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Short communication

Hesperidin Plays Neuroprotective Effects Against Quinolinic Acid in Human SH-SY5Y Cells: Focusing on ROS Levels and Cell Cycle Arrest

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Abstract

Background and objectives: In some neurodegenerative diseases, an aberrant accumulation of quinolinic acid is frequently associated with the loss of nerve cells and a condition known as neuritis. This is typically caused by an excessive production of free radicals. Studies have shown that hesperidin has potent antioxidant effects, but nothing is known about how it protects against the neurotoxicity induced by quinolinic acid. This study aimed to evaluate the protective effect of hesperidin against quinolinic acid-induced neurotoxicity in the SH-SY5Y neuroblastoma cell line. **Methods:** The MTT test was used to determine cell viability and protective dosage of hesperidin. Flow cytometry using propidium iodide (PI) staining was used to determine the cell cycle of SH-SY5Y cells after exposure to quinolinic acid in combination with hesperidin. Reactive oxygen species (ROS) levels within cells were also measured using 2', 7'-dichlorodihydrofluorescein diacetate (H2DCFDA) in the mentioned groups. **Results:** Our results demonstrated that hesperidin had a protective effect against quinolinic acid-induced toxicity at nontoxic concentrations ($p<0.001$). Moreover, the percentage of apoptotic cells in the sub-G1 phase increased significantly ($p<0.001$). Hesperidin pretreatment significantly decreased sub-G1 arrest that was induced by quinolinic acid ($p<0.001$). Hesperidin significantly decreased ROS levels generated by quinolinic acid ($p<0.001$). **Conclusion:** The current study showed that hesperidin exerts its effect through antioxidant activity and can be considered a promising neuroprotectant agent against quinolinic acid-induced neurotoxicity in neurodegenerative disorders; however, more research is necessary in this area for the treatment.

Keywords: hesperidin; oxidative stress; quinolinic acid; SH-SY5Y neuroblastoma cell line

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Introduction

Kynurenine is produced via the kynurenine pathway, which produces quinolinic acid, niacin, and nicotinamide adenine dinucleotide (NAD) [1]. Tryptophan-2, 3 dioxygenase (TDO), which catalyzes the kynurenine pathway's earliest steps, is primarily found in the liver. Nevertheless, the more abundantly expressed indoleamine dioxygenases 1 and 2 (IDO1, IDO2) are also found in the brain. The enzymatic activity of IDO may produce reactive oxygen species (ROS) [2]. TNF-alpha and INF-gamma both boost IDO expression. The activated Fe (II) forms of IDOs

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might produce ROS [3]. Moreover, some metabolites have neurotoxic characteristics, such as quinolinic acid in the kynurenine pathway [4]. Many neurodegenerative diseases have been connected to the accumulation of quinolinic acid including Parkinson's disease, Alzheimer's disease, HIV-related cognitive loss, and multiple sclerosis [5]. Controlling oxidative harm in the neurological system is therefore crucial. The potential of multiple natural compounds to modulate redox in various clinical conditions has recently attracted the attention of numerous researchers. Research suggests that natural substances may reduce or even stop neurodegeneration while enhancing cognition and memory [6]. Hesperidin is a natural flavonoid from the *Citrus* L. plants. This glycoside is a common component of fruits, and vegetables, and exhibits several medicinal properties, including anti-inflammatory and antioxidant properties [7]. An in vitro model widely used in research is the SH-SY5Y neuroblastoma cell line. Therefore, the aims of this study are determination of the role of kynurenine pathway metabolites, the impact of reactive oxygen species (ROS), and the potential neuroprotective effects of hesperidin, particularly in the context of neurodegenerative diseases and oxidative damage.

Materials and Methods

Ethical considerations

The ethics and animal care committee of Mashhad University of Medical Sciences approved the study with the code IR.MUMS.MEDICAL.REC.1400.742.

Chemicals

Gol Exir Pars (Iran) supplied hesperidin (purity > 97%). We obtained 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), quinolinic acid (QuA), and RNase A from Sigma-Aldrich (USA). Trypsin, fetal calf serum (FCS), Dulbecco's modified Eagle's medium (DMEM/F12), and penicillin/streptomycin were provided from Gibco (UK). Mojallali Co. (Iran) supplied TritonTM X-100, DMSO, and ethanol. We purchased the cellular ROS assay kit (Cat. No. ab113851) from Abcam, UK.

Cell culture

The SH-SY5Y neuroblastoma cell line was obtained from Iran's Pasteur Institute (Tehran, Iran). We used undifferentiated cells because they have been used to assess neurotoxicity in various previous studies [8].

Cell viability measurement using the MTT assay

The MTT test was carried out according to a previous study. The cells were treated for the required exposure duration with different concentrations of quinolinic acid (0–16 mM) and hesperidin (15.625–1000 μM). These drugs were dissolved in DMSO to prepare stock solutions (2M quinolinic acid and 10 mM hesperidin). Hesperidin dissolved at a concentration of 100 mM by adding 51.6 mg to 1 mL of DMSO. These stock solutions were then diluted to working concentrations using DMEM/F12 medium [9].

The protective effects of hesperidin against quinolinic acid-induced cytotoxicity

According to a previous study, treatment was carried out [10]. Finally, each group's cell viability was tested in triplicate using the MTT assay.

Flow cytometry assay

According to a previous study, treatment was carried out [11].

Intracellular reactive oxygen species analysis

According to a previous study, treatment was carried out [10]. Each sample was examined three times.

Statistical analysis

GraphPad Prism 8 was used to analyze the statistical data (San Diego, USA). The normal distribution was verified using the Shapiro-Wilk normality test. Statistical variances between the groups were calculated using a one-way analysis of variance (ANOVA). The data were presented as mean ± SD, and $p<0.05$ was considered significant.

Results and Discussion

Results from the MTT assay in the current study showed that quinolinic acid toxicity to the SH-SY5Y cell line decreased cell viability in a dose-dependent manner in such a way that 48h exposure to the dose of 6 mM resulted in a 50% reduction in cell viability. As a result, the IC_{50} value of 6 mM was chosen for subsequent investigations.

Based on MTT test results, none of the hesperidin concentrations from 15.625 to 1000 μM declined SH-SY5Y cell viability after 24 h; therefore, these amounts were deemed safe for use in subsequent tests. According to the results, hesperidin significantly decreased quinolinic acid-induced cytotoxicity at 62.5, 125 μM, and 250 μM ($p< 0.001$, Figure 1). According to Nakai et al., free radical scavengers reduced the neuronal damage caused by quinolinic acid-induced oxidative stress and apoptosis in the rat striatum [10]. We showed that QuA causes apoptosis in SH-SY5Y cells by enhancing the number of cells in the sub-G1 phase, which is consistent with the results of the oxidative stress assessment.

The findings of this study demonstrated that, compared with the untreated control group, the number of apoptotic cells was significantly increased after quinolinic acid treatment of SH-SY5Y cells at the dose of 6 mM (i.e., cells in the sub-G1 phase). Pre-treatment with hesperidin could reduce this effect (from 29.6% of apoptotic cells in the QuA-treated group to 17% and 22.3% in the 62.5 μM and 125 μM hesperidin pre-treated groups, respectively; $p<0.001$). As a result of quinolinic acid treatment, there was a dramatic increase in the percentage of apoptotic cells in the sub-G1 phase (from 9.37% in the untreated control to 29.6% in the 6 mM QuA-treated cells; $p<0.001$) (Table 1). Data has shown that hesperidin has neuroprotective benefits in research models of neurodegenerative disorders [12].

We also assessed the effect of the antioxidant hesperidin on the toxicity caused by quinolinic acid in SH-SY5Y cells. Our results showed that hesperidin reduced ROS production and improved the survival of neuroblastoma cells treated with quinolinic acid. Compared with the untreated control group, the findings of the ROS experiment demonstrated that QuA treatment of SH-SY5Y cells markedly increased the generation of ROS (Figure 2).

Treatment with tert-butyl hydroperoxide (TBHP) alone as a positive control considerably elevated intracellular levels of ROS compared with the control group. Furthermore, it became clear that pre-treatment with hesperidin remarkably reduced the amount of ROS caused by quinolinic acid in comparison with the group that received only quinolinic acid.

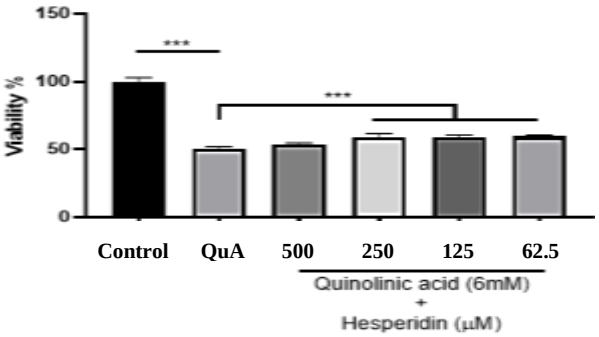

Figure 1. The protective benefits of 24 h pretreatment with hesperidin against 6 mM quinolinic acid (QuA)-induced cytotoxicity; untreated cells are the control; The results are the mean ± SD and are typical of three independent studies; ***$p< 0.001$: significant differences with the untreated control group

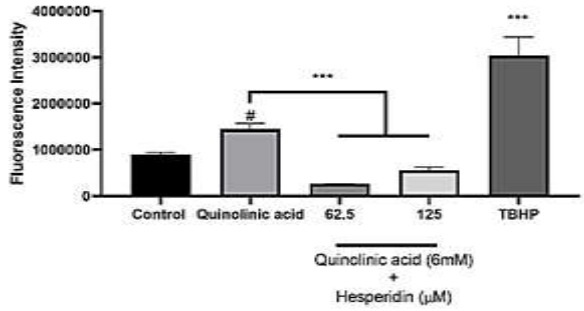

Figure 2. The effect of hesperidin on ROS levels within cells induced by quinolinic acid; Intracellular levels of ROS were measured (H_2DCFDA) using the fluorescent ROS marker dye 2', 7'-dichloro dichlorofluorescein diacetate; The positive control was tert-butyl hydroperoxide (TBHP), which significantly boosted the formation of ROS; The results are displayed as the mean±SD of triplicate tests; Compared to the control group, a significant difference was considered with $p<0.05$; and ***$p <0.001$ compared to the QuA-treated group.

Table 1. The ratio of cells in each treatment's sub-G1 phase

Cell treatment	Proportion of cells in the sub-G1 phase (% of total cell count)
Control (untreated)	9.37
6 mM QuA only	29.6^{***}
125 μM Hesperidin + 6 mMQuA	$22.3^{###}$
62.5 μM Hesperidin + 6 mMQuA	$17.0^{###}$

The table displays the ratio of cells in each treatment's sub-G1 phase; ###: $p<0.001$, *** $p<0.001$ significant deviations from the quinolinic acid- treated and untreated control groups, respectively

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Author contributions

Farzaneh Abbasinezhad-Moud and Emad Azimi contributed to the manuscript design, conception, acquisition, and drafting; Mehdi Rostami and Elaheh Gheybi contributed to the interpretation of data and critically revised the manuscript; Mohammad Soukhtanloo contributed to the idea and drew on his respective expertise to contribute. All writers have read and given their consent to the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

QuA: quinolinic acid; ROS: reactive oxygen species; NAD: nicotinamide adenine dinucleotide; TDO: tryptophan-2,3 dioxygenase; IDO1: indoleamine dioxygenases 1; IDO2: indoleamine dioxygenases 2; IL-1β: interleukin-1 beta; MAPK: mitogen-activated protein kinase; Nrf2: nuclear factor erythroid 2-related factor 2; BBB: blood-brain barrier, SOD: superoxide dismutase; CAT: catalase; GSH-Px: glutathione peroxidase; GST: glutathione-S-transferase; HMC: hesperidin methyl chalcone