Original Article

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Eriodictyol attenuates hydrogen peroxide-induced cell death in neuronal SH-SY5Y cells

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ABSTRACT

Background: Eriodictyol, a flavonoid isolated from *Dendrobium ellipsophyllum*, provides health benefits in several oxidative stress-related diseases. **Objective:** This study aimed to determine the protective effect and mechanisms of eriodictyol on hydrogen peroxide (H_2O_2)-induced cell death in neuronal SH-SY5Y cells. **Methods:** The protective effect of eriodictyol against H_2O_2 was evaluated by resazurin assay. Reactive oxygen species (ROS) and the mitochondrial membrane potential were detected by 2'-7' dichlorodihydrofluorescein diacetate and tetramethylrhodamine ethyl ester assays, respectively. Finally, the effects of eriodictyol on antioxidant enzyme expression and the mitochondria-mediated apoptotic cascade were determined using Western blot analysis. **Results:** Pre-treatment with eriodictyol suppressed cell death mediated by H_2O_2. Intracellular ROS and the loss of mitochondrial membrane potential induced by H_2O_2 were significantly reduced following eriodictyol treatment. Western blot analysis revealed that H_2O_2 enhanced the expression of BAX/Bcl-2, cytochrome c, cleaved caspase-3, and caspase-3, which was, in turn, attenuated in the presence of eriodictyol. Furthermore, pre-treatment with eriodictyol extensively promoted the expression of antioxidant enzymes including superoxide dismutase and catalase, indicating the significant neuroprotective effect of eriodictyol through an antioxidant-dependent mechanism. **Conclusion:** Eriodictyol shows a protective effect against H_2O_2 -induced neuronal cell death in SH-SY5Y cells through a decrease of ROS production, an increase in antioxidant enzyme expression, the protection of mitochondrial function, and the inhibition of the mitochondria-mediated apoptotic cascade. Our data provide scientific information for the development of potent therapeutic agents for neurodegenerative diseases.

Keywords: *Dendrobium ellipsophyllum*, Eriodictyol, hydrogen peroxide, reactive oxygen species, SH-SY5Y

INTRODUCTION

Neurodegenerative diseases are associated with progressive and irreversible neuronal death[1] and oxidative stress plays a major role in the pathogenesis of these diseases.[2] Excessive oxidative stress is caused by an imbalance in the generation of reactive oxygen species (ROS) and antioxidants and antioxidant enzymes that have a protective effect on neurons.[3] ROS mediate cellular lipid, protein, and DNA damage, leading to mitochondrial dysfunction and cell death.[3-5] Hydrogen peroxide (H_2O_2), one of the major ROS, can diffuse through the plasma membrane[6] and is readily converted into highly toxic hydroxyl radicals through Fenton's reaction.[3] H_2O_2 caused an increase in intracellular ROS, lipid peroxidation, and mitochondrial dysfunction and a decrease in antioxidant enzymes such

as superoxide dismutase (SOD) and catalase (CAT)[7] and consequently promoted mitochondrial-mediated apoptosis in several cell types including neurons.[8,9] Therefore, either the suppression of ROS levels or the upregulation of antioxidant enzymes might be an intriguing strategy for the treatment of neurodegenerative disorders.

Eriodictyol is a flavonoid isolated from the whole *Dendrobium ellipsophyllum* Tang and Wang (locally known in Thai as Ueang Thong) plant [Figure 1]. The previous studies reported its pharmacological activities including its antimetastatic,[10] antioxidant,[11] and anti-inflammatory[12] properties. In addition, eriodictyol attenuated β-amyloid (25–35)-induced oxidative cell death in primary neurons by activating Nrf2.[13] This study shows the effect of eriodictyol against H_2O_2-induced apoptotic cell death in SH-SY5Y cells, a neuronal cell culture model, and the underlying mechanism of this effect. This study supports the use of eriodictyol for further drug research and development in the treatment of neurodegenerative diseases.

MATERIALS AND METHODS

Cell Culture

Human neuroblastoma SH-SY5Y cells were purchased from the ATCC (Manassas, VA, USA). SH-SY5Y cells were cultured in DMEM-F12 medium containing 10% fetal bovine serum and 0.1% penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. The medium was changed every 2 days and subcultured once the cell confluence was 80–90%.

Chemicals and Reagents

Eriodictyol was extracted from the whole *D. ellipsophyllum* Tang and Wang plant as previously described (Tanagornmeatar *et al*., 2014). Resazurin, 2'-7' dichlorodihydrofluorescein diacetate (DCFH-DA), tetramethylrhodamine ethyl ester (TMRE), phenylmethanesulfonyl fluoride (PMSF), sodium orthovanadate, and NP-40 were purchased from Sigma Chemicals (MO, USA). Dimethyl sulfoxide was purchased from Fisher Scientific (Loughborough, UK). H_2O_2 was purchased from Ajax Finechem (Auckland, New Zealand). Rabbit monoclonal anti-SOD, anti-CAT, and anti-cytochrome c antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit monoclonal anti-BAX, anti-Bcl-2, and anti-caspase-3 antibodies were purchased from Cell Signaling Technology (Frankfurt, Germany). Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibodies were purchased from Millipore (Billerica, MA, USA). A protease inhibitor cocktail was purchased from Roche Diagnostics GmbH (Mannheim, Germany).

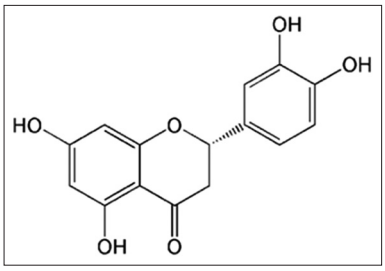

Figure 1: Chemical structure of (2S)-eriodictyol

Cell Viability Assay

SH-SY5Y cells were seeded onto 96-well plates at a density of 5×10^4 cells/well. To select the toxic concentration of H_2O_2, cells were incubated with H_2O_2 (100–1000 μM) for 12 h. To investigate the protective effect of eriodictyol, cells were seeded and pretreated with eriodictyol at 0.1, 1, and 10 μM for 24 h. Then, the medium was removed and replaced with a toxic concentration of H_2O_2 for 12 h. Cell viability was determined using a resazurin assay. Ten microliters of resazurin (0.05 mg/ml) was added to each well 4 h before the end of the incubation time. Fluorescence intensity was then measured at 530 nm excitation and 590 nm emission wavelengths using a microplate reader (CLARIOstar®, BMG LABTECH, Germany).

Intracellular ROS Levels

Intracellular ROS were detected by DCFH-DA assay. SH-SY5Y cells were seeded onto 96-well plates at a density of 5×10^4 cells/well. Cells were incubated with eriodictyol at 0.1, 1, and 10 μM for 24 h followed by H_2O_2 (200 μM) for 1 h. Cells were rinsed with phosphate-buffered saline (PBS) and DCFH-DA (10 μM) was added. After incubation for 30 min, the fluorescence intensity was quantified using a microplate reader with 488 nm excitation and 575 nm emission wavelengths.

Mitochondrial Membrane Potential

The TMRE assay was used to determine the mitochondrial membrane potential. SH-SY5Y cells were seeded onto 96-well plates at a density of 5×10^4 cells/well and incubated with eriodictyol at 0.1, 1, and 10 μM for 24 h. The medium was removed and H_2O_2 (200 μM) was added and incubated for 12 h. After these treatments, the cells were rinsed with PBS solution, and 100 nM TMRE was added to each well and incubated for 15 min. Fluorescent intensity was determined by a microplate reader at an excitation wavelength of 585 mm and an emission wavelength of 535 nm.

Western Blot Analysis

Cells were seeded onto 6-well plates at a density of 2×10^6 cells/well and incubated with eriodictyol at 0.1, 1, and 10 μM for 24 h. The medium was removed and H_2O_2 (200 μM) was added and incubated further for 12 h. After treatment, the cells were washed with PBS and lysed in lysis buffer containing 1 mM PMSF, protease inhibitor cocktail, sodium fluoride, sodium orthovanadate, and 1% NP-40 for 30 min on ice. Supernatants were separated by centrifugation at 16,000× g for 15 min at 4°C. The protein concentration was determined using a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Equal amounts of protein in each sample were dissolved in a 15% SDS-polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane. Non-specific proteins in the membrane were blocked with 5% skim milk containing 0.5 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 7.5), 150 mM NaCl, and 0.1% Tween-20 for 1 h at room temperature, and the membrane was subsequently incubated with primary antibodies against BAX (1:1000), Bcl-2 (1:1000), caspase-3 (1:1000), cytochrome c (1:1000), GAPDH (1:1000), SOD (1:500), and CAT (1:500) overnight

at 4°C. After three washes with tris-buffered saline with 0.1% Tween-20 (TBST), the blots were incubated with the corresponding HRP-conjugated secondary antibody (1:1000) in TBST with 5% skim milk for 2 h at room temperature. The blots were then washed 4 times in TBST buffer and developed using the enhanced chemiluminescence method by a Luminescent Image Analyzer (ImageQuant™ LAS 4000, GE Healthcare, Sweden). Protein bands were quantified by densitometric analysis using ImageJ software (NIH, USA).

Statistical Analysis

The data are presented as the mean ± standard error of the mean from three independent experiments. Data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by LSD *post hoc* was used to compare the means between groups. Differences were considered statistically significant if $P < 0.05$.

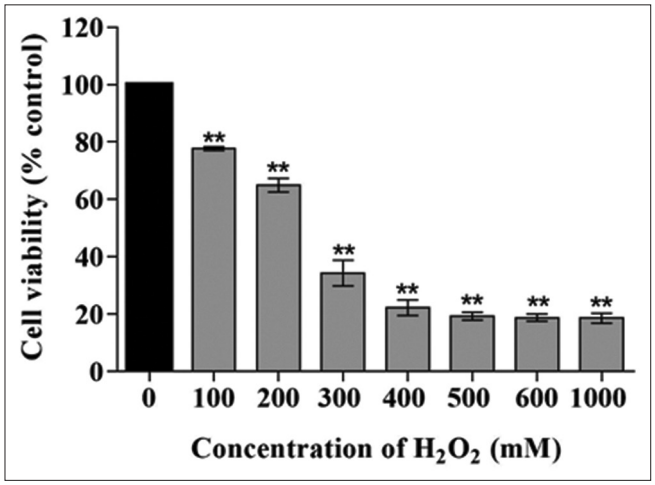

Figure 2: The effect of hydrogen peroxide (100–1000 μM) on cell viability. Data are presented as mean ± standard error of the mean of three independent experiments. $^{**}P < 0.01$ compared to control

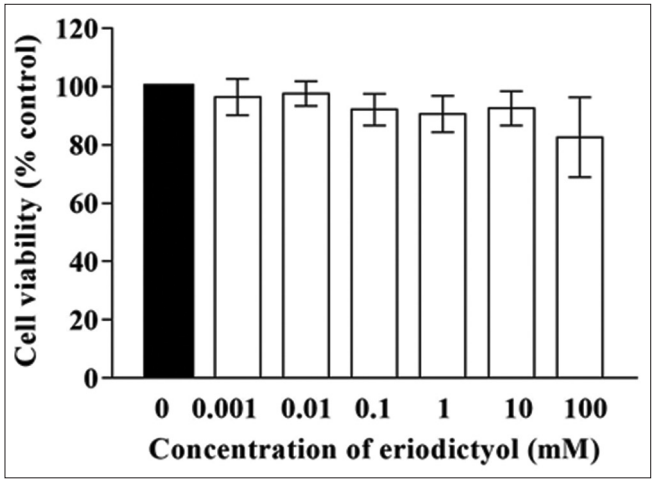

Figure 3: The effect of eriodictyol (0.001–100 μM) on cell viability. Data are presented as mean ± standard error of the mean of three independent experiments

RESULTS

Effect of Eriodictyol on H_2O_2-induced Cell Death

Following H_2O_2 incubation, viable cells were gradually reduced in number in a concentration-dependent manner ($P < 0.01$, Figure 2). H_2O_2 at a concentration of 200 μM significantly decreased the percentage of viable cells to approximately 50% ($P < 0.01$ vs. control). Eriodictyol (0.001–100 μM) did not affect cell viability [Figure 3]. However, the highest concentration of eriodictyol (100 μM) slightly decreased cell viability by 17.36%. Therefore, three concentrations of eriodictyol, 0.1, 1, and 10 μM, were selected for further study. We then pretreated the cells with eriodictyol (0.1, 1, and 10 μM) followed by H_2O_2. Eriodictyol pre-treatment increased cell viability up to 80% compared to that observed with H_2O_2 treatment alone ($P < 0.01$, Figure 4), indicating the protective effect of eriodictyol against H_2O_2-induced cell death.

Effect of Eriodictyol on Intracellular ROS and Antioxidant Enzyme Levels in H_2O_2-treated SH-SY5Y Cells

To investigate the intracellular H_2O_2-mediated ROS levels, cells were treated with the indicated concentration of H_2O_2. As shown in Figure 5a, the intracellular ROS levels were markedly increased by 1.4-fold compared to those in the control ($P < 0.05$). Furthermore, pre-treatment with eriodictyol (0.1, 1, and 10 μM) clearly suppressed intracellular ROS mediated by H_2O_2 compared to H_2O_2 treatment alone ($P < 0.05$, Figure 5a), suggesting the antioxidant effect of eriodictyol.

Since SOD and CAT are important enzymes in maintaining a proper intracellular oxidative status,[14] we investigated whether the antioxidant activity of eriodictyol occurred through increased antioxidant enzyme levels. Western blot analysis revealed that SOD and CAT expression were reduced after H_2O_2 treatment ($P < 0.05$ vs. control, Figure 5b), and eriodictyol (0.1 and 1 μM) pre-treatment reversed this increase

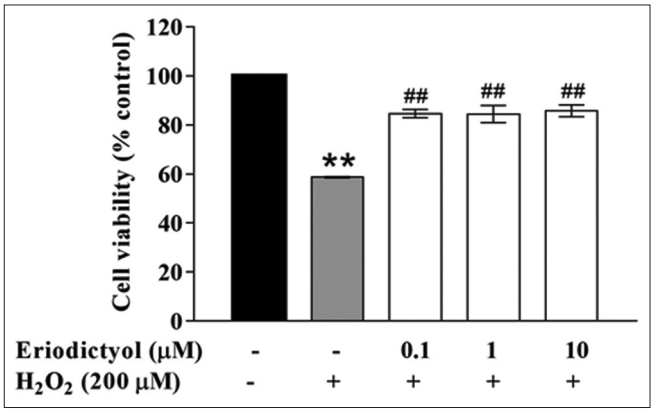

Figure 4: The protective effects of eriodictyol on hydrogen peroxide (H_2O_2)-induced cell death in SH-SY5Y cells. Data are presented as mean ± standard error of the mean of three independent experiments. $^{**}P < 0.01$ compared to control; $^{\#\#}P < 0.01$ compared to H_2O_2 treatment alone

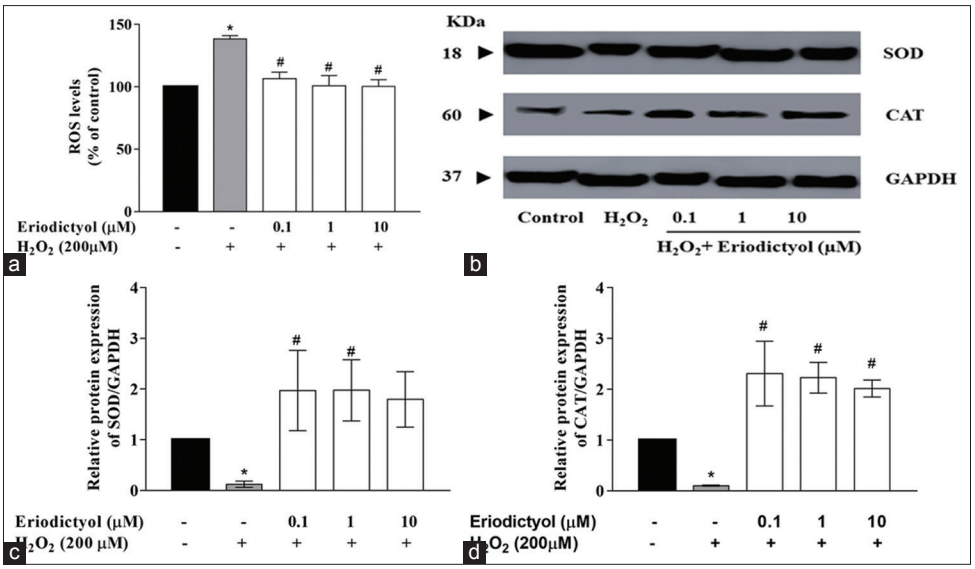

Figure 5: The effects of eriodictyol on intracellular reactive oxygen species levels (a) and superoxide dismutase (SOD) and catalase (CAT) expression (b). SOD (c) and CAT (d) expression were quantified and expressed as SOD/glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and CAT/GAPDH. Data are presented as mean ± standard error of the mean of three independent experiments. *$P < 0.05$ compared to control; #$P < 0.05$ compared to hydrogen peroxide treatment alone

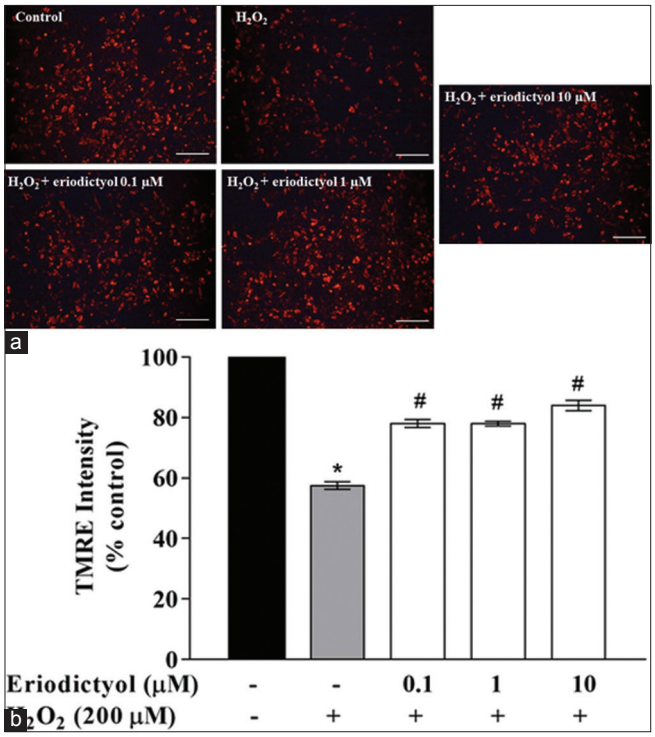

Figure 6: The effects of eriodictyol on mitochondrial membrane potential in SH-SY5Y cells. (a) Fluorescent images of the cells stained with tetramethylrhodamine ethyl ester (TMRE). (b) Quantification of fluorescence in the cells stained with TMRE. Data are presented as mean ± standard error of the mean of three independent experiments. *$P < 0.05$ compared to control; #$P < 0.05$ compared to hydrogen peroxide treatment alone. The scale bar is 100 μM

in expression compared to H_2O_2 treatment alone ($P < 0.05$, Figure 5c and d). This indicates that the antioxidant effect of eriodictyol might be through the upregulation of SOD and CAT -dependent mechanism.

Effect of Eriodictyol on the Mitochondrial Membrane Potential in H_2O_2-treated SH-SY5Y Cells

A reduction in the mitochondrial membrane potential is an important characteristic of H_2O_2-mediated neuronal death.[1] We then tested whether eriodictyol could protect against dysregulation of the mitochondrial membrane potential induced by H_2O_2. H_2O_2-treated cells exhibited a significantly lower in mitochondrial membrane potential than the control cells ($p < 0.05$). Importantly, eriodictyol (0.1, 1, and 10 μM) clearly elevated the mitochondrial membrane potential in H_2O_2-treated cells compared to that observed following treatment with H_2O_2 alone ($P < 0.05$, Figure 6), indicating that eriodictyol protects against mitochondrial membrane potential dysfunction caused by H_2O_2.

Effect of Eriodictyol on Apoptotic Regulatory Protein Expression

The expression levels of apoptotic regulatory proteins were determined. As shown in Figure 7, the ratio between the proapoptotic protein BAX and the anti-apoptotic protein Bcl-2 was clearly higher in H_2O_2-treated cells than in the non-treated group ($P < 0.05$). Interestingly, the Bcl-2/BAX ratio was significantly lower with eriodictyol pre-treatment (0.1, 1, and 10 μM) than without it ($P < 0.05$, Figure 7), suggesting that the protective effect of eriodictyol was through the regulation of BAX and Bcl-2 expression.

Furthermore, the levels of the cytosolic apoptotic markers cytochrome c and cleaved caspase-3 were investigated by Western blot analysis. H_2O_2 increased the expression of cytosolic cytochrome c, caspase-3, and cleaved caspase-3 ($P < 0.05$, Figure 8) compared to their expression without H_2O_2 treatment, which substantially decreased in response to eriodictyol (0.1, 1, and 10 μM) pre-treatment ($P < 0.05$). These data indicate that H_2O_2 enhanced cell death through apoptosis, which could be rescued by eriodictyol.

DISCUSSION

Our study provides an intriguing evidence of the effect of eriodictyol on H_2O_2-induced neuronal cell death. ROS have been identified as a major cause of neurodegenerative diseases that are involved in the impairment or loss of neuronal cells.[15] Oxidative stress caused by ROS damages various cell components, disturbing mitochondrial function, and consequently triggering programmed cell death.[1] H_2O_2, a highly toxic free radical, causes ROS generation, oxidative cell stress, and mitochondrial dysfunction imitating the molecular events involved in neuronal apoptosis in neurodegenerative diseases.[9] Consistent with this, our data also demonstrated that the number of viable cells was extensively reduced in response to H_2O_2 exposure, together with an increase in intracellular ROS [Figure 5a]. ROS might, therefore, be an interesting target for the treatment of neurodegenerative disorders.

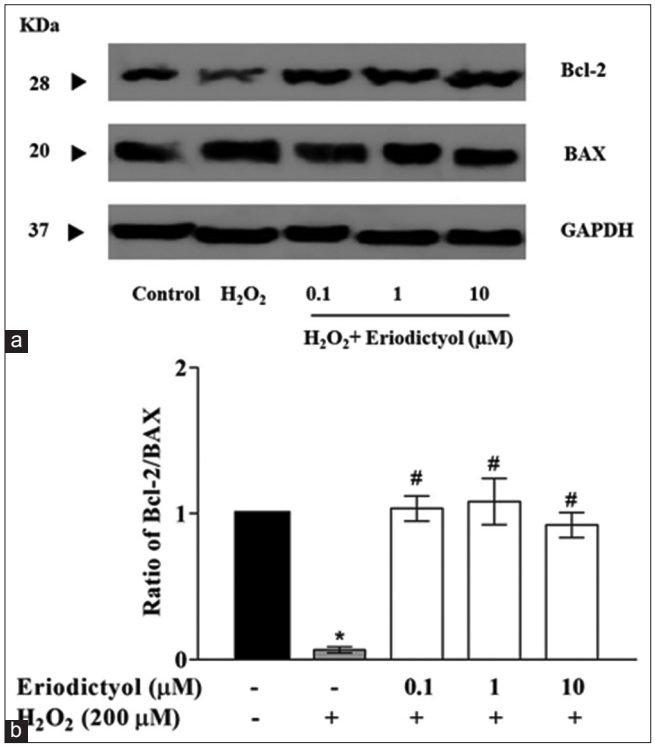

Figure 7: Effects of eriodictyol on BAX and Bcl-2 expression (a) and the BAX/Bcl-2 ratio (b). Data are presented as mean ± standard error of the mean of three independent experiments. *$P < 0.05$ compared to control; #$P < 0.05$ compared to hydrogen peroxide -treated alone

Many attempts have been made to discover new compounds with potent antioxidant activity. Flavonoids are a group of compounds that are well known for their radical scavenging properties. Flavonoids directly scavenge ROS and decrease oxidative stress levels by donating a hydrogen atom or an electron to ROS and deactivating them.[16,17] Our data also reported that eriodictyol suppresses intracellular ROS production mediated by H_2O_2. The direct ROS scavenging effect of eriodictyol was previously reported in the DPPH assay.[18] As eriodictyol reduces intracellular ROS levels at 1 h after H_2O_2 treatment, we proposed that eriodictyol scavenges ROS and consequently reduces ROS levels.

Since the ROS levels were determined in the presence of the intracellular antioxidant system and ROS production,[19] we found that eriodictyol shifted this balance favorably and upregulation of SOD and CAT expression, thereby reducing intracellular ROS and preventing cell death [Figure 5]. It has been reported that the Nrf2 pathway contributes to the defense mechanism against ROS at the transcriptional level through the activation of cytoprotective related genes,[20] and a previous study demonstrated that eriodictyol activated this

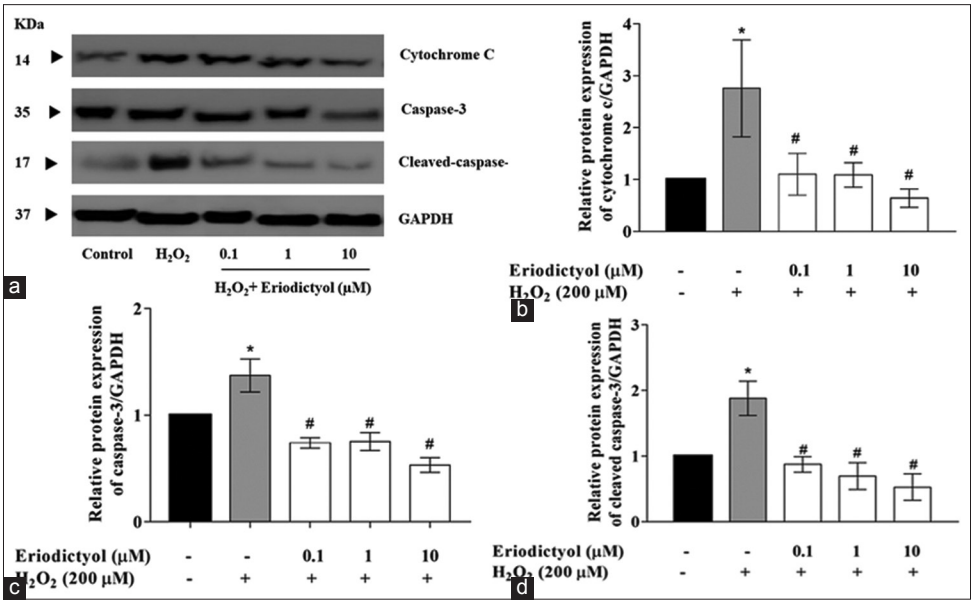

Figure 8: Effects of eriodictyol on cytochrome c, caspase-3, and cleaved caspase-3 expression (a). The expression levels of cytochrome c (b), caspase-3 (c), and cleaved caspase-3 (d) were calculated and expressed as cytochrome c/glyceraldehyde 3-phosphate dehydrogenase (GAPDH), caspase-3/GAPDH, and cleaved caspase-3/GAPDH. Data are presented as mean ± standard error of the mean of three independent experiments. *$P < 0.05$ compared to control; #$P < 0.05$ compared to hydrogen peroxide -treated alone

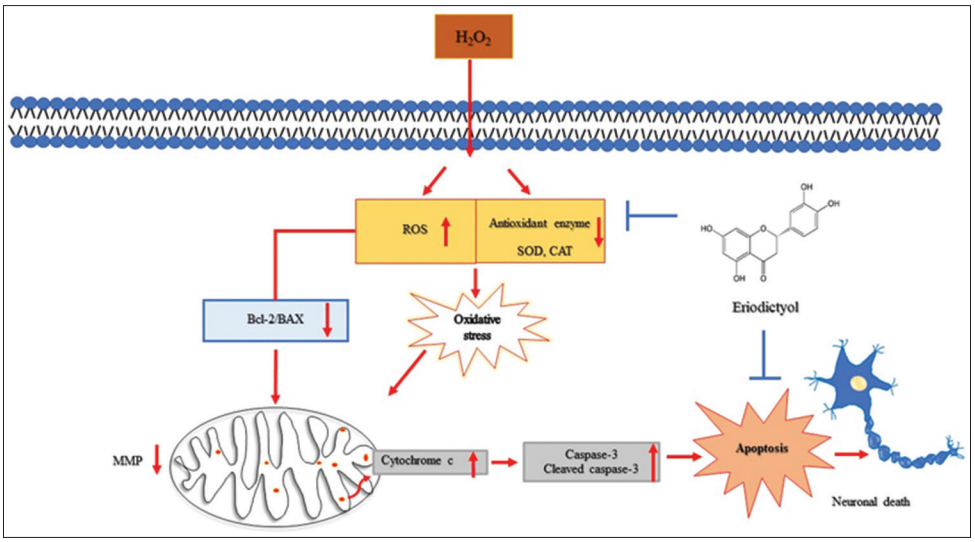

Figure 9: The proposed protective mechanism of eriodictyol against hydrogen peroxide-induced cytotoxicity in SH-SY5Y cells

pathway. The neuroprotective effect of eriodictyol in our study might be due to the stimulation of the Nrf2 mechanism by eriodictyol.

Mitochondrial dysfunction as a consequent event of oxidative stress was implicated in neurodegenerative diseases. Mitochondrial deficits are present in the brains of patients with Alzheimer's and/or Parkinson's disease.[21] Excessive ROS generation caused by H_2O_2 leads to hyperpolarization of the mitochondrial membrane, a decrease in adenosine triphosphate levels, and the release of cytochrome c and apoptosis activation.[22,23] Our study demonstrated that eriodictyol protected against mitochondrial membrane potential loss and cytochrome c release [Figures 6 and 8]. Apoptotic cell death by the mitochondrial pathway is mainly regulated by Bcl-2 family proteins.[23] A previous study showed that H_2O_2 increased the expression of BAX, cleaved caspase-3, and cleaved caspase-9, whereas it decreased Bcl-2 protein expression.[17] Feng *et al.* (2016) found that lycopene, another flavonoid, protected against SH-SY5Y cell death through inhibiting pro-apoptotic proteins and promoting the expression of antiapoptotic proteins.[24] The present study also found that eriodictyol inhibited H_2O_2-induced apoptosis by minimizing BAX expression in contrast to Bcl-2, an underlying mechanism of the cytoprotective effect of this compound.

CONCLUSION

Our results suggested that eriodictyol exerted protective effects against H_2O_2-induced neurotoxicity in SH-SY5Y cells. These protective effects were mediated through the inhibition of ROS generation and an increase in the expression of antioxidant enzymes, preventing mitochondrial dysfunction and inhibiting neuronal apoptosis [Figure 9]. These results highlight the potential benefit of eriodictyol as a compound for use in neurodegenerative disease management.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Wang X, Wang W, Li L, Perry G, Lee HG, Zhu X, *et al.* Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta 2014;1842:1240-7.
2. Andersen JK. Oxidative stress in neurodegeneration: Cause or consequence? Nat Med 2004;10 Suppl: S18-25.
3. Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. Exp Neurobiol 2015;24:325-40.
4. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and parkinson's disease. Front Neuroanat 2015;9:91.
5. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
6. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biol 2017;11:613-9.
7. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014;24:R453-62.
8. Whittemore ER, Loo DT, Watt JA, Cotman CW. A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. Neuroscience 1995;67:921-32.
9. Zhou L, Yao GD, Song XY, Wang J, Lin B, Wang XB, *et al.* Neuroprotective effects of 1,2-diarylpropane type phenylpropanoid enantiomers from red raspberry against H2O2-induced oxidative stress in human neuroblastoma SH-SY5Y cells. J Agric Food Chem 2018;66:331-8.
10. Tanagornmeatar K, Chaotham C, Sritularak B, Likhitwitayawuid K, Chanvorachote P. Cytotoxic and anti-metastatic activities of phenolic compounds from *Dendrobium ellipsophyllum*. Anticancer Res 2014;34:6573-9.
11. Lv P, Yu J, Xu X, Lu T, Xu F. Eriodictyol inhibits high glucose-induced oxidative stress and inflammation in retinal ganglial cells. J Cell Biochem 2019;120:5644-51.
12. Wang Y, Chen Y, Chen Y, Zhou B, Shan X, Yang G, *et al.* Eriodictyol inhibits IL-1β-induced inflammatory response in human osteoarthritis chondrocytes. Biomed Pharmacother 2018;107:1128-34.
13. Sasaki N, Toda T, Kaneko T, Baba N, Matsuo M. Protective effects of flavonoids on the cytotoxicity of linoleic acid hydroperoxide

toward rat pheochromocytoma PC12 cells. Chem Biol Interact 2003;145:101-16.
14. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv 2015;5:27986-8006.
15. Mattson MP. Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 2000;1:120-9.
16. Kwon SH, Ma SX, Lee SY, Jang CG. Sulfuretin inhibits 6-hydroxydopamine-induced neuronal cell death via reactive oxygen species-dependent mechanisms in human neuroblastoma SH-SY5Y cells. Neurochem Int 2014;74:53-64.
17. Suematsu N, Hosoda M, Fujimori K. Protective effects of quercetin against hydrogen peroxide-induced apoptosis in human neuronal SH-SY5Y cells. Neurosci Lett 2011;504:223-7.
18. Chanput W, Krueyos N, Ritthiruangdej P. Anti-oxidative assays as markers for anti-inflammatory activity of flavonoids. Int Immunopharmacol 2016;40:170-5.
19. Pavelescu LA. On reactive oxygen species measurement in living systems. J Med Life 2015;8 Spec Issue:38-42.
20. Johnson J, Maher P, Hanneken A. The flavonoid, eriodictyol, induces long-term protection in ARPE-19 cells through its effects on nrf2 activation and phase 2 gene expression. Invest Ophthalmol Vis Sci 2009;50:2398-406.
21. Sullivan PG, Brown MR. Mitochondrial aging and dysfunction in alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:407-10.
22. Alvarino R, Alonso E, Tribalat MA, Gegunde S, Thomas OP, Botana LM, *et al*. Evaluation of the protective effects of sarains on H_2O_2-induced mitochondrial dysfunction and oxidative stress in SH-SY5Y neuroblastoma cells. Neurotox Res 2017;32:368-80.
23. Waseem M, Sahu U, Salman M, Choudhury A, Kar S, Tabassum H, *et al.* Melatonin pre-treatment mitigates SHSY-5Y cells against oxaliplatin induced mitochondrial stress and apoptotic cell death. PLoS One 2017;12:e0180953.
24. Feng C, Luo T, Zhang S, Liu K, Zhang Y, Luo Y, Ge P. Lycopene protects human SHSY5Y neuroblastoma cells against hydrogen peroxideinduced death via inhibition of oxidative stress and mitochondria associated apoptotic pathways. Mol Med Rep 2016;13:4205-214.