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ORIGINAL ARTICLE

Apigenin-7-Glucoside Attenuates Hydrogen Peroxide-Induced Oxidative Stress and Neuronal Death in SH-SY5Y Cells Via Activation of Antioxidant Enzymes System and Inhibition of Caspases Genes Expression

Apigenin-7-Glikozit Antioksidan Enzim Sisteminin Aktivasyonu ve Kaspaz Genlerinin Ekspresyonunun İnhibisyonu Yoluyla SH-SY5Y Hücrelerinde Hidrojen Peroksit İndüklü Oksidatif Stresi ve Nöronal Ölümü Azaltır

[1]Ebru Güçlü, [1]İlknur Çınar Ayan

[1]Department of Medical Biology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Correspondence

Ebru Güçlü, Necmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Biology, Konya/ Türkiye

E-Mail: ebruavc.87@gmail.com

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ABSTRACT

Aims: In this study, it was aimed to investigate the possible neuroprotective effect of Apigenin-7-Glycoside (AP7Glu), a natural product with known anticancer and anti-inflammatory activity, against hydrogen peroxide (H_2O_2)-induced oxidative stress in SH-SY5Y cells.
Methods: The effect of H_2O_2 and AP7Glu on cell viability was determined by XTT analysis. SOD and catalase analyses were performed to determine antioxidant enzyme activities. Expression levels of antioxidant defense system-related genes and caspase genes were evaluated by qRT-PCR analysis.
Results: After treatment of 300 µM H_2O_2 to SH-SY5Y cells for 24 hours, cell viability was determined as 63.18% and this dose was used to induce oxidative stress. H_2O_2 treatment in the presence of different concentrations of AP7Glu increased cell viability compared to H_2O_2-treated cells. And, H_2O_2 decreased SOD and catalase enzyme activities compared to the control group. With the treatment of H_2O_2 in the presence of AP7Glu, an increase was observed in these enzyme activities compared to H_2O_2-treated cells. In addition, H_2O_2 decreased the expression levels of genes associated with the antioxidant defense system and increased the expression levels of Caspase genes. And, H_2O_2 treatment in the presence of AP7Glu increased the expression levels of genes associated with the antioxidant defense system and decreased CASP8 expression compared to H_2O_2-treated cells.
Conclusion: AP7Glu has a neuroprotective effect against H_2O_2-induced oxidative stress and neuronal cell death.

Keywords: Apigenin-7-Glycoside, Hydrogen peroxide, Oxidative stress, SH-SY5Y

ÖZ

Amaç: Bu çalışmada antikanser ve anti-inflamatuar etkinliği bilinen bir doğal ürün olan Apigenin-7-Glikozitin (AP7Glu), SH-SY5Y hücrelerinde hidrojen peroksit (H_2O_2)-indüklü oksidatif strese karşı olası nöroprotektif etkisinin araştırılması amaçlandı.
Yöntem: H_2O_2 ve AP7Glu'nun hücre canlılığı üzerine etkisi XTT analizi ile belirlendi. Antioksidan enzim aktivitelerinin belirlenmesine yönelik olarak SOD ve katalaz analizleri gerçekleştirildi. Antioksidan savunma sistemi ile ilişkili genler ile Kaspaz genlerinin ekspresyon seviyeleri qRT-PCR analizi ile değerlendirildi.
Bulgular: SH-SY5Y hücrelerine 24 saat süresince 300 µM H_2O_2 uygulaması sonrasında hücre canlılığı % 63.18 olarak belirlendi ve oksidatif stresin indüklenmesi için bu doz kullanıldı. Farklı konsantrasyonlarda AP7Glu varlığında H_2O_2 uygulaması sadece H_2O_2 ile muamele edilmiş hücreler ile karşılaştırıldığında hücre canlılığını artırdı. H_2O_2 SOD ve katalaz enzim aktivitelerini kontrol grubuna göre azalttı. H_2O_2'nin AP7Glu varlığında uygulanması ise sadece H_2O_2 ile muamele edilmiş hücreler ile karşılaştırıldığında bu enzimlerin aktivitelerini artırdı. Ayrıca, H_2O_2 antioksidan savunma sistemi ile ilişkili genlerin ekspresyon seviyelerini azaltırken Kaspaz genlerinin ekspresyon seviyelerini artırdı. Sadece H_2O_2 ile muamele edilmiş hücreler ile karşılaştırıldığında ise AP7Glu varlığında H_2O_2 uygulaması antioksidan savunma sistemi ile ilişkili genlerin ekspresyon seviyelerini arttırdı ve CASP8 ekspresyonunu azalttı.
Sonuç: AP7Glu H_2O_2 ile indüklenmiş oksidatif stres ve nöronal hücre ölümüne karşı nöroprotektif bir etkiye sahiptir.

Anahtar Kelimeler: Apigenin-7-Glikozit, Hidrojen peroksit, Oksidatif stres, SH-SY5Y

Introduction

Although neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) are characterized by various disease-specific pathologies, it is also known that oxidative stress contributes to the development of these diseases (1,2). Reactive oxygen species (ROS) are produced in the cell depending on the functions of the mitochondrial transport chain, endoplasmic reticulum, and plasma membrane (3-5). Cellular antioxidant defense systems are involved in the detoxification of ROS. Oxidative stress occurs as a result of the insufficiency of these systems against increased ROS production (6). Accordingly, ROS accumulates in the cell and damages electron-rich biomolecules such as nucleic acids and lipids (7). The brain is heavily affected by the increased ROS production because it needs a great deal supply of oxygen and is rich in peroxide-sensitive lipids (8). As a result, increased ROS production also damages neuronal cell functions and induces neuronal cell death. This may result in the development of neurodegenerative

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Complaints: geneltip@selcuk.edu.tr

diseases associated with oxidative stress (9). ROS that are effective in the neurodegeneration process are hydrogen peroxide (H2O2), superoxide anion (O2-), and hydroxyl radical (HO□) (10). Also, treatment of H2O2 to neuronal culture cells causes an imbalance between ROS production and antioxidant defense systems. Therefore, H2O2 is frequently used to induce oxidative stress and cell death in neuronal cell lines (11,12).

Antioxidants are very important in the prevention and treatment of various chronic diseases induced by oxidative stress, especially neurodegenerative diseases. The antioxidant defense system in the cell consists of antioxidants such as glutathione, vitamin C, and vitamin E, as well as antioxidant enzyme systems such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) (13). In addition, recent studies draw attention to the antioxidant effects of various natural products with pharmacological properties and their protective roles against oxidative stress. These natural products may also have a potential role in the prevention and treatment of neurodegenerative diseases, especially in terms of preventing neurogeneration and supporting the antioxidant defense systems in the cell (14,15).

Apigenin is a flavonoid found in vegetables such as celery, artichokes, and parsley, and has been shown to have anticancer (16), antioxidant (17), antibacterial (18), and anti-inflammatory (19) effects in various studies. And, apigenin-7-glucoside (AP7Glu), a glycoside form of apigenin, is a natural product with similar properties to apigenin, but with better solubility compared to other flavonoids such as apigenin. It has been reported that AP7Glu, which has an anti-inflammatory effect like apigenin, is especially effective against upper respiratory infections (20).

Considering the role of oxidative stress in neurodegenerative diseases and the antioxidant and neuroprotective effects of natural compounds, it is very important to elucidate the molecular mechanisms of the effects, such as supporting antioxidant defense systems or preventing neuronal cell death. In this study, we aimed to investigate the possible neuroprotective effect of AP7Glu against H2O2-induced oxidative stress. For this, we evaluated the effects of H2O2 and AP7Glu treatments on cell viability, SOD, and catalase enzyme activities in SH-SY5Y human neuroblastoma cells. We also investigated the changes in the expression levels of genes associated with apoptosis and antioxidant defense system at the mRNA level.

Materials and Methods

Cell culture and treatments

SH-SY5Y (ATCC® CRL-2266™) human neuroblastoma cells were cultured in DMEM: F12 (Sigma-Aldrich, Cat. no: D6421) supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 1% Penicillin-Streptomycin at 37°C in a humidified incubator with 5% CO2. H2O2 was freshly prepared from 30% stock solution with the medium before each experiment. AP7Glu (Sigma-Aldrich, 578-74-5) dissolved in a final concentration of 0.1% DMSO.

Cell viability

The effect of H2O2 and AP7Glu on cell viability was determined by the XTT cell proliferation test. Cells were seeded at 2x104 cells/well in 96-well plates and allowed to adhere for 24 hours. For the determination of the neurotoxic effect of H2O2 on SH-SY5Y cells, cells were treated with 100, 200, 300, 400, and 500 µM H2O2 for 24 hours. To determine the effect of AP7Glu on cell viability, cells were exposed to 5, 10, 15, 20, 40, 50, 75, and 100 µM AP7Glu for 24 hours. To determine the possible protective effect, pre-treatment was performed with 5, 10, 15, 20, 40, 50, 75, and 100 µM AP7Glu for 6 hours before treatment with 300 µM H2O2 for 24 hours. After all treatments, 100 µl of XTT solution was added to each well (Biological Industries - Cat. No.:20-3001000). At the end of 4 hours, the absorbance value in each well was measured at 450 nm wavelength in a microplate reader. Cells not treated with H2O2 or AP7Glu were used as a control group. The percentage of cell viability was determined by dividing the dose absorbance values by the control absorbance values and multiplying by 100.

Determination of SOD enzyme activity

The possible protective effect of AP7Glu against H2O2-induced oxidative stress was evaluated by determining SOD enzyme activity according to the manufacturer's instructions (Biovision - Cat. No.:K335-100). For this, control and dose group cells (5x105) were lysed with 0.1 M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM β-ME, 0.1 mg/ml PMSF on ice. Then, centrifugation was performed at 14.000 x g for 5 minutes and supernatants were collected. Sample, Blank 1, Blank 2, and Blank 3 wells were prepared in 96-well plates. The supernatant containing 20 µl of SOD was added to each sample and Blank 2 wells, and 20 µl of H2O was added to Blank 1 and Blank 3 wells. Then, 200 µl of WST working solution included in the kit was added to each well. Finally, 20 µl of dilution buffer was added to Blank 2 and Blank 3 wells, and 20 µl of enzyme working solution was added to the sample and Blank 1 wells. Plates were incubated for 20 minutes at 37oC, and after incubation, a reading was performed at 450 nm in a microplate reader. SOD activity was calculated according to the following formula:

Determination of catalase enzyme activity

The possible protective effect of AP7Glu against H2O2-induced oxidative stress was evaluated by determining the activity of catalase, another important antioxidant enzyme, according to the manufacturer's instructions (Biovision - Cat. No.:K773-100). Briefly, control and dose group cells (1x106) were centrifuged with buffer included in the kit at 10.000 x g for 15 minutes and the supernatant was collected. For sample wells, 50 µl of supernatant was added to a 96-well plate and the total volume was made up to 78 µl with buffer. Sample high control (HC) wells were prepared by adding 28 µl of

buffer to the same amount of supernatant. 10 µl of stop solution was added to HC wells and catalase activity of HC samples was inhibited by incubation at 25°C for 5 minutes. Meanwhile, 1 mM H2O2 was formed from 0.88 M H2O2 contained in the kit, and H2O2 standards (0, 2, 4, 6, 8, 10 nmol/well) were prepared from this concentration. For the catalase reaction, 12 µl of 1 mM H2O2 was added to the sample and HC wells and the reaction was started with incubation at 25°C for 30 minutes. After incubation, 10 µl of stop solution was added to sample wells and the reaction was stopped. Then, 50 µl of a mixture of buffer, OxiRed probe, and HRP solution was added to the sample, HC, and standard wells. After 10 minutes of incubation at 25°C, readings were taken at 570 nm. In this analysis, catalase first reacts with H2O2 to produce water and oxygen, and then unconverted H2O2 forms a product with an OxiRed probe that can be measured at 570 nm. As a result, catalase activity is inversely proportional to the generated signal. For the determination of catalase activity, the difference between the HC absorbance value and sample absorbance value was determined and the amount of converted H2O2 was calculated using the H2O2 standard curve. Catalase activity was determined according to the following formula:

Catalase activity = $\frac{B}{30 \times V}$ x Sample dilution factor = nmol/min/ml = mU/ml

B is the decomposed H2O2 amount from H2O2 standard curve

V is the sample volume

30 is the reaction time (30 minutes)

RNA isolation, cDNA synthesis, and Quantitative Real-Time PCR (qRT-PCR)

The possible neuroprotective effect of AP7Glu against H2O2-induced oxidative stress was determined at the molecular level by evaluating the expression levels of CASP3, CASP7, CASP8, and CASP9 genes associated with apoptosis and the expression levels of SOD1, SOD2, SOD3 and CAT genes, which encode important antioxidants, by qRT-PCR analysis. For this, total RNA was isolated from control and dose group cells and cDNA was synthesized (Bio-Rad - Cat. No.:170–8891). Primers for target and reference genes were designed using IDT Primer Quest (https://eu.idtdna.com/ Prime request/ Home/ Index) program, and forward and reverse primer sequences are presented in Table 1. The reaction components and PCR protocol were as previously described (Güçlü et al., 2022). Threshold (Ct) values of each reaction were recorded for analysis and fold changes were determined using the 2–ΔΔCT method in "RT2 Profiler ™ PCR Array Data Analysis" software.

Statistical analysis

Results were presented as means ± S.E.M. Statistical analyses were performed by GraphPad Prism software (Version 8.0.2, San Diego, CA) and the comparison between groups was analyzed by using One-way ANOVA. $P < 0.05$ was considered statistically significant.

Table 1. Primer sequences of target and reference genes

Gene	Forward primer (5'->3')	Reverse primer (5'->3')
CASP3	CTCTGGAATATCCCTGGACAAC	ACATCTGTACCAGACCGAGA
CASP7	GTCACCATGCGATCCATCAA	CGCCCATACCTGTCACTTTATC
CASP8	GATTCAGAGGAGCAACCCTATT	AGCAGAAAGTCAGCCTCATC
CASP9	CGACCTGACTGCCAAGAAA	GACAGCCGTGAGAGAGAATG
SOD1	GATGAAGAGAGGCATGTTGGAG	CTCCTGAGAGTGAGATCACAGA
SOD2	GGAGATGTTACAGCCCAGATAG	CGTTAGGGCTGAGGTTTGT
SOD3	TGTTCCTGCCTGCTCCT	ACATGTCTCGGATCCACTCC
CAT	AGGGTGGTGCTCCAAATTAC	TGTTGAATCTCCGCACTTCTC
ACTB	GGACCTGACTGACTACCTCAT	CGTAGCACAGCTTCTCCTTAAT

Results

Effect of H2O2 and AP7Glu on cell viability of SH-SY5Y cells

In this study, we investigated the protective effect of AP7Glu against H2O2-induced oxidative stress in SH-SY5Y cells. For this, firstly, we treated SH-SY5Y cells with different concentrations of H2O2 for 24 hours and determined percent of cell viability. Accordingly, after treatment with 100, 200, 300, 400, and 500 µM H2O2, % cell viability was 81.87%, 73.69%, 63.18%, 57.32%, and 54.58%, respectively. In addition, % cell viability significantly decreased compared to the control group at all doses ($P < 0.0001$) (Figure 1). Since cell viability is inhibited by approximately 40-50% at doses of 300 µM and higher, we decided to treat the cells with 300 µM H2O2 for 24 hours to induce oxidative stress and neurotoxicity in SH-SY5Y cells in subsequent analysis.

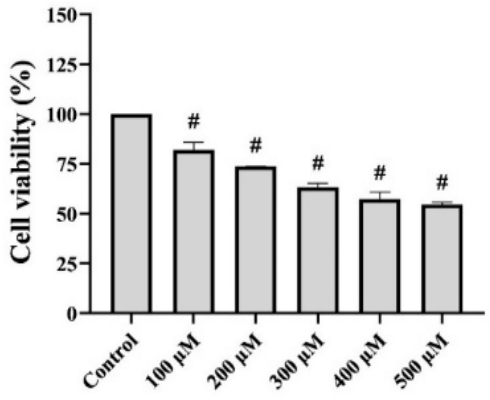

Figure 1. The Effect of H2O2 on the viability of SH-SY5Y cells. Cells were treated with different concentrations of H2O2 for 24 hours. Absorbance values were determined by XTT analysis and percent of cell viability was calculated. # $P < 0.0001$.

Similarly, to evaluate the effect of AP7Glu on cell viability, we treated cells with different concentrations of AP7Glu for 24 hours and determined % cell viability. Accordingly, after treatment with 5, 10, 15, 20, 40,

50, 75, and 100 µM AP7Glu for 24 hours, percent of cell viability was 93.53%, 99.33%, 96.18%, 85.14%, 85.12%, 85.41%, 82.18%, and 72.27%, respectively. The decrease in the percent of cell viability was significant after treatment with 20, 40, 50, 75, and 100 µM AP7Glu (P = 0.0079 for 20 µM, P = 0.0078 for 40 µM, P = 0.0092 for 50 µM, P = 0.0014 for 75 µM, P < 0.0001 for 100 µM) (Figure 2).

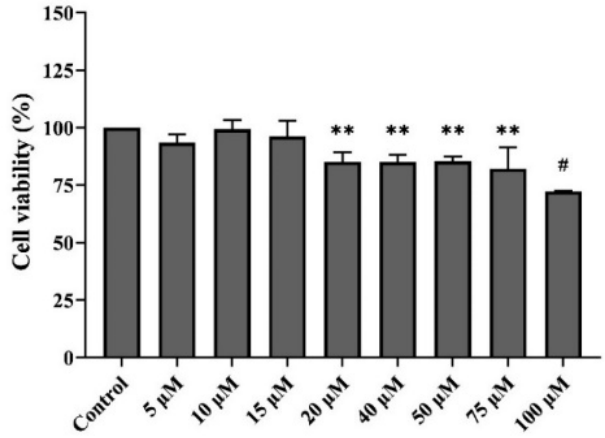

Figure 2. Effect of AP7Glu on the viability of SH-SY5Y cells. Cells were treated with different concentrations of AP7Glu for 24 hours. Absorbance values were determined by XTT analysis and percent of cell viability was calculated. ** P < 0.01, **#** P < 0.0001.

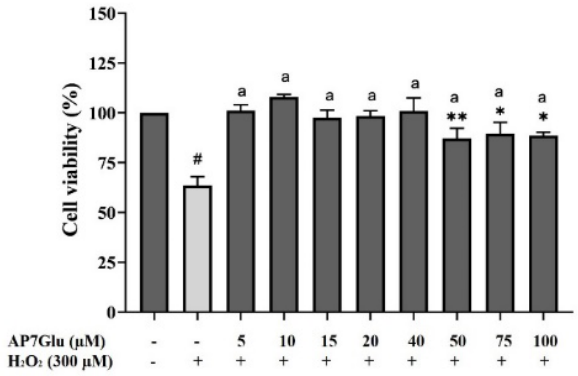

Figure 3. The effect of H2O2 and AP7Glu on the viability of SH-SY5Y cells. Cells were pre-treated with different concentrations of AP7Glu for 6 hours. Then, 300 µM H2O2 was added to the wells. Absorbance values were determined by XTT analysis and percent of cell viability was calculated. * P < 0.05, ** P < 0.01, **#** P < 0.0001 vs control, a P < 0.0001 vs H2O2-treated cells.

To determine the possible protective effect of AP7Glu against H2O2-induced neurotoxicity, cells were pre-treated with 5, 10, 15, 20, 40, 50, 75, and 100 µM AP7Glu for 6 hours. Then, 300 µM H2O2 was added to the cells and the cells were incubated with AP7Glu and H2O2 for 24 hours. According to XTT analysis, no significant change was observed in cell viability after H2O2 treatment in the presence of 5, 10, 15, 20, and 40 µM AP7Glu when compared to the control group (P □ 0.05). After H2O2 treatment in the presence of 50, 75, and 100 µM AP7Glu, the cell viability decreased significantly compared to the control group, and it was determined as 81.12% (P = 0.0064), 89.40% (P = 0.0325) and 88.67% (P = 0.0194), respectively. In addition, after the cells were treated with only 300 µM H2O2, cell viability (%)significantly decreased to 63.60% compared to the control group (P < 0.0001). On the other hand, H2O2 treatment in the presence of AP7Glu significantly increased cell viability compared to H2O2-treated cells (P < 0.0001) (Figure 3). This result shows the neuroprotective effect of AP7Glu against H2O2-induced neurotoxicity.

Effect of H2O2 and AP7Glu on SOD and catalase enzyme activities

As shown in Figure 2, the treatment of 5, 10, and 15 µM AP7Glu has a nontoxic effect on SH-SY5Y cells. Since 5 µM was the lowest nontoxic dose of these, it might not have caused as significant an effect as other doses in subsequent analysis. On the other hand, 15 µM was a concentration close to 20 µM, which was considered a toxic dose for SH-SY5Y cells. For this reason, it was considered that it would be more appropriate to use 10 µM AP7Glu as an intermediate dose. Accordingly, in subsequent analysis, cells were treated with 300 µM H2O2 for 24 hours in the presence and absence of 10 µM AP7Glu. Then, the effects of H2O2 and AP7Glu on SOD and catalase enzyme activities were evaluated. According to SOD analysis results, treatment of H2O2 to cells alone or in the presence of AP7Glu significantly reduced percent of SOD activity compared to the control group (64.21% in H2O2-treated cells, P < 0.0001; 79.9% in cotreated cells with H2O2 and AP7Glu, P = 0.0025). On the other hand, the increase observed in SOD activity after the cotreatment of H2O2 and AP7Glu was significant when compared to cells treated with H2O2 alone (P = 0.0025) (Figure 4A).

According to catalase activity results, this enzyme activity was 8.94 mU/mL in the control group while it was 5.07 mU/mL and 8.49 mU/mL in the groups where H2O2 was treated alone and together with AP7Glu, respectively. While H2O2 treatment significantly decreased catalase enzyme activity compared to the control group (P < 0.0001), cotreatment H2O2 and AP7Glu did not cause a significant change in this enzyme activity compared to the control group (P = 0.4884). On the other hand, the increase in catalase activity was significant after the cotreatment of H2O2 and AP7Glu when compared to H2O2-treated cells (P < 0.001) (Figure 4B).

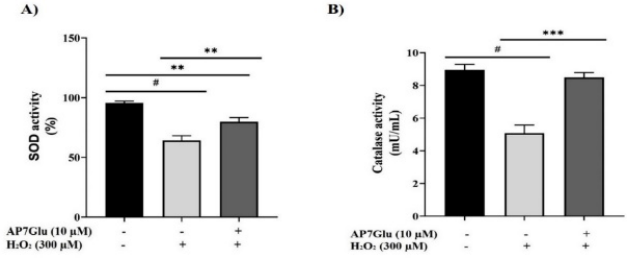

Figure 4. Effect of H2O2 and AP7Glu on A) SOD and B) catalase enzyme activities in SH-SY5Y cells. ** P < 0.01, *** P < 0.001, # P < 0.0001.

Effect of H2O2 and AP7Glu on expression levels of caspases and antioxidant enzyme activity-related genes

The effects of H2O2 and AP7Glu on the expression levels of CASP3, CASP7, CASP8, and CASP9 apoptotic genes, and SOD1, SOD2, SOD3, CAT genes associated with antioxidant enzyme activity were evaluated by qRT-PCR analysis. And, the data were normalized with the ACTB housekeeping gene, and the results were presented as fold change compared to the control group. Accordingly, when compared to the control group, H2O2 treatment significantly decreased SOD1, SOD2, and CAT expression levels at 2.53 (P = 0.001720), 1.75 (P = 0.035174) and 5.37 (P = 0.000025) fold, respectively and significantly increased CASP7, CASP8, and CASP9 expression levels as 1.47 (P = 0.038901), 2.93 (P = 0.044876) and 1.7 (P = 0.049646) fold, respectively. On the other hand, AP7Glu treatment did not cause a significant change in the expression level of these genes (Figure 5).

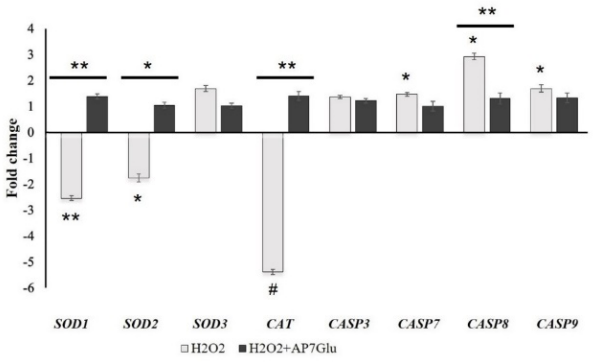

Figure 5. Effect of H2O2 and AP7Glu on expression levels of caspases and antioxidant enzyme activity-related genes in SH-SY5Y cells. Ct values of each gene were normalized with Ct values of the *ACTB* housekeeping gene. * P < 0.05, ** P < 0.01, *** P < 0.001, # P < 0.0001. ACTB is used as the housekeeping gene.

In addition, when compared to H2O2-treated cells after cotreatment of H2O2 and AP7Glu, expression levels of SOD1, SOD2, and CAT genes significantly increased as 3.49 (P = 0.004214), 1.83 (P = 0.038316) and 7.52 (P = 0.002587) fold, respectively, and, CASP8 gene expression significantly decreased as 2.24 (P = 0.048676) fold (Figure 6).

Discussion

In this study, we investigated the protective effect of AP7Glu against H2O2-induced oxidative stress and neuronal cell death in neuroblastoma cells. SH-SY5Y human neuroblastoma cells are widely used for in vitro oxidative stress model, and oxidative stress and neuronal cell death are induced by H2O2 (11,12).

Firstly, we performed cytotoxicity analysis to find the dose that inhibited cell viability by approximately 40%-50% in SH-SY5Y cells, and as a result, we found that 300 µM H2O2 treatment for 24 hours significantly inhibited cell viability by approximately 40% in SH-SY5Y cells. In other studies, aimed at inducing oxidative stress in SH-SY5Y cells, cells were generally treated with 200 µM or 300 µM H2O2 for 24 hours. And, in these studies, it has been shown that H2O2 treatment at these doses inhibits cell viability by 40-50%, similar to our study (22,23). As a result, we treated SH-SY5Y cells with 300 µM H2O2 for 24 hours to induce oxidative stress and neuronal cell death in subsequent analyses.

Natural products may have anticancer effects as well as many pharmacological properties. AP7Glu has also been reported to have anticancer effects on colon cancer and cervical cancer cells. The IC50 values of AP7Glu for 48 hours in HCT116 colon cancer and HeLa cervical cancer cells were 15 µM and 47.26 µM, respectively (24,25). However, the dose that has a neuroprotective effect against oxidative stress-induced neuronal cell death should be non-toxic. According to the cytotoxicity analysis results, the treatment of 5, 10, and 15 µM AP7Glu for 24 hours has no significant effect on SH-SY5Y cells viability. However, the treatment of AP7Glu at doses of 20 µM and above significantly reduced cell viability compared to the control group. When compared to H2O2-treated cells, H2O2 treatment in the presence of different concentrations of AP7Glu significantly increased cell viability. This finding shows that AP7Glu may have a neuroprotective effect against H2O2-induced neurotoxicity. And, we observed the highest % viability in cells treated with H2O2 in the presence of 10 µM AP7Glu. Considering the nontoxic effect of 10 µM AP7Glu treatment on SH-SY5Y cells, the cells were treated with 300 µM H2O2 in the presence of 10 µM AP7Glu for 24 hours in subsequent analyses.

As an oxidative stress inducer, H2O2 causes the formation of hydroxyl radicals that damage structural molecules such as lipid, protein, and DNA. As a result, an imbalance occurs between antioxidant and cellular oxidants, and the increase in cellular ROS production leads to oxidative stress (26). GPx, SOD, and catalase are antioxidant enzymes that inhibit ROS and thus protect the cell against oxidative stress. Among them, SOD catalyzes the dismutation of superoxide anion free radical (O2-) to molecular oxygen and H2O2 (27,28). Catalase enzyme found in peroxisomes reduces cellular damage of free radicals by breaking down H2O2 into water and molecular oxygen (29). It is known that H2O2, which is used to induce oxidative stress, reduces antioxidant enzyme activities (30-32). Similarly, in our study, H2O2 treatment significantly decreased SOD and catalase enzyme activities in SH-SY5Y cells compared to the control group. On the other hand, an increase was detected in the presence of AP7Glu in decreasing SOD and catalase enzyme activities after H2O2 treatment.

In this study, we also evaluated the effects of H2O2 and AP7Glu on the antioxidant defense system at the molecular level and determined the expression levels of SOD1, SOD2, SOD3, and CAT genes by qRT-PCR analysis. Among them, the SOD1 gene encodes the monomeric SOD1 protein of homodimeric metalloprotein containing copper and zinc, which is the major cytoplasmic antioxidant enzyme found

mainly in the cytoplasm, but also detected in the nucleus, lysosome, and mitochondria (33,34). The SOD2 gene encodes manganese superoxide dismutase (Mn-SOD), an antioxidant enzyme found in the mitochondrial matrix (35). And, SOD3 gene encodes extracellular matrix SOD, which is rarely found in most tissues (36,37). CAT gene, located on chromosome 11 in humans, encodes the tetrameric catalase protein, each subunit consisting of four domains (38). According to qRT-PCR analysis results, H2O2 treatment significantly decreased mRNA levels of SOD1, SOD2, and CAT genes compared to the control group in SH-SY5Y cells. This finding supports our results of enzyme activity analysis and reveals the oxidative stress-inducing effect of H2O2 at the molecular level. On the other hand, this effect of H2O2 was inhibited by AP7Glu treatment, and an increase in mRNA levels of SOD1, SOD2, and CAT genes was detected when compared to H2O2-treated cells. When this finding is evaluated together with our antioxidant enzyme activity results, it supports the protective role of AP7Glu against H2O2-induced oxidative stress.

There are not many studies on the protective effect of AP7Glu against oxidative stress. However, AP7Glu has been shown to have a protective role against lipopolysaccharide-induced acute lung injury in mice. Researchers explained this effect with the increase in antioxidant enzyme activity after AP7Glu pre-treatment, similar to our results (20).

Oxidative stress is known to cause DNA damage in cells. In particular, the accumulation of DNA damage in the promoter regions of protein-coding genes prevents the transcription of active genes. Thus, cellular function is impaired and ultimately apoptosis is induced (39). The main proteins that are at the center of apoptosis and are responsible for the transmission of cell death signals are caspase family members consisting of cysteine proteases. The caspase family is divided into two subfamilies as proapoptotic and proinflammatory caspases. Proapoptotic caspases associated with cell death are Caspase 2, -3, -6, -7, -8, -9, and -10 (40,41).

In this study, the protective effect of AP7Glu on H2O2-induced oxidative stress and neuronal cell death was evaluated at the molecular level by determining the expression levels of CASP3, -7, -8, and -9 genes associated with apoptosis. According to qRT-PCR analysis results, H2O2-induced oxidative stress increased the expression levels of CASP7, CASP8, and CASP9 genes. And, H2O2 treatment in the presence of AP7Glu did not cause a significant change in the expression levels of these genes when compared to the control group. In addition, the cotreatment of AP7Glu and H2O2 significantly decreased the mRNA level of CASP8 compared to H2O2-treated cells.

When all the results are evaluated together, this study shows that AP7Glu has a neuroprotective effect against H2O2-induced oxidative stress and neuronal cell death by increasing antioxidant enzyme activities and decreasing the expression of Caspase genes. Considering the role of oxidative stress in the development of neurodegenerative diseases, this result suggests that AP7Glu may have a potential effect on the protection of neurodegeneration.

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Conflict of Interest

The authors declare no competing interests.

Authorship Contributions

EG: Investigation, methodology, formal anaysis, writing—original draft, visualization. İÇA: Investigation, methodology, formal anaysis, visualization.

References

1.Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol 2003;53 Suppl 3:S26-36

2.Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000;1502(1):139-144

3.Overmyer K, Brosché M, Kangasjärvi J. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci 2003;8(7):335-342

4.Crane FL, Low H. Reactive oxygen species generation at the plasma membrane for antibody control. Autoimmun Rev 2008;7(7):518-522

5.Bhandary B, Marahatta A, Kim HR, Chae HJ. Involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. Int J Mol Sci 2012;14(1):434-456

6.Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev 2017;2017:8416763

7.Yavarpour-Bali H, Ghasemi-Kasman M, Pirzadeh M. Curcumin-loaded nanoparticles: a novel therapeutic strategy in the treatment of central nervous system disorders. Int J Nanomedicine 2019;14:4449-4460

8.Collin F. Chemical Basis of Reactive Oxygen Species Reactivity and Involvement in Neurodegenerative Diseases. Int J Mol Sci 2019;20(10):2407

9.Annunziato L, Amoroso S, Pannaccione A, Cataldi M, Pignataro G, D'Alessio A, Sirabella R, Secondo A, Sibaud L, Di Renzo GF. Apoptosis induced in neuronal cells by oxidative stress: the role played by caspases and intracellular calcium ions. Toxicol Lett 2003;139(2-3):125-133

10.Singh A, Kukreti R, Saso L, Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules 2019;24(8):1583

11.Jantas D, Chwastek J, Grygier B, Lasoń W. Neuroprotective Effects of Necrostatin-1 Against Oxidative Stress-Induced Cell Damage: an Involvement of Cathepsin D Inhibition. Neurotox Res 2020;37(3):525-542

12.Ruankham W, Suwanjang W, Wongchitrat P, Prachayasittikul V, Prachayasittikul S, Phopin K. Sesamin and sesamol attenuate H2O2-induced oxidative stress on human neuronal cells via the SIRT1-SIRT3-FOXO3a signaling pathway. Nutr Neurosci 2021;24(2):90-101

13.Asaduzzaman Khan M, Tania M, Zhang DZ, Chen HC. Antioxidant enzymes and cancer. Chin J Cancer Res 2010;22:87-92

14.Zhang H, Rong T. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion in Food Science 2016, 8: 33-42

15.Uddin MS, Mamun AA, Rahman MM, Jeandet P, Alexiou A, Behl T, Sarwar MS, Sobarzo-Sánchez E, Ashraf GM, Sayed AA, Albadrani GM, Peluso I, Abdel-Daim MM. Natural Products for Neurodegeneration:

Regulating Neurotrophic Signals. Oxid Med Cell Longev 2021;2021:8820406

16.Perrott KM, Wiley CD, Desprez PY, Campisi J. Apigenin suppresses the senescence-associated secretory phenotype and paracrine effects on breast cancer cells. Geroscience 2017;39(2):161-173.

17.Sánchez-Marzo N, Pérez-Sánchez A, Ruiz-Torres V, Martínez-Tébar A, Castillo J, Herranz-López M, Barrajón-Catalán E. Antioxidant and Photoprotective Activity of Apigenin and its Potassium Salt Derivative in Human Keratinocytes and Absorption in Caco-2 Cell Monolayers. Int J Mol Sci 2019;20(9):2148

18.Nayaka HB, Londonkar RL, Umesh MK, Tukappa A. Antibacterial Attributes of Apigenin, Isolated from Portulaca oleracea L. Int J Bacteriol 2014;2014:175851

19.Sadraei H, Asghari G, Khanabadi M, Minaiyan M. Anti-inflammatory effect of apigenin and hydroalcoholic extract of Dracocephalum kotschyi on acetic acid-induced colitis in rats. Res Pharm Sci 2017;12(4):322-329

20.Li KC, Ho YL, Hsieh WT, Huang SS, Chang YS, Huang GJ. Apigenin-7-glycoside prevents LPS-induced acute lung injury via the downregulation of oxidative enzyme expression and protein activation through the inhibition of MAPK phosphorylation. Int J Mol Sci 2015;16(1):1736-1754

21.Güçlü E, Çınar Ayan İ, Dursun HG, Vural H. Tomentosin induces apoptosis in pancreatic cancer cells through increasing reactive oxygen species and decreasing mitochondrial membrane potential. Toxicol In Vitro;84:105458.

22.Wang J, Zhao YM, Zhang B, Guo CY. Protective effect of total phenolic compounds from Inula helenium on hydrogen peroxide-induced oxidative stress in SH-SY5Y cells. Indian journal of pharmaceutical sciences 2015;77(2):163.

23.Tian W, Heo S, Kim DW, Kim IS, Ahn D, Tae HJ, Kim MK, Park BY. Ethanol Extract of Maclura tricuspidata Fruit Protects SH-SY5Y Neuroblastoma Cells against H2O2-Induced Oxidative Damage via Inhibiting MAPK and NF-κB Signaling. Int J Mol Sci 2021;22(13):6946

24.Smiljkovic M, Stanisavljevic D, Stojkovic D, Petrovic I, Marjanovic Vicentic J, Popovic J, Golic Grdadolnik S, Markovic D, Sankovic-Babice S, Glamoclija J, Stevanovic M, Sokovic M. Apigenin-7-O-glucoside versus apigenin: Insight into the modes of anticandidal and cytotoxic actions. EXCLI J 2017;16:795-807

25.Liu MM, Ma RH, Ni ZJ, Thakur K, Cespedes-Acuña CL, Jiang L, Wei ZJ. Apigenin 7-O-glucoside promotes cell apoptosis through the PTEN/PI3K/AKT pathway and inhibits cell migration in cervical cancer HeLa cells. Food Chem Toxicol 2020;146:111843

26.Tian W, Zhao J, Lee JH, Akanda MR, Cho JH, Kim SK, Choi YJ, Park BY. Neuroprotective Effects of Cornus officinalis on Stress-Induced Hippocampal Deficits in Rats and H2O2-Induced Neurotoxicity in SH-SY5Y Neuroblastoma Cells. Antioxidants (Basel) 2019;9(1):27

27.Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 2018;12(3):88-93

28.Islam MN, Rauf A, Fahad FI, Emran TB, Mitra S, Olatunde A, Shariati MA, Rebezov M, Rengasamy KRR, Mubarak MS. Superoxide dismutase: an updated review on its health benefits and industrial applications. Crit Rev Food Sci Nutr 2022;62(26):7282-7300

29.Ighodaro OM, Akinloye OA. First-line defense antioxidants-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. Alexandria journal of medicine 2018;54(4): 287-293.

30.Feng C, Luo T, Zhang S, Liu K, Zhang Y, Luo Y, Ge P. Lycopene protects human SH-SY5Y neuroblastoma cells against hydrogen peroxide-induced death via inhibition of oxidative stress and mitochondria-associated apoptotic pathways. Mol Med Rep 2016;13(5):4205-4214

31.Gao J, Xu Y, Zhang J, Shi J, Gong Q. Lithocarpus polystachyus Rehd. leaves aqueous extract protects against hydrogen peroxide-induced SH-SY5Y cell injury through activation of the Sirt3 signaling pathway. Int J Mol Med 2018;42(6):3485-3494

32.Ureña-Vacas I, González-Burgos E, Divakar PK, Gómez-Serranillos MP. Lichen Extracts from Cetrarioid Clade Provide Neuroprotection against Hydrogen Peroxide-Induced Oxidative Stress. Molecules 2022;27(19):6520

33.Nojima Y, Ito K, Ono H, Nakazato T, Bono H, Yokoyama T, Sato R, Suetsugu Y, Nakamura Y, Yamamoto K, Satoh J, Tabunoki H, Fugo H. Superoxide dismutases, SOD1 and SOD2, play a distinct role in the fat body during pupation in silkworm Bombyx mori. PLoS One 2015;10(2):e0116007

34.Pansarasa O, Bordoni M, Diamanti L, Sproviero D, Gagliardi S, Cereda C. SOD1 in Amyotrophic Lateral Sclerosis: "Ambivalent" Behavior Connected to the Disease. International Journal of Molecular Sciences 2018; 19(5):1345

35.Alateyah N, Gupta I, Rusyniak RS, Ouhtit A. SOD2, a Potential Transcriptional Target Underpinning CD44-Promoted Breast Cancer Progression. Molecules 2022;27(3):811

36.Erdman VV, Nasibullin TR, Tuktarova IA, Timasheva YR, Danilko KV, Viktorova, TV, Mustafina OE. The Study of Association of Polymorphic Markers of the SOD1, SOD2, and SOD3 Genes with Longevity. Russian Journal of Genetics 2020;56(12): 1504-1511

37.Sah SK, Agrahari G, Kim TY. Insights into superoxide dismutase 3 in regulating biological and functional properties of mesenchymal stem cells. Cell Biosci 2020;10:22

38.Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxid Med Cell Longev 2019;2019:9613090

39.Méndez-Armenta M, Nava-Ruíz C, Juárez-Rebollar D, Rodríguez-Martínez E, Gómez PY. Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. Oxid Med Cell Longev 2014;2014:293689

40.Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene 2008;27(48):6194-6206

41.Fiandalo MV, Kyprianou N. Caspase control: protagonists of cancer cell apoptosis. Exp Oncol 2012;34(3):165-175