Annals of Clinical and Analytical Medicine
Original Research

Modulation of apoptotic pathways by curcumin and resveratrol conveys neuroprotection against rotenone-induced toxicity in SH-SY5Y cells

Apoptotic modulation by curcumin and resveratrol in SH-SY5Y cells

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Abstract

Aim: This study investigates the neuroprotective properties of the polyphenolic compounds resveratrol and curcumin against rotenone-induced toxicity in SH-SY5Y neuronal cells.

Material and Methods: The cytotoxic threshold (LD50) of rotenone was established through a dose-response assessment in SH-SY5Y cells. Cells were pre-incubated with varying concentrations of resveratrol (1-100 µM) and curcumin (10-1000 nM), followed by a 24-hour rotenone challenge (200 nM). Cell survival was gauged using the MTS assay, while caspase-3 levels, as a marker of apoptosis, were quantified via ELISA. The capability of cells to form colonies post-treatment was determined through a clonogenic survival assay. Moreover, the Western blot analysis revealed that pre-treatment with resveratrol and curcumin significantly modulated the protein expression levels of Bax and Bcl-2.

Results: Pretreatment with resveratrol and curcumin significantly enhanced cell viability and clonogenic survival in the presence of rotenone, with notable decreases in caspase-3 levels, implying reduced apoptosis ($p < 0.05$). These compounds also modulated the expression of Bax and Bcl-2, contributing to their protective effects.

Discussion: Resveratrol and curcumin exhibit promising neuroprotective effects by improving cell survival and modulating apoptotic markers in SH-SY5Y cells subjected to rotenone-induced toxicity. These findings support the potential therapeutic role of these polyphenols in neurodegenerative disease management, pending further validation through comprehensive pre-clinical and clinical studies.

Keywords

Curcumin, Resveratrol, Rotenone, Neuroprotection, Apoptosis, SH-SY5Y Cells

DOI: 10.4328/ACAM.22183 Received: 2024-03-18 Accepted: 2024-05-27 Published Online: 2024-07-06 Printed: 2024-08-01 Ann Clin Anal Med 2024;15(8):570-574
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Introduction

Neurodegenerative disorders are a diverse group of chronic diseases characterized by severe morbidity, cognitive decline, and impaired motor functions. These diseases include Alzheimer's, Parkinson's, Huntington's disease, and Amyotrophic lateral sclerosis (ALS), all contributing significantly to global health burdens [1]. Characterized by the progressive loss of neurons, neurodegenerative diseases are increasing and severely impacting life quality [2]. Their causes are complex, involving genetic susceptibilities and environmental factors such as toxin exposure and oxidative stress [3]. Additionally, aging, genetic predispositions, exposure to pesticides, and diseases like diabetes and hypertension further increase the risk of these disorders [4].

Pesticides, especially rotenone, are linked to serious health impacts. Rotenone, extracted from the roots of plants in the East Indies, inhibits mitochondrial complex I, causing increased oxidative stress and subsequent neuronal damage, which can lead to neurodegenerative diseases [5]. There is no cure for such diseases, including Parkinson's. Rotenone exposure specifically harms dopaminergic neurons, replicating Parkinson's behavioral and neuropathological traits in experimental models, thus highlighting the importance of finding preventive strategies against rotenone's effects [6].

Recent research highlights natural compounds like curcumin and resveratrol as potential neuroprotective agents. Curcumin, from turmeric, is noted for its antioxidant and anti-inflammatory properties. Studies show it can mitigate rotenone-induced damage in neurons by regulating oxidative stress and inflammation [7]. Curcumin works by neutralizing free radicals and boosting detoxifying enzymes like Glutathione S-transferase and Heme oxygenase 1 [8, 9]. Similarly, resveratrol, found in grapes and other fruits, protects neurons from rotenone by reducing oxidative stress and inflammation. It also inhibits inflammatory gene expression and cytokines like sirtuin, adenosine monophosphate kinase, and nuclear factor-κB [10, 11].

Our study explores the effects of curcumin and resveratrol on rotenone-induced neurotoxicity in SH-SY5Y cells, a neuroblastoma model. We aim to determine their neuroprotective properties and their impact on apoptotic pathways.

Material and Methods

Reagents and Materials

SH-SY5Y cells were acquired from ATCC, while curcumin, resveratrol, rotenone, and the MTS test were sourced from Sigma-Aldrich. DMEM, Fetal Bovine Serum, Trypsin-EDTA (0.25%), and PBS were purchased from Biochrom. A Caspase 3 ELISA kit was obtained from Sigma-Aldrich.

Cell Culture

SH-SY5Y cells were grown in DMEM/F-12 supplemented with 1% Penicillin-Streptomycin and 10% FBS, maintained at 37 °C in a 5% CO2 incubator at 96% humidity. Cells were harvested at 80% confluence using 2 mL of Trypsin-EDTA, then seeded at $5x10^3$ cells/100 µL per well in 96-well plates. After 24 hours, cells were pretreated with varying curcumin (10nM to 1000nM) and resveratrol (1 nM to 100 nM) concentrations for another 24 hours, followed by 200 nM rotenone exposure for the clonogenic assay.

Cell Viability Assay

Post-rotenone treatment, cells were washed with PBS and incubated in fresh medium for 48 hours. The MTS solution was prepared by mixing MTS powder with PBS at a 20:1 ratio. MTS solution was added to each well, incubated for an hour, and the optical density was measured at 490 nm using a BIOTEK ELISA reader.

Determination of Rotenone's LD50

Rotenone concentrations ranging from 1 nM to 500 nM were tested to determine its cytotoxic effects. The LD50 was established at 200 nM.

Clonogenic Survival Assay

Cells were seeded at 5x104 cells/well in 6-well plates and treated with resveratrol and curcumin for 24 hours, then with 200 nM rotenone for another 24 hours. After treatment, cells were washed, cultured for 1-3 weeks, fixed with glutaraldehyde, stained with crystal violet, and colonies counted under a microscope.

Caspase-3 Levels Assessment

Caspase-3 levels were measured using the Sigma-Aldrich ELISA kit, following the manufacturer's instructions. Optical density was read at 450 nm, using a standard curve for caspase-3 level determination.

Western Blot Analysis

Cells were cultured, treated, and lysed at 4°C. Lysates were centrifuged at 13,000 g for 10 minutes at 4°C. Protein concentrations were determined using a Bio-Rad assay. Proteins (40 µg/sample) were separated on a 4–15% SDS-PAGE gel and transferred to PVDF membranes. Membranes were blocked, then incubated with primary antibodies against Bax, Bcl-2, and Beta-Actin overnight at 4°C. After washing, membranes were exposed to secondary antibodies and visualized using a FluorChem 8900 imager.

Statistical Analysis

The data from the three separate in vitro experiments were presented as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. P values less than 0.05 were deemed significant and indicated with an asterisk.

Results

Our study demonstrated that curcumin and resveratrol significantly mitigate rotenone-induced damage in SH-SY5Y cells. We examined their effects on cell proliferation, colony formation, and apoptosis in cells exposed to rotenone.

Determination of Rotenone LD50

We evaluated the dose-response relationship of SH-SY5Y cells to rotenone in 96-well plates. Cell survival was measured using an MTS assay after exposure to rotenone concentrations from 1 nM to 500 nM for 24 hours. As shown in Figure 1A, rotenone reduced cell viability in a dose-dependent manner, with an LD50 of 200 nM, which was used for subsequent experiments (***$p<0.0001$).

Cell Viability

Cells were pretreated with various concentrations of resveratrol (1, 5, 10, 50, and 100 µM) and curcumin (10, 50, 100, 500, and 1000 nM) for 24 hours before 200 µM rotenone exposure. An MTS assay assessed the protective effects of these compounds on cell survival. Pretreatment with 1 nM resveratrol and 10

nM curcumin raised cell viability to 98% and 87%, respectively (###p<0.0001). While both compounds improved survival across most tested concentrations, high levels of resveratrol (100 µM) were associated with decreased cell viability (Figure 1B).

Caspase-3 Levels

Rotenone exposure significantly increased caspase-3 levels, indicating enhanced apoptosis. However, treatment with either resveratrol or curcumin at all tested concentrations significantly reduced caspase-3 expression to near-control levels (###p<0.0001) (Figure 1C).

Colony Formation Capability

The impact of resveratrol and curcumin on the colony-forming capacity of cells under rotenone-induced stress was significant. Treatment with 200 nM rotenone substantially reduced colony formation compared to untreated cells (***p<0.0001). However, pre-treatment with 1 µM resveratrol and 10 nM curcumin significantly enhanced colony formation, indicating robust protective effects against rotenone toxicity (###p<0.0001) as depicted in Figure 2.

Bax and Bcl-2 levels

Our study revealed significant changes in Bax and Bcl-2 expression levels upon treatment with resveratrol and curcumin (Figure 3A). Notably, the 200 nM rotenone group exhibited a significant increase in Bax expression (***p<0.0001) compared to the non-treated (NT) control group. In contrast, all concentrations of resveratrol and curcumin resulted in a significant decrease in Bax levels when compared to the 200 nM rotenone group (###p<0.0001) (Figure 3B). Additionally, Bcl-2 expression analysis indicated a marked reduction in the 200 nM rotenone-treated group (***p<0.0001) relative to the NT group. However, all concentrations of resveratrol and curcumin, except for 10 µM Resveratrol, significantly increased Bcl-2 levels compared to the 200 nM rotenone group (###p<0.0001) (Figure 3C). Furthermore, when examining the Bax/Bcl-2 ratio, a significant elevation was noted in the 200 nM rotenone group (***p<0.0001) in comparison to the NT group. Inversely, each concentration of resveratrol and curcumin led to a significant reduction in the Bax/Bcl-2 ratio when measured against the 200 nM rotenone group (###p<0.0001) (Figure 3D). These findings highlight the modulatory effects of resveratrol and curcumin on apoptosis-related proteins in the context of rotenone treatment.

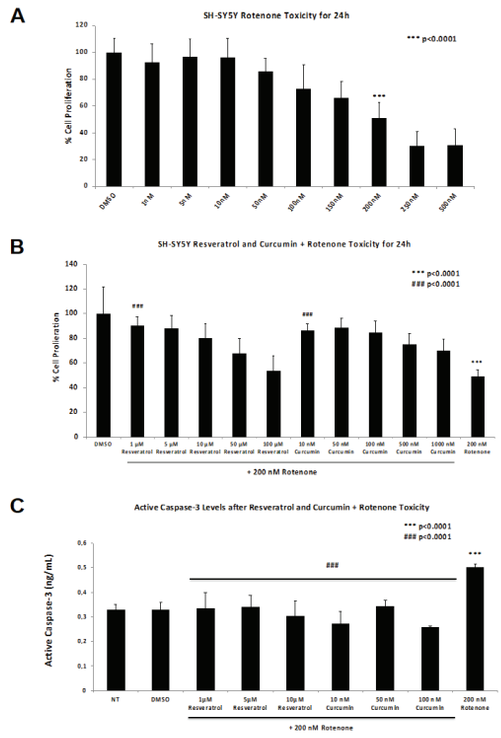

Figure 1. A represents the dose-dependent relationship of rotenone with cellular viability. A significant difference (***p<0.0001) is observed when comparing treatment groups with the DMSO control group. B shows The impact of resveratrol and curcumin on cell viability in the context of rotenone-induced toxicity was assessed using an MTS assay. A highly significant difference was observed when compared to the non-treated (NT) control (***p<0.0001), and likewise when compared to the 200 nM rotenone-treated group across all concentrations (###p<0.0001). C shows that assessing the influence of resveratrol and curcumin on caspase-3 levels revealed significant changes. When compared to the non-treated (NT) group, there was a notable increase in 200 nM rotenone group (***p<0.0001). However, in comparison to the 200 nM rotenone group, all concentrations of resveratrol and curcumin showed a significant reduction in caspase-3 levels (###p<0.0001)

Discussion

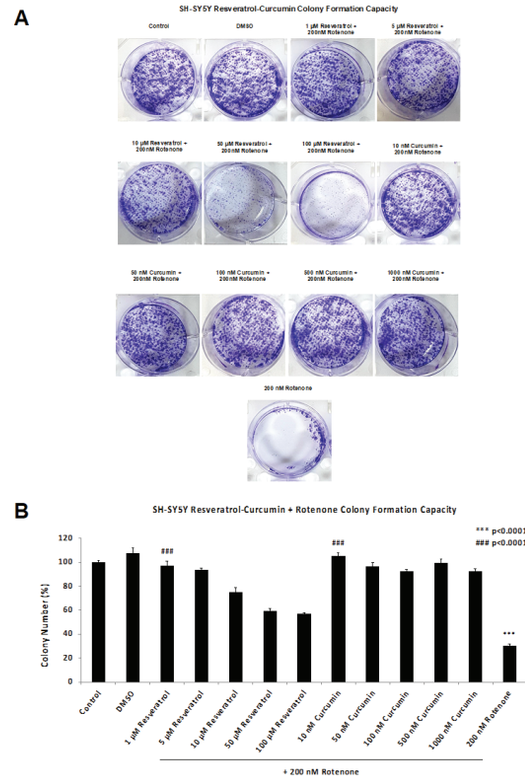

Figure 2. Colony Formation Results. In Figure 2A, the impact of resveratrol and curcumin on the colony formation ability was investigated. A significant alteration was noted in the group exposed to 200 nM rotenone compared to the non-treated (NT) group (***p<0.0001) (Figure 2B). Importantly, protective effects against 200 nM rotenone toxicity were significantly demonstrated following treatments with 1 µM resveratrol and 10 nM curcumin (###p<0.0001) (Figure 2B)

Rotenone, a widely used insecticide, has been increasingly associated with Parkinson's disease (PD) due to its capacity to disrupt mitochondrial function, enhance inflammation, stimulate apoptotic pathways, and imbalance antioxidant-oxidant levels, contributing to neurodegeneration [12, 13]. The limited regenerative ability of neurons underscores the importance of preventative strategies, highlighting the need for exploring both natural and synthetic substances that could mitigate rotenone's toxic effects.

Natural compounds like polyphenols have shown protective potential against diseases, including PD. Among them, resveratrol and curcumin, derived from plants, have been studied for their antioxidant properties. In our research, we evaluated their neuroprotective roles in an SH-SY5Y dopaminergic neuronal model exposed to rotenone. Notably, PD diagnoses often coincide with the degeneration of approximately 50% of dopaminergic neurons in the nigro-striatal pathway [14]. Our studies used rotenone concentrations that replicated this level of cell death, thereby providing relevant disease modeling.

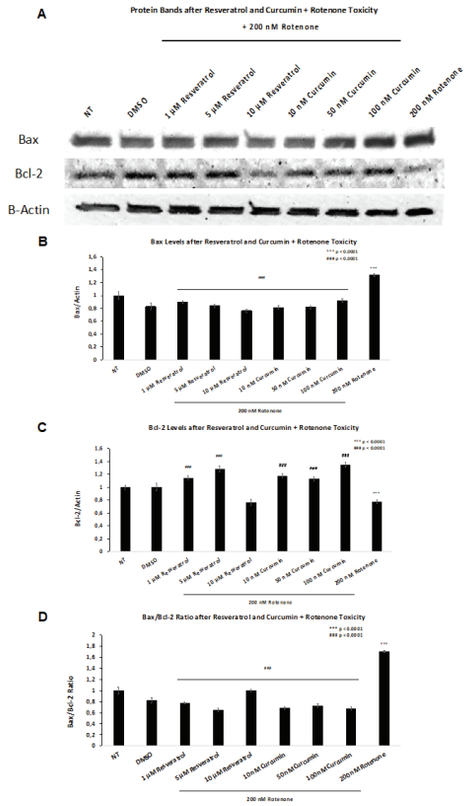

Figure 3. Bax and Bcl-2 levels. A: Significant alterations were found when resveratrol and curcumin were evaluated for their impact on Bax and Bcl-2 levels. B:The 200 nM rotenone group showed a significant increase in Bax expression (***p<0.0001) in comparison to the non-treated (NT) group. However, all concentrations of resveratrol and curcumin demonstrated a significant decrease in Bax levels when compared to the 200 nM rotenone group (###p<0.0001). C: Regarding Bcl-2 expression, the data indicated a notable reduction in the 200 nM rotenone-treated group (***p<0.0001) relative to the non-treated (NT) control. In contrast, with the exception of the 10 µM Resveratrol concentration, all other tested concentrations of resveratrol and curcumin (###p<0.0001) demonstrated a statistically significant elevation in Bcl-2 levels when compared to the 200 nM rotenone group. D: In the context of the Bax/Bcl-2 ratio, the 200 nM rotenone group (***p<0.0001) exhibited a significant elevation in this ratio compared to the non-treated (NT) group. In contrast, each concentration of resveratrol and curcumin showed a significant reduction (###p<0.0001) in the Bax/Bcl-2 ratio when measured against the 200 nM rotenone group

Our findings indicate that resveratrol at 1, 5, 10, and 50 µM doses demonstrates neuroprotective effects. Specifically, a 1 µM pre-treatment with resveratrol increased cell proliferation by 87%, although higher doses reduced its protective efficacy and resulted in toxicity at 100 µM [15]. This dose-response relationship aligns with other studies indicating that resveratrol's protective effects begin at low micromolar concentrations and diminish with higher doses [14]. Similar patterns were observed in studies using the PC12 cell line, which reported neuroprotection between 1.5 µM and 60 µM [30] and in animal models, which found optimal effects at 5 and 20 µM [16].

Moreover, resveratrol has been noted for its broader biological benefits, including cardiovascular, antidiabetic, and neuroprotective properties. Our results, consistent with these findings, suggest that even lower doses may be effective, potentially enhancing the feasibility of clinical applications [17]. Curcumin also showed significant neuroprotective effects across various concentrations, with the highest efficacy observed at 50 µM, which increased cell viability by 98%. This supports other findings that report curcumin's dose-dependent protective effects [18]. The ability of curcumin to increase the colony-forming capacity of cells aligns with our MTS assay results, further validating its role in enhancing cell proliferation and survival.

Studies have highlighted the involvement of apoptosis and caspase activation in neurodegenerative diseases. Rotenone is known to induce dopaminergic neuronal apoptosis via the mitogen-activated protein kinase (MAPK) pathway and caspase-dependent pathways [19]. In our study, caspase-3 levels increased significantly upon rotenone exposure, confirming the induction of apoptosis. Interestingly, both resveratrol and curcumin effectively reduced these increases, suggesting their potential to interfere with apoptotic processes.

Previous research has also emphasized the anti-apoptotic pathways of polyphenols. For instance, studies have shown that resveratrol protects against brain injury and neurodegeneration by activating autophagy and inhibiting apoptosis pathways like Akt/mTOR and SIRT1-AMPK [20]. Similarly, curcumin derivatives such as Dethoxycurcumin and CNB-001 have been reported to inhibit rotenone-induced apoptosis, highlighting their antioxidant and anti-inflammatory effects in PD models [21].

Resveratrol and curcumin have been shown to modulate apoptosis-related proteins, potentially through interactions with apoptotic pathways [22,23]. This study's findings suggest that the neuroprotective effects of polyphenols may be partly attributed to their ability to modulate these pathways, emphasizing the need for further research to explore these mechanisms in greater depth.

The profound effects of resveratrol and curcumin on rotenone-

induced caspase-3 expression underscore their neuroprotective potential. This study, building on the extensive literature linking rotenone to PD pathogenesis, suggests these polyphenols as viable candidates for mitigating rotenone's harmful effects.

Furthermore, our study explored the modulation of Bax and Bcl-2 expression by resveratrol and curcumin, revealing significant regulatory effects on these apoptotic markers. Resveratrol's influence on Bcl-2 and Bax expression has been documented in various cellular contexts, supporting its role in apoptotic modulation [24]. Curcumin's impact on these proteins was also evident in our findings, consistent with studies demonstrating its efficacy in reducing oxidative stress and regulating apoptosis in diabetic rat models [25].

In summary, our study supports the neuroprotective roles of resveratrol and curcumin against rotenone-induced toxicity. The dose-response relationship and their effects on apoptotic pathways highlight their potential therapeutic benefits in neurodegenerative conditions like PD. While promising, further research is needed to fully understand their mechanisms and optimize their clinical application, considering bioavailability and pharmacokinetics in human settings. This necessitates moving beyond cellular models to in vivo and clinical studies to validate these compounds' effectiveness and safety.

Conclusion

Rotenone, commonly used in agriculture, poses risks as an environmental neurotoxin linked to Parkinson's disease (PD). Recognizing this risk is crucial for developing effective preventative strategies. Polyphenols like resveratrol and curcumin, known for their antioxidant properties, have shown promise in protecting against rotenone-induced neurotoxicity in SH-SY5Y cells, a dopaminergic neuronal model. Although our results did not show statistically significant changes in caspase-3 levels, they align with other studies suggesting the neuroprotective potential of these compounds. This study supports the use of natural polyphenols in combating rotenone's effects, highlighting the need for further research to understand their therapeutic roles in preventing and treating neurodegenerative diseases.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

Funding: *This study was supported by grant TYL-2018-20049 from the Ege University Scientific Research Fund.*

Conflict of Interest

The authors declare that there is no conflict of interest.

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How to cite this article:

Betul Genc, Meltem Yılmaz Cosar, Mumin Alper Erdogan, Ozlem Yılmaz. Modulation of apoptotic pathways by curcumin and resveratrol conveys neuroprotection against rotenone-ınduced toxicity in SH-SY5Y cells. Ann Clin Anal Med 2024;15(8):570-574