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Research Article

Neuroprotective Effects of Alpha-Tocopherol and Naringenin Against Amyloid-β (1-42)- Induced Neurotoxicity in SH-SY5Y Cells

Chanchal Yadav[1], Nalini Kanta Sahoo[1,*]

[1]Faculty of Pharmaceutical Sciences, Rama University, Kanpur, India
The authors declare that there is no conflict of interest
Corresponding Author
Nalini Kanta Sahoo
Dean,
Faculty of Pharmaceutical Sciences, Rama University, Kanpur, India
Mob No.: +91-9396772373
Email: sahoo.nalini@gmail.com

Abstract
Oxidative stress plays a central role in amyloid-β (Aβ)-mediated neurotoxicity and the progression of Alzheimer's disease. Targeting oxidative damage using antioxidant compounds has emerged as a promising therapeutic strategy. The present study aimed to evaluate the neuroprotective effects of α-tocopherol and naringenin, individually and in combination, against Aβ (1-42)-induced neurotoxicity in SH-SYSY cells.

Methods:
Cell viability was initially assessed to determine the cytotoxicity of α-tocopherol (25 µM) and naringenin (25 µM), individually and in combination, using the MTT assay. Neurotoxicity was induced using Aβ (1-42) (10 µM). Cells were pretreated with test compounds prior to Aβ exposure, and cell viability was evaluated. Intracellular oxidative stress was assessed by measuring Reduced glutathione (GSH) levels using Ellman's method.

Results:
α-Tocopherol and naringenin, individually and in combination, did not exhibit cytotoxicity at the tested concentrations. Exposure to Aβ significantly reduced cell viability and depleted intracellular GSH levels, confirming induction of oxidative stress. Pretreatment with α-tocopherol and naringenin markedly improved cell viability and restored GSH levels compared to the Aβ-treated group. Notably, the combination treatment demonstrated superior protective effects, indicating a possible synergistic interaction between the two compounds.

Conclusion:
The findings suggest that alpha-tocopherol and naringenin exert significant neuroprotective effects against Aβ-induced oxidative damage, primarily through restoration of antioxidant defenses. The enhanced efficacy observed with the combination treatment highlights the potential of multi-target antioxidant strategies in mitigating Aβ-mediated neurotoxicity. These results support further investigation of this combination as a promising therapeutic approach for AD.

Keywords: Amyloid-β, Neuroprotection, α-Tocopherol, Antioxidant, Naringenin

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and neuronal loss. It is the most common cause of dementia worldwide and poses a significant global health burden. The pathological hallmarks of AD include extracellular deposition of amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles, synaptic dysfunction, and progressive neuronal degeneration [1]. Among the various isoforms, Aβ (1-42) is considered the most neurotoxic species due to its high aggregation propensity and ability to induce oxidative stress, mitochondrial dysfunction, and apoptotic cell death [2]. Accumulating evidence suggests that oxidative stress plays a central role in Aβ-mediated neurotoxicity, where excessive generation of reactive oxygen species (ROS) disrupts cellular redox balance and leads to lipid peroxidation, protein oxidation, and DNA damage [3]. In neuronal cells, Aβ (1-42) has been shown to impair mitochondrial function, reduce endogenous antioxidant defenses such as reduced glutathione (GSH), and activate apoptotic pathways, ultimately contributing to neuronal death [4]. Therefore, targeting oxidative stress and restoring antioxidant capacity has emerged as a promising therapeutic strategy for mitigating AD progression.

Alpha-Tocopherol, the most biologically active form of vitamin E, is a potent lipid-soluble antioxidant known for its ability to protect cellular membranes from oxidative damage. It acts by scavenging lipid peroxyl radicals and interrupting lipid peroxidation chain reactions [5]. Several studies have demonstrated that α-Tocopherol can attenuate Aβ-induced oxidative stress, improve neuronal survival, and preserve membrane integrity in neurodegenerative conditions [6]. However, its efficacy as a standalone therapeutic agent remains limited, necessitating exploration of complementary compounds with multi-target actions.

Naringenin, a naturally occurring flavonoid predominantly found in citrus fruits, has gained attention for its strong antioxidant, anti-inflammatory, and neuroprotective properties. It has been reported to modulate multiple signaling pathways, including nuclear factor erythroid 2-related factor 2 (Nrf2), mitogen-activated protein kinase (MAPK), and apoptotic cascades, thereby enhancing cellular antioxidant defenses and reducing neuronal damage [7]. Furthermore, naringenin has shown potential in attenuating Aβ-induced toxicity by reducing ROS generation, restoring mitochondrial function, and preventing apoptosis in neuronal models [8].

In vitro models play a crucial role in elucidating the molecular mechanisms of neurodegeneration and evaluating potential therapeutic agents. The SH-SY5Y human neuroblastoma cell line is widely used as a neuronal model due to its ability to differentiate into neuron-like cells and exhibit key biochemical and functional characteristics of dopaminergic neurons [9]. These cells are particularly suitable for studying Aβ-induced neurotoxicity, oxidative stress, and neuroprotective interventions under controlled experimental conditions.

2. Materials and Methods

2.1. Chemicals and Reagents

Amyloid beta peptide (Aβ1-42), α-Tocopherol, and naringenin were procured from standard commercial suppliers such as Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, and trypsin-EDTA were obtained from Gibco. All other reagents and chemicals used in the study were of analytical grade.

2.2. Cell Culture

The human neuroblastoma cell line SH-SY5Y was obtained from a National Center

of Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% (v/v) FBS and 1% penicillin-streptomycin solution. The cells were maintained in a humidified incubator at 37°C with 5% CO_2 atmosphere. Cells were sub-cultured upon reaching 80-90% confluency using trypsinization [10, 11].

2.3. Preparation of Amyloid-β (1-42)

Amyloid-beta peptide (Aβ1-42) was initially dissolved in hexafluoroisopropanol (HFIP) to obtain a monomeric peptide solution and incubated at room temperature for complete solubilization. The solvent was then evaporated under a gentle stream in a vacuum desiccator to obtain a thin peptide film. The dried peptide film was reconstituted in dimethyl sulfoxide (DMSO) to prepare a concentrated stock solution, which was subsequently diluted with culture medium and incubated at 37ºC for 24-48 hours to allow aggregation. The aggregated Aβ (1 - 42) was used at a final concentration of 10 µM to induce neurotoxicity in SH-SY5Y cells [3, 4, 9].

2.4. Preparation of Test Compounds

Alpha-Tocopherol was dissolved in absolute ethanol to prepare a stock solution and vortexed thoroughly to ensure uniform dispersion. Naringenin was dissolved separately in dimethyl sulfoxide (DMSO) to obtain its stock solution [12, 13]. Both stock solutions were further diluted with culture medium to achieve a final working concentration of 25 µM for individual treatments. For combination treatment, appropriate volumes of α-Tocopherol and naringenin stock solutions were mixed and diluted in culture medium to yield a final concentration of 25 µM each. The final concentration of organic solvents (ethanol and DMSO) in all treatment groups was maintained below 0.1% (v/v) to prevent solvent-induced cytotoxicity.

2.5. Experimental Design (MTT Assay)

The experimental design was structured to evaluate the effects of α-Tocopherol and naringenin on cell viability under both normal and Aβ-induced neurotoxic conditions in SH-SY5Y cells. Initially, the cytotoxic effects of α-Tocopherol and naringenin were evaluated individually and in combination to determine their safety profile in SH-SY5Y cells. Cells were treated with 25 µM α-Tocopherol, 25 µM naringenin, and their combination (25 µM + 25 µM) for 24 hours. Cell viability was assessed using the MTT assay to confirm that the selected concentrations were non-toxic and suitable for further experimentation. Following confirmation of non-cytotoxic concentrations, the neuroprotective potential of the test compounds was evaluated against Aβ (1-42)-induced neurotoxicity. Cells were pre-treated with α-Tocopherol, naringenin, or their combination for 2-4 hours, followed by exposure to 10 µM Aβ (1-42) for 24 hours [9, 14].

2.6. Measurement of Reduced Glutathione (GSH)

Intracellular levels of Reduced glutathione (GSH) were estimated using Ellman's method with slight modifications (Ellman, 1959). Following treatment, SH-SY5Y cells from different experimental groups were harvested and washed twice with cold phosphate-buffered saline (PBS, pH 7.4) to remove residual media. The cells were then lysed using an appropriate lysis buffer (e.g., phosphate buffer or 5% trichloroacetic acid) and centrifuged at 10,000 rpm for 10 minutes at 4°C to obtain the supernatant. An aliquot of the supernatant was mixed with Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB], and the reaction mixture was incubated at room temperature for 10-15 minutes. The formation of the yellow-colored chromophore (5-thio-2-nitrobenzoic acid) was measured spectrophotometrically at 412 nm using a microplate reader. The GSH content was quantified using a standard

calibration curve and expressed as µg/mg protein [15, 16].

2.7. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Newman-Keuls multiple comparison test using GraphPad Prism. The value of $p << 0.05$ was considered statistically significant.

3. Results

3.1. Effect of A-Tocopherol and Naringenin on Cell Viability

The cytotoxic effects of α-Tocopherol and naringenin, individually and in combination, were evaluated in SH-SY5Y cells using the MTT assay. As shown in figure 1, treatment with ATF (25 µM), NRG (25 µM), and their combination did not produce any significant reduction in cell viability compared to the control group. Cell viability in all treated groups remained close to 100%, indicating that neither α-Tocopherol nor naringenin exerted cytotoxic effects at the tested concentrations. A slight, non-significant increase in cell viability was observed in the treated groups, suggesting a possible supportive effect on cellular metabolic activity. These findings confirm that the selected concentrations of ATF and NRG are safe and non-toxic to SH-SY5Y cells and are therefore suitable for further evaluation of their neuroprotective effects against Aβ (1-42)-induced toxicity.

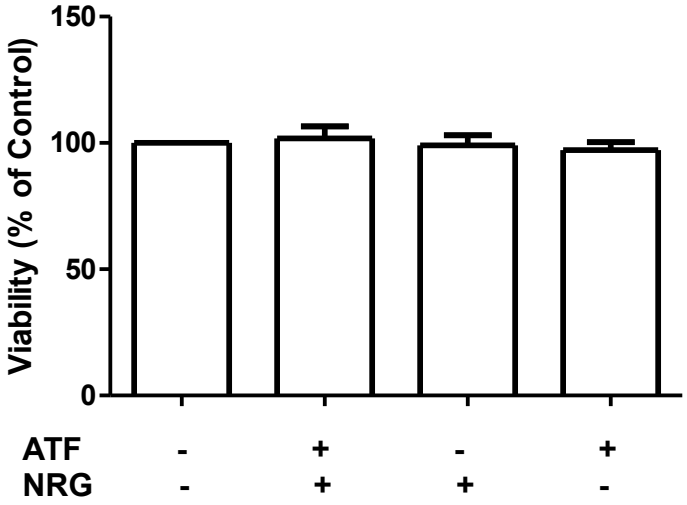

Figure 1. Effect of α-Tocopherol (ATF) and naringenin (NRG), individually and in combination, on cell viability in SH-SY5Y cells. Data are expressed as mean ± SEM from three independent experiments (n=3). Statistical analysis was conducted using one-way ANOVA followed by Newman-Keuls post hoc test using Graphpad Prism software. A value of $p < 0.05$ was considered statistically significant.

3.2. Effect of α-Tocopherol and Naringenin on Aβ-Induced Cytotoxicity

The neuroprotective effects of α-Tocopherol and naringenin, individually and in combination, were evaluated against Aβ (1-42)-induced cytotoxicity in SH-SY5Y cells using the MTT assay. Exposure to Aβ (1-42)

(10 µM) resulted in a significant reduction in cell viability compared to the control group, confirming successful induction of neurotoxicity. Pretreatment with α-Tocopherol (25 µM) and naringenin (25 µM), both individually and in combination, significantly improved cell viability compared to the Aβ-treated group. Among the treatments, the combination of α-Tocopherol and naringenin exhibited the most pronounced protective effect, restoring cell viability closer to normal levels. Statistical analysis revealed that all treatment groups showed highly significant protection ($^{***}p < 0.001$) when compared to the Aβ-induced group. Furthermore, the combination treatment demonstrated a significantly greater protective effect compared to individual treatments ($^{###}p < 0.001$), indicating a possible synergistic interaction between α-Tocopherol and naringenin. These findings suggest that both compounds possess significant neuroprotective properties, with their combination offering enhanced, protection against Aβ-induced cytotoxicity.

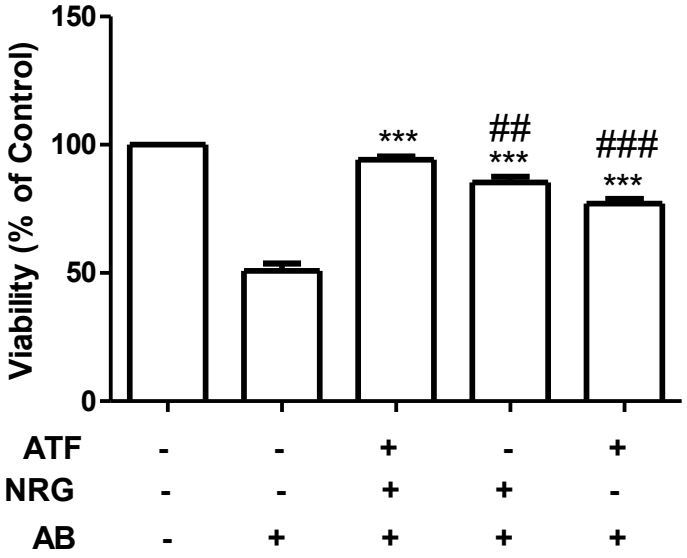

Figure 2. Protective effect of α-Tocopherol (ATF) and naringenin (NRG), individually and in combination, against Aβ (1-46)-induced cytotoxicity in SH-SY5Y cells Data are expressed as mean ± SEM from three independent experiments (n=3). Statistical analysis was conducted using one-way ANOVA followed by Newman-Keuls post hoc test using Graphpad Prism software. $^{***}p < 0.001$ vs Aβ-treated group $^{###}p < 0.001$ vs combination group.

3.3. Effect of Alpha-Tocopherol and Naringenin on Intracellular GSH Levels

The effect of alpha-tocopherol and naringenin, individually and in combination, on intracellular Reduced glutathione (GSH) levels was evaluated in SH-SY5Y cells following Aβ (1-42) exposure. Treatment with Aβ (1-42) (10 μM) resulted in a significant depletion of intracellular GSH levels compared to the control group, indicating the induction of oxidative stress. Pretreatment with α-Tocopherol (25 μM) and naringenin (25 μM), both individually and in combination, significantly restored GSH levels compared to the Aβ -treated group. Among the treatments, the combination of α-Tocopherol and NRG exhibited the most pronounced restoration of GSH levels, approaching near-control values.

Statistical analysis demonstrated that the combination treatment showed highly significant improvement (**p < 0.001) compared to the Aβ group. Individual treatments with naringenin (p < 0.01) and α-Tocopherol (*p < 0.01) also significantly increased GSH levels; however, their effects were significantly lower than the combination treatment, as indicated by the statistical comparison (#p<0.05, ##p < 0.01). These results suggest that Aβ-induced oxidative stress leads to depletion of intracellular antioxidant defenses, while treatment with α-Tocopherol and naringenin effectively restores GSH levels, with the combination showing enhanced antioxidant capacity.

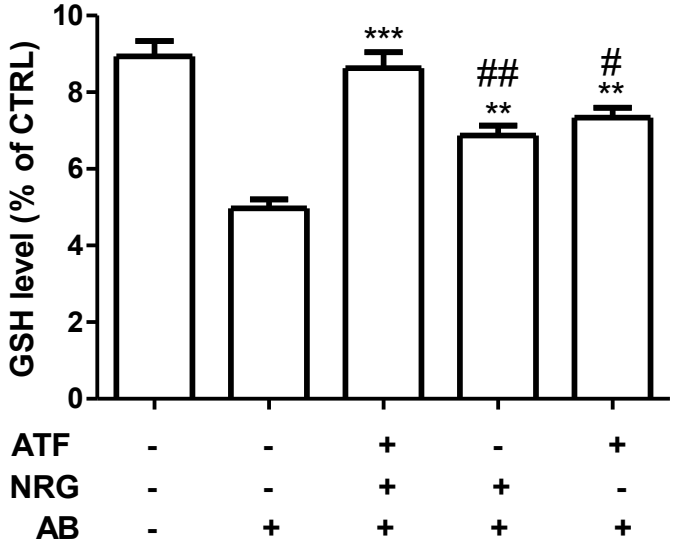

Figure 3. Effect of alpha-tocopherol (ATF) and naringenin (NRG), individually and in combination, on intracellular GSH levels in Aβ (1-42)-treated SH-SY5Y cells. Data are expressed as mean ± SEM from three independent experiments (n=3). Statistical analysis was conducted using one-way ANOVA followed by Newman-Keuls post hoc test using Graphpad Prism software. A value of $p < 0.05$ was considered statistically significant. $^{*}p < 0.001$, $^{*}p < 0.01$ vs Aβ treated -group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs combination group.

4. Discussion

The present study investigated the neuroprotective potential of α-tocopherol and naringenin, individually and in combination, against Aβ (1-42)-induced neurotoxicity in SH-SY5Y cells. The findings demonstrate that both compounds significantly attenuate Aβ-induced cytotoxicity and oxidative stress, with the combination treatment exhibiting enhanced protective efficacy.

Amyloid-β (1-42) is widely recognized as a major contributor to the pathogenesis of AD, primarily through induction of oxidative stress, mitochondrial dysfunction, and neuronal apoptosis [17, 18]. In the present study, exposure to Aβ resulted in a marked reduction in cell viability along with significant depletion of intracellular reduced glutathione (GSH), confirming successful establishment of an oxidative stress-mediated neurotoxicity model. These findings are consistent with previous reports demonstrating that Aβ disrupts redox homeostasis by enhancing ROS generation and impairing endogenous antioxidant systems [19, 20].

The restoration of cell viability observed following treatment with α-tocopherol and naringenin suggests their protective role against Aβ-induced neuronal damage. α-Tocopherol, a lipid-soluble antioxidant, is known to localize within cellular membranes and protect against lipid peroxidation by scavenging peroxyl radicals [21]. Previous studies have reported that vitamin E effectively mitigates Aβ-induced oxidative damage and preserves neuronal integrity [22, 23]. In agreement with these findings, the present study demonstrates that ATF significantly improves cell viability and restores GSH levels, indicating its role in maintaining membrane stability and redox balance.

Naringenin, a flavonoid with potent antioxidant properties, has been shown to exert neuroprotective effects through multiple mechanisms, including activation of endogenous antioxidant pathways and inhibition of apoptotic signaling [24]. It has

been reported to activate the Nrf2 signaling pathway, leading to upregulation of antioxidant enzymes and restoration of cellular redox homeostasis [25, 26]. The observed increase in GSH levels following NRG treatment in the present study supports its role in enhancing intracellular antioxidant defenses and protecting neuronal cells from oxidative stress-induced damage.

A key finding of this study is the superior protective effect observed with the combination of α-tocopherol and naringenin compared to their individual treatments. The combination treatment resulted in greater restoration of cell viability and GSH levels, suggesting a possible synergistic interaction. This enhanced effect may be attributed to the complementary mechanisms of action of the two compounds. While α-tocopherol primarily acts as a chain-breaking antioxidant within lipid membranes, naringenin exerts broader cytoprotective effects by modulating intracellular signaling pathways and boosting endogenous antioxidant systems. Similar synergistic antioxidant effects have been reported in studies combining lipid-soluble and polyphenolic antioxidants, highlighting the advantage of multi-target therapeutic strategies [28, 29]

The depletion of GSH observed in Aβ-treated cells is a critical indicator of oxidative stress and neuronal vulnerability, as GSH plays a central role in detoxifying reactive oxygen species and maintaining cellular redox balance [16]. Restoration of GSH levels by α-tocopherol and naringenin further supports their antioxidant potential and underscores their relevance in counteracting Aβ-induced oxidative damage. These findings are in line with previous studies demonstrating that enhancement of intracellular GSH levels is associated with neuroprotection in various models of neurodegeneration [3, 29].

Overall, the results of the present study suggest that targeting oxidative stress through antioxidant therapy may represent a promising strategy for mitigating Aβ-induced neurotoxicity. The combination of α-tocopherol and naringenin, by simultaneously protecting membrane integrity and enhancing endogenous antioxidant defenses, offers a more effective approach compared to individual treatments. This multi-targeted strategy aligns with current therapeutic perspectives in AD, which emphasize the need for interventions addressing multiple pathological pathways.

5. Conclusion

The present study demonstrates that α-tocopherol and naringenin effectively attenuate Aβ (1-42)-induced neurotoxicity in SH-SY5Y cells. Exposure to Aβ significantly

reduced cell viability and depleted intracellular antioxidant defenses, as evidenced by decreased reduced glutathione (GSH) levels. Pretreatment with α-tocopherol and naringenin, both individually and in combination, markedly improved cell survival and restored GSH levels, highlighting their potent antioxidant and neuroprotective properties.

Notably, the combination treatment exhibited. Superior efficacy compared to individual treatments, suggesting a possible synergistic interaction through complementary mechanisms involving membrane stabilization and enhancement of endogenous antioxidant systems. These findings indicate that targeting oxidative stress using a combination of lipid-soluble and flavonoid antioxidants may represent a promising therapeutic strategy for mitigating Aβ-induced neuronal damage. Further studies, including in vivo investigations and mechanistic pathway analyses, are warranted to validate their potential in the management of AD.

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