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Hesperidin protects against MPP$^+$-induced neurotoxicity in SH-SY5Y cells

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ABSTRACT

Dopaminergic neuron loss and alpha-synuclein aggregation brought on by an excess of reactive oxygen species (ROS) are linked to Parkinson's disease (PD). The pharmacological characteristics of hesperidin (HES), including its anti-inflammatory, anti-atherosclerosis, anti-diabetic, anti-cancer effects have been the subject of much recent research. However, its possible neuroprotective effects on SH-SY5Y cells, a cellular PD model, caused by the neurotoxic MPP$^+$, have not been studied. In this investigation, we postulated that antioxidant activities may scavenge ROS, hence mediating neuroprotective benefits against MPP$^+$-induced oxidative stress parameters. Here, HES pre-treatment could attenuate MPP$^+$-induced neuronal cell death, which was reflected by decreasing the levels of ROS, Total Oxidant Status (TOS) and inflammation. Moreover, HES led to increased levels of the antioxidant enzyme GSH and TOS. According to this study, HES demonstrated notable neuroprotective benefits against MPP$^+$-induced SH-SY5Y cell death *in vitro*. Our findings demonstrate that HES may be a valuable adjunct for Parkinson's disease prevention.

Keywords: Hesperidin, Parkinson’s disease, Cytotoxicity, Oxidative stress, SH-SY5Y cells

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INTRODUCTION

The etiology of neurodegenerative illnesses, which impact 15% of the global population, is significantly influenced by neurodegeneration. A physiological process known as neurodegeneration causes neurons to eventually die after losing their structural integrity or functional capacities [1, 2]. The symptoms of Parkinson's disease (PD), one of the most significant neurodegenerative diseases, include tremors, rigid muscles, poor mobility, and problems with balance and coordination. We do not yet know the primary harmful process of the illness [3, 4].

Numerous studies have shown how vital neuro-inflammatory, oxidative stress (OS), intracellular ion build-up, and mitochondrial dysfunction are in the development of neurodegeneration in PD [5, 6]. In vitro PD models frequently employ MPP^+ (1-methyl-4-phenylpyridinium), a neurotoxin that causes PD-associated alterations in mitochondrial function, including complex-1 inhibition and elevated reactive oxygen species (ROS) [7, 8]. Increasing MPP^+ concentrations resulted in a dose-dependent reduction in cell viability as an increase in intracellular ROS levels and Ca^{2+} accumulations, according to research examining the neuroprotective impact of insulin on neurotoxicity in MPP^+-induced SH-SY5Y cells [9]. In their MPP^+/MPTP-induced in vitro and in vivo neurotoxicity model study, Song et al. found that MPP^+ caused SH-SY5Y cells to have higher MDA and ROS levels and lower GSH levels. They also proposed that suppressing OS could stop MPP^+-induced neurotoxicity [10]. MPP^+-induced cells showed reduced cell viability and elevated intracellular ROS levels, caspase-3 activation, and mitochondrial dysfunction in SH-SY5Y cells, according to Wang et al. [8].

Citrus fruits are the primary source of hesperidin (HES). This flavonoid has garnered much interest because of its possible health advantages, including lipid-lowering, anti-inflammatory, anti-apoptotic, and antioxidant properties [11]. Recent studies indicate that by lowering oxidative stress, regulating inflammatory responses, and inhibiting apoptosis, HES may help lessen some of the diabetes-related problems [12, 13, 14]. Because of its ability to lower oxidative stress, inflammation, and apoptosis, HES is a prospective therapy option for Parkinson's disease. Studies using experimental PD models have shown that HES therapy has an excellent safety profile with little toxicity [15]. Thus, a deeper comprehension of the processes via which HES demonstrates its neuroprotective qualities might further enhance treatment strategies.

In this investigation, we induced stress parameters in MPP^+-treated SH-SY5Y cells to examine the impact of HES on oxidative stress, inflammation, and ROS accumulation.

MATERIAL AND METHOD

Cell Culture and Study Groups

The cells were cultured at 37 °C in a humidified incubator with 5% CO_2 in DMEM/F12 basal medium combination that contained 10% fetal bovine serum and 1% penicillin-streptomycin (Sigma-Aldrich, USA). To be used in the plate reader, the cells were planted in four flasks at a density of 1×10^6 cells each. Every group received a treatment. Sigma-Aldrich was the supplier of the HEP and MPP (Sigma Aldrich, USA).

The following four groups were created from the SH-SY5Y cells:

CON group: No treatment was applied to the cells in this group.

MPP group: For 24 hours, the cells in this group were treated with 0.5 mM MPP^+ [16].

HES group: For 24 hours, the cells in this group were treated with 20 µM HES [17].

HES+MPP group: For 24 hours, the cells in this group were treated with 0.5 mM MPP^+ and 20 µM HES.

Measurement of Biochemical Parameters

The samples were then centrifuged at 1000 rpm for 20 minutes according to the respective kit protocols. Following centrifugation, the supernatants were discarded, and the resulting cell pellets were resuspended in phosphate-buffered saline (PBS; pH 7.4) to achieve a final concentration of approximately 1×10^6 cells/ml. Cell lysis was performed to release intracellular contents, utilizing repeated freeze-thaw cycles. The supernatants were incubated for 60 minutes at 37°C following the guidelines. Standard samples and the supernatant were moved to 96-well plates and allowed to incubate. After washing, staining solutions were added, and the mixture was incubated for 15 minutes at 37°C. The stop solution was applied at the conclusion of each of these processes. The levels of TAS (Total Antioxidant Status, Rel-Assay, Türkiye), TOS (Total Oxidant Status, Rel-Assay, Türkiye), ROS, TNF-α, and GSH in the SH-SY5Y cell supernatants were quantified using ELISA kits obtained from SunRed-Biotech Com. Ltd (China). The manufacturer's instructions recorded absorption readings using a BioTek ELx808™ microplate reader.

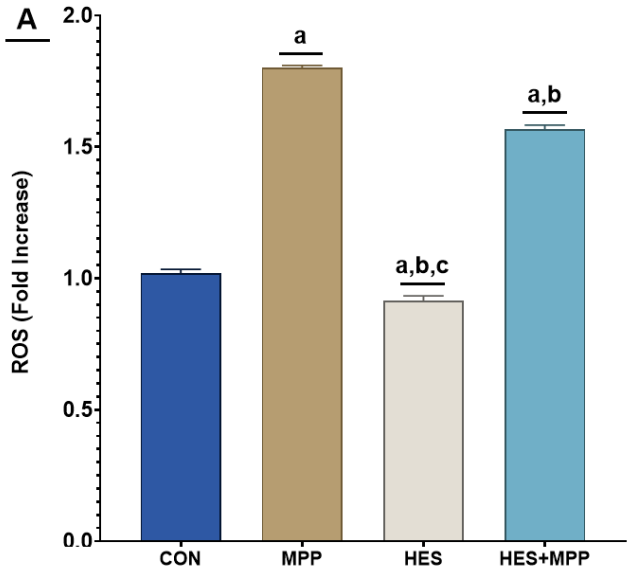
Statistical Analysis

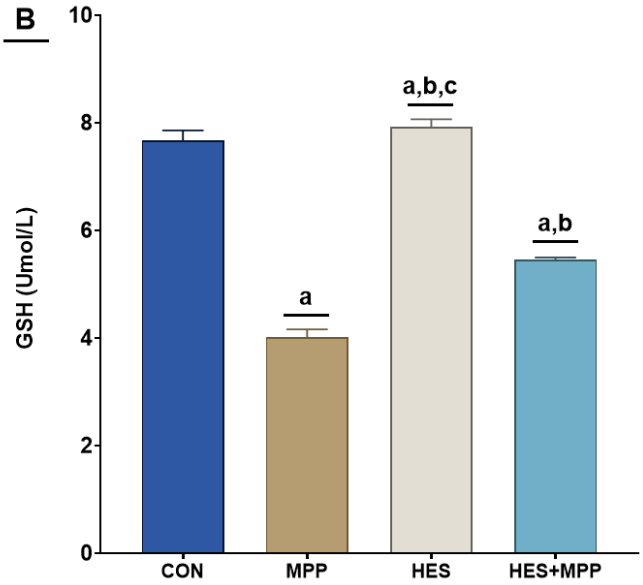
Data were expressed as the mean ± standard deviation (SD). For statistical analysis, one-way analysis of variance (ANOVA) was employed to assess differences among groups with normally distributed data, utilising SPSS statistical software. Tukey's post hoc test was applied when significant differences were detected to identify pairwise group differences. Statistical significance was defined as $p < 0.05$ for all analyses.

RESULTS

Modulatory Effect of HES on ROS, TNF-α, and GSH Levels on MPP-induced Cytotoxicity in SH-SY5Y Cells

ELISA kits were used to measure ROS, TNF-α, and GSH levels to investigate the effect of MPP on oxidative stress in SH-SY5Y cell lysates. When ROS and TNF-α levels were analyzed between the groups in the study, it was determined that ROS (Figure 1A) and TNF-α (Figure 1C) levels of the MPP group were significantly higher and GSH levels (Figure 1B) were significantly lower than the other groups ($p < 0.001$). On the other hand, ROS and TNF-α levels were significantly lower and GSH levels were higher in the HES-treated MPP group (HES+MPP group) than in the MPP group ($p < 0.001$). While there was a statistically significant difference between the ROS and TNF-α levels of the control and HES groups ($p<0.001$), there was no statistically significant difference between the GSH levels ($p >0.05$).





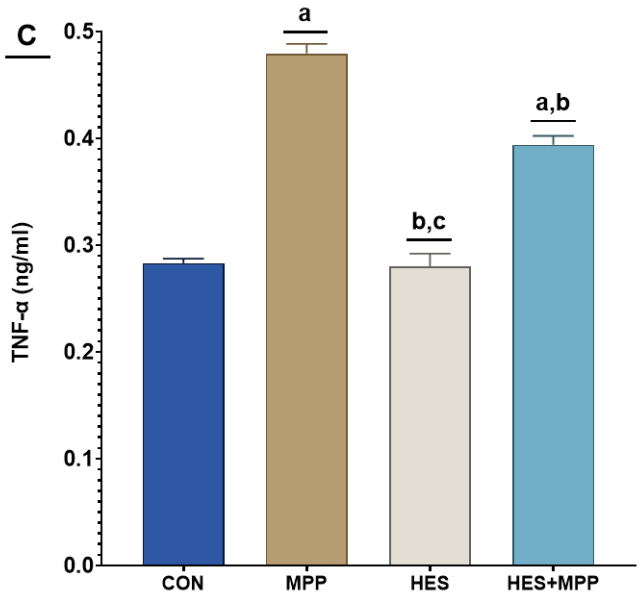


Figure 1. Effect of HES on (A) ROS, (B) GSH, and (C) TNF-α levels in SH-SY5Y cells after MPP-induced cytotoxicity. ([a]p <0.001 vs CON group, [b]p <0.001 vs MPP group, [c]p <0.001 vs HES+MPP group).

Modulatory Effect of HES on TOS and TAS Levels on MPP-induced Cytotoxicity in SH-SY5Y Cells

ELISA kits were used to measure TOS and TAS levels to investigate the effect of MPP on oxidative stress in SH-SY5Y cell lysates. When TOS levels were analyzed between the groups in the study, it was determined that TOS (Figure 2A) levels of the MPP group were significantly higher and TAS levels (Figure 2B) were significantly lower than the other groups ($p < 0.001$). On the other hand, TOS levels were significantly lower and TAS levels were higher in the HES-treated MPP group (HES+MPP group) than in the MPP group ($p < 0.001$). There was no statistically significant difference

between the TOS and TAS levels of the control and HES groups (p >0.05).

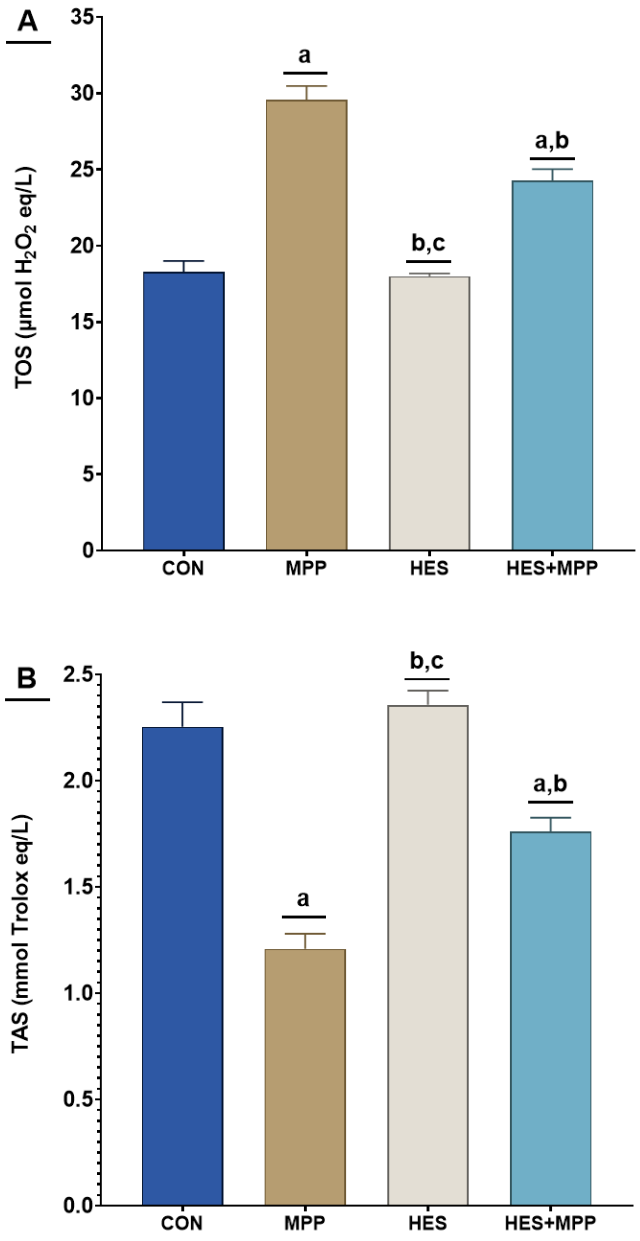


Figure 2. Effect of HES on (A) TOS, and (B) TAS levels in SH-SY5Y cells after MPP-induced cytotoxicity. ([a]p <0.001 vs CON group, [b]p <0.001 vs MPP group, [c]p <0.001 vs HES+MPP group).

DISCUSSION

A frequent neurodegenerative illness with both motor and non-motor symptoms is PD [3, 4]. In *in vitro* PD model investigations, the neuroblastoma (SH-SY5Y) cell line is frequently employed. Additionally, it was found that a PD model was created in the SH-SY5Y cell line using the MPP^+ agent, which breaks the electron transport chain in the cell and results in cell death by forming free radicals [5, 6, 8, 9]. In the present investigation, we examined the impact of HES on ROS, inflammation, and oxidative stress in MPP^+-induced neuronal damage in neuroblastoma cells.

Overproduction of OS in cells is directly correlated with an increase in ROS, which can harm proteins, lipids, or DNA, among other biological targets, resulting in various diseases. Atherosclerosis, cancer, hypertension, diabetes autoimmune diseases, and age related illnesses are all known to be influenced by excessive mitochondrial ROS production [18]. OS is brought on by MPP^+'s binding to the mitochondria's NADH dehydrogenase, which halts the electron transport chain [19]. Yan et al. looked at how artemisinin protects OS, which is crucial to the pathophysiology of PD. According to this study, when MPP^+ was applied to SH-SY5Y cells, ROS levels rose but antioxidant (SOD, GSH) levels fell. Furthermore, it was noted that the MPP^+ treatment group had higher levels of caspase-3 protein expression [20]. Liu et al. found that in the PD model SH-SY5Y cells, MPP^+-induced ROS build-up was followed by a depolarization of mitochondrial membrane potential and reduced cell survival. [21]. Ahlatçi reported that TOS, MDA and caspase-3 levels decreased and TAS levels increased in C6 cells treated with GA before glutamate incubation compared to the glutamate group [22]. Following MPP^+ administration, this study found that the levels of ROS (Figure 1A) and TOS (Figure 2A) in SH-SY5Y cells significantly increased. On the other hand, the HES+MPP group's ROS and TOS levels were much lower than those of the MPP group. Accordingly, in the literature, we discovered that HES therapy may be a key therapeutic agent for the elevated ROS and TOS levels with MPP^+ administration.

In MPP^+-treated neuroblastoma cells, Wang et al. observed elevated ROS generation, up-regulated NADPH oxidase-2 expression, down-regulated SOD activity, and reduced GSH levels [23]. Tectorigenin's impact on MPP+-induced neurotoxicity in SH-SY5Y cells was investigated by Gong et al. In their study, MPP treatment caused a significant decrease in cell viability and increased apoptosis, as evidenced by upregulation of cytochrome c expression and caspase-3 activity in cells. They also noted a notable drop in glutathione peroxidase, catalase, and superoxide dismutase levels [5]. When Song et al. examined how baicalein, a bioactive flavone molecule, prevented MPP+-induced neurotoxicity, they found that MPP+-treated SH-SY5Y cells had significantly lower GSH levels and higher levels of MDA and ROS [10]. Pirunkaset et al. reported that ROS production increased and SOD and CAT activities were down-regulated in SH-SY5Y neuroblastoma cells in which rotenone induced Parkinson's disease model. It also reported increased mRNA expression

levels of the oxidative and inflammatory genes inducible nitric oxide synthase (iNOS) and Interleukin 1β. Diacetylcurcumin manganese complex treatment down-regulated ROS levels and increased SOD and CAT activities, showing anti-inflammatory effect [24]. In SH-SY5Y cells, cadmium chlorine-induced neurotoxicity caused a decrease in cell viability and GSH levels and an increase in MDA and ROS levels. Selenium treatment reversed this situation [25]. MPP reduced the GSH and TAS levels in SH-SY5Y cells to the lowest level compared to other groups, according to our study's analysis of the groups' GSH (Figure 1B) and TAS (Figure 2B) densities. In comparison to the MPP group, it was shown that administering HES therapy in addition to MPP raised the GSH and TAS levels. Compared to the other groups, MPP considerably raised TNF-α levels in SH-SY5Y cells, according to an analysis of TNF-α levels between the groups. Compared to the MPP group, it was shown that administering HES therapy and MPP reduced TNF-α levels (Figure 1C). Thus, we demonstrated that HES's antioxidant qualities may control the low level produced by MPP.

CONCLUSION

Our study highlights the protective role of HES in attenuating MPP^+-induced neurotoxicity through attenuation of ROS, oxidative stress and inflammatory responses in an in vitro model of PD. These findings suggest that in SH-SY5Y cells, HES shows significant promise as a neuroprotective agent, especially in diseases such as Parkinson's disease characterized by MPP^+ cytotoxicity and redox imbalance. HES treatment ameliorated MPP^+-induced oxidant and apoptotic effects by increasing the glutathione redox system. Further mechanistic and translational studies are needed to elucidate its therapeutic potential fully.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Conflicts of Interest

There is no conflict of interest for the publication of this article.

Ethics committee approval

This research was carried out using cells propagated through commercially available cell culture. Ethics committee approval is not required in this study. The study was conducted following the international declaration, guidelines, etc.

Referee Evaluation Process

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