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Research Article

Neuroprotective Effects of Urolithins A and B against H_2O_2-induced toxicity in PC12 cells

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ABSTRACT

Oxidative stress has been consistently implicated in a range of neurodegenerative diseases (NDDs). This detrimental condition is caused by exaggerated reactive oxygen species (ROS) generation which results in damage to neuronal cells. Urolithin A and B (UA and UB) are natural compounds derived from ellagitannins, which are abundant in pomegranate and other fruits. Herein, neuroprotective potential and underlying mechanisms of UA and UB against H_2O_2-induced oxidative injury of PC12 cells were investigated in this study. We found that pretreatment with UA and UB significantly rescued H_2O_2-induced toxicity in a dose-dependent manner, as demonstrated by increased cell viability and reduced ROS production. The pretreatment of PC12 cells with UA and UB could also modulate the expression of apoptotic-related proteins thereby inhibiting H_2O_2-induced apoptosis. Moreover, UA and UB activated the Nrf2 signaling and upregulated the expression of antioxidant genes including catalase, heme oxygenase-1 and superoxide dismutase 1 (SOD1). Consistently, the activity of SOD1 was increased after pretreatment of PC12 with UA and UB in the presence of H_2O_2. Overall, our study provides supporting evidence on the neuroprotective potential of UA and UB against oxidative stress-induced neuronal damage. Together, the findings suggest that both UA and UB could have potential health benefits for NDDs therapy and prevention.

Keywords: Urolithin A, Urolithin B, Neuroprotective, Nrf2, Antioxidant enzyme

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Introduction

Reactive oxygen species (ROS) are a class of chemically reactive molecules that are generated as byproducts of normal cellular metabolism. ROS play a critical role in various biological processes such as signaling, cell proliferation, differentiation, and apoptosis. These molecules are necessary for maintaining normal cellular functions. On the other hand, an imbalance caused by the overproduction and accumulation of these molecules can lead to a state of oxidative stress, which consequently led to damaging biological macromolecules such as lipids, proteins, and nucleic acids [1, 2]. Oxidative stress is extensively implicated in a wide range of pathological conditions, including cancer, aging, and neurodegenerative diseases (NDDs) [3]. ROS can cause DNA damage and mutations, leading to genetic instability and cancer development. In the brain, ROS are known as one of the contributing factors to the pathogenesis of NDDs, including Alzheimer's (AD) and Parkinson's disease (PD) [4]. Furthermore, ROS-mediated oxidative damage is one of the main factors that contribute to the aging process [5]. To counteract the detrimental effects of ROS, cells have evolved a complex network of antioxidant defense mechanisms. One of the key players in this defense system is the nuclear factor-erythroid 2-related factor 2 (Nrf2), that transcriptionally controls the expression of numerous antioxidant genes, including superoxide dismutase-1 (SOD1) and heme oxygenase-1 (HO-1), which are crucial for neutralization of ROS and maintenance of cellular redox homeostasis [6].

Hydrogen peroxide (H_2O_2) is a chemical that is commonly used to induce oxidative stress in cell culture studies [7, 8]. At low concentrations, H_2O_2 can act as a signaling molecule and participate in physiological processes such as cell proliferation and differentiation [9]. However, at high concentrations, H_2O_2 can cause oxidative stress by reacting with cellular components such as lipids, DNA, and proteins, leading to cell damage and death. Excessive amounts of H_2O_2 can increase intracellular ROS levels, leading to oxidative stress. The generated ROS can subsequently cause damage to mitochondria, accompanied by the release of pro-apoptotic factors and the activation of apoptotic pathways [10].

Nowadays, using medicinal plants extracts or bioactive compounds derived from natural sources are gained much of attention owing to their potential benefits to human health and minimal side effects [11, 12]. Urolithins are a group of polyphenolic compounds derived from ellagic acid, which is found in various fruits such as pomegranates, strawberries, and certain nuts. Urolithins are produced in the gut microbiota through the conversion of ellagitannins, which are present in those diet [13]. Urolithins have been reported in previous studies to exhibit a wide range of pharmacological activities, including anti-inflammation and anticancer [14, 15]. Additionally, urolithins have shown to modulate several fundamental signaling pathways including the NF-κB and the PI3K/Akt [16, 17]. Urolithin A and B (UA and UB) are two of the most abundant and well-studied urolithins. They have been shown to possess potent antioxidant activities and protect against oxidative damage in several cell types [18, 19]. Moreover, recent studies have shown neuroprotective properties of UA and UB in various models of NDDs, particularly AD and PD models [20, 21]. However, the protective mechanisms of UA and UB against H_2O_2-induced neurotoxicity in PC12 cells have not been fully explored. Therefore, this study

aimed to investigate neuroprotective properties of UA and UB against oxidative stress in PC12 cells induced by H_2O_2. The study's findings would provide a valuable insight into the potential therapeutic and preventive applications of these natural compounds in NDDs.

Materials and Methods

Chemicals and reagents

UA, UB and protease inhibitor cocktail were obtained from MedChemExpress, USA. Phosphate buffered saline (PBS) solution, RIPA lysis buffer, and poly-d-lysine (PDL) were from Thermo Fisher Scientific (MA, USA). Phenylmethylsulfonyl fluoride (PMSF) was from Biobasic (Toronto, Canada). Annexin V-FITC apoptosis detection assay kit with propidium iodide (PI) was purchased from BioLegend (CA, USA). Primary antibodies against Bax and Bcl2 were purchased from Santa Cruz Biotechnology (CA, USA). Anti-rabbit secondary antibody, anti-HO-1, anti-Nrf2, anti-SOD1 and anti-caspase-3 antibodies were from Cell Signaling Technology (MA, USA). Bradford reagent and 2',7'-dichlorofluorescin diacetate (DCFH-DA) were from Sigma-Aldrich (MO, USA). TRIzol reagent was purchased from Invitrogen (CA, USA). Skim milk powder and polyvinylidene fluoride (PVDF) membrane were brought from Merck Millipore (Darmstadt, Germany). The Acrylamide/Bis solution was from Bio-Rad Laboratories (CA, USA). The Amersham ECL Prime detection reagent was obtained from GE Healthcare (Freiburg, Germany).

Cell culture

PC12 cell line derived from rat adrenal pheochromocytoma cells, was obtained from American Type Culture Collection (ATCC, number; CRL-1721). The cells were cultured in RPMI-1640 medium supplemented with 10% horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin solution and maintained at 37°C in a humidified atmosphere of 5% CO_2. Cultured cells were changed with freshly prepared medium every 2 to 3 days or transferred to a new vessel when it reaches 80 to 90% confluency.

Cell viability assay

Cell viability was analyzed using the thiazolyl blue tetrazolium bromide (MTT) reagent (BioChemica, Sauerlach, Germany). PC12 cells were seeded in a PDL-coated 96-well plate at $2x10^4$ cells per well and allowed to grow for 24 h. Then the cells were pretreated with various doses of UA and UB (2.5, 5, 10, and 20 μM) for 18 h followed by 200 μM of H_2O_2 for 6 h. After the treatment, 10 μL of 5 mg/mL MTT was added to the well and further incubated for 3 h to induce formation of purple formazan crystal products. Then 1% SDS solution were added to solubilize MTT formazan overnight. The absorbance at 570 nm was read through a UV-VIS spectrophotometer (PerkinElmer, MA, USA).

Detection of Intracellular ROS

The intracellular ROS generation was analyzed with DCFH-DA, the cell-permeable oxidation-sensitive fluorescent probe (Sigma-Aldrich). Briefly, the cells were incubated with 10 μM DCFH-DA prepared in PBS for 30 min at 37°C in the dark. After washing with PBS, the cells were resuspended in fresh RPMI 1640 medium and the fluorescence intensity was measured using a fluorescence microplate reader (PerkinElmer) at an excitation and emission wavelength of 485 nm and 535 nm, respectively. The results were reported as relative fluorescence compared to the control.

Detection of Apoptosis

The Annexin V-FITC apoptosis detection assay kit with PI was used to assess apoptotic cells. Briefly, after 18 h pretreatment with UA and UB followed by 6 h incubation of 200 μM H_2O_2, the cells were harvested, washed with ice-cold PBS, and resuspended in 100 μL of binding buffer containing Annexin V-FITC and PI prior to incubation at room temperature for 15 min under the dark condition. The samples were then added with an additional 400 μL of binding buffer and subsequently analyzed using a flow cytometer (BD FACSCaliburTM), followed by data analysis with CellQuest Pro software.

RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

The mRNA expression levels of antioxidant genes were determined using qRT-PCR. Total RNA was achieved by TRIzol reagent (Invitrogen) and determined its quality and quantity by a NanoDrop spectrophotometer (Thermo Scientific). Then, 1 μg of total RNA and the RT Premix kit with oligo dT18 primer (Intron Biotechnology, Seoul, Korea) were used for synthesis of complementary DNA (cDNA). The qRT-PCR of the obtained cDNA was conducted with RealMOD™ Green W2 2x qPCR kit (Intron Biotechnology) and gene-specific primers (Table 1) on an ExiCycler™ 96 PCR system (Bioneer Daejeon, Korea). The PCR cycling condition starts with an initial denaturation step (a cycle of 95°C for 10 min), followed by the 40 cycles of amplification at 95°C for 20 sec and 58°C for 40 sec. The relative gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method and normalized with β-actin expression.

Table 1 Primer sequences of specific genes used in qRT-PCR.

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
CAT	TTTTCACCGACGAGATGGCA	CCCACAAGGTCCCAGTTACC
SOD1	ACCACTGCAGGACCTCATTT	CCTTTCCAGCAGCCACATTG
HO-1	TTAAGCTGGTGATGGCCTCC	GTGGGGCATAGACTGGGTTC
NRF2	ATTTGTAGATGACCATGAGTCGC	GCCAAACTTGCTCCATGTCC
β-actin	AGTACAACCTTCTTGCAGCTC	GACAATGCCGTGTTCAATGG

Western blotting

After treatment completion, the cells were washed twice with ice-cold PBS and lysed in RIPA lysis buffer containing protease inhibitor cocktail and PMSF. The supernatant was collected from cell

lysates after centrifugation at 13,800 xg at 4°C for 15 min, then protein concentration was measured by Bradford reagent (Sigma-Aldrich). Equivalent amount of protein samples was resolved in 10% or 12% sodium dodecyl sulfate-polyacrylamide gel (Bio-Rad Laboratories) and transferred onto PVDF membrane (Merck Millipore). The membrane was placed in 5% skim milk powder solution (Merck Millipore) in Tris-buffered saline with 0.1% Tween 20 (TBST) for an hour prior incubating with specific primary antibodies at 4°C for overnight. On the next day, the membrane was washed again with TBST and subsequently incubated with the horseradish peroxidase-conjugated secondary antibodies for an additional hour at room temperature. Protein blots were detected using Western blotting detection reagent and visualized by ChemiDoc imaging system (Bio-Rad Laboratories). Quantification of blot density was performed with ImageJ software (NIH, Bethesda, Maryland, USA)

Statistical analysis

Data was represented as the mean ± standard deviation (SD) from three independent experiments. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied to analyze the mean difference among group. Statistical significance is considered at p-value of less than 0.05.

Results and Discussion

UA and UB rescue H_2O_2-induced cell toxicity

To assess initial toxicity by MTT assay, different concentrations of UA and UB (2.5, 5, 10, 20, and 30 μM) were used to treat for 24 h. Resveratrol (Resv) and NAC were used as positive control and 0.1% DMSO was used as vehicle control (Veh). It was observed that most of the treatment concentrations were not toxic to the cells as indicated by a non-significant difference in percent cell viability when compared to both untreated and vehicle control (Figure 1-A). To evaluate the potential protective effects of UA and UB on H_2O_2-induced cell toxicity, we performed MTT assays on PC12 cells. As shown by Figure 1-B, H_2O_2 treatment resulted in a significant reduction in cell viability compared to the control group ($p < 0.001$). However, pretreatment with UA and UB was able to significantly increase the percent cell viability in a concentration-dependent manner.

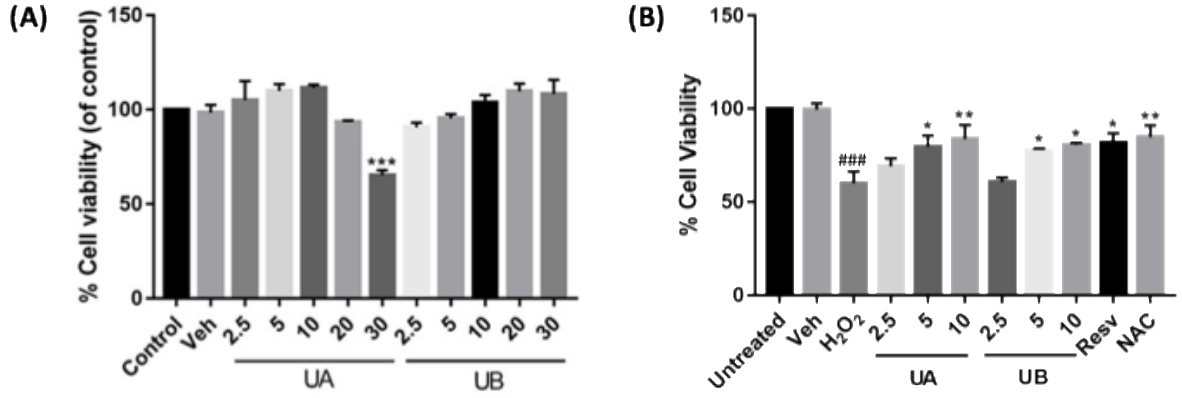

Figure 1 Cytotoxicity and neuroprotective effects of UA and UB in PC12 cells. For cytotoxicity assessment, (A) the cells were treated with UA or UB at various concentrations of 2.5, 5, 10, 20, and 30 μM for 24 h. For neuroprotective assessment, (B) cells were pretreated with UA or UB at the concentrations of 2.5, 5 and 10 μM for 18 h followed by 200 μM of H_2O_2 for another 6 h. Pretreatment with resveratrol or NAC was used as positive controls. For (A), ***$p < 0.001$ vs. Veh. For (B), *$p < 0.05$, **$p < 0.01$ vs. H_2O_2 and $^{###}p < 0.001$ vs. Veh.

UA and UB reduce H_2O_2-induced intracellular ROS

In order to determine intracellular ROS, the staining probe DCFH-DA was utilized and the result showed that H_2O_2 treatment markedly increased intracellular ROS ($p < 0.001$) as shown in Figure 2. However, at a concentration of 5 μM, UA and UB pretreatment showed a trend of ROS decrease and demonstrated a significant intracellular ROS reduction at a concentration of 10 μM as compared to the H_2O_2-exposed cells ($p < 0.05$). Resveratrol and NAC were used as positive controls.

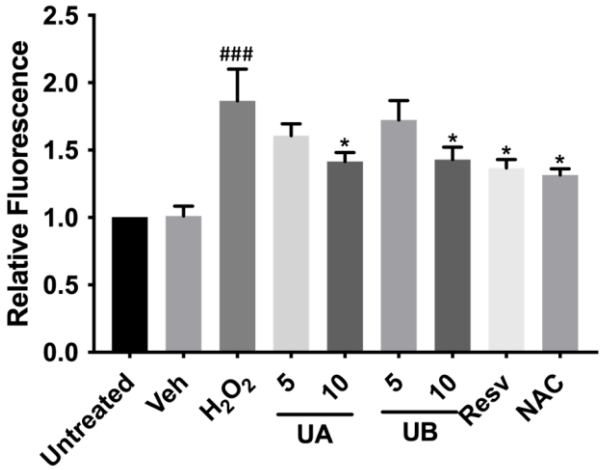

Figure 2 Protective effects of UA and UB against H_2O_2-induced ROS increase. Intracellular ROS was assessed using DCFH-DA staining method. Cells were pretreated with UA or UB at the concentration of 5 and 10 μM for 18 h, then treated with 200 μM of H_2O_2 for 15 min. Pretreatment with resveratrol or NAC was used as positive controls. *$p < 0.05$ vs. H_2O_2 and $^{###}p < 0.001$ vs. Veh.

UA and UB inhibit cell apoptosis induced by H_2O_2

Flow cytometry analysis was used to investigate apoptotic cell death. Resveratrol and NAC were used as positive controls. The result revealed that the percentage of apoptotic cells in the H_2O_2-treated group was significantly higher than that of control group ($p < 0.05$). However, this increased percentage was found significantly reduced after pretreatment with UA and UB at a concentration of 10 μM compared to the H_2O_2-treated group as shown in Figure 3-A. Additionally, the Western blot analysis showed that pretreatment with UA and UB inhibited the reduction of the apoptosis-related caspase-3 protein induced by H_2O_2 (Figure 3-B). When compared to the control group, the ratio of Bax/Bcl-2 expression was found significantly increased in the H_2O_2-treated group ($p < 0.05$). Whereas pretreatment with UA and UB at a concentration of 10 μM could significantly decrease Bax/Bcl-2 ratio expression compared to H_2O_2-treated group as shown in Figure 3-C.

Effects of UA and UB on antioxidant gene expression

The present study investigated the effect of UA and UB on the expression of antioxidant genes in PC12 cells. The qRT-PCR analysis was done to evaluate the mRNA expression levels of three antioxidant genes, namely, SOD1, HO-1, and catalase (CAT). The results showed that treatment with 10 μM UA and UB significantly reduced the mRNA expression level of HO-1 ($p < 0.05$) compared to the control group. At this concentration, both UA ($p < 0.01$) and UB ($p < 0.001$) also significantly increased SOD1 expression, whereas only UA was able to significantly upregulate CAT expression ($p < 0.05$).

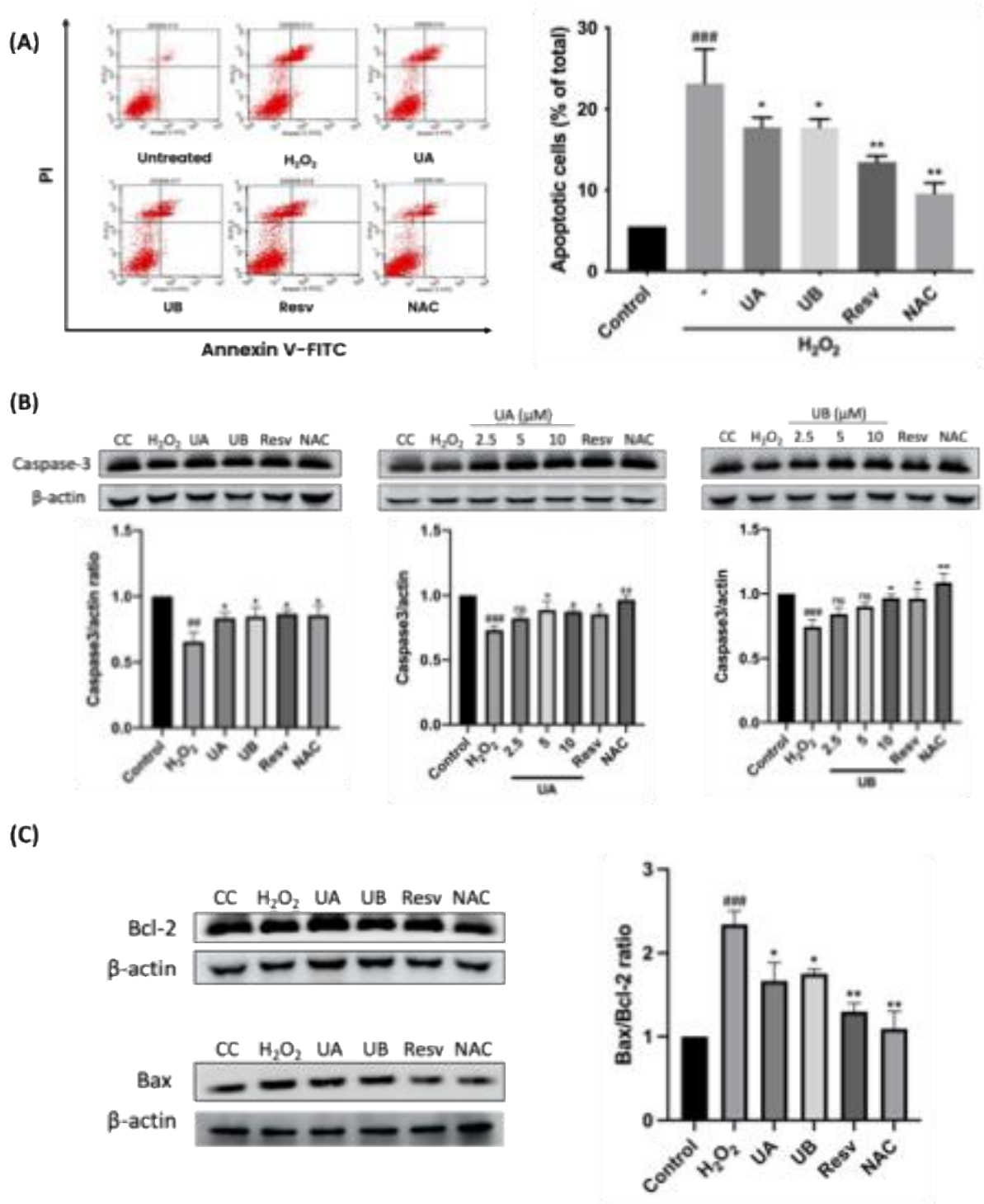

Figure 3 UA and UB reduced H_2O_2-induced cell death by modulating apoptotic proteins. Cells were pretreated with UA or UB for 18 h, followed by an additional 6 h incubation with 200 μM of H_2O_2. Resveratrol or NAC was used as positive controls. (A) Apoptotic cell death was assessed using flow cytometry. The protein levels of (B) caspase-3, (C) bax and bcl-2 were determined using Western blotting and normalized to the levels of β-actin. *$p < 0.05$, **$p < 0.01$, ^{ns} not significant vs. H_2O_2 and $^{###}p < 0.001$ vs. control.

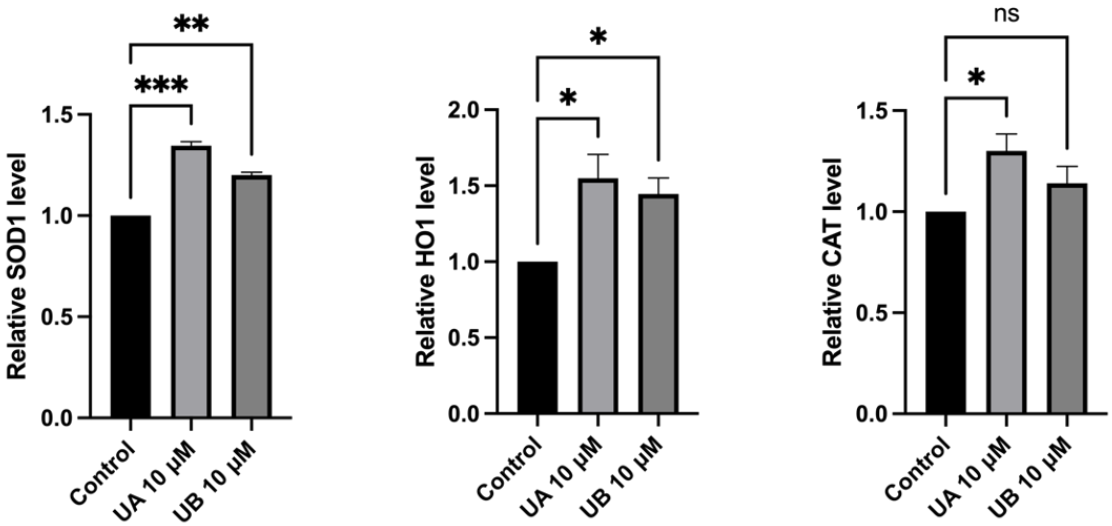

Figure 4 UA and UB increased gene expression levels of antioxidant genes. Cells were treated with UA or UB for 18 h and the mRNA expression levels of SOD1, HO-1 and CAT were performed by qRT-PCR. *p < 0.05, **p < 0.01 and ***p < 0.001, ^{ns} not significant vs. control.

Effects of UA and UB on Nrf2 pathway

To investigate the effects of UA and UB on the Nrf2 pathway in PC12 cells, the protein expression levels of Nrf2 and its downstream target SOD1 were evaluated by Western blotting. It was shown that cell treatment with H_2O_2 alone slightly increased Nrf2 protein expression level, indicating oxidative stress-induced activation of the Nrf2 pathway. However, treatment with UA and UB at concentrations of 5 and 10 μM significantly upregulated the Nrf2 levels compared to the untreated and H_2O_2-treated groups in a dose-dependent manner (Figure 5-A). Moreover, the change of SOD1, a downstream target of Nrf2, was further assessed. Treatment with H_2O_2 alone significantly downregulated the level of SOD1. However, pretreatment of UA and UB could dose-dependently rescue the downregulation of SOD1 compared to the H_2O_2-exposed group as shown in Figure 5-B.

UA and UB increase SOD1 enzymatic activity

Enzymatic activity assay was performed to assess the function of SOD1. It was observed that H_2O_2 treatment significantly reduced SOD1 activity compared to untreated control group. However, pretreatment with UA and UB significantly and dose-dependently increased SOD1 enzymatic activity when compared to the H_2O_2-treated group (Figure 6). Resveratrol and NAC were used as positive controls in this experiment.

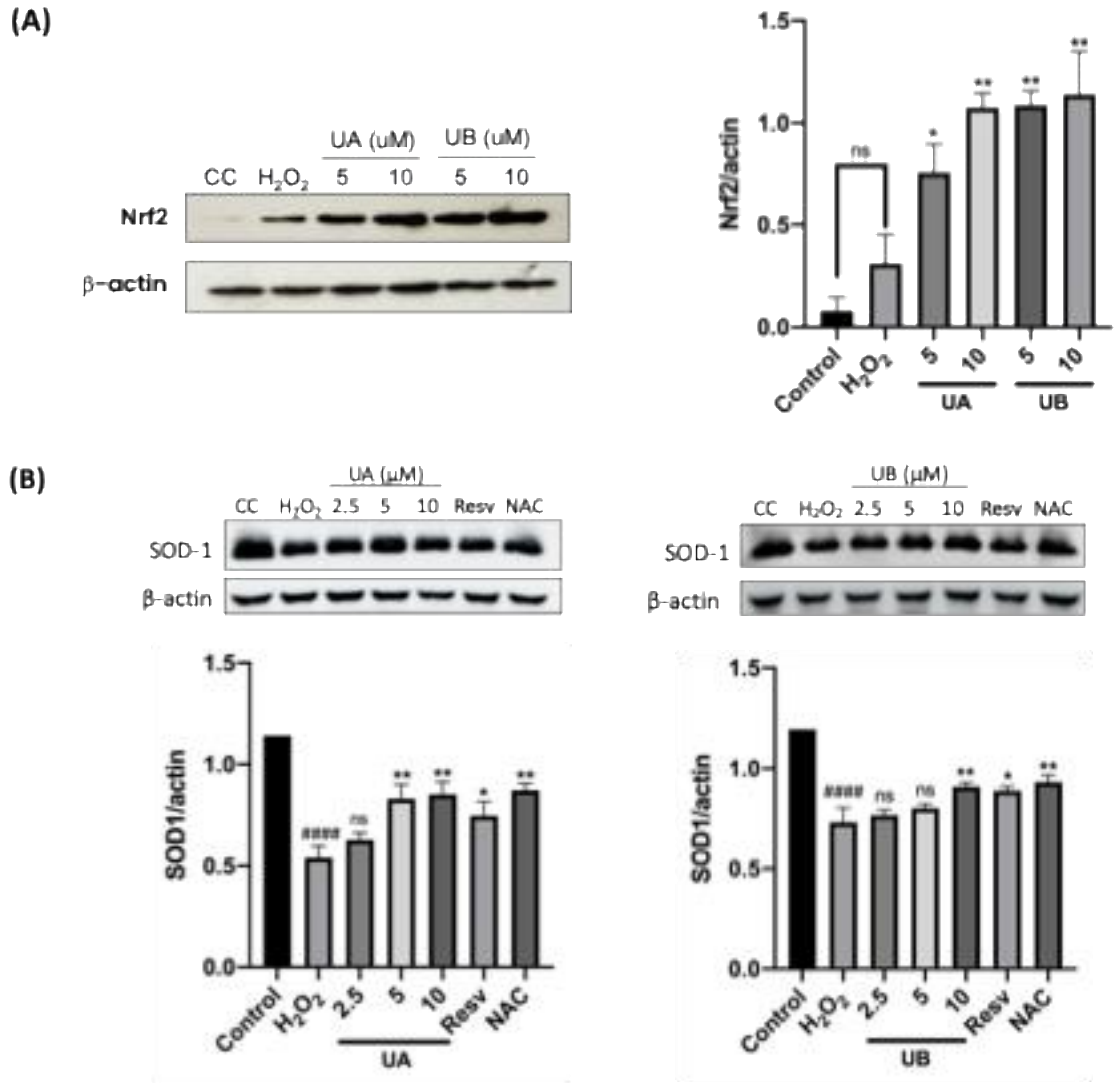

Figure 5 UA and UB increase expression of Nrf2 and its downstream target. Nrf2 proteins and its downstream target was examined using Western blot analysis. Cells were treated with UA or UB at indicated concentrations for 18 h, followed by treated with 200 μM of H_2O_2 for 6 h. The expression of Nrf2 and its downstream target, SOD1, was determined. Resveratrol and NAC were used as positive controls. β-actin was used as endogenous control. For (A), *$p < 0.05$, **$p < 0.01$ vs. H_2O_2, ^{ns} not significant vs. control. For (B), *$p < 0.05$, **$p < 0.01$, ^{ns} not significant vs. H_2O_2, $^{####}p < 0.0001$ vs. control.

This study used PC12 cells which are a widely used model system to study neuronal differentiation, neurotransmitter release, and neuroprotection. These cells are derived from a rat pheochromocytoma and exhibit many properties of sympathetic neurons, including the ability to synthesize and release catecholamines in response to stimuli. PC12 cells are sensitive to oxidative stress and undergo apoptotic cell death when exposed to high levels of ROS, making them an ideal model system to study the neuroprotective effects of natural compounds [22].

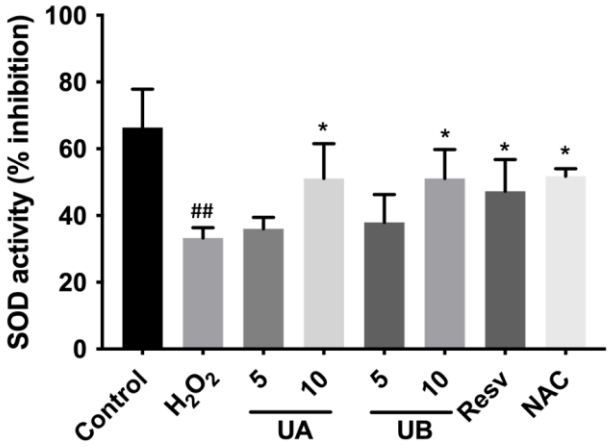

Figure 6 UA and UB increase SOD1 enzymatic activity. SOD1 enzymatic activity was determined using SOD Assay Kit-WST. Cells were pretreated with UA and UB at the concentrations of 5 and 10 μM for 18 h, followed by treating with 200 μM of H_2O_2 for 6 h. Pretreatment with resveratrol and NAC was used as positive controls. *$p < 0.05$ vs. H_2O_2 and $^{\#\#}p < 0.01$ vs. control.

The present study investigated the neuroprotective effects of UA and UB against H_2O_2-induced toxicity in PC12 cells. The results indicated that UA and UB treatment rescued H_2O_2-induced toxicity and reduced intracellular ROS levels, thereby inhibiting cell apoptosis. The protective effect of UA and UB on H_2O_2-induced toxicity was demonstrated through the MTT assay, which measures cell viability. The results showed that UA and UB treatment at concentrations of 5 and 10 μM significantly rescued the cell viability that was reduced by H_2O_2. These findings suggest that UA and UB can protect against oxidative stress-induced cell damage. The reduction of intracellular ROS levels by UA and UB was demonstrated by the DCFH-DA assay. UA and UB treatment significantly reduced H_2O_2-induced ROS production in a dose-dependent manner. Similarly, a study by Kim et al. using a neuroblastoma SK-N-MC cell line exposed to 300 μM of H_2O_2 for oxidative stress induction showed that pretreatment of UA at a concentration of 1.25, 2.5, and 5 μM could inhibit cytotoxicity and reduce H_2O_2-induced intracellular ROS [23]. It is well established that ROS can harm cellular components, leading to cellular impaired function and programmed cell death. Typically, cells under physiological conditions possess endogenous antioxidant defense system which may not be sufficient to protect cells against oxidative stress under aging or pathological conditions. Thus, the reduction of intracellular ROS levels by UA and UB suggests their potential as neuroprotective agents for maintaining cellular homeostasis and preventing cellular damage. Moreover, the inhibition of cell apoptosis by UA and UB was demonstrated through the Annexin V and PI staining as the percentage of apoptotic cells was significantly lower after UA and UB treatment when compared to cells induced by H_2O_2. Several studies have reported that UA could mitigate apoptosis in various experimental models of diseases. For example, UA administration attenuates neuronal apoptosis in a murine model of traumatic brain injury [24]. The administration of UA also prevents focal cerebral ischemia by inhibiting apoptosis and neuroinflammation in a mice model [25]. Apoptosis is known to play a crucial role in the pathological mechanisms of neuronal death in NDDs,

therefore the potential ability of UA and UB to inhibit apoptosis suggests their potential as therapeutic agents in counteracting these diseases.

Furthermore, UA and UB were found to upregulate antioxidant gene expression, particularly SOD1, HO-1, CAT, GPX1, and Nrf2, as demonstrated through real-time PCR analysis. Nrf2 functions as a transcription factor that regulates the transcription of genes involved in antioxidant defense systems, xenobiotic detoxification, and cellular protection, in which the genes include HO-1 and SOD1. The activation of the Nrf2 pathway has been shown to protect against oxidative stress-induced cell damage and has been extensively implicated in the pathogenesis of several NDDs. In the present study, UA and UB pretreatment upregulated the protein expression of Nrf2 and its downstream target, SOD1. It is also reported that UA and UB can activate Nrf2 pathway in several other studies. For example, UA could suppress myocardial fibrosis through Nrf2 activation as demonstrated its protective effect in both primary rat cardiac fibroblasts and rat model [26]. Similarly, UB could mitigate myocardial ischemia/reperfusion injury through the activated Nrf2 signaling cascade [27]. Moreover, the enzymatic assay indicated that pretreatment of UA and UB could increase SOD1 enzymatic activity, indicating the functional ability of Nrf2 activation on alleviating oxidative stress caused by H_2O_2. These findings suggest that the neuroprotective effects of UA and UB may be mediated, at least in part, by the activation of the Nrf2 pathway. Still, ongoing studies are essential to further elucidate the neuroprotective mechanisms of both compounds and also evaluate their effectiveness using cell lines and animal models of NDDs [28-30].

Conclusions

In conclusion, the present study demonstrates the neuroprotective effects of UA and UB against H_2O_2-induced toxicity in PC12 cells. The results suggest that UA and UB attenuate H_2O_2-induced ROS generation and oxidative damage, which ultimately leads to the protection of PC12 cells from apoptotic cell death. The findings suggest that both compounds exert their neuroprotective activities by activation of Nrf2 pathway, resulting in the upregulated mRNA and protein expressions of several key antioxidant enzymes including SOD1, that can scavenge ROS and prevent oxidative damage to cellular macromolecules. Overall, this study provides valuable insights into the potential therapeutic applications of UA and UB and contributes to supporting evidence of the beneficial effects of natural compounds on human health. The use of dietary polyphenols and their metabolites as neuroprotective agents represents a promising approach for therapy and prevention of NDDs.

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References

1. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.
2. Bardaweel SK, Gul M, Alzweiri M, Ishaqat A, HA AL, Bashatwah RM. Reactive oxygen species: the dual role in physiological and pathological conditions of the human body. Eurasian J Med. 2018;50(3):193-201.
3. Klaunig JE. Oxidative stress and cancer. Curr Pharm Des. 2018;24(40):4771-8.
4. Cassidy L, Fernandez F, Johnson JB, Naiker M, Owoola AG, Broszczak DA. Oxidative stress in alzheimer's disease: a review on emergent natural polyphenolic therapeutics. Complement Ther Med. 2020;49:102294.
5. Maldonado E, Morales-Pison S, Urbina F, Solari A. Aging hallmarks and the role of oxidative stress. Antioxidants (Basel). 2023;12(3):651.
6. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284(20):13291-5.
7. Kang J, Wang Y, Guo X, He X, Liu W, Chen H, et al. N-acetylserotonin protects PC12 cells from hydrogen peroxide induced damage through ROS mediated PI3K/AKT pathway. Cell Cycle. 2022;21(21):2268-82.
8. Jiang HH, Yan FS, Shen L, Ji HF. Silymarin versus Silibinin: differential antioxidant and neuroprotective effects against H_2O_2-induced oxidative stress in PC12 cells. Nat Prod Commun. 2016;11(5):633-6.
9. Di Marzo N, Chisci E, Giovannoni R. The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells. Cells. 2018;7(10).
10. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977-92.
11. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytobioactive compounds as therapeutic agents for human diseases: a review. Food Sci Nutr. 2023;11(6):2500-29.
12. Khot KB, Gopan G, Bandiwadekar A, Jose J. Current advancements related to phytobioactive compounds based liposomal delivery for neurodegenerative diseases. Ageing Res Rev. 2023;83:101806.
13. Cerda B, Tomas-Barberan FA, Espin JC. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. J Agric Food Chem. 2005;53(2):227-35.
14. Tomas-Barberan FA, Gonzalez-Sarrias A, Garcia-Villalba R, Nunez-Sanchez MA, Selma MV, Garcia-Conesa MT, et al. Urolithins, the rescue of "old" metabolites to understand a "new" concept:

metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. Mol Nutr Food Res. 2017;61(1).

15. Espin JC, Larrosa M, Garcia-Conesa MT, Tomas-Barberan F. Biological significance of urolithins, the gut microbial ellagic acid-derived metabolites: the evidence so far. Evid Based Complement Alternat Med. 2013;2013:270418.
16. Fu X, Gong LF, Wu YF, Lin Z, Jiang BJ, Wu L, et al. Urolithin A targets the PI3K/Akt/NF-kappaB pathways and prevents IL-1beta-induced inflammatory response in human osteoarthritis: in vitro and in vivo studies. Food Funct. 2019;10(9):6135-46.
17. Abdelazeem KNM, Kalo MZ, Beer-Hammer S, Lang F. The gut microbiota metabolite urolithin A inhibits NF-kappaB activation in LPS stimulated BMDMs. Sci Rep. 2021;11(1):7117.
18. Kallio T, Kallio J, Jaakkola M, Maki M, Kilpelainen P, Virtanen V. Urolithins display both antioxidant and pro-oxidant activities depending on assay system and conditions. J Agric Food Chem. 2013;61(45):10720-9.
19. Djedjibegovic J, Marjanovic A, Panieri E, Saso L. Ellagic Acid-derived urolithins as modulators of oxidative stress. Oxid Med Cell Longev. 2020;2020:5194508.
20. Li HL, Zhang SY, Ren YS, Zhou JC, Zhou YX, Huang WZ, et al. Identification of ellagic acid and urolithins as natural inhibitors of Abeta(25-35)-induced neurotoxicity and the mechanism predication using network pharmacology analysis and molecular docking. Front Nutr. 2022;9:966276.
21. Liu J, Jiang J, Qiu J, Wang L, Zhuo J, Wang B, et al. Urolithin A protects dopaminergic neurons in experimental models of Parkinson's disease by promoting mitochondrial biogenesis through the SIRT1/PGC-1alpha signaling pathway. Food Funct. 2022;13(1):375-85.
22. Xie D, Deng T, Zhai Z, Sun T, Xu Y. The cellular model for Alzheimer's disease research: PC12 cells. Front Mol Neurosci. 2022;15:1016559.
23. Kim KB, Lee S, Kim JH. Neuroprotective effects of urolithin A on H(2)O(2)-induced oxidative stress-mediated apoptosis in SK-N-MC cells. Nutr Res Pract. 2020;14(1):3-11.
24. Gong QY, Cai L, Jing Y, Wang W, Yang DX, Chen SW, et al. Urolithin A alleviates blood-brain barrier disruption and attenuates neuronal apoptosis following traumatic brain injury in mice. Neural Regen Res. 2022;17(9):2007-13.
25. Lin XH, Ye XJ, Li QF, Gong Z, Cao X, Li JH, et al. Urolithin A prevents focal cerebral ischemic injury via attenuating apoptosis and neuroinflammation in Mice. Neuroscience. 2020;448:94-106.
26. Chen P, Pei J, Wang X, Tai S, Tang L, Hu X. Gut bacterial metabolite Urolithin A inhibits myocardial fibrosis through activation of Nrf2 pathway in vitro and in vivo. Mol Med. 2022;28(1):19.
27. Zheng D, Liu Z, Zhou Y, Hou N, Yan W, Qin Y, et al. Urolithin B, a gut microbiota metabolite, protects against myocardial ischemia/reperfusion injury via p62/Keap1/Nrf2 signaling pathway. Pharmacol Res. 2020;153:104655.
28. Kittimongkolsuk P, Sukjamnong S, Janpaijit S, Rangsinth P, Chumpolphant S, Chaikhong K, et al. Neuroprotective properties of wild mango (*Mangifera caloneura Kurz*) leaves on alleviating urban

air pollutant toxicity: insights from transcriptome analysis of a human neuronal cell model. Food Frontiers. 2025; 6:1987-2010.

29. Rangsinth P, Zheng C, Shiu PH-T, Wang W, Kwong TC, Choy CT, et al. *Amauroderma rugosum* extract improves brain function in d-Galactose-Induced aging mouse models via the regulatory effects of its polysaccharides on oxidation, the mTOR-Dependent pathway, and gut microbiota. Food Frontiers. 2025;6(2):872-90.
30. Kumaree KK, Brimson JM, Verma K, Chuchawankul S, Tencomnao T, Prasansuklab A. Agarwood leaf ethanol extract provides neuroprotective properties and promotes cholinergic differentiation of HT22 hippocampal neurons. Sci Rep. 2025;15(1):10230.