toxicity and minimal side effects

compared with various synthetic antioxidants. *Acer* species have been used in traditional medicine against neuralgia, rheumatism, bruises, hepatic disorders, eye diseases, hemostasis, abnormal urination, constipation, and gastroenteric disorders for many years in East Asia and North America (Bae, 2000; Bi et al., 2016). In addition, various pharmacological studies have shown antioxidant, anticancer, anti-inflammatory and neuro-protective effects (Bi et al., 2016). *Acer okamotoanum* (*A. okamotoanum*) is a maple species found on the Korean island of Ulleungdo (Moon and Kwon, 2004). Several studies reported antioxidant, anti-cancer, immune improvement, and skin whitening activity of extracts from *A. okamotoanum* (Jeong et al., 2009; Jin et al., 2008; Qadir et al., 2007). Especially, *A. okamotoanum* demonstrated in vitro antioxidant activities via nitrite scavenging, superoxide dismutase-like activity, and glutathione S-transferase activity. In addition, active components from *A. okamotoanum*, such as flavonol glycosides and phenolic compounds, reported anti-oxidant and anti-cancer activity, as well as inhibition of HIV-1 integrase (Jin et al., 2006; Jin et al., 2007; Kim et al., 1998; Lee et al., 2018). In addition, we previously demonstrated that an ethyl acetate (EtOAc) fraction from *A. okamotoanum* improves cognition and memory function in Alzheimer's disease mouse model (Choi et al., 2017b). However, the neuro-protective activity and mechanism of *A. okamotoanum* in neuronal cells has not been investigated. Therefore, this study was designed to examine the protective effects and mechanisms of the EtOAc fraction from *A. okamotoanum* against H_2O_2-induced oxidative stress in SH-SY5Y neuronal cells.

Materials and methods

Reagents

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, and trypsin EDTA solution were purchased from Welgene (Daegu, Korea). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Bio Basic (Toronto, Canada) and dimethyl sulfoxide (DMSO) was purchased from Bio Pure (Ontario, Canada). Dichlorofluorescein diacetate (DCF-DA) was purchased from Sigma (St. Louis, MO, USA) and H_2O_2 was purchased from Junsei (Tokyo, Japan). RIPA buffer was purchased from Elpics Biotech (Daejeon, Korea), protease inhibitor cocktail from Calbiochem (Cambridge, MA, USA), and polyvinylidene fluoride (PVDF) membranes from Millipore (Bedford, MA, USA). Sample buffer and enhanced chemiluminescence (ECL) substrate solution were purchased from Bio-Rad (CA, USA). Primary antibodies such as iNOS, COX-2, IκBα, Bax, and Bcl-2 were obtained from Santa Cruz (CA, USA). Phospho-nuclear factor-kappaB (p-NF-κB), phosho IκBα (p-IκBα), β-Actin, caspase-3, -9, poly ADP ribose polymerase (PARP), and secondary antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

Sample preparation

Acer okamotoanum samples were collected from Ulleungdo, Republic of Korea. A voucher specimen has been deposited at the Department of Integrative Plant Science, Chung-Ang University, Anseong, Republic of Korea (Voucher No. LEE 2014-04). Dried aerial parts of *A. okamotoanum* were extracted five times in methanol (MeOH), and then dried using a rotary evaporator. The MeOH extract was fractionated with EtOAc and used in our study.

Cell culture

SH-SY5Y cells were obtained from the American Type Culture Collection (OH, USA). The cells were grown in DMEM containing FBS (10% v/v) and penicillin–streptomycin (1% v/v) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. The SH-SY5Y cells were seeded at a density of 5×10^4 cells/mL in 96-well plate, and then incubated for 24 h. Afterward, the test wells were treated with EtOAc fraction of *A. okamotoanum* at various concentrations (1, 5, 10, 25 and 50 μg/mL). After 2 h incubation at 37 °C, cells were treated with 300 μM H_2O_2 for 24 h.

Cell viability

Cell viability was determined using a MTT colorimetric assay (Mosmann, 1983). Cell culture media was replaced with medium containing MTT (5 mg/mL) in the wells. After 4 h of incubation, formazan crystals were dissolved in 200 μL of DMSO and the absorbance of each well was then read at 540 nm using a microplate reader (Thermo Fisher Scientific, Vantaa, Finland).

Reactive oxygen species (ROS) scavenging activity

Intracellular ROS production was measured using DCF-DA fluorescence assay (Wang and Zhu, 2003). After the culture supernatant had been removed, the cells were treated with the medium containing DCF-DA (80 μM). After 30 min of incubation at 37 °C, the fluorescence intensity was measured at an excitation − 485 nm and an emission − 535 nm using a fluorescence spectrophotometer (FLUOstar OPTIMA, BMG Labtech, Ortenberg,

Germany). Representative fluorescence images were obtained using fluorescent microscopy (Olympus BX50, Tokyo, Japan).

Lactate dehydrogenase (LDH) release activity

LDH release activity was measured using an LDH cytotoxicity detection kit (Clontech laboratories Inc., CA, USA), according to the manufacturer's protocol. Briefly, 100 µL of culture supernatant from each well were allowed to react with 100 µL of the LDH reaction solution, and then incubated at room temperature for 30 min. The optical densities of the samples were measured at 490 nm using a microplate reader (Racher et al., 1990).

Western blot analysis

The cells were harvested and washed with PBS. After centrifugation, cells were lysed in 100 µL of RIPA buffer containing a protease inhibitor cocktail. The lysate was incubated on ice for 30 min and centrifuged at 12,000 rpm for 30 min at 4 °C. After the supernatant was collected, protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad, CA, USA), and added to an equivalence volume of sample buffer. Equal amounts of protein (30 µg) in each sample were electrophoresed using 10% or 13% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes. The membranes were blocked with 5% skim milk in PBS-T for 1 h at room temperature, and then incubated overnight with primary antibodies at 4 °C. After washing the membrane with PBS-T, it was incubated with the appropriate HRP-conjugated secondary antibodies at room temperature for 1 h. The membrane was activated with ECL substrate solution, according the manufacturer's instructions and visualized with the Davinch-chemi^{TM} Chemiluminescence Imaging System (Core Bio, Seoul, Korea).

Statistics analysis

Results are presented as the mean ± standard deviation (SD). The statistical significance of data among the group differences was tested using one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test and Student's *t*-test. Statistical significance was considered for P values < 0.05.

Results and discussion

Recently, many researchers have shown that oxidative stress is the cause of neurodegenerative diseases, and natural antioxidants have attracted much attention for use in the therapeutic strategy for neurodegenerative diseases. To search for natural antioxidants, ROS-induced in vitro cellular models are commonly used (González-Sarrías et al., 2016; Zhou et al., 2014). H_2O_2 is widely known as one of the most important toxic inducers of oxidative stress, which

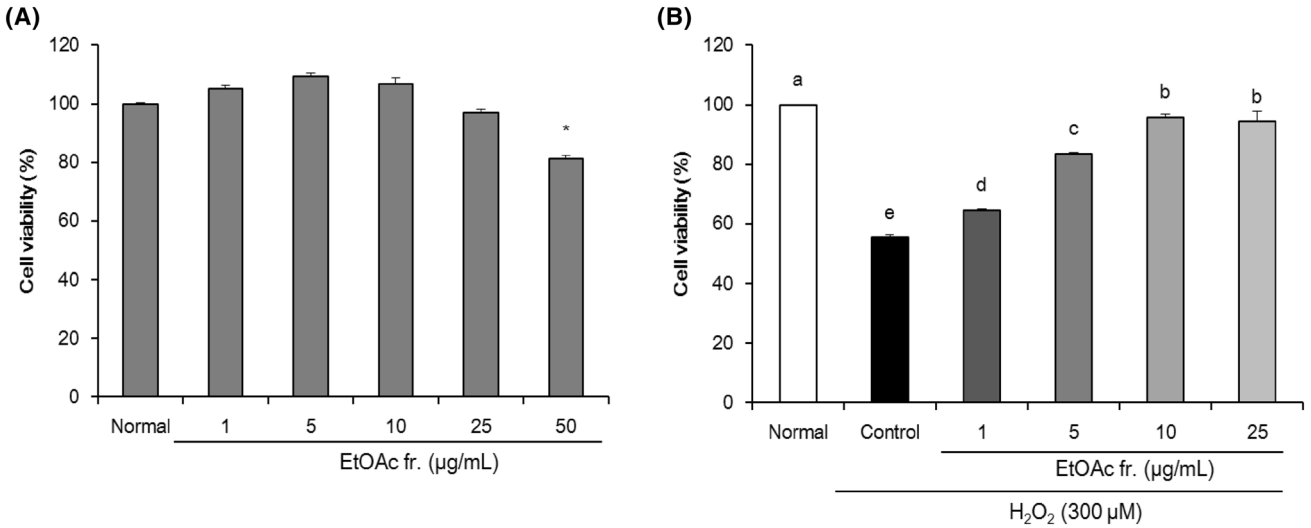

Fig. 1 Effects of the EtOAc fraction from *A. okamotoanum* on SH-SY5Y cell viability. (**A**) SH-SY5Y cells were seeded and cultured for 24 h. Cells were treated with the various concentrations (1, 5, 10, 25, 50 µg/mL) of the EtOAc fraction from *A. okamotoanum* for 24 h. Values are expressed as the mean ± SD. *$P < 0.05$ compared to the normal group by Student's *t*-test. (**B**) SH-SY5Y cells were seeded and cultured for 24 h. Cells were treated with various concentrations (1, 5, 10, 25 µg/mL) of EtOAc fraction from *A. okamotoanum*, and then with 300 µM H_2O_2 for 24 h. Values are expressed as the mean ± SD. [a–e]Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

produces highly reactive hydroxyl radicals, resulting in cellular injury (Tabner et al., 2001). In addition, accumulation of H_2O_2 has been observed in neurodegenerative disease with inflammation, necrosis, and apoptosis in the brain (Finkel and Holbrook, 2000; Li et al., 2003). Human neuroblastoma SH-SY5Y cells are widely used as a cellular model for research on H_2O_2-induced oxidative stress (Nirmaladevi et al., 2014; Shin et al., 2007).

Natural plants contain various bioactive compounds. Especially, potential antioxidant activities are dependent on hydroxyl groups of hydrophilic bioactive compounds (Miller and Rice-Evans, 1997). Therefore, to extract a large quantity of hydrophilic bioactive compounds, we used MeOH and EtOAc. In addition, we previously reported that the EtOAc fraction from *A. okamotoanum* has higher in vitro free radical scavenging activities against DPPH and O_2^- and higher total phenolic and flavonoid contents, compared with n-BuOH, methylene chloride and n-hexane fractions, and the crude MeOH extract (Choi et al., 2017a). Furthermore, the EtOAc fraction of *A. okamotoanum* led to a significant protective effect in oxidative stress-induced C6 glial cells among other extracts and fractions (Choi et al., 2017a). The most active compounds from the EtOAc fraction of *A. okamotoanum* leaves were flavonol glycosides such as quercetin-3-O-β-D-galactopyranoside and quercetin-3-O-α-L-arabinopyranoside (Choi et al., 2017b). Kim et al. reported that many flavonoid glycosides containing galloyl isolated from the EtOAc fraction of *A. okamotoanum* leaves strongly inhibit HIV-1 integrase. Furthermore, two coumarins, cleomiscosin A and C, isolated from the EtOAc fraction from leaves and twigs of *A. okamotoanum*, led to strong anti-oxidant activities (Jin et al., 2007). In addition, we also isolated and identified active components such as afzelin, quercitrin, and isoquercitrin from the EtOAc fraction of *A. okamotoanum*

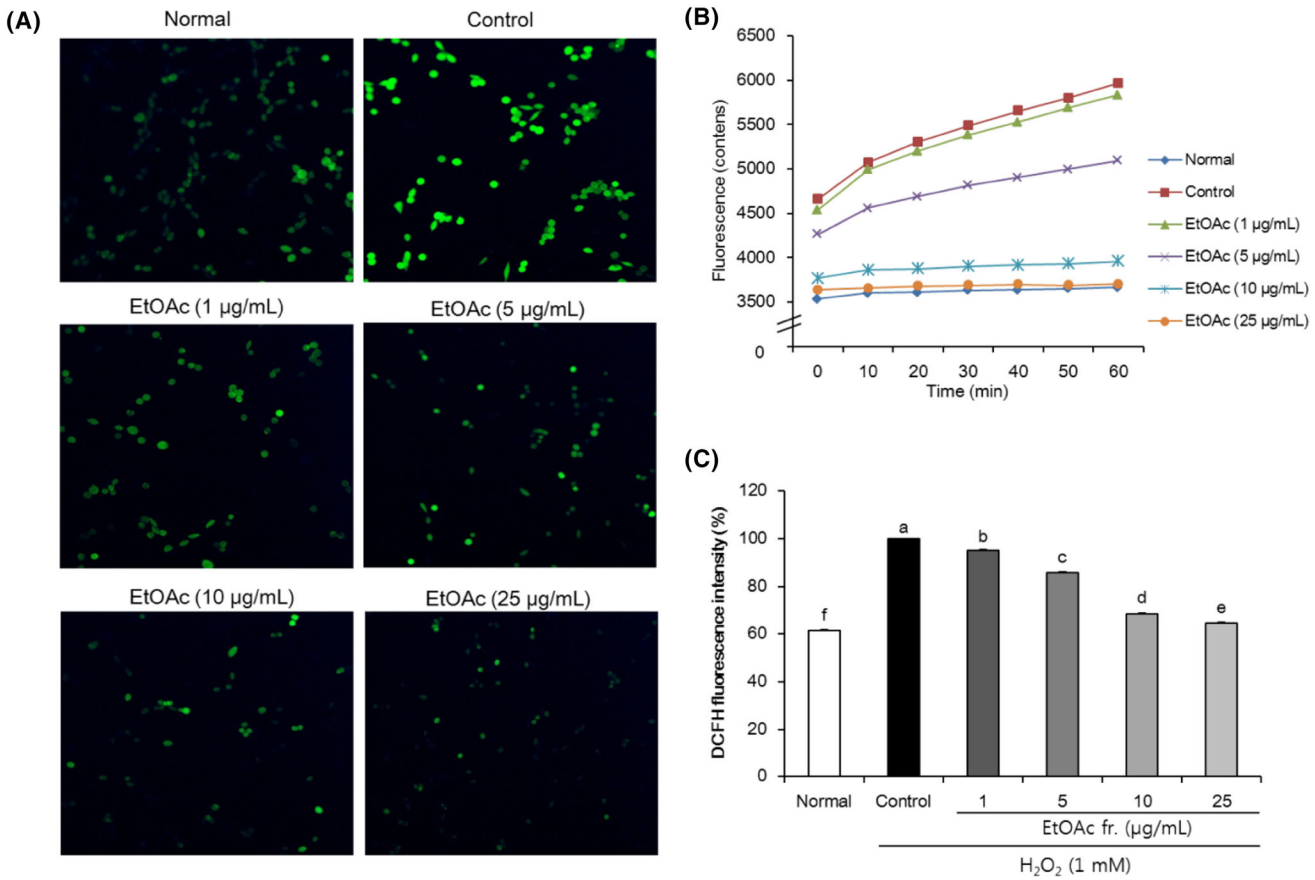

Fig. 2 Effects of the EtOAc fraction from *A. okamotoanum* on ROS levels in SH-SY5Y cells treated with H_2O_2. SH-SY5Y cells were seeded and cultured for 24 h. Cells were treated with various concentrations (1, 5, 10, 25 μg/mL) of the EtOAc fraction from *A. okamotoanum*, and then with 1 mM H_2O_2 for 24 h. ROS production was determined using the DCF-DA assay followed by fluorescence spectrophotometry (excitation—485 nm; emission—535 nm). (**A**) The representative fluorescence images under observation by fluorescent microscopy (×100 magnification). (**B**) Time course of change in intensity of ROS fluorescence within 60 min. (**C**) The intensity of ROS fluorescence at 60 min. Values are mean ± SD. $^{a–f}$Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

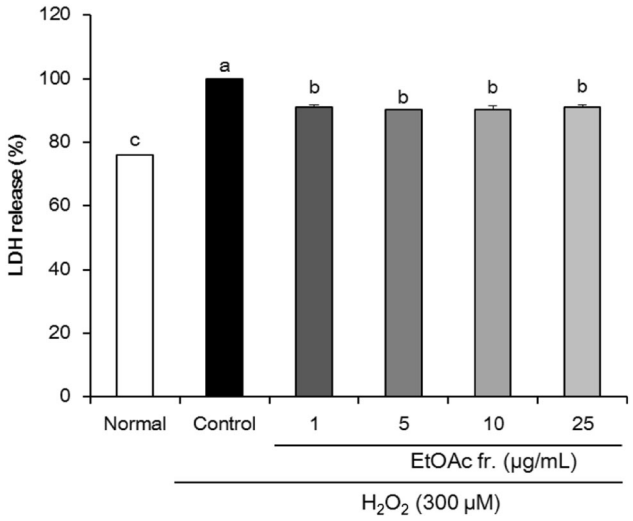

Fig. 3 Effects of the EtOAc fraction from *A. okamotoanum* on LDH release in SH-SY5Y cells treated with H_2O_2. SH-SY5Y cells were treated with various concentrations (1, 5, 10, 25 μg/mL) of the EtOAc fraction from *A. okamotoanum*, and then with 300 μM H_2O_2 for 24 h. LDH release was determined using an LDH assay. Values are expressed as the mean ± SD. [a–c]Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

(Lee et al., 2018). On the basis of these findings, the EtOAc fraction was used.

In this study, we demonstrated the protective activity of the EtOAc fraction from *A. okamotoanum* against H_2O_2-induced inflammation and apoptosis in SH-SY5Y cells via regulation of inflammatory and apoptotic gene expression. As shown in Fig. 1(A), cytotoxicity in SH-SY5Y cells was not observed up to the EtOAc fraction concentration of 25 μg/mL. At 50 μg/mL, cell viability was decreased to 81.31% of control cells. Therefore, we tested the EtOAc fraction from *A. okamotoanum* at concentrations up to 25 μg/mL in SH-SY5Y cells exposed to H_2O_2, and measured cell viability using the MTT assay. As illustrated in Fig. 1(B), the H_2O_2-treated control group showed significantly decreased cell viability of 55.31% compared with the non-treated normal group of 100.00%. However, treatment with the EtOAc fraction from *A. okamotoanum* showed dose-dependent increase in cell viability compared with H_2O_2-treated SH-SY5Y cells. In particular, the 10 and 25 μg/mL concentrations of the EtOAc fraction from *A. okamotoanum* increased cell viability from 55.31 to 95.77 and 94.43%, respectively. Li et al., (2003) demonstrated that SH-SY5Y cell viability was decreased by treatment with H_2O_2 (Li et al., 2003). Our results showed that H_2O_2 treatment led to a loss of cell viability in SH-SY5Y cells. However, cell viability was dose-dependently elevated by the treatment of various concentrations of EtOAc fraction from *A. okamotoanum.*

To evaluate the inhibition of ROS production associated with the EtOAc fraction from *A. okamotoanum* in H_2O_2-exposed SH-SY5Y cells, the DCF-DA assay was performed. As shown in Fig. 2, the H_2O_2-treated control group showed significantly increased ROS production. Figure 2(B) showed that ROS production was steadily increased in the control group by H_2O_2 exposure in a time-dependent manner during 60 min, indicating that H_2O_2 led to oxidative stress. Figure 2(C) showed the amount of ROS production at 60 min. However, the EtOAc fraction of *A. okamotoanum*, at the concentrations of 5, 10 and 25 μg/mL, produced dose-dependent declines in H_2O_2-induced accumulation of ROS, averaging 85.83, 68.33, and 64.70%, respectively. We also showed that H_2O_2 could evoke ROS-induced cytotoxicity in SH-SY5Y cells. Previous research found that SH-SY5Y cells increase ROS production mediated by H_2O_2 (Garcimartín et al., 2014; Park et al., 2015). However, the presence of the EtOAc fraction from *A. okamotoanum* alleviated H_2O_2 neurotoxicity by a reduction of ROS.

Figure 3 shows the protective effects of EtOAc fraction from *A. okamotoanum* against toxicity in the LDH assay. LDH release in the H_2O_2-treated control group was significantly elevated to 100.00%, compared with the non-treated normal group with release of 76.13%. However, treatment with the EtOAc fraction from *A. okamotoanum* in H_2O_2-induced SH-SY5Y cells significantly decreased H_2O_2-induced LDH release. LDH is an intracellular enzyme present in cells. Increased LDH in culture medium indicates damage to cellular membranes and is a marker for cell death (Hansen et al., 1989). Consistent with another study (Garcimartín et al., 2014), our data demonstrated that LDH leakage into the culture medium was increased in H_2O_2-induced SH-SY5Y cells. Treatment with the EtOAc fraction from *A. okamotoanum* significantly reduced LDH in the medium of SH-SY5Y cells exposed to H_2O_2. Therefore, we confirmed the protective effects of the EtOAc fraction from *A. okamotoanum* in H_2O_2-exposed SH-SY5Y cells, through measured concentration-dependent increases in cell viability, decline in ROS, and decreased LDH release.

To further evaluate the protective molecular mechanisms of the EtOAc fraction from *A. okamotoanum*, we measured inflammation-related protein expressions such as iNOS, COX-2, p-IκBα, and p-NF-κB. As shown in Fig. 4, the H_2O_2-treated control group showed significantly up-regulated expressions of iNOS, COX-2, p-NF-κB, and an increase in the ratio of p-IκBα/IκBα. However, treatment with the EtOAc fraction from *A. okamotoanum* in H_2O_2-induced SH-SY5Y cells showed significant and dose-dependent decreased expression of iNOS, COX-2, p-NF-κB, and a decrease in the ratio of p-IκBα/IκBα, compared with the control group. The NF-κB pathway is widely known as

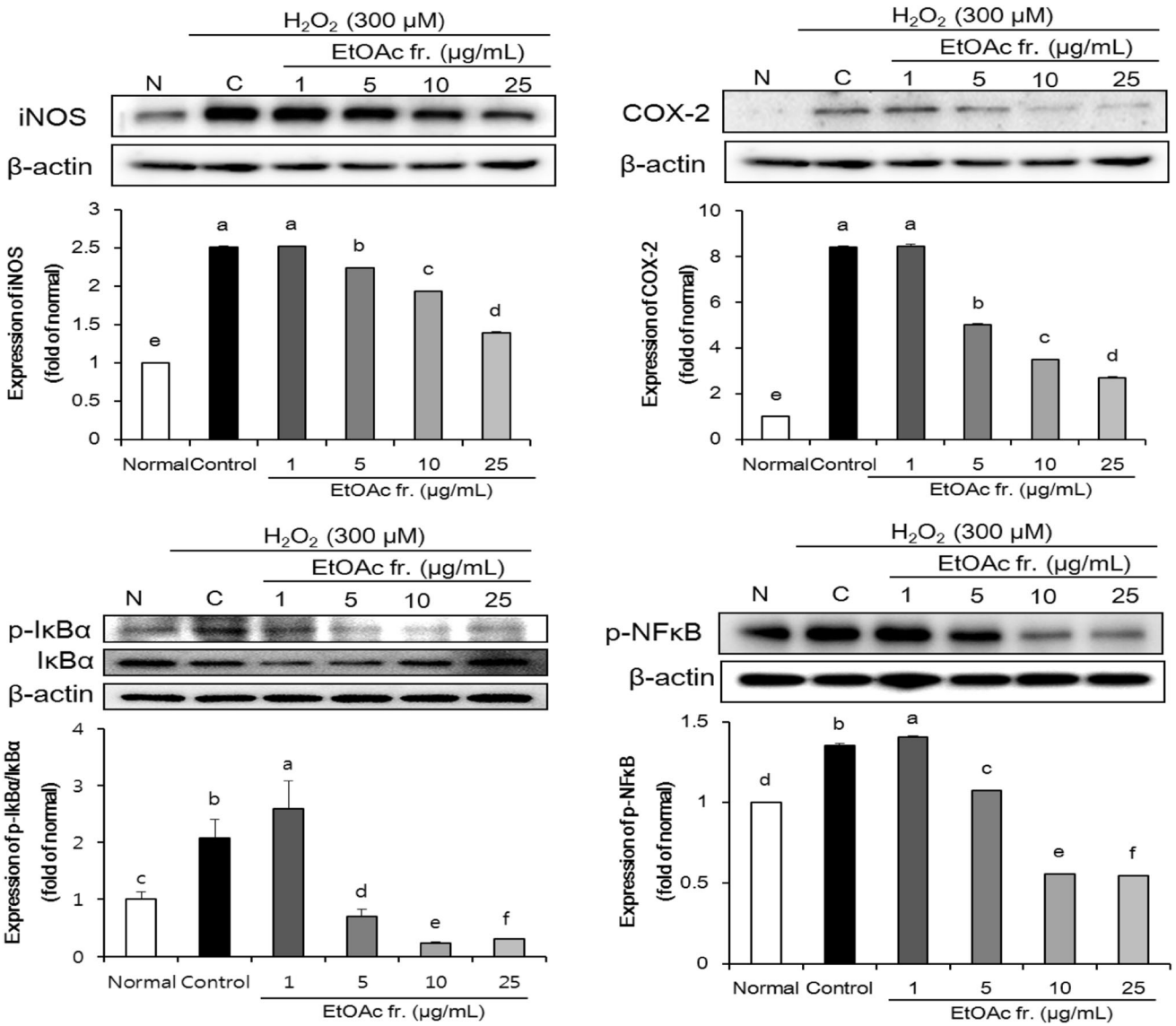

Fig. 4 Effects of the EtOAc fraction from *A. okamotoanum* on iNOS, COX-2, p-IκBα, IκBα, and p-NFκB protein expressions in H_2O_2-induced SH-SY5Y cells. SH-SY5Y cells were treated with concentrations (1, 5, 10, 25 μg/mL) of the EtOAc fraction from *A. okamotoanum*, and then with 300 μM H_2O_2 for 24 h. Protein expressions were measured using western blotting. Values are expressed as the mean ± SD. [a–e]Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. β-actin was used as a loading control. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

an important pro-inflammatory mediator and plays a role in neurodegenerative diseases (Amor et al., 2010; Tilstra et al., 2014). Under normal conditions, NF-κB is present in the cytosol bound to IκB inhibitors (NF-κB/IκB complex). However, when cells were stimulated by oxidative stress, activated IκB-α activates the translocation of NF-κB from the cytosol to the nuclei (Amor et al., 2010). Activated NF-κB can induce over-expression of inflammatory mediators, including iNOS and COX-2 (Jung et al., 2009). The over expression of COX-2 and iNOS leads to production of various inflammatory cytokines that are associated with degenerative diseases (Minghetti, 2004). Therefore, we observed expressions of iNOS, COX-2, IκBα, p-IκBα, and p-NF-κB in H_2O_2-induced SH-SY5Y cells. Our study demonstrated that the EtOAc fraction from *A. okamotoanum* significantly attenuated expressions of iNOS and COX-2 via the NF-κB pathway. Especially, gene expression levels of iNOS and COX-2 in H_2O_2-induced SH-SY5Y cells were reduced in a concentration-dependent manner by treatment with the EtOAc fraction from *A. okamotoanum*.

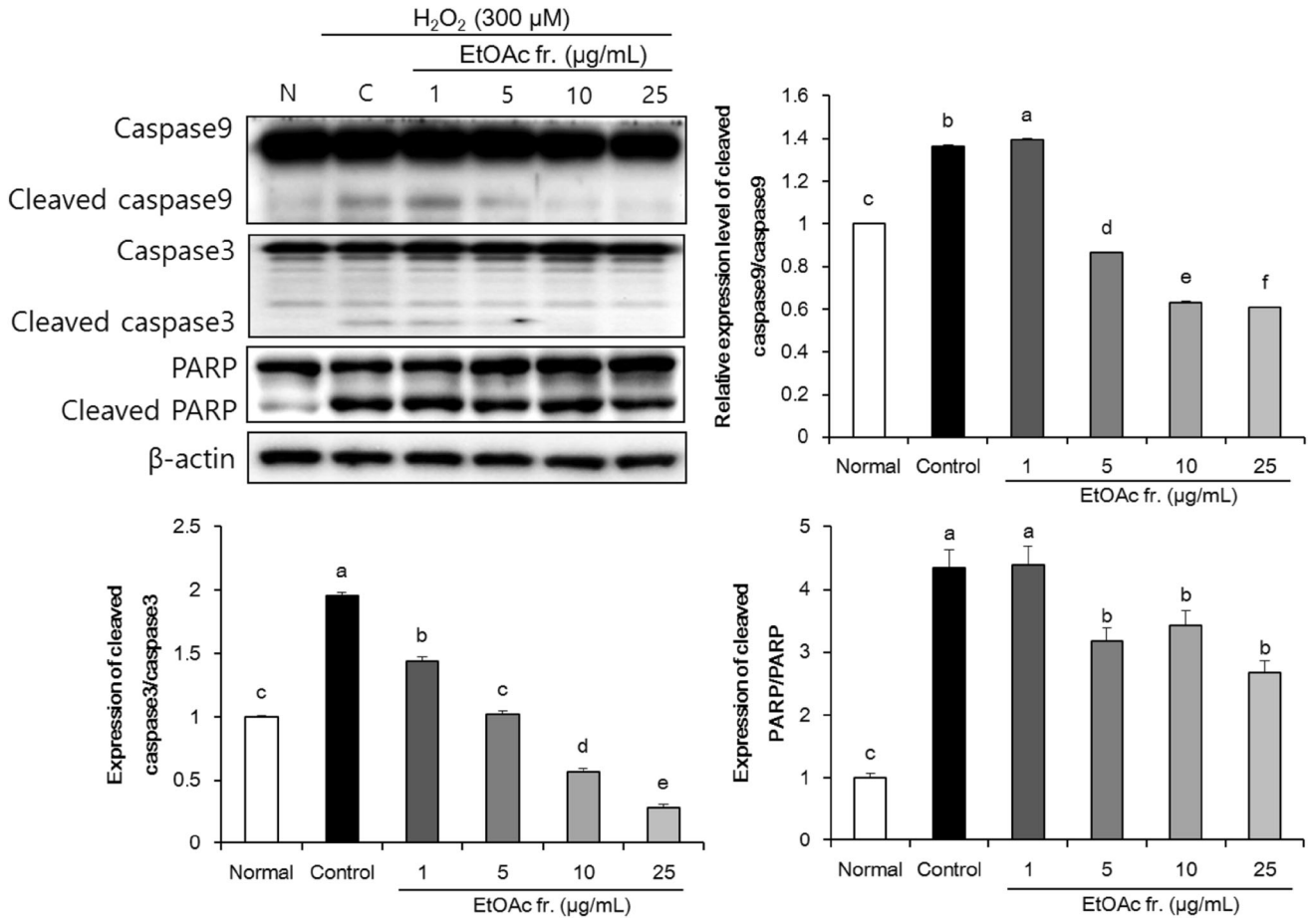

Fig. 5 Effects of the EtOAc fraction from *A. okamotoanum* on caspase-9, caspase-3 and PARP protein expressions in H_2O_2-induced SH-SY5Y cells. Cells were treated with concentrations (1, 5, 10, 25 μg/mL) of the EtOAc fraction from *A. okamotoanum*, and then with 300 μM H_2O_2 for 24 h. Protein expressions were measured using western blotting. Values are expressed as the mean ± SD. $^{a–f}$Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. β-actin was used as a loading control. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

To identify the protective mechanisms of the EtOAc fraction from *A. okamotoanum* against H_2O_2-induced apoptosis, we investigated protein expression of pro- and anti- apoptotic genes using western blotting. As shown in Fig. 5, the expressions of cleaved caspase-9, caspase-3, and PARP were increased in the H_2O_2-exposed control group compared with the untreated normal group. However, in the groups treated with the EtOAc fraction from *A. okamotoanum,* dose-dependent down-regulation of the over-expression of cleaved caspase-9, caspase-3, and PARP was observed. In addition, Fig. 6 illustrates the expression of the pro-apoptotic gene, Bax, and the anti-apoptotic gene, Bcl-2. The H_2O_2-treated control group showed an increased ratio of Bax to Bcl-2 via enhancement of the expression of Bax and decreasing the expression of Bcl-2, compared with the normal group. However, the EtOAc fraction from *A. okamotoanum* significantly decreased the ratio of Bax to Bcl-2. Oxidative stress-induced neuronal cell death is associated with the mitochondria-related apoptotic pathway in H_2O_2-induced SH-SY5Y cells, and is an important mechanism in neurodegenerative diseases (Hu et al., 2015). The overproduction of ROS leads to increased permeability of the mitochondrial membrane, thus resulting in release of cytochrome C. Released cytochrome C activates pro-apoptosis factors, such as caspase-9, caspase-3 and PARP, eventually inducing cell death (Hu et al., 2015; Wang et al., 1998). Our results suggest up-regulation of proteolytic cleavage of caspase-9, caspase-3 and PARP following treatment with H_2O_2. However, the EtOAc fraction from *A. okamotoanum* protects neuronal cells against H_2O_2 by down-regulation of caspase-9, caspase-3, and PARP. In addition, mitochondrial membrane injury by oxidative stress leads to neuronal apoptosis by modulating expression of the Bcl-2 gene

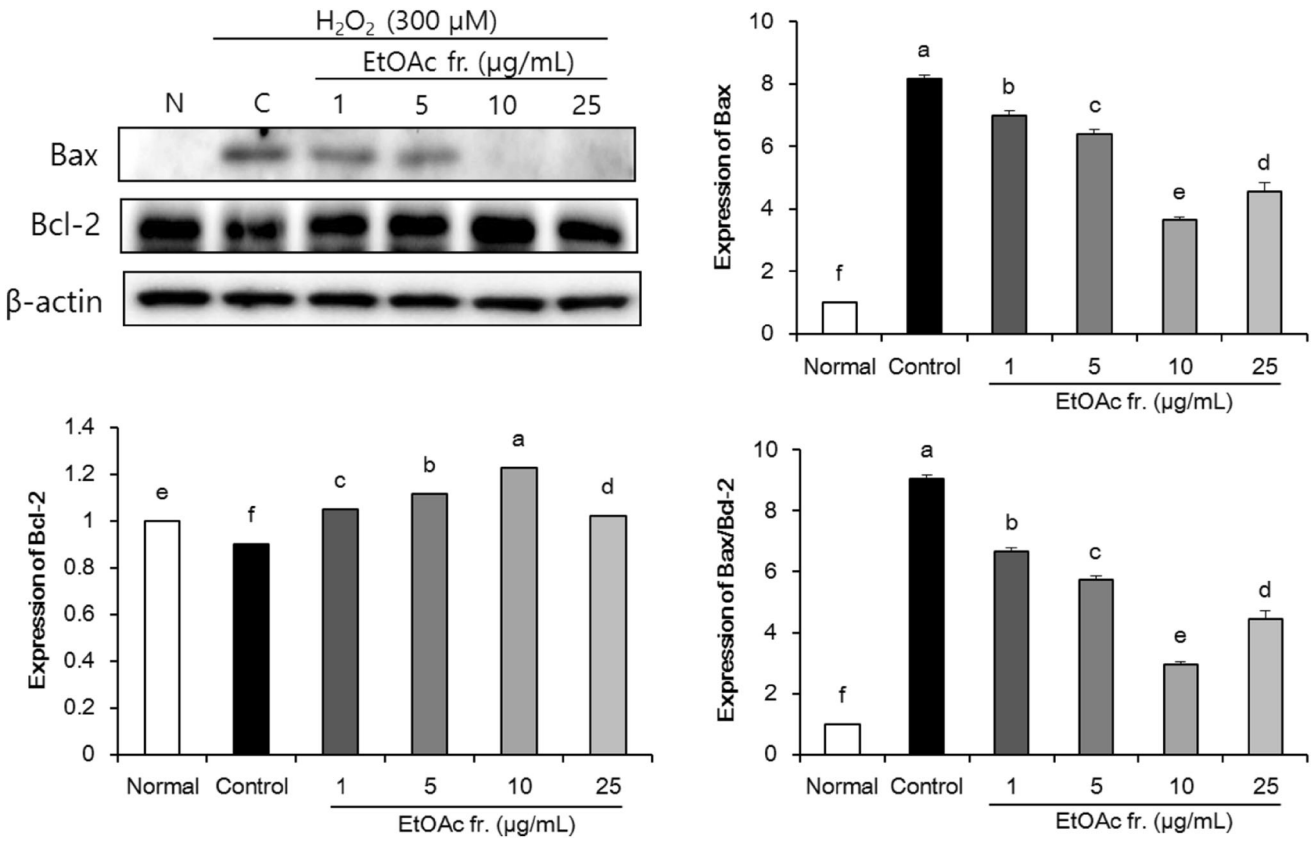

Fig. 6 Effects of the EtOAc fraction from *A. okamotoanum* on Bax and Bcl-2 protein expressions in H_2O_2-induced SH-SY5Y cells. Cells were treated with concentrations (1, 5, 10, 25 μg/mL) of the EtOAc fraction from *A. okamotoanum*, and then with 300 μM H_2O_2 for 24 h. Protein expressions were measured using western blotting. Values are expressed as the mean ± SD. [a–f]Means with different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. β-actin was used as a loading control. Normal group indicates untreated cells, whereas control group indicates the cells exposed to H_2O_2 alone

family (Pastorino et al., 1998; Zhang et al., 2007). Similar to our results, previous research showed that H_2O_2-treated SH-SY5Y cells increased pro-apoptotic gene expression such as Bax, and lowered anti-apoptotic gene such as Bcl-2 (Zhang et al., 2007). The present results indicate that the EtOAc fraction from *A. okamotoanum* could not only significantly reduce Bax, but also increase Bcl-2. Therefore, the EtOAc fraction from *A. okamotoanum* protects oxidative stress by regulation of inflammation and apoptosis in SH-SY5Y cells.

In conclusion, this study demonstrated that the EtOAc fraction from *A. okamotoanum* prevents H_2O_2-induced oxidative stress in SH-SY5Y cells. The treatments with the EtOAc fraction elevated cell viability by decreasing ROS production and LDH release. In addition, the EtOAc fraction from *A. okamotoanum* protects SH-SY5Y cells from oxidative stress-induced inflammation and apoptosis by modulating the NF-κB pathway, inhibiting caspase activity and regulating the Bax/Bcl-2 ratio. The results suggest that *A. okamotoanum* may possess neuroprotective potential, but further study is necessary to elucidate the pharmacological effects in neurodegenerative diseases.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interests.

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