NEURAL REGENERATION RESEARCH
www.nrronline.org

# Hydroxytyrosol and Parkinson's disease: protective actions against alpha-synuclein toxicity

**Ruth Hornedo-Ortega, Ana M. Espinosa-Oliva***

Downloaded from http://journals.lww.com/nrronline by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 02/23/2024

**Parkinson's disease:** Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, after Alzheimer's disease, affecting 1% of the general population over the age of 65 years. According to data from the World Health Organization (WHO), its prevalence has doubled in the past 25 years. In 2019, global estimates indicated over 8.5 million individuals with PD and it is suggested that PD caused 329000 deaths, an increase of over 100% since 2000 (WHO, 2022). Although there are pharmacological interventions that improve the quality of life of PD patients, this brain disorder still has no cure.

The accumulation of misfolded α-synuclein (αsyn) protein in dopaminergic neurons is the main histopathological hallmark of PD. αsyn is a small protein (140 amino acids) that presents a spectrum of various species, including monomers, oligomers, and fibrils. Both oligomers and fibrils have been shown to be toxic in different contexts. However, their different sizes and shapes determine differences in their reactivity and toxicity. For this reason, whether αsyn oligomers or fibrils are more toxic keeps on intense discussion (Alam et al., 2019). What is clear is that the formation of αsyn aggregates is toxic to dopaminergic neurons, so it must be avoided to prevent neuronal death.

Dysfunction and death of dopaminergic neurons are also associated with an increase in microglial activity. This is due to the increased microglial activation can result in the upregulation of pro-inflammatory cytokines (neuroinflammation) and reactive oxygen species (ROS) (oxidative stress). These processes seem to play a key role in the development and progression of PD, hence suppression of microglial activation can be considered as an important strategy for PD prevention.

Recently, some non-pharmacological strategies to reduce the incidence or slow down PD progression have been under development. In this sense, since it was demonstrated that PD incidence could be influenced by environmental factors such as diet, much attention has been directed toward dietary interventions. Thus, emerging and promising therapeutic approaches based on diet are focused on avoiding important processes associated with this disorder including the formation and propagation of αsyn oligomers and fibrils, and microglial activation, among others.

**Hydroxytyrosol:** Among the different diets, increasing evidence supports that adherence to the traditional Mediterranean diet is related to a significant reduction in the risk of age-related pathologies such as dementia, Alzheimer's disease, and PD (Gardener and Caunca, 2018). This dietary pattern is characterized by a considerable consumption of plant foods (grains, fruits, legumes, vegetables, and nuts); moderate intake of poultry, fish, and wine (especially red wine); low intake of processed and red meat; and extra-virgin olive oil as the primary fat source. Extra-virgin olive oil is rich in polyphenols, particularly hydroxytyrosol (HT) (3,4-dihydroxyphenylethanol; also known as DOPAC). This polyphenol and its derivatives are one of the most relevant bioactive compounds, and they are recognized for their cardioprotective properties. In fact, in 2010, the European Food Safety Authority (EFSA) authorized a health claim for olive oil polyphenols (HT and its derivatives; containing at least 5 mg/20 g of olive oil), substantiating their contribution to the protection of blood lipids from oxidative stress (Commission Regulation EU 432/2012) (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). Thus, given the number of beneficial properties of HT, its neuroprotective effects are being increasingly studied because of its ability to easily cross the blood-brain barrier (Wu et al., 2009). Specifically, regarding HT properties as an anti-parkinsonian agent, more and more research has been carried out in recent years, which we will discuss below.

**HT–anti-aggregation effect and prevention of the formation of toxic α-syn oligomers and fibrils:** The first evidence of the anti-aggregative effect of HT on the formation of αsyn fibrils dates to 2018. In this sense, and using different physicochemical techniques (thioflavin T, transmission electronic microscopy, and electrophoresis), it was demonstrated that HT (25–200 μM) can very actively inhibit (between 70–90%) αsyn fibril formation (70 μM) (Hornedo-Ortega et al., 2018). These results were later confirmed by Gallardo-Fernandez and his co-workers, proving that HT alone and in combination with other bioactive compounds from food (protocatechuic acid and melatonin) counteracts αsyn fibrillation (80%, HT: 100 μM/αsyn: 70 μM) (Gallardo-Fernández et al., 2019a). These observations were also corroborated by Palazzi et al. (2020), noting that HT completely abolished αsyn aggregation at the 1:1 protein/HT ratio (Palazzi et al., 2020). Regarding the potential mechanism underlying this anti-aggregative effect, it was demonstrated that HT stabilizes the monomeric αsyn and induces the formation of non-toxic oligomeric species due to its ability to chemically modify αsyn by the oxidation of methionine and the formation of covalent adducts in the N-terminal region. Additionally, HT catechol moiety possesses the capacity to specifically bind to the non-Aβ component of the amyloid plaques (NAC) region (a central region responsible for protein fibrillation) and the C-terminal region of the αsyn protein through non-covalent hydrophobic interactions (Palazzi et al., 2020; **Figure 1**). These protein modifications caused by HT prevent the growth of amyloid-like fibrils. Interestingly, with the use of wild-type and E46K mutant αsyn (which develops species that are more toxic than wild-type αsyn), it has been demonstrated that HT interacts differently with each of these proteins. In fact, E46K mutant αsyn requires higher concentrations of HT for its complete inhibition than wild-type αsyn. The interaction with HT led to the formation of structures different from the fibrils, which were identified as off-pathway aggregates (stable oligomers that lead to fibril formation inhibition) (Fongaro et al., 2022). In addition to inhibiting aggregation and preventing the formation of toxic αsyn fibrils, HT has been shown to destabilize preformed fibrils *in vitro*. In this way, HT can effectively (30–80%; HT: 25–100 μM/αsyn fibrils: 70 μM) destabilize preformed αsyn oligomers/fibrils, reversing the neurotoxic effects of these ordered structures (Hornedo-Ortega et al., 2018). Very recently, this fact was confirmed and the potential destabilizing mechanism of action and the interaction between HT and the αsyn trimer were elucidated by molecular dynamics simulations (Kaur et al., 2023). A significant decrease in the β-sheet content (40–63%) of the residues of the pre-NAC and NAC regions of the αsyn trimer was observed after the incorporation of HT. Hydrogen bond interactions of the hydroxyl groups of HT with these residues lead to the weakening of interchain interactions in the αsyn trimer and result in the disruption of the αsyn oligomer (Kaur et al., 2023; **Figure 1**).

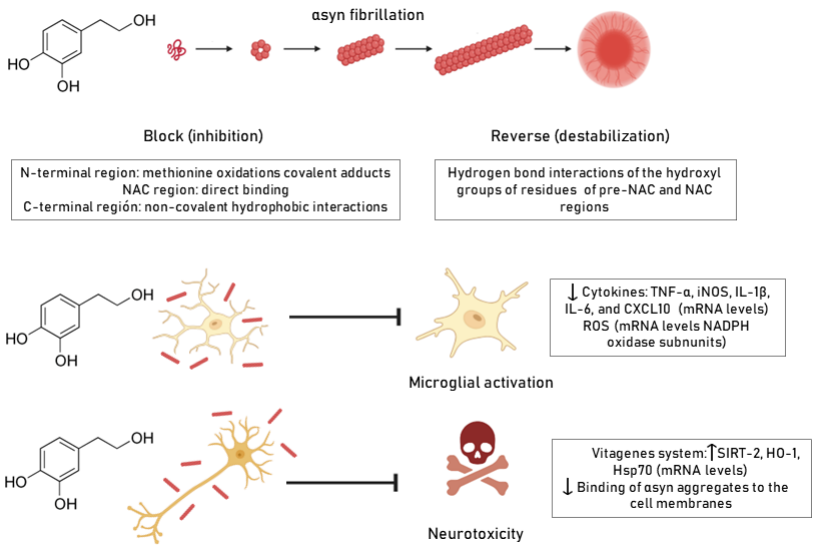


**Figure 1** | **Representative scheme of the molecular mechanisms by which HT could exert its anti-aggregative, anti-inflammatory, antioxidant, and neuroprotective actions against the toxic effects of αsyn oligomers and fibrils.** Created with BioRender.com and ChemDraw 20.1.1. αsyn: α-Synuclein; CXCL10: interferon-γ-inducible protein 10; HO-1: heme oxygenase-1; HSP-70: heat shock protein 70; IL: interleukin; iNOS: inducible nitric oxide synthase; NAC: non-Aβ component of amyloid plaques; NADPH: nicotinamide adenine dinucleotide phosphate; ROS: reactive oxygen species; SIRT-2: sirtuin-2; TNF-α: tumor necrosis factor α.

Downloaded from http://journals.lww.com/nrronline by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 02/23/2024

**HT–anti-inflammatory, antioxidant, and neuroprotective effects against αsyn-induced toxicity:** The aggregation of αsyn is known to trigger the activation of glial cells such as microglia, inducing neuroinflammation by releasing cytokines such as interleukin (IL)-1β, IL-18, IL-6, and tumor necrosis factor-α, among others (Du et al., 2020). The pro-inflammatory microglia also release ROS, reactive nitrogen species, and nitric oxide by activating the enzymes inducible nitric oxide synthase or nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). Hence, aggregated αsyn is capable of inducing an inflammatory and oxidative environment that also results in neuronal death (Du et al., 2020). HT is known to possess anti-inflammatory and antioxidant activities in different cell lines and animal models of PD, using neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/1-methyl-4-phenylpyridinium ($MPP^+$), 6-hydroxydopamine, paraquat, and rotenone (DiRosa et al., 2020; Abdul-Latif et al., 2021). However, the anti-inflammatory and antioxidant actions of HT against αsyn-induced microglial activation have not been studied.

Recently, it has been demonstrated for the first time in microglial cells that HT is capable of reducing inflammation and the oxidative environment induced by αsyn (Gallardo-Fernández et al., 2019b). Thus, BV2 murine microglial cells were treated with αsyn fibrils (5 μM), with and without HT (1–50 μM) for 6 hours. A reduction in the expression levels of pro-inflammatory mediators tumor necrosis factor-α, inducible nitric oxide synthase, IL-1β, IL-6, and interferon-γ-inducible protein 10 (CXCL10) was observed after HT treatment (**Figure 1**). Moreover, the levels of AKT and p-p38 measured by Western Blot were also reduced by HT. These results suggest the potential molecular pathways that could be implicated in the anti-inflammatory effect of HT (Gallardo-Fernández et al., 2019b).

Regarding the antioxidant properties in an oxidative environment induced by αsyn, treatment with HT prevents the association of the different subunits of NADPH oxidase (mRNA expression of p22, p47, and gp91), affecting its activation and consequent ROS production (Gallardo-Fernández et al., 2019b; **Figure 1**).

Concerning the neuroprotective activity of this bioactive compound, the co-treatment of HT with neuronal cells (PC12 cell line) exposed to αsyn as an inducer of toxicity, produced a significant increase in cell viability. Interestingly, HT was able to reverse the toxic effect of αsyn (140 μM), reaching control viability values (100%) at 25 μM (Hornedo-Ortega et al., 2018). Emerging evidence has proved the high potential of the vitagene system as a target for neuroprotective strategies. Vitagenes are a group of genes (including heat shock proteins (Hsp) Hsp32, Hsp70, the thioredoxin, and the sirtuin protein systems) involved in preserving cellular homeostasis under stressful conditions. In addition, these genes are also involved in the cellular degradation of misfolded αsyn, thus protecting against αsyn-induced toxicity (Calabrese et al., 2011). The modulation of this system may be responsible for the neuroprotective effects of HT since an increase in the expression of sirtuin-2, heme oxygenase-1, Hsp-70 was observed in the neurons exposed to αsyn and co-treated with this bioactive (Gallardo-Fernández et al., 2019a; **Figure 1**).

Palazzi et al. (2020) also evaluated the neuroprotective effect of HT on SH-SY5Y human neuroblastoma cells. They observed that cell viability increased between 20–30% when the cells were treated with αsyn aggregated (5 μM) grown in the presence of HT during 48 hours or 168 hours (1:1 and 1:4 αsyn/HT), in comparison to cells treated with αsyn aggregates grown in the absence of HT. Besides, an important reduction of ROS production and in the binding of αsyn aggregates to the cell membranes was observed when cells were exposed to αsyn aggregates grown in the presence of HT, a fact that could also explain HT neuroprotective effect (Palazzi et al., 2020; **Figure 1**). The PD-model UA440 of *Caenorhabditis elegans* is characterized by the expression of αsyn in dopaminergic neurons. Also supporting the neuroprotective activity of HT, a significant reduction in the number of degenerated neurons was observed in this model by HT treatment (250 μg/mL; DiRosa et al., 2020).

**Perspectives:** Based on the above, we can conclude that HT has important potential properties (anti-aggregative, anti-inflammatory, antioxidant, and neuroprotective) to combat the toxic effects of αsyn oligomers and fibrils, which, consequently, may be beneficial in counteracting PD. These activities are strictly related to the chemical structure of HT and, specifically, to the presence of the catechol functional group that makes this molecule highly reactive. But elucidation of the molecular mechanisms underlying these potential effects of HT is essential, and to date, has not been sufficiently demonstrated. The existing literature on this subject is interesting, but further studies are necessary to support these observations. For example, there are few *in vivo* studies using animals overexpressing αsyn, and no human studies (cohort or randomized control trials) have evaluated the effect of this compound against PD. Despite the potential role of HT as an anti-parkinsonian agent, a great deal of effort and time is needed from the scientific community before it can be considered as a preventive or therapeutic strategy for PD.

*We apologize to colleagues whose work has not been cited due to space limitations.*

*Authors are also grateful to RHO's postdoctoral contract (Ayuda IJC2020-045695-I financed by MCIN/AEI/10.13039/501100011033, European Union NextGeneration EU/PRTR).*

**Ruth Hornedo-Ortega, Ana M. Espinosa-Oliva***
Departamento de Nutrición y Bromatología, Toxicología y Medicina Legal, Facultad de Farmacia, Universidad de Sevilla, Seville, Spain (Hornedo-Ortega R)
Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain; and Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain (Espinosa-Oliva AM)
***Correspondence to:** Ana M. Espinosa-Oliva, PhD, anaespinosa@us.es.
https://orcid.org/0000-0002-2442-767X (Ana M. Espinosa-Oliva)

**Date of submission:** July 10, 2023
**Date of decision:** September 1, 2023
**Date of acceptance:** September 14, 2023
**Date of web publication:** November 8, 2023

**https://doi.org/10.4103/1673-5374.387987**
**How to cite this article:** *Hornedo-Ortega R, Espinosa-Oliva AM (2024) Hydroxytyrosol and Parkinson's disease: protective actions against alpha-synuclein toxicity. Neural Regen Res 19(7):1427-1428.*

**Open access statement:** *This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.*

**Open peer reviewer:** *Vittorio Calabrese, University of Catania, Italy.*
**Additional file:** *Open peer review report 1.*

## References

Abdul-Latif R, Stupans I, Allahham A, Adhikari B, Thrimawithana T (2021) Natural antioxidants in the management of Parkinson's disease: review of evidence from cell line and animal models. J Integr Med 19:300-310.
Alam P, Bousset L, Melki R, Otzen DE (2019) Alpha-synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. J Neurochem 150:522-534.
Calabrese V, Cornelius C, Cuzzocrea S, Iavicoli I, Rizzarelli E, Calabrese EJ (2011) Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. Mol Aspects Med 32:279-304.
Di Rosa G, Brunetti G, Scuto M, TrovatoSalinaro A, Calabrese EJ, Crea R, Schmitz-Linneweber C, Calabrese V, Saul N (2020) Healthspan enhancement by olive polyphenols in C. elegans wild type and Parkinson's models. Int J Mol Sci 21:3893.
Du XY, Xie XX, Liu RT (2020) The role of α-synuclein oligomers in Parkinson's disease. Int J Mol Sci 21:8645.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011) Scientific opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 9:2033.
Fongaro B, Cappelletto E, Sosic A, Spolaore B, Polverino de Laureto P (2022) 3,4-Dihydroxyphenylethanol and 3,4-dihydroxyphenylacetic acid affect the aggregation process of E46K variant of α-synuclein at different extent: Insights into the interplay between protein dynamics and catechol effect. Protein Sci 31:e4356.
Gallardo-Fernández M, Hornedo-Ortega R, Cerezo AB, Troncoso AM, García-Parrilla MC (2019a) Melatonin, protocatechuic acid and hydroxytyrosol effects on vitagenes system against alpha-synuclein toxicity. Food Chem Toxicol 134:110817.
Gallardo-Fernández M, Hornedo-Ortega R, Alonso-Bellido IM, Rodríguez-Gómez JA, Troncoso AM, García-Parrilla MC, Venero JL, Espinosa-Oliva AM, de Pablos RM (2019b) Hydroxytyrosol decreases LPS- and α-synuclein-induced microglial activation in vitro. Antioxidants (Basel) 9:36.
Gardener H, Caunca MR (2018) Mediterranean diet in preventing neurodegenerative diseases. Curr Nutr Rep 7:10-20.
Hornedo-Ortega R, Cerezo AB, Troncoso AM, Garcia-Parrilla MC (2018) Protective effects of hydroxytyrosol against α-synuclein toxicity on PC12 cells and fibril formation. Food Chem Toxicol 120:41-49.
Kaur G, Mankoo OK, Goyal D, Goyal B (2023) Unveiling how hydroxytyrosol destabilizes α-syn oligomers using molecular simulations. J PhysChem B 127:5620-5632.
Palazzi L, Leri M, Cesaro S, Stefani M, Bucciantini M, Polverino de Laureto P (2020) Insight into the molecular mechanism underlying the inhibition of α-synuclein aggregation by hydroxytyrosol. Biochem Pharmacol 173:113722.
World Health Organization (2022) Parkinson disease. Available from: https://www.who.int/news-room/fact-sheets/detail/parkinson-disease. Accessed September 7, 2023.
Wu YT, Lin LC, Tsai TH (2009) Measurement of free hydroxytyrosol in microdialysates from blood and brain of anesthetized rats by liquid chromatography with fluorescence detection. J Chromatogr A 1216:3501-3507.

*P-Reviewer: Calabrese V; C-Editors: Zhao M, Sun Y, Qiu Y; T-Editor: Jia Y*