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Apigenin Ameliorates Oxidative Stress-induced Neuronal Apoptosis in SH-SY5Y Cells

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The overproduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS) causes oxidative damage to neuronal cells, leading to the progression of neurodegenerative diseases. In this study, we determined the nitric oxide radical (NO), hydroxyl radical (·OH), and superoxide anion radical (O_2^-) scavenging activities of apigenin. Our results showed that apigenin exhibited remarkable, concentration-dependent ·OH, O_2^-, and NO radical scavenging activities. Particularly, apigenin indicated the strongest ·OH radical scavenging activity with 93.38% in the concentration of 100 μM. Furthermore, we also investigated the protective effects of apigenin against hydrogen peroxide (H_2O_2)-induced oxidative stress in SH-SY5Y cells. The H_2O_2 treatment resulted in a significant decrease in cell viability, as well as an increase in lactate dehydrogenase (LDH) release and ROS production compared with the H_2O_2-nontreated SH-SY5Y cells. However, the cell viability significantly increased in the apigenin-treated group, as well as inhibited ROS generation and LDH release compared with the H_2O_2-induced control group. To elucidate the protective mechanisms of apigenin against oxidative stress in SH-SY5Y, we analyzed the apoptosis-related protein expression. The apigenin treatment resulted in the downregulated expression of apoptosis-related protein markers, such as cytochrome C, cleaved caspase-3, poly (ADP)-ribose polymerase (PARP), and B-cell lymphoma 2-associated X (Bax), as well as the upregulated expression of anti-apoptosis markers such as B-cell lymphoma 2 (Bcl-2). In this study, we report that apigenin exhibits a neuroprotective effect against oxidative stress in SH-SY5Y cells. These results suggest that apigenin may be considered as a potential agent for neurodegenerative disease prevention.

Keywords: **Hydrogen peroxide, neuronal, neurodegenerative disease, neuroprotection, oxidative stress**

Introduction

Oxidative stress is defined as out of balance between the elimination and production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. ROS and RNS, such as hydroxyl radical (·OH), nitric oxide radical (NO), hydrogen peroxide (H_2O_2), and superoxide anion radical (O_2^-) are produced by external environmental factors and biochemical reactions [2]. Moderate levels of ROS and RNS play an essential role, either directly or indirectly, in health through mechanisms including cellular signaling, biosynthetic processes, and host defenses [3]. However, overproduction of ROS and RNS is thought to be responsible for damages to lipids, proteins, and DNA, leading to oxidative stress [4]. Oxidative stress can induce neuronal apoptosis and neuroinflammation in brain cells, resulting in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [5, 6]. The brain is especially more vulnerable to oxidative stress than other organs, on account of its high oxygen consumption and

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amount of polyunsaturated fatty acids. The neurons are especially sensitive to free radicals, which can lead to cell death [7].

SH-SY5Y neuronal cells have been widely used to characterize neuron-like behavior when investigating potential neuroprotective activity [8]. Recently, studies have confirmed that oxidative stress contributes to apoptosis and inflammation in SH-SY5Y neuronal cells [9, 10]. H_2O_2 is related to pathological processes of chronic and acute neuronal toxicity [11]. ROS production and apoptosis-related protein expression of Bax, caspase-3 and cytochrome C are induced by H_2O_2 in SH-SY5Y cells [12]. Therefore, H_2O_2-induced SH-SY5Y cells are well used to research the promising agents with protective effects from oxidative stress.

Apigenin (4′,5,7-trihydroxyflavone), non-mutagenic and non-toxic natural plant flavonoid, is found widely in plants, vegetables, tea leaves, and fruits [13, 14]. Previous studies demonstrated that apigenin has beneficial effects, such as anti-oxidant [15], anti-inflammation [16], anti-apoptosis activity [17], and anti-cancer properties [18]. In addition, administration of apigenin improved the memory deficit of amyloid beta (Aβ) transgenic mice [19]. Apigenin attenuated lipopolysaccharide-induced myocardial damage by inhibiting inflammatory cytokines and their regulatory factor, nuclear factor kappa B [20]. Apigenin has also been reported to inhibit apoptosis through the regulation of mitogen-activated protein kinase (MAPK) induced by Aβ in neuronal cells [21]. However, the protective effect of apigenin for H_2O_2-induced neurotoxicity and neuronal apoptosis in SH-SY5Y cells have not been examined yet. Thus, the current study investigated the O_2^-, ·OH, and NO radical scavenging effects of apigenin *in vitro*. In addition, the protective effect and underlying mechanisms of apigenin against oxidative damage induced by H_2O_2 were confirmed using SH-SY5Y neuronal cells.

Materials and Methods

Reagents

Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 2′,7′-dichloro-fluorescein diacetate (DCF-DA) were obtained from Sigma-Aldrich Inc. (USA). Sodium pentacyanonitrosylferrate (III) dihydrate (SNP) and H_2O_2 were supplied by Junsei Chemical Co. (Japan). The LDH cytotoxicity detection kit was obtained from Takara Bio (Japan). Trichloroacetic acid (TCA) was purchased from Biosesang Inc. (Korea). Radioimmunoprecipitation assay (RIPA) buffer was purchased from Elpis Biotech. (Korea). Phenazine methosulfate (PMS) and NADH were obtained from Bio Basic Inc. (USA). Polyvinylidene fluoride (PVDF) membrane was obtained from Millipore Co. (USA). Thiobarbituric acid (TBA) was purchased from Acros Organics Inc. (USA).

Sample preparation

Apigenin (4′,5,7-trihydroxyflavone) was obtained from Sigma-Aldrich Inc. and had a purity > 95% based on HPLC analysis. Apigenin was dissolved in DMSO as a stock solution and diluted with dulbecco's modified eagle's medium (DMEM) prior to use.

·OH scavenging assay

·OH radical scavenging activity was determined follow to the method reported by Chung *et al.* [22]. Various concentrations of apigenin (25, 50, 100, and 250 μM) were added to the reaction mixture containing 10 mM H_2O_2 and 10 mM $FeSO_4$·H_2O-EDTA with 10 mM 2-deoxyribose solution and incubated at 37℃ for 4 h. After incubation, 1.0% TBA and 2.8% TCA were added to the mixture and boiled for 20 min at 100℃. ·OH radical scavenging ability was measured at 490 nm by an RT-6100 microplate reader (Rayto Life and Analytical Sciences Co. Ltd., China).

O_2^- scavenging assay

O_2^- radical scavenging activity was estimated in accordance of the method reported by Nishikimi *et al.* [23]. Various concentrations of apigenin (25, 50, 100, and 250 μM) were added to the reaction mixture containing 100 μl of 0.1 M Tris-HCl (pH 7.4), 200 μl of 100 mM PMS, 400 μl of 500 mM NADH and 200 μl of 0.5 mM NBT. After 10 min, the absorbance was read at 560 nm utilizing an RT-6100 microplate reader (Rayto Life and Analytical Sciences Co. Ltd.).

NO scavenging assay

The NO radical scavenging activity of apigenin was measured following to the method reported by Marcocci *et al.* [24]. Various concentrations of apigenin (25, 50,

100, and 250 μM) diluted in MeOH were mixed with 10 mM SNP, and incubated at room temperature for 150 min. Griess reagent was added at a ratio of 1:1 in a 96-well plate for 30 min at room temperature. The absorbance was measured at 540 nm using an RT-6100 microplate reader (Rayto Life and Analytical Sciences Co. Ltd.).

Cell culture

SH-SY5Y cells were purchased from KCLB (Korea Cell Line Bank, Korea). The cells were cultured with 100 units/ml penicillin-streptomycin and 10% fatal bovine serum based DMEM at 37℃ with 5% CO_2 in an incubator. The culture medium was replaced three times per week. Cells were sub-cultured with 0.05% trypsin-EDTA in phosphate buffered saline (PBS). Cells were used for experimentation when confluence was ~80%.

MTT assay

Cells were seeded in a 96-well plate that at a density of 5×10^4 cells/ml and cultured for 24 h. The cells were treated with different concentrations of apigenin (0.1, 0.5, and 1 μM) for 3 h, followed by 300 μM of H_2O_2 treatment for 24 h to induce oxidative stress. After incubation, 5 mg/ml MTT solution was added and allowed to react for 4 h. The incorporated formazan crystals in the viable cells were solubilized using DMSO. The absorbance was read at 540 nm using an RT-6100 microplate reader (Rayto Life and Analytical Sciences Co. Ltd.).

DCF-DA assay

To measure the ROS scavenging activity, the DCF-DA assay was used [25]. SH-SY5Y cells were seeded in 96-well plate at a density of 5×10^4 cells/ml and cultured for 24 h. Apigenin was added to each well in diverse concentrations (0.1, 0.5, and 1 μM) and incubated for 3 h. To induce oxidative stress, 300 μM of H_2O_2 was added for 24 h. After incubation, 80 μM DCF-DA was added and left to react for 30 min. Fluorescence was read using a fluorescence spectrophotometer (FLUOstar OPTIMA, BMG Labtech, Germany) at an excitation of 480 nm and an emission of 535 nm.

LDH release assay

SH-SY5Y cells were seeded in a 96-well plate at a density of 5×10^4 cells/ml. Apigenin (0.1, 0.5, and 1 μM) was added for 3 h, followed by treatment with 300 μM H_2O_2. The supernatant and reaction mix were added to a 96-well plate and incubated at room temperature for 30 min. Absorbance was read at 540 nm by an RT-6100 microplate reader (Rayto Life and Analytical Sciences Co. Ltd.).

Western blotting analysis

SH-SY5Y cells were seeded in a cell culture dish and incubated for 24 h. After incubation, the cells were pretreated with apigenin (0.1, 0.5, and 1 μM) for 3 h and treated with 300 μM of H_2O_2 for another 24 h. Protein extracts from the SH-SY5Y cells were prepared using lysis buffer. Proteins were separated by electrophoresis using 10% or 13% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE), then transferred to the PVDF membranes. Proteins were blocked using 5% skimmed milk for 60 min, and washed with PBS. Membranes were incubated with the primary antibody overnight at 4℃ [each antibody at a dilution of 1:1000; poly (ADP)-ribose polymerase (PARP), cleaved caspase-3, cytochrome C, B-cell lymphoma 2-associated X (Bax), B-cell lymphoma 2 (Bcl-2), and β-actin]. Membranes were then incubated with the appropriate HRP-conjugated secondary antibodies for 1 h. Expression of protein was visualized utilizing a chemiluminescent imaging system (Davinci Chemi, Korea).

Statistical analysis

Results were stated as means ± standard deviation (SD). SPSS (version 25; SPSS Inc., USA) was used to perform analysis of variance (ANOVA), followed by

Table 1. ·OH, O_2^-, and NO radical scavenging activity of apigenin.

Treatment (μM)	Scavenging activity (%)		
	·OH radical	O_2^- radical	NO radical
25	91.72 ± 0.35[b]	3.33 ± 0.79[d]	42.15 ± 0.29[d]
50	93.00 ± 0.46[a]	9.62 ± 1.00[c]	46.32 ± 0.57[c]
100	93.38 ± 0.25[a]	17.41 ± 1.11[b]	48.93 ± 0.57[b]
250	92.75 ± 0.56[a]	32.14 ± 1.35[a]	50.34 ± 0.35[a]

Values are mean ± SD.
[a-d]Means are significantly different ($p < 0.05$) by Duncan's multiple range test.

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Duncan's post-hoc tests. Significance was set at $p < 0.05$.

Results

Effect of apigenin on ·OH, O_2^-, and NO radical scavenging activity

The ·OH, O_2^-, NO radical scavenging activity of apigenin is shown in Table 1. All concentrations of apigenin (25, 50, 100, and 250 μM) showed more than 90% ·OH radical scavenging activities. Of note, the strongest ·OH radical scavenging activity was 93.38% at the concentration of 100 μM. Apigenin also showed O_2^- radical scavenging ability concentration-dependently. Apigenin revealed dose-dependent radical scavenging activity against NO. Furthermore, apigenin showed a NO radical scavenging effect of 50.34% at 250 μM. Therefore, apigenin has antioxidant capabilities by the scavenging of ·OH, O_2^-, and NO radicals.

Protective effect of apigenin on cell viability in SH-SY5Y cells treated with H_2O_2

The effect of apigenin on cell viability of SH-SY5Y cells treated with H_2O_2 was investigated by the MTT assay (Fig. 1). The results indicated that H_2O_2-treated cells were significantly decreased cell viability to 50.26% compared to H_2O_2-non treated cells (100%). However, treatment with apigenin increased cell viability to 66.28%, 82.69%, and 85.16% at 0.1, 0.5, and 1 μM, respectively.

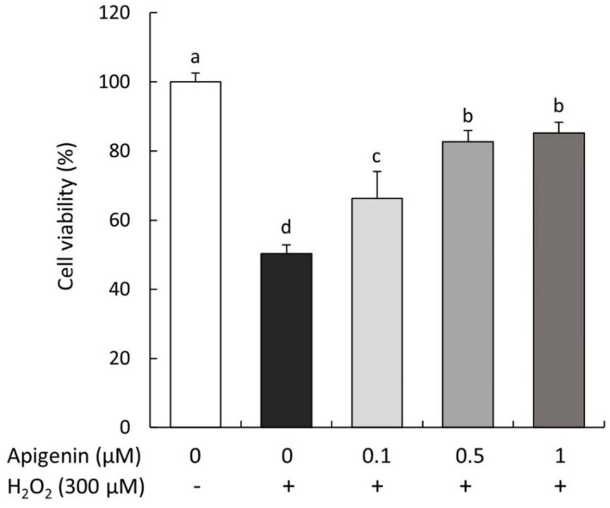

Fig. 1. Effect of apigenin on cell viability in SH-SY5Y cells treated with H_2O_2. Values are mean ± SD. [a-d]Means are significantly different ($p < 0.05$) by Duncan's multiple range test.

These results indicated that apigenin had protective effect from H_2O_2-induced cell death in SH-SY5Y cells.

Protective effect of apigenin on ROS release in SH-SY5Y cells treated with H_2O_2

To establish the protective effect of apigenin on ROS generation in H_2O_2-induced SH-SY5Y cells, the DCF-DA assay was performed. After 60 min, ROS production was increased in the H_2O_2-treated cells compared to the H_2O_2-non treated cells, indicating that H_2O_2 induced ROS overproduction (Fig. 2A). Compared to 100% of the

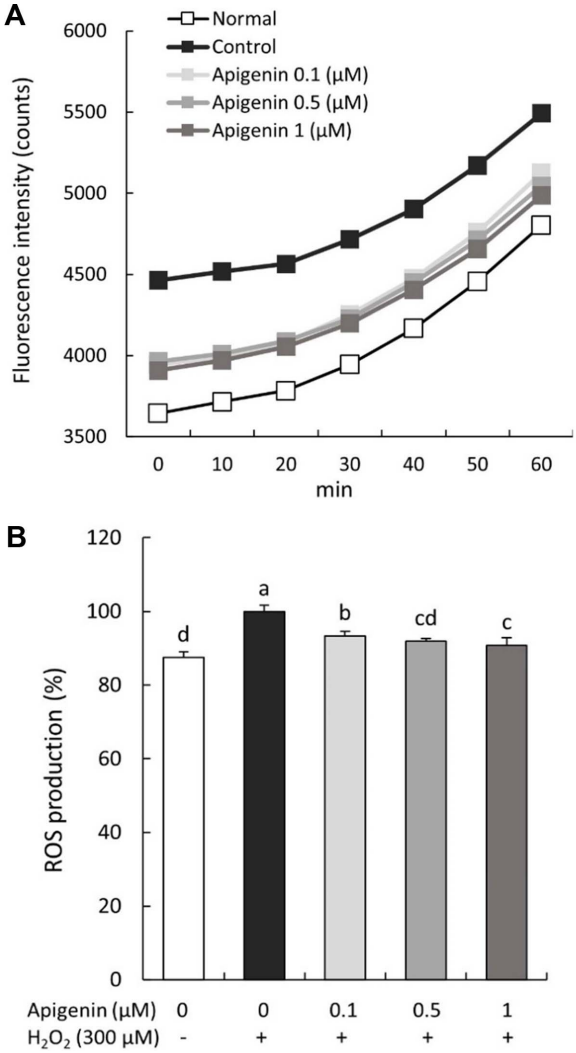

Fig. 2. Effect of apigenin on ROS production in SH-SY5Y cells treated with H_2O_2. Time course of change in intensity of DCF fluorescence during 60 min (A). Intensity of ROS production at 60 min (B). Values are mean ± SD. [a-d]Means are significantly different ($p < 0.05$) by Duncan's multiple range test.

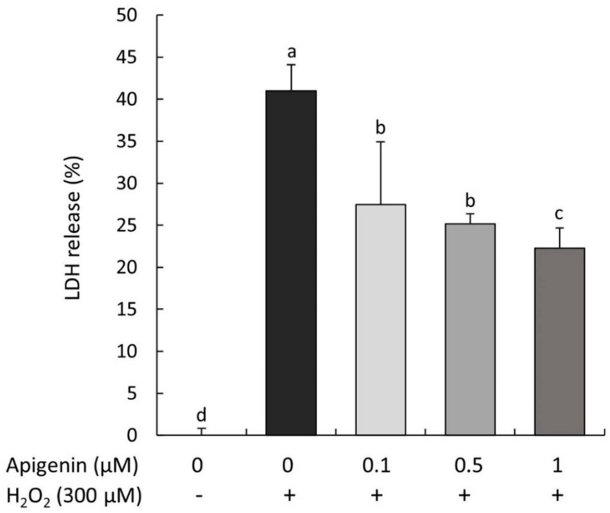

Fig. 3. Effect of apigenin on LDH release in SH-SY5Y cells treated with H_2O_2. Values are mean ± SD. [a-d]Means are significantly different ($p < 0.05$) by Duncan's multiple range test.

300 μM H_2O_2-only treated group, ROS production in the non-treated group was 87.47% (Fig. 2B). Whereas treatment of the cells with apigenin showed inhibitory effects on ROS formation, showing 93.33%, 91.89%, and 90.82% at concentrations of 0.1, 0.5, and 1 μM, respectively. These results confirmed that ROS generation in SH-SY5Y neuronal cells treated with H_2O_2 was attenuated by apigenin.

Protective effect of apigenin on LDH elimination in SH-SY5Y cells treated with H_2O_2

The inhibitory effect of apigenin on LDH release in H_2O_2-treated SH-SY5Y cells was shown in Fig. 3. The levels of LDH were significantly increased in the group treated with only H_2O_2 (40.99%) compared to non-treated group (0.84%). However, treatment with apigenin (0.1, 0.5, and 1 μM) significantly inhibited LDH release by 27.49%, 25.18%, and 22.31%, respectively. These finding suggest that apigenin has protective effect against neuronal oxidative damage caused by H_2O_2.

Protective effect of apigenin on apoptosis-related protein expressions in SH-SY5Y cells treated with H_2O_2

To confirm the protective mechanisms of apigenin against H_2O_2-induced apoptosis, we examined cytochrome C, cleaved caspase-3, cleaved PARP/PARP, and Bax/Bcl-2 protein expression by Western blotting in SH-SY5Y cells treated with H_2O_2 (Fig. 4). In our results, the increase of Bax expression and decrease of Bcl-2 expression were observed, in H_2O_2-treated control groups, resulting in significant increase in the Bax/Bcl-2 protein ratio. However, apigenin-treated groups showed a down-regulation of the Bax/Bcl-2 levels, which was similar to the H_2O_2-non treated group. In addition, the protein levels of cleaved PARP/PARP, cytochrome C, and cleaved caspase-3 were significantly increased in the H_2O_2-induced control cells compared with the H_2O_2-non induced cells. Nevertheless, the levels of cytochrome C, cleaved PARP/PARP, and cleaved caspase-3 protein expression were significantly decreased in apigenin-treated groups. In this study, our results indicated that apigenin protected from oxidative stress-induced neuronal cell death *via* regulation of the apoptosis signaling pathway.

Discussion

ROS and RNS play an important part in physiological functions, acting as molecular signals in the body. Overproduction of ROS and RNS are causative of harmful reactions, which are associated with a high risk of oxidative damage to organs, resulting in the progression of degenerative diseases [26]. In particular, oxidative stress in the brain is strongly connected in neurodegenerative diseases which are AD, PD, and Huntington's disease [27]. The brain is insufficient for oxidative defense system and is more vulnerable to oxidative stress than other organs. Therefore, lipids, proteins, and DNA can be damaged by excessive production of ROS and RNS in the brain [7, 28]. Many previous studies have indicated that supplements of anti-oxidant may prevent the progression of neurodegenerative diseases, and may mitigate ROS-induced neuronal damage [27, 29]. Notably, natural anti-oxidants isolated from various plants are receiving more attention for the prevention and treatment of neurodegenerative disorders [30, 31]. Apigenin, one of the flavonoids with low toxicity, is found widely in plants, such as parsley, onions, oranges, and herb teas [13, 14]. Apigenin has been reported to have various beneficial effects including anti-apoptotic, anti-inflammatory, and anti-bacterial effects [14, 16, 32]. According to Balez *et al.* [33], apigenin possessed neuroprotective effects against inflammation, apoptosis in

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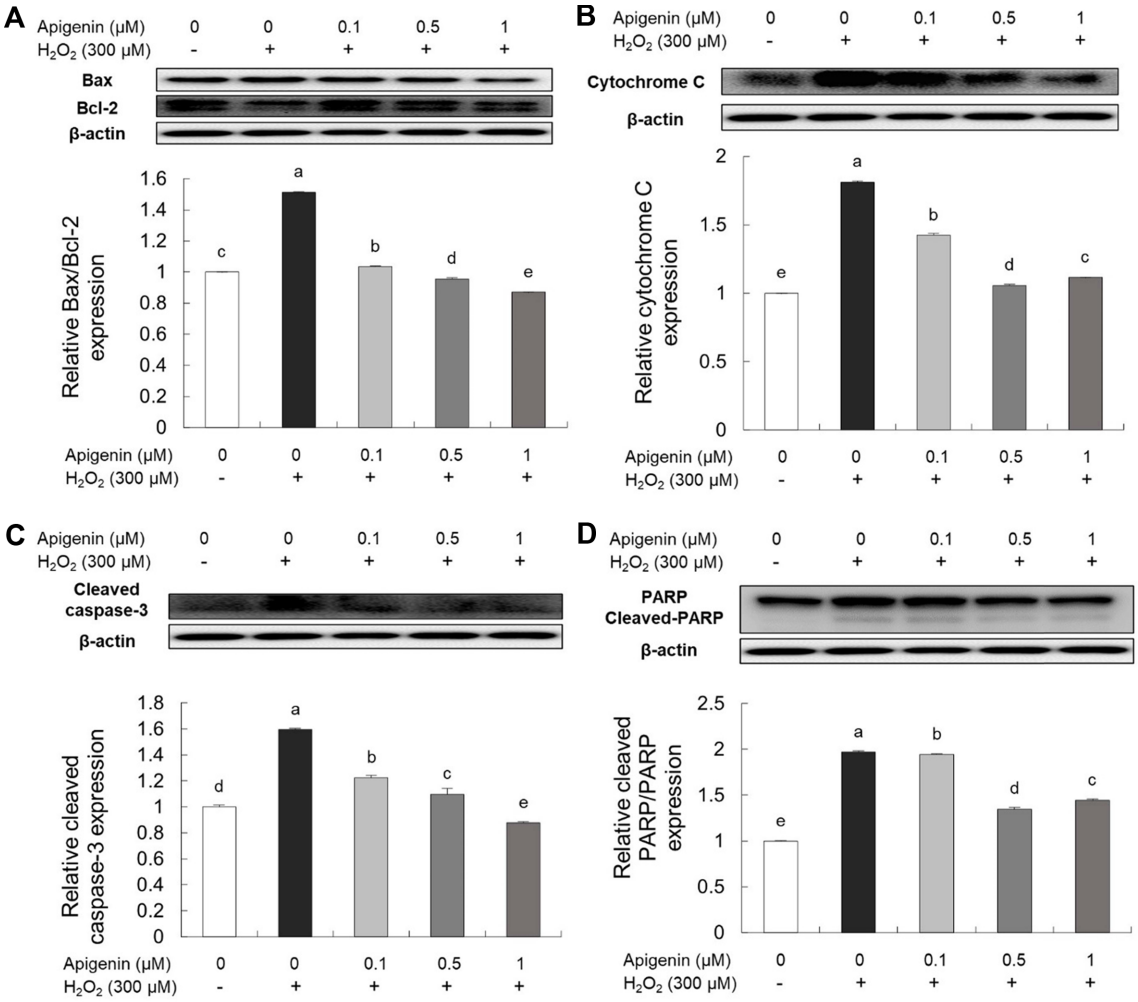

Fig. 4. Effects of apigenin on the levels of Bax/Bcl-2 (A) cytochrome C (B) cleaved caspase-3 (C) cleaved PARP/PARP (D) protein expression in SH-SY5Y cells treated with H_2O_2. Bax, B-cell lymphoma 2-associated X; Bcl-2, B-cell lymphoma 2; cytochrome C; cleaved caspase-3; PARP, poly (ADP)-ribose polymerase. Values are mean ± SD. $^{a-e}$Means are significantly different ($p < 0.05$) by Duncan's multiple range test. β-actin was used as a loading control.

induced pluripotent stem cell-derived neurons by attenuating Ca^{2+} signaling and caspase-3/7 activity. In addition, apigenin protected neuronal apoptosis by inhibiting the caspase-3 activity in H_2O_2-treated SH-SY5Y neuronal cells [34]. As mentioned above, oxidative stress is an imbalance of oxidant and antioxidant defense systems caused by stressors of external and internal in the body. In addition, neurons in the brain are confirmed fastest degenerated, due to the lack of an antioxidant defense system than other institutions and susceptible to oxidative stress [35, 36]. Consistent with these reports, that oxidative stress caused by ROS interferes with cell function and ultimately leads to cell death [37]. Previous study has confirmed that AD patients had increased levels of oxidative stress in the cells which the most vulnerable areas of the brain tissue [38]. Furthermore, oxidative stress affects synaptic transmission and ultimately could leads to a memory dysfunction due to oxidative stress [39]. Therefore, recent researches have been promoted on the prevention of diseases and protective effect of neuronal cells based on the preventing or delaying of cell death. However, the protective effect of apigenin that inhibiting mitochondria-mediated apoptotic signaling pathway in SH-SY5Y neuronal cells has not been studied. The present study is focused on the radical scavenging of apigenin and its neuroprotective

effects on oxidative stress-induced neuronal cell death by analyzing protein expressions participate in these effect.

The ·OH radical, which is the most reactive ROS, can be formed by a Fenton-type reaction, leading to severe damage in adjacent biomolecules [40]. The O_2^- radical is a precursor of other major ROS such as ·OH radical and H_2O_2, which can damage biomolecules and alter physiological functions [41, 42]. Furthermore, overproduction of NO radical has been reported to induce DNA fragmentation, cell membrane damage, and neuronal cell death [43]. In this study, apigenin exerted strong ·OH, O_2^-, and NO radical scavenging activities in a concentration-dependently. Although lacking a hydroxyl group at position 3 and a catechol structure in the B-ring, apigenin has a double bond at the 2, 3 carbon that leads to a more reactive structure possessing anti-oxidant capacities [32, 44, 45]. Therefore, the strong radical scavenging ability imply attributed to the double bond at the 2, 3 carbon position.

SH-SY5Y cells are phenotypically and genetically homogeneous cells that can be used to study the relationship between molecules and cell physiology [8]. Therefore, SH-SY5Y cells induced by oxidative stress are extensively used as a model system for investigating neuroprotective properties [9, 10]. Previous studies have indicated that H_2O_2 treatment significantly decreased cell viability via an oxidative-dependent apoptotic process [46, 47]. In this study, SH-SY5Y neuronal cells treated with H_2O_2 were used as an experimental model to examine the role of apigenin to protect against apoptosis induced by oxidative stress. Apigenin-treated SH-SY5Y cells had an antioxidant effect by increasing cell viability and reducing ROS production. In addition, treatment with apigenin significantly decreased levels of LDH release, indicating that apigenin had protective effects against oxidative damage caused by H_2O_2.

The relative ratios of anti-apoptosis and pro-apoptosis Bcl-2 family proteins have been linked to various pathological conditions characterized by cell death [48]. Related proteins of the Bcl-2 family include anti-apoptosis members (Bcl-2, Bcl-w, and Bcl-$_{XL}$) and pro-apoptosis members (Bax, Bak, Bad, Bik, and Bid) [49]. The BH3 protein is a Bcl-2 homology protein and functions as a trans-dominant inhibitor of Bcl-2. However, the BH3 domain of Bax is heterodimerized with Bcl-2 for migration to the mitochondria *via* oligomerization to release cytochrome C, thereby causing apoptosis [50, 51]. Therefore, the ratio of the pro-apoptosis protein Bax to the anti-apoptosis protein Bcl-2 is important for regulating the release of caspase activity-inducing factors from mitochondria. Apigenin attenuated doxorubicin-induced cardiotoxicity *via* the reduction of oxidative stress and apoptosis in male rats, showing a marked decrease in the apoptotic protein Bax and caspase-3 as well as an increase in the anti-apoptosis marker Bcl-2 [52]. Moreover, apigenin inhibited patulin-induced apoptosis in HEK 293 cells *via* up-regulation of Bcl-2 and down-regulation of Bax, leading to inhibition of caspase activation [53]. In present study, apigenin significantly reduced Bax level and increased Bcl-2 level. Therefore, these results showed that apigenin exerted neuroprotective effects against oxidative stress *via* the regulation of apoptosis-related protein in SH-SY5Y cells.

Cytochrome C is released into the cytosol from the mitochondrial intermembrane space, promoting caspase-3 activation and eventually causing neuronal apoptosis [54]. In addition, caspase-3 is the most important end-cleaving enzyme in the process of apoptosis, cleaving the DNA repair enzyme, PARP, and inducing cell apoptosis [55]. When caspases are activated, that bind with PARP and cleave it, thereby preventing DNA repair and inducing apoptosis. Thus, PARP protein is considered as a marker of apoptosis. The results indicated that apigenin protected against H_2O_2 by down-regulation of cytochrome C, cleaved caspase-3, and PARP protein expression in neuronal cells. A previous study indicated that apigenin protects against proteasome inhibitor-induced apoptosis in neuronal cells through regulation of cytochrome C, Bax/Bcl-2, caspases (-8, -9 and -3), and PARP [56]. Apigenin has been also reported to show neuroprotective effects on subarachnoid hemorrhage-induced neuronal apoptosis *via* down-regulation of Bax and caspase-3 expression levels [57]. In present study, cytochrome C, PARP, and cleaved caspase-3 were up-regulated by treatment with H_2O_2 in SH-SY5Y cells. However, apigenin treatment inhibited H_2O_2-induced cytochrome C, cleaved PARP/PARP, and cleaved caspase-3 protein expressions, suggesting that apigenin exerted its neuroprotective effect through down-regulation of the apoptotic signaling pathway. Apoptosis refers to a complex process involving condensation of the

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nucleus, cytoplasm, and chromatin [58, 59]. Early and late apoptosis can be distinguished by the time of occurrence of apoptosis in the central nervous system. In addition, early apoptosis is related to the proliferation site, whereas late apoptosis is related to the post-mitosis site [60]. Early cell death is characterized by exposure of the translocated phosphatidylserine (PS) to the outer plasma membrane surface [61]. Therefore, flow cytometric analysis using annexin V and propidium iodide (PI), which is an externalized binding of PS, can confirm an indicator of early cell death. This staining can differentiate between early, late apoptotic and viable cells. Previous study has confirmed that apigenin prevented rate of early apoptosis through double staining with annexin V-FITC and PI in oxygen and glucose deprivation/reperfusion-induced neuronal cell injury [62]. Although neuroprotective of apigenin on early-apoptosis and underlying mechanism should be further studied in H_2O_2-induced SH-SY5Y neuronal cells, apigenin may contribute to protecting neuronal cells against oxidative stress by regulation of apoptosis signaling pathway involving Bax/Bcl-2, cytochrome C release, and caspase activation.

In conclusion, apigenin showed effective ·OH, O_2^-, and NO radical scavenging activity. In particular, apigenin (25, 50, 100, and 250 μM) showed strong ·OH radical scavenging ability, more than 90%. Furthermore, apigenin increased the cell viability and decreased the ROS generation in H_2O_2-treated SH-SY5Y cells. The release of LDH induced by H_2O_2 was also reduced by treatment with apigenin. Moreover, apigenin exhibited anti-apoptotic effects against H_2O_2, as shown by down-regulation of cytochrome C, cleaved capase-3, cleaved PARP, and Bax expression levels, and up-regulation of Bcl-2 expression level. In present study, we suggest that apigenin exerts a neuro-protective effect against oxidative stress in SH-SY5Y neuronal cells, and could potentially be used as a preventative agent in neurodegenerative disease.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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