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ARTICLE

Olive Polyphenols as Modulators of Amyloid Aggregation: Mechanisms and Implications for Neurodegenerative Diseases.

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The Mediterranean diet is well known for its role in promoting healthy aging and reducing the risk of chronic diseases, with extra virgin olive oil (EVOO) recognized as a key contributor to these benefits. Among EVOO's constituents, minor phenolic compounds have emerged as principal mediators of its biological activity. Given the pivotal role of amyloid aggregation in protein misfolding disorders (PMDs), considerable research over the past two decades has focused on amyloidogenic proteins and the discovery of natural compounds capable of modulating their aggregation. This review summarizes current evidence on the anti-amyloidogenic properties of olive-derived polyphenols, emphasizing their mechanisms of action and therapeutic relevance in two major neurodegenerative diseases, Alzheimer's and Parkinson's. Additionally, insights from molecular modeling studies are discussed to elucidate the structural basis of interactions between these polyphenols and amyloid proteins, shedding light on their influence on aggregation pathways.

1. Introduction

Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease are characterized by the progressive loss of neuronal structure and function, ultimately leading to cognitive decline, motor dysfunction, disability and death. These disorders represent a growing global health burden, especially in aging populations, with limited effective therapeutic options currently available.1 Despite decades of research, pharmacological treatments remain largely symptomatic and do not halt or reverse the neurodegenerative processes. In this sense, current treatments for AD (donepezil, rivastigmine, galantamine and memantine) provide only modest benefits, with meta-analyses and clinical trials consistently reporting small improvements in cognition, daily functioning or global assessments.2 For PD, the standard treatment remains the administration of levodopa in combination with decarboxylase inhibitors such as carbidopa, either orally or through intestinal infusion formulations. Other widely used symptomatic medications include dopamine agonists such as pramipexole and ropinirole, monoamine oxidase B inhibitors such as rasagiline and safinamide and catechol-O-methyltransferase (COMT) inhibitors.3, 4 Although effective in the early stages, these treatments gradually lose efficacy over time and do not modify the underlying pathogenic mechanisms.

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A common pathophysiological feature shared among many neurodegenerative diseases is the interplay between oxidative stress, chronic neuroinflammation and the accumulation of misfolded proteins such as amyloid-β (Aβ), tau and α-synuclein (α-syn). These interconnected mechanisms contribute to mitochondrial dysfunction, synaptic loss, glial activation and, ultimately, neuronal degeneration.5, 6 As a result, there is growing interest in multifunctional therapeutic agents capable of simultaneously targeting oxidative damage, inflammation and protein aggregation as a more holistic strategy for neuroprotection.

Amyloids are highly ordered protein aggregates, approximately 100–200 Å in diameter, composed of cross-β sheet structures in which β-strands run perpendicularly to the fibril axis. While early studies suggested that large fibrillar aggregates were the primary neurotoxic species, emerging evidence points to smaller, soluble oligomeric intermediates as the major contributors to neurodegeneration. These oligomers are structurally heterogeneous, ranging from dimers to protofibrillar assemblies composed of hundreds of monomers and they remain in dynamic equilibrium with monomers and fibrils. Some of these species act as on-pathway intermediates in amyloid fibrillogenesis, while others may represent off-pathway end products, some of which exhibit significant neurotoxicity.7

In recent years, plant-derived polyphenols have gained increasing attention for their ability to modulate amyloid aggregation. Their appeal lies in several advantageous properties, including low cytotoxicity compared to most synthetic agents, cost-effective availability, dietary accessibility and multifunctional bioactivity. These compounds have been shown to inhibit fibril formation, destabilize preformed fibrils or promote the disaggregation of amyloid assemblies.8 It has been proposed that small aromatic polyphenols are able to interfere with amyloid aggregation by binding to aggregation-prone intermediates or by redirecting the aggregation process toward non-toxic species.9, 10 Specifically, the phenolic rings in these compounds may disrupt π-π stacking interactions between aromatic residues in amyloidogenic proteins, thereby inhibiting the self-assembly into fibrillar structures.11

Among natural bioactive compounds, olive polyphenols, particularly those found in extra virgin olive oil (EVOO), have emerged as promising candidates for the prevention and modulation of neurodegenerative disorders. Epidemiological evidence suggests that adherence to the Mediterranean Diet (MD) and other specific diets enriched in EVOO such as the MIND diet (Mediterranean-DASH Diet Intervention for Neurodegenerative Delay), is associated with improved cognitive performance and a reduced risk of neurodegenerative diseases.12-14 These protective effects are increasingly attributed to specific phenolic constituents such as hydroxytyrosol (HT), oleuropein, oleocanthal (OC) and tyrosol (TYR). While most studies on olive polyphenols have focused on their antioxidant and anti-inflammatory roles, recent research increasingly highlights their anti-amyloidogenic effects. 15-17 18-20

Compared to other extensively studied polyphenols such as curcumin, epigallocatechin gallate or resveratrol, investigations into the role of olive phenolics in amyloid-related disorders began relatively recently. Nevertheless, emerging data, mainly involving oleuropein aglycone (OA), HT and OC are promising and suggest that these compounds may offer a valuable avenue for future therapeutic development, as they can directly target the molecular mechanisms underlying neurodegeneration, in contrast to currently available treatments.

This review provides a comprehensive and up-to-date overview of the effects of olive polyphenols on amyloid protein aggregation processes implicated in the pathogenesis of major neurodegenerative diseases, with a primary focus on AD and PD. Emphasis is placed on their potential to inhibit fibril formation, promote disaggregation and modulate key molecular pathways involved in protein misfolding. Additionally, recent advances in molecular modeling studies using established olive-derived ligands are discussed as a framework for exploring the structural interactions of emerging compounds with amyloidogenic proteins.

2. Olive Polyphenols: Bioactivity and nutraceutical potential

The olive tree (Olea europaea L.) constitutes a rich reservoir of bioactive compounds known for their significant health-promoting properties, found throughout various parts of the plant. Olive-derived products, particularly EVOO, olive leaves and table olives are rich sources of bioactive polyphenols. These compounds are secondary plant metabolites known for their potent biological activity, which make them particularly valuable for incorporation into functional foods, nutraceuticals and therapeutic formulations.21, 22

Among olive-derived products, olive leaves and EVOO are the most extensively investigated sources of bioactive compounds with potential nutraceutical applications. Olive leaves are particularly rich in polyphenols, with secoiridoid derivatives, especially oleuropein, representing the most abundant constituents. Additional phenolic compounds include the phenolic alcohols HT, TYR and oleoside, as well as flavones such as luteolin and luteolin-7-O-glucoside, all of which have been associated with antioxidant, anti-inflammatory and cytoprotective activities.23 24 Also, EVOO presents a high concentration of phenolic compounds that contribute to its functional properties. Prominent among these are secoiridoid aglycones derived from oleuropein and ligstroside and their biologically active metabolites, oleacein and OC.25 These compounds are mechanistically linked to modulation of redox-sensitive pathways and inflammatory mediators relevant to ageing processes.26 HT and TYR further enhance EVOO's bioactivity through free radical scavenging and have been

implicated in cardiovascular and neuroprotective mechanisms.27, 28 Although present in lower concentrations, flavonoids such as luteolin and apigenin, along with phenolic acids, contribute to the overall biological effects. The synergistic interplay among these phenolic constituents is thought to underlie EVOO's protective effects, supporting its classification as a functional food with relevance to healthy ageing.29

Due to their well-characterized structures and extensively studied bioactivities, oleuropein, OA, HT, OC and TYR have been selected as the primary phenolic compounds explored in this review. Their chemical structure and their compared abundance in EVOO and in olive leaves are shown in Figure 1. This section will summarize the general features of these polyphenols, including formation, biological properties and bioavailability profiles. While the safety of these olive polyphenols within the context of dietary intake is well established, the evidence base for long-term use of concentrated, high-dose supplements remains limited. Accordingly, available data on long-term safety, adverse effects and possible drug interactions are also discussed in this section.

2.1. Oleuropein and OA

Oleuropein is the main polyphenolic compound found in olive leaves and unripe olives.30, 31 As a secoiridoid glycoside, it contributes to the characteristic bitterness of olives and olive oil. Structurally, oleuropein is an ester formed between HT and oleoside, a secoiridoid glucoside derived from elenolic acid. Its biosynthesis proceeds via the mevalonate pathway, characteristic of secoiridoid derivatives.32 Upon enzymatic or chemical hydrolysis, oleuropein is degraded into various bioactive components including OA, HT, elenolic acid and glucose, depending on the degradation pathway involved.33

OA, also known as 3,4-dihydroxyphenylethanol elenolic acid (3,4-DHPEA-EA), is a secoiridoid derivative of oleuropein produced through the action of β-glucosidase released from olive fruits during the crushing process.34 Its concentration in EVOO is highly variable and influenced by multiple factors, including the olive cultivar, degree of fruit ripeness, oil extraction method and storage conditions.35 Furthermore, the methodology used to extract and quantify OA from oil samples significantly impacts the reported concentration values.

OA exhibits a wide range of bioactivities, including antioxidant, anti-inflammatory, antimicrobial and anticancer effects, also contributing to the stabilization of LDL cholesterol and improvement of endothelial function.36-39 Human studies investigating the bioavailability of OA have consistently demonstrated that its oral intake predominantly results in the appearance of conjugated HT metabolites, primarily glucuronidated and sulfated derivatives, in both plasma and urine. These metabolites constitute the main circulating and excreted forms following OA ingestion, reflecting rapid and extensive first-pass metabolism and biotransformation of OA into HT. Conjugated metabolites were typically detectable in urine within 8 hours post-consumption, confirming efficient systemic clearance via renal excretion. Substantial inter-individual variability in metabolite levels has also been reported, likely attributable to differences in gut microbiota composition, metabolic capacity, and intestinal absorption efficiency. Notably, peak plasma concentrations of intact OA were higher when administered in a liquid formulation compared to encapsulated forms, indicating that the delivery matrix significantly affects absorption kinetics.40 In this sense, when OA and other phenolics are consumed as part of EVOO, urinary excretion of OA and its phase II metabolites remains prominent.41 These findings suggest that aglycone forms of secoiridoids are more efficiently absorbed than their glycosylated counterparts and that the EVOO matrix may enhance gastrointestinal stability and facilitate absorption of phenolic compounds.

Regarding its safety as nutraceutical, Oleuropein, most commonly administered in the form of standardized olive leaf extract (OLE), has been evaluated in several randomized controlled trials (RCT), typically at doses of around 50-500 mg/day over periods of 8–14 weeks.42-44 These short- to medium-term studies generally report good tolerability, although long-term safety data extending over several years remain limited. Reported adverse events are usually mild and include gastrointestinal discomfort or dizziness. Notably, OLE has been shown in some trials to lower blood pressure and blood glucose, effects that may be therapeutically beneficial in populations with hypertension or type 2 diabetes mellitus.45 However, these pharmacodynamic properties raise the possibility of additive hypotensive or hypoglycemic effects when OLE is combined with conventional antihypertensive or antidiabetic medications, highlighting the need for clinical monitoring of blood pressure and glucose levels in such contexts. Overall, findings from small-to-moderate sized RCTs, supported by systematic reviews and meta-analyses, indicate that OLE is generally safe in the short term although more rigorous long-term safety evaluations, particularly in patients receiving multiple medications are needed.

2.2. HT

HT (3,4-Dihydroxyphenyl Ethanol; DOPET) is a phenolic compound considered one of the most potent antioxidants found in olive-derived products. It is a key component of the minor polar fraction of EVOO, where it is primarily derived from the hydrolysis of oleuropein during olive fruit ripening and oil storage. HT is also present in olive fruit, leaves and in high concentrations in the waste fraction following olive oil production.46 It exhibits strong free radical scavenging activity, inhibits lipid peroxidation and possesses metal-chelating properties. Due to its high bioavailability, HT has been extensively associated with various health benefits, including improvements in serum lipid profile, cardioprotective, anti-diabetic, anti-neoplastic and anti-inflammatory effects.47 In addition, HT has shown neuroprotective properties and potential benefits in the prevention of neurodegenerative diseases.48

Clinical studies using olive oils enriched with natural or added phenolics have shown that HT is rapidly absorbed in the intestine, undergoes extensive phase I and II metabolism in the gut and liver, and is efficiently excreted via the kidneys. Free HT is rarely detected in plasma or urine, as it is predominantly present as glucuronidated, sulfated or methylated derivatives. Major metabolites include HT-3-

O-sulfate (HT-3-S), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), the latter formed via alcohol and aldehyde dehydrogenase activity and further methylated to HVA. Despite their lower abundance, oleuropein and TYR can also serve as precursors to HT following metabolism, further contributing to circulating HT levels. Animal studies corroborate these findings, showing rapid, dose-dependent absorption and distribution of HT to organs such as the liver, kidneys, heart and brain, with high urinary excretion of its main metabolites.49 It has been shown that targeted glycosylation enhances HT stability while allowing regeneration of the active compound, representing a promising strategy to improve its bioavailability and applicability in nutraceuticals.50

HT has been classified as 'Generally Recognized as Safe' (GRAS) by the European Food Safety Authority (EFSA) for inclusion in food supplements at specified doses.51 Human intervention studies administering approximately 5-25 mg/day for several weeks have consistently demonstrated good tolerability, with no reports of serious adverse effects.52 At dietary intake levels, HT is therefore considered to pose a low risk. Nonetheless, until further evidence is available, caution may be warranted when HT is co-administered with drugs that have a narrow therapeutic index. Overall, current findings support its safety for the proposed uses, but additional long-term studies are needed to establish its risk profile under chronic high-dose exposure.

2.3. TYR

TYR (2-(4-Hydroxyphenyl)ethanol) is a simple phenolic alcohol, structurally related to HT but with lower antioxidant potential. It is found in both free and conjugated forms in EVOO and is a product of the hydrolysis of secoiridoids. Despite its moderate antioxidant activity, TYR contributes to vascular protection and may enhance mitochondrial function and cellular longevity.26

Although TYR differs from HT by only one hydroxyl group, their bioavailability profiles are notably distinct. TYR exhibits good intestinal permeability, as shown by its absorption across Caco-2 monolayers and rat intestinal tissues.53 However, its biotransformation appears limited. *In vitro* studies report slow conjugation in Caco-2/TC7 cells, with methylated and sulfated metabolites only quantifiable after 24 hours.54 Unlike HT, TYR does not undergo methylation, likely due to the absence of a catechol moiety and hepatic metabolism is also minimal, with less than 10% glucuronidation observed in HepG2 cells after 18 hours.55 Overall, despite efficient absorption, TYR undergoes relatively low metabolic conversion, possibly attributed to its simpler structure and reduced reactivity compared to HT.56

Regarding its safety, human studies have reported good tolerability, with no evidence of serious adverse effects at doses achievable through a MD. Compared with HT, TYR exhibits lower antioxidant activity but a similarly favorable safety profile.57, 58 Data on high-dose or long-term supplementation remain limited, although no major toxicological concerns have been identified to date.

2.4. OC

Oleocanthal (3,4-HPEA-EDA) is the dialdehydic form of (−)deacetoxy-ligstroside aglycone, formed by TYR linked to elenolic acid and was first identified in virgin olive oil in 1993.59 Its concentration in EVOO varies significantly from as low as 0.2 mg/kg to as high as 498 mg/kg depending on factors such as olive cultivar, fruit maturity, geographic origin, agricultural practices, and processing, storage or heating conditions.60 Despite accounting for only about 10% of EVOO's total polyphenol content, OC contributes notably to its sensory profile, producing the characteristic throat irritation and pungency through activation of the transient receptor potential ankyrin 1 (TRPA1) receptor.61, 62

OC exhibits anti-inflammatory activity comparable to that of ibuprofen, primarily through selective inhibition of cyclooxygenase enzymes (COX-1 and COX-2), leading to reduced prostaglandin synthesis.63 Additionally, OC has demonstrated neuroprotective and anticancer effects in various experimental models.64

In animal models, OC displays limited bioavailability compared to other phenolic compounds in EVOO, such as HT. Once absorbed, it undergoes phase I metabolic transformations producing primarily hydrated, as well as hydrogenated and hydroxylated metabolites, before being processed through glucuronidation.65 In rats, only approximately 16% of the orally administered dose was detected in systemic circulation, with substantial intestinal metabolism reported. Conversely, human studies indicate the potential for higher absorption rates.66 Recent investigations in mice found no detectable levels of OC in plasma following oral administration, suggesting a rapid *in vivo* degradation. However, thirteen metabolites were identified, notably oleocanthalic acid and TYR sulfate, which have been proposed as biomarkers of OC exposure and are likely contributors to its observed biological activities.67 Optimized OC formulations, such as OC powder and OC-solid dispersions with erythritol, have been shown to improve oral bioavailability while retaining its neuroprotective activity.68 Such approaches highlight the potential of tailored OC delivery systems as nutraceuticals for mitigating amyloid-driven AD pathology.

Clinical trials on OC supplementation are limited, with most safety data derived from dietary intake and preclinical studies. Human evidence does not show consistent adverse effects from EVOO consumption, though *in vitro* COX-inhibition raises theoretical bleeding or gastrointestinal risks at high doses.69 Potential interactions with antiplatelet or anticoagulant medications are largely theoretical, and *in vitro* data suggest possible CYP enzyme modulation, though clinical relevance remains unclear. Overall, safety evidence for OC is primarily preclinical or limited in humans and recent narrative and systematic reviews highlight significant gaps.

3. Olive Polyphenols as modulators of protein aggregation in AD

AD is the most common form of dementia in the ageing population, representing a major public health challenge worldwide. Its increasing prevalence and the lack of effective disease-modifying treatments results in a growing burden on patients, caregivers and healthcare systems. Consequently, considerable research efforts have been devoted to identifying molecules and interventions capable of preventing, treating or delaying the onset and progression of AD. 18 One of the main neuropathological hallmarks of AD include the extracellular deposition of amyloid plaques, primarily composed of fibrillar networks of aggregated Aβ peptides, particularly Aβ (1–40), Aβ (1–42) and the highly aggregation-prone pyroglutamylated forms Aβ (3–42) and Aβ (11–42).70 These peptides are generated via sequential cleavage of the amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase. Aβ peptides are intrinsically disordered and self-assemble into increasingly ordered aggregates, oligomers, protofibrils and insoluble fibrils, characterized by a β-sheet-rich amyloid structure, which resists degradation and clearance.71 72 In parallel, neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein represent the second major pathological hallmark of AD. Tau, a microtubule-associated protein involved in axonal stability and transport, exists in six isoforms and is regulated through multiple post-translational modifications, including phosphorylation, glycosylation, ubiquitination and truncation.73 In AD, tau becomes abnormally hyperphosphorylated, promoting its aggregation into NFTs and neuropil threads.74

Emerging evidence supports the “toxic oligomer hypothesis” which proposes that small, soluble oligomeric intermediates, rather than mature fibrils, are the primary neurotoxic agents in amyloid diseases.75 These oligomers disrupt cellular homeostasis through mechanisms such as membrane destabilization, calcium dyshomeostasis, oxidative stress and impairment of protein quality control systems (e.g., the proteasome, autophagy pathways). Accordingly, tau oligomers also exert neurotoxic effects, particularly through mitochondrial and synaptic dysfunction, both early events in AD and other tauopathies.76 Recent studies also suggest that Aβ fibrils themselves may contribute directly to neurotoxicity, potentially acting as a reservoir for toxic oligomers or exerting independent harmful effects.77

In recent years, plant-derived natural extracts and phenolic compounds have attracted significant attention as potential inhibitors of amyloid aggregation. In the following sections, the principal olive-derived polyphenols will be examined for their direct modulatory effects on amyloid aggregation processes implicated in AD (Figure 2). The reported activities of these polyphenols and the specific mechanisms of action involved are also summarized in Table 1.

3.1. Oleuropein and OA

The majority of studies examining oleuropein as an inhibitor of Aβ aggregation have utilized its aglycone form, OA, due to evidence from *in vitro* experiments indicating its ability to interfere with amyloid formation by stabilizing intermediate species along the aggregation pathway.18, 78 In such studies, monomeric Aβ is generally incubated at 25 °C to facilitate amyloid fibril formation under *in vitro* conditions. The progression of fibrillization is then assessed using various analytical methods, including dynamic light scattering, Fourier transform infrared spectroscopy and Thioflavin T (ThT) fluorescence binding assays.

Leri et al. found that when pretreating Aβ with OA at equivalent concentrations, the formation of toxic oligomeric species was prevented. These results point to a modulatory role of OA in halting the assembly of Aβ (1-42) oligomers and blocking their growth into mature fibrils potentially through initial binding to the N-terminus of the monomeric and/or oligomeric peptide. Interestingly, the presence of OA during fibril formation led to a diminished ability of the resulting fibrils to seed aggregation of monomeric Aβ1−42.18

It has been suggested that the phenolic ring present in polyphenols such as OA, can interact with the aromatic residues in amyloidogenic proteins, disturbing the π-stacking between monomeric units and effectively preventing the self-assembly into fibrils.11, 79 In this sense, Rigacci et al. found that OA inhibits cytotoxic Aβ aggregation and delays the transition of Aβ (1-42) into a ß-sheet-rich structure possibly by reducing the exposure of the hydrophobic regions in Aβ (1-42). Moreover, these authors propose that OA interferes with Aβ (1-42) aggregation in two different, yet not mutually exclusive, ways: by binding the monomeric peptide and by binding the nascent aggregates, generating complexes with different aggregation propensities.78

Several studies using Electrospray Ionization (ESI), Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) had previously identified the 17-28 hydrophobic α helix region of Aβ as the responsible for the noncovalent interaction between the oleuropein molecule and Aβ (1-42)/Aβ (1-40) peptides, although in these studies only the glycosylated form of oleuropein was considered. 80 81, 82 More recently, this interaction has been assessed by long-time molecular dynamics simulation revealing that OA is able to target a key motif in Aβ peptide known to be relevant for stabilizing the assembled fibrils and penetrate within the fibril structure. By this mechanism OA would induce a structural instability of preformed Aβ fibrils, determining the effective Aβ fibril disaggregation.83

The *in vitro* and *in silico* data are consistent with findings from cellular models of Aβ fibrillization. In particular, a study using SH-SY5Y neuroblastoma cells treated with copper and L-DOPA to induce toxicity demonstrated that oleuropein (in its

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glycosylated form) markedly suppressed Aβ aggregation.84 The proposed mechanism underlying this inhibitory effect involves fibril disassembly and modulation of aggregation kinetics and Aβ conformational preferences, thereby impeding further aggregation. Aβ toxicity has also been linked to its interaction with cell membranes and interference with signaling pathways.85 In this sense, another study using RA-SH-SY5Y cells reported that Aβ (1-42) aggregates grown in the presence of OA exhibited poor cytotoxicity mainly as a consequence of their inability to bind the cell membrane at the GM1 level.18

Building on initial *in vitro* data, subsequent studies in model organisms have reinforced the notion that OA provides protection against both the formation of amyloid aggregates and their toxicity. The nematode C. elegans has been extensively used as a simplified invertebrate model of AD. The CL2006 transgenic C. elegans strain constitutively expresses cytoplasmic human Aβ3-42 in the body wall muscle cells and exhibits an age-related progressive reduction of muscle-specific motility which is associated to the accumulation of both Aβ3–42 fibrils and oligomers.86 Its consistent, observable phenotype and feasibility for large-scale screening approaches have established this model as a valuable tool in neurodegenerative research.87 To date, only a limited number of studies have examined the effects of OA in this transgenic C. elegans model. The available data suggest that OA supplementation confers neuroprotective effects, as evidenced by a reduction in Aβ plaque accumulation and toxic oligomer formation, attenuation of Aβ-induced locomotor deficits, and a statistically significant extension of lifespan in CL2006 nematodes.88, 89 It is worth noting that the observed effects do not stem from the well-characterized antioxidant properties of this polyphenol, implying that OA directly disrupts Aβ aggregation pathways, circumventing the generation of neurotoxic species as already shown *in vitro* for Aβ (1-42).88 In line with this, a more recent study reported that OLEs delayed amyloidogenic toxicity in the temperature-sensitive C. elegans CL4176 strain, which expresses human amyloid β1-42 peptide in muscle cells, causing progressive paralysis. This effect was accompanied by a dose-dependent reduction of Aβ aggregates, that can be attributed to the high content of oleuropein in the tested OLEs.90

In the context of AD rodent models, Grossi et al. reported the neuroprotective effects of OA in CRND8 mice, a transgenic model of Aβ deposition. In their study, they examined the effects of dietary supplementation with OA (50 mg/kg of diet) for 8 weeks in young and aged mice. OA treatment notably counteracted the neurotoxic effects of Aβ and Aβ-induced cognitive impairment. This effect was accompanied by a reduction in Aβ plaque load (size and density of the aggregates) due to enhanced autophagy, which restored the lysosomal system and activated microglia to migrate to Aβ deposits for plaque disassembly.91 These findings suggest that OA, besides interfering with de novo amyloid deposition, favors preformed plaque disassembly *in vivo*. Similar results were obtained in more recent studies using different mouse AD models and OLEs supplementation (containing both oleuropein and OA). In particular, 3 to 4-month OLE administration significantly reduced the Aβ plaques in APPswe/PS1dE9 and 5xFAD mice compared to control groups, suggesting that oleuropein/OA can cross the BBB and inhibit the production of Aβ fibrils also disrupting preformed fibrils.84, 92 In 5xFAD mice, the oleuropein-rich OLE-enriched diet also enhanced synaptic markers and improved memory performance, indicating its potential to prevent or slow AD progression.92 In addition to studies in mice, when the nucleus basalis magnocellularis (NBM) of adult male Wistar rats was injected with Aβ (1-42) oligomers, differences were observed depending on the presence of OA. Specifically, the NBM injected with Aβ (1-42) aggregated in the presence of OA showed a marked reduction in soluble amyloid oligomers compared to those injected with Aβ (1-42) alone. This suggests that OA effectively inhibits the formation of toxic Aβ species. Furthermore, Aβ (1-42) aggregates formed in the presence of OA did not cause toxicity to cholinergic neurons in the NBM and unlike Aβ aggregated alone, they also failed to trigger an inflammatory response.93

Taken together, these findings support the conclusion that oleuropein, and particularly OA, not only stabilizes monomeric amyloid proteins and prevents fibril maturation and seeding activity, but is also capable of disrupting preformed fibrils.

In parallel to Aβ accumulation, tau fibrillization is recognized as a key contributor to the formation and deposition of insoluble aggregates in the AD brain, leading to intraneuronal and glial pathology. An *in vitro* analysis revealed that OA at 10 µM reduced tau protein aggregation by 84%, while the reduction was of 67% with oleuropein at its glycosylated form.94 The proposed mechanistic pathway of OA in the prevention of tau fibrillization seems to be derived from a 3,4-dihydroxyphenyl moiety, also found on other polyphenols previously shown to inhibit Aβ fibril formation.95 These results are supported by a recent study that used computational modeling and classical MD simulations to analyze the interaction of OA with the small nucleating segment PHF6 (paired helical filaments), responsible for tau aggregation. They found that PHF6 monomers collapse in water to form β-sheet rich structures and OA is able to significantly prevent peptide aggregation.96

The ability of oleuropein to modulate tau aggregation was also examined in the BR5706 C. elegans strain, which expresses aggregation-prone human tau throughout the nervous system and exhibits characteristic features of tauopathy, including tau deposits and motor deficits. Overall, worms treated with OLE enriched in oleuropein showed better locomotive parameters, with a higher speed and wavelength and a lower stretching effort compared with control groups, suggesting that oleuropein was able to ameliorate proteotoxicity related to tau aggregation.89, 90

3.2. HT

HT, a strong antioxidant, is the polyphenol moiety resulting from enzymatic hydrolysis of OA and its glycoside in the mature drupe or in the stomach.53 This polyphenol has been detected in the

brains of OA-fed CRND8 mice, a model of Aβ (1-42) deposition, where it exerts protective effects comparable to those of the whole OA molecule.97, 98

Through various *in vitro* analyses, Leri et al. demonstrated that HT influences the aggregation pathway of Aβ (1-42) via a mechanism distinct from that of OA, promoting the rapid formation of ThT-negative, SDS-soluble, non-toxic fibrils with antiparallel β-sheet conformation. Although the precise mechanisms are not ascertained, the authors suggest that this different action could be due to its increased hydrophilic character compared to OA and the ability to form π-stacking interactions with the central region of Aβ (1-42) peptides leading to more efficient intermolecular interactions and aggregation. HT could also promote the off-pathway aggregation of Aβ (1-42) via alternative hydrogen bonding facilitated by the presence of several hydroxyl groups.18 Romanucci et al. also reported an inhibitory effect of HT on Aβ (1-40) aggregation *in vitro* and went further by performing molecular dynamics simulations to shed light on the molecular details of the interaction between Aβ (1-40) and HT. Their results showed that HT has a higher probability of interacting with the amino acid portion 16–19 of Aβ (1-40), which is known to be responsible for amyloidogenic aggregation. Furthermore, the hydroxyl group at the C-3 position of HT appears to play a pivotal role in stabilizing its interaction with Aβ (1-40). It likely acts as an initial recruitment motif by forming multiple hydrogen bonds with GLU22, which may facilitate subsequent π-stacking interactions with PHE19 and PHE20. Ultimately, the stronger binding affinity between HT and Aβ (1-40) is proposed to inhibit the amyloidogenic motif by stiffening the 16–19 region, thereby preventing aggregation along the on-pathway route.99

Although OA and HT appear to modulate Aβ aggregation through distinct mechanisms, their effects on Aβ (1-42)-induced cytotoxicity in RA-SH-SY5Y cells are remarkably similar. This similarity may arise from a shared ability to prevent Aβ (1-42) binding to the cell membrane at the monosialotetrahexosylganglioside (GM1) ganglioside level.18

Regarding *in vivo* studies, HT effects on Aβ (1-42) aggregation have been assessed also in *C. elegans* models of AD in two different studies. In this sense, HT treatment in the strain CL2331 induced a decrease in amyloid plaques by 43%. HT neuroprotection in this study was corroborated by using the transgenic strain CL2355 that expresses the human Aβ peptide in the neurons, showing a chemotaxis improvement by 240% when the neuron-impaired animals were treated with 1 mM HT.100 Similarly, Romero-Marquez et al. reported a delay in Aβ-induced paralysis related with a lower presence of Aβ aggregates in CL4176 nematodes after a HT-enriched extract treatment.46

In a study involving four-month-old TgCRND8 and wild-type mice, animals were administered a low-fat diet (5%) supplemented with HT at a dose of 50 mg/kg of diet over an 8-week period. HT supplementation led to a significant improvement in cognitive performance in TgCRND8 mice. Moreover, a marked reduction in both the area and number of Aβ (1-42) and pyroglutamate-modified Aβ (pE3-Aβ) plaques was observed in the cortex and in the hippocampus of HT-treated TgCRND8 mice.98

While direct evidence on HT's influence on tau aggregation is still limited, Daccache et al. (2011) demonstrated that HT inhibits the *in vitro* aggregation of the P301L tau mutant, which aggregates more rapidly than the wild-type protein and thus serves as a useful model for early aggregation studies.94

3.3. OC

OC, an amphipathic molecule structurally related to oleuropein and sourced from EVOO, has been proposed to possess neuroprotective effects, potentially through interactions with Aβ and subsequent modulation of oligomeric conformations or activity. An *in vitro* study using primary hippocampal neuron cultures investigated the ability of OC to alter the structure of Aβ-derived diffusible ligands (ADDLs), which are considered the toxic species implicated in Alzheimer's disease pathology. The results demonstrated that OC treatment led to structural modifications of Aβ oligomers, increased immunoreactivity and reduced binding and synaptic toxicity.101

Most studies investigating the effects of OC in the context of AD have focused on its role in tau aggregation. OC inhibits tau fibrillization by stabilizing the protein in its naturally unfolded state. Using the PHF6 peptide segment (VQIVYK), a hexapeptide within the third repeat domain of tau critical for fibril formation, it has been shown that OC forms an adduct with lysine residues via initial Schiff base formation. Structural and functional analyses indicate that both aldehyde groups of OC are essential for this inhibitory activity.101, 102 In a separate study, the interaction between OC and full-length wild-type tau (tau-441) was examined using a combination of circular dichroism, surface plasmon resonance, fluorescence spectroscopy and MS. The results revealed that OC induces stable conformational changes in tau's secondary structure, thereby interfering with its aggregation.103

In animal models, a growing body of evidence from transgenic AD mouse models demonstrates that OC exerts both direct and indirect anti-amyloidogenic effects. Multiple studies have demonstrated that OC reduces cerebral Aβ burden *in vivo*. A recent investigation using 5xFAD mice reported that a low dose of OC (0.5 mg/kg) significantly reduced Aβ levels following a 3-month dietary intervention. This reduction was attributed to OC's suppression of neuroinflammatory signaling, particularly through inhibition of the NF-κB pathway and the NLRP3 inflammasome, both of which are known to exacerbate Aβ aggregation and deposition.104 Expanding on these findings, Tajmim et al. evaluated two novel OC formulations: OC powder and an erythritol-based solid dispersion in 5xFAD female mice. Both formulations significantly attenuated Aβ plaque deposition in the brain and concurrently reduced tau phosphorylation. Importantly, these effects were accompanied by improvements in behavioral outcomes, suggesting that the mitigation of Aβ pathology had functional relevance. The study highlights the

importance of formulation strategies in enhancing OC's bioavailability and therapeutic efficacy.68 A similar study investigated the effects of OC-rich EVOO in combination with donepezil, a standard cholinesterase inhibitor used in AD treatment. The co-treatment led to a pronounced reduction in Aβ load and associated pathological features, although this effect was linked to enhanced Aβ clearance through upregulation of BBB transport systems and increased enzymatic degradation, alongside a shift in APP processing toward the non-amyloidogenic pathway.105

In this sense, several studies have attributed OC's influence on Aβ dynamics to enhancement of cerebral clearance mechanisms. Qosa et al. demonstrated that four weeks of OC administration in TgSwDI mice led to a significant reduction in amyloid deposits within both the hippocampal parenchyma and cerebral microvasculature. These findings were ascribed to improved Aβ efflux across the BBB, a critical pathway that is often impaired in AD and contributes to Aβ accumulation.106 Complementing these results, Al Rihani et al. examined the impact of long-term consumption of OC-rich EVOO in TgSwDI mice at an advanced disease stage. Treatment resulted in decreased vascular and parenchymal Aβ deposition, which was associated with restoration of BBB integrity. Additionally, the study identified activation of the AMPK/ULK1 autophagy pathway and inhibition of NLRP3-mediated inflammation as key mechanisms underlying the reduction in Aβ burden, suggesting that OC not only promotes extracellular clearance but also enhances intracellular degradation of Aβ aggregates.107 Interestingly, in TgSwDI mice, an EVOO-enriched diet (with OC as the main polyphenol) reduced Aβ and tau levels and improved cognition when administered early, but delayed treatment only reduced Aβ, with no effect on tau or cognition, indicating limited efficacy once tau pathology is established.108

Collectively, these findings provide compelling preclinical evidence that OC and OC-rich EVOO reduce Aβ aggregation and deposition through a multifaceted mechanism of action. OC inhibits amyloid plaque formation directly and indirectly by suppressing neuroinflammatory pathways, enhancing clearance of Aβ across the BBB, promoting intracellular autophagy-mediated degradation and modulating APP processing to reduce Aβ production. The convergence of these mechanisms reinforces OC's potential as a disease-modifying agent in AD, particularly in targeting early and advanced amyloid pathology.

4. Olive polyphenols as modulators of protein aggregation in PD.

PD is a chronic, age-related neurodegenerative disorder and the second most prevalent neurodegenerative disease after AD.109 The hallmark pathology of PD involves the accumulation of Lewy bodies and Lewy neurites, neuronal inclusions composed predominantly of amyloid fibrils formed by α-syn, a protein involved at multiple levels in the development of PD and related neurodegenerative disorders.110, 111 α-syn progressive aggregation into amyloid fibrils with age is considered a key pathogenic event and autosomal dominant forms of PD have been attributed to SNCA gene mutations and multiplications.19 Despite extensive research, the precise mechanisms of α-syn misfolding and aggregation as well as the specific role of these fibrils in PD pathogenesis remain unclear.

α-syn is a small protein (~14.4 kDa) composed of three distinct structural domains. The highly conserved N-terminal domain (residues 1-60) contains repeats of an 11-residue sequence (KTKEGV) which facilitates α-helical folding upon membrane binding.112 The central hydrophobic region (residues 61-95), known as the non-Aβ component of amyloid plaques (NAC), is responsible for the protein's amyloidogenic properties.113 The C-terminal domain (residues 96-140) forms a highly acidic and hydrophilic tail that may mediate protein interactions. Although α-syn is natively unfolded in the cytoplasm 114, it demonstrates structural plasticity depending on its environment 115 especially by forming α-helices in the presence of lipid-rich environments such as micelles, vesicles and membranes 116 117, 118, suggesting it may serve different functions depending on its cellular localization.119 This structural adaptability is believed to be functionally important 120, with evidence suggesting roles in regulating presynaptic vesicle dynamics, facilitating SNARE-complex formation and modulating dopaminergic signaling.121, 122

α-syn aggregation *in vitro* follows a nucleation-dependent pathway involving a lag phase, subsequent fibril elongation and a final steady state. This process is sensitive to the protein's variant (wild-type or mutant) as well as environmental influences such as pH, temperature, metal ions and toxins like pesticides.123 Although in Lewy bodies and Lewy neurites α-syn is found mainly as insoluble fibrils enriched in cross-β-sheet architecture 111, current evidence points to soluble oligomeric α-syn species as the primary mediators of neurotoxicity, disrupting cellular homeostasis through interactions with synaptic and other intracellular targets.124 Among the potentially pathogenic species, small oligomers, ranging from dimers to hexamers (20-100 kDa), are considered particularly neurotoxic.125 Given the identification of structurally and chemically diverse oligomer species in pathological tissues, understanding the structural determinants of toxic oligomers is crucial to elucidating α-syn-driven neurodegenerative processes.126 Therapeutic approaches under investigation include inhibiting fibril formation and stabilizing oligomers in non-toxic conformational states.122 Some of the natural phenolic compounds reviewed here can inhibit α-syn misfolding and aggregation by more than one of these mechanisms as described next (Table 2). Figure 3 illustrates the protein sequence of α-syn and its aggregation pathway. Regions interacting with various olive polyphenols are highlighted in the peptide sequence (Figure 3A) and their effects on specific aggregation steps are shown in Figure 3B.

4.1. Oleuropein and OA

In line with its effects on amyloid aggregation in AD, the primary inhibitory activity of oleuropein on α-syn aggregation is mostly ascribed to its deglycosylated form, OA. In particular, OA has been shown to inhibit α-syn fibrillation *in vitro*, likely through interactions with both monomeric and oligomeric species and to mitigate the toxicity of α-syn amyloid aggregates in SH-SY5Y cells by promoting the formation of harmless off-pathway oligomers. The same study reported that OA modifies the biophysical properties of preformed α-syn assemblies and characterized an oligomeric species of α-syn grown in the presence of OA.19 Interestingly, α-syn aggregates generated in the presence of OA showed diminished binding to GM1-rich membrane regions, possibly reflecting changes in their surface properties. Supporting evidence from another *in vitro* study showed that an OA-rich extract from olive fruit suppressed α-syn fibrillation by promoting the formation of small, low-toxicity oligomers, thereby interrupting the fibrillogenesis pathway.127 Consistent with these results, a recent investigation found that OA, not only diminishes early α-syn aggregate formation in neuroblastoma cells, but also counteracts the aggregation and toxicity of administered preformed fibrils.20

MD simulations have been also used to explore the impact of OA on α-syn's conformational behavior and aggregation tendency. A recent analysis revealed that OA binding stabilizes the NAC and C-terminal regions of α-syn increasing the intramolecular distance and reducing long-range hydrophobic interactions between these regions.128 These interactions have been shown to favor amyloid aggregation.19 Additionally, OA was proposed to interact with the N-terminal domain, rendering it less available for membrane and lipid interactions necessary for toxic aggregate formation. Moreover, binding free energy calculations confirmed a strong affinity between OA and α-syn. Collectively, these findings suggest that OA stabilizes the α-syn monomer structure and promotes the formation of stable, non-toxic aggregates.129 Additionally, a more recent molecular modelling study identifies three potential modes of interaction between OA and the α-synuclein trimer, with the most plausible involving OA insertion into the trimer structure and engagement with specific peptide chains. This interaction, primarily targeting the pre-NAC (residues 47–56) and NAC (residues 61–95) regions, critical for trimer stability and aggregation, suggests a mechanism for trimer destabilization.20 These theoretical insights align with previous experimental evidence indicating OA's ability to disrupt small aggregates.

Despite the *in vitro* evidence, relatively few *in vivo* studies have investigated the effects of OA on α-syn aggregation. Parkinsonian features in C. elegans can be induced either by rotenone exposure or by expressing human α-synuclein, leading to movement deficits.130, 131 In this sense, OA has been shown to effectively decrease α-syn accumulation in the muscle cells of PD model strains OW13 and NL5901 and to restore motor function, as evidenced by improved swim performance.20, 132

In rodent models, oleuropein treatment has been shown to alleviate motor impairments in a rotenone-induced PD mouse model, primarily through modulation of the BDNF/CREB/Akt signaling pathway.133 However, the specific effects of OA on α-syn aggregation have not yet been investigated in these models.

4.2. HT and metabolites

The observation that OA interferes with α-syn amyloid aggregation has led to growing interest in HT, the key phenolic metabolite of oleuropein, as a possible modulator of α-syn pathology.

The interaction between α-syn and HT have been investigated using a range of *in vitro* assays, including ThT fluorescence, transmission electron microscopy (TEM), electrophoresis and MTT cytotoxicity testing. These analyses demonstrate that HT exhibits a potent inhibitory effect on α-syn aggregation and is also capable of destabilizing pre-formed fibrils. Furthermore, HT effectively mitigates α-syn-induced cytotoxicity.134

Palazzi et al. employed a combination of biophysical and cellular approaches to investigate the molecular mechanisms by which HT inhibits α-syn aggregation. Their findings indicate that HT inhibits α-syn aggregation in a dose-dependent manner through both covalent and non-covalent interactions. Importantly, HT does not alter α-syn's intrinsically disordered structure but instead stabilizes specific regions of the protein, thereby preventing fibril formation.79 In cellular assays, HT was shown to reduce the toxicity of α-syn aggregates. Additionally, the interaction of these aggregates with the cell membrane, critical for the prion-like behavior of on-pathway oligomers, was significantly diminished when aggregates were formed in the presence of HT.134

The structural impact of HT on α-syn oligomers and its potential binding mechanism was also explored through MD simulations in a recent study. Secondary structure analysis revealed that HT significantly decreases β-sheet content while increasing the coil regions within the α-syn trimer, indicative of a destabilizing effect. Clustering analysis of representative conformations showed that HT forms hydrogen bonds via its hydroxyl groups with residues in both the N-terminal and NAC regions of the α-syn trimer. These interactions weaken interchain contacts, ultimately leading to oligomer disruption. Binding free energy calculations further confirmed that HT binds favorably to the α-syn trimer and significantly reduces interchain binding affinity, supporting its potential to destabilize and disaggregate α-syn oligomers.135

HT, also known as DOPET, is not only produced through the hydrolysis of oleuropein but also arises as a by-product of dopamine oxidative metabolism. Monoamine oxidase (MAO) catalyses the oxidative-deamination of dopamine, giving rise to the aldehyde metabolite DOPAL (3,4-dihydroxyphenylacetaldehyde), that subsequently is oxidized by

aldehyde dehydrogenase to the corresponding carboxylic acid, 3,4-Dihydroxyphenylacetic acid (DOPAC) or, to a lesser extent, reduced to HT by aldehyde or aldose reductase.136 Several studies have also examined the effect of HT derivatives on α-syn aggregation pathways. A recent study examined the ability of three HT metabolites to inhibit α-syn aggregation and toxicity using *in vitro* assays including ThT, TEM, electrophoresis, MTT, and RT-PCR. Among them, DOPAL showed the strongest effect, fully inhibiting fibril formation at low concentrations and destabilizing preformed fibrils. DOPAL also significantly reduced α-syn-induced neurotoxicity.137 In addition, DOPAC has demonstrated potent inhibitory effects on α-syn fibrillation by stabilizing monomeric forms and facilitating the generation of off-pathway oligomers.138 At the molecular level, the catechol group in HT and its derivative DOPAC has been shown to interact with α-syn through both covalent and noncovalent bonds. Notably, noncovalent interactions play a key role in inhibiting fibril formation by altering the balance between soluble/insoluble and monomeric/oligomeric α-syn species.139 Recent MD simulations suggest that catechols initially bind noncovalently to the N-terminal and NAC regions of α-syn, but may subsequently form covalent bonds due to proximity and chemical reactivity. This transition disrupts long-range electrostatic interactions, enhancing the inhibitory effect of these catechol containing compounds. These findings highlight the importance of preserving noncovalent interactions when designing fibril-inhibiting compounds, as covalent modifications at specific residues can alter α-syn structure and aggregation dynamics.140

In C. elegans models of PD, while HT-acetate, a derivative of HT, has demonstrated stronger anti-aggregation properties than HT in the NL5901 C. elegans PD model HT itself remains effective, as evidenced by its ability to reduce α-syn levels and improve swim performance in OW13 nematodes.141, 142 132

While different studies have investigated the effects of HT in murine models of PD, these have predominantly focused on its modulatory roles in neuroinflammation, oxidative stress and apoptotic pathways, rather than on its potential anti-aggregation properties.143, 144

4.3. TYR

Although the literature TYR is more limited compared to the previous compounds, some studies have investigated its impact on α-syn aggregation. *In vitro* assessments of fibril formation and destabilization suggest that TYR has a minimal inhibitory effect, with HT showing substantially greater efficacy in preventing fibril formation and promoting fibril disassembly.134 142

In C. elegans models of PD, TYR demonstrated efficacy in reducing α-syn inclusions *in vivo* at specific concentrations, which correlated with reduced neurotoxicity and increased longevity. Additionally, TYR delayed the α-syn-dependent progressive loss of dopaminergic neurons.145 Despite these promising findings, the specific molecular mechanisms driving these TYR's protective effects have yet to be elucidated.

While detailed studies on the molecular interaction between TYR and α-syn are currently lacking, insights can be drawn from research in the context of AD. In particular, a structure-activity relationship analysis of three TYR-based ligands, HT, TYR, and HVA, revealed their distinct effects on the self-assembly of Aβ peptides. While TYR and HVA both contain a conserved hydroxyl group at the C4 position, the additional hydroxyl group at C3 of HT was found to be critical for stabilizing ligand-Aβ (1-40) interactions through hydrogen bonding near residue Glu22. 99

5. Therapeutic perspectives and future directions

Given the well-established link between protein misfolding and cytotoxicity in PMDs, targeting amyloidogenic peptides and proteins has emerged as a promising therapeutic strategy. Ongoing efforts in AD and PD research include approaches aimed at reducing amyloidogenic protein expression, enhancing the clearance of misfolded species, stabilizing native conformations and inhibiting the formation of toxic oligomers and fibrils. Although several amyloid-targeting compounds have progressed to clinical trials, most have yielded inconclusive results. Elucidating the fundamental mechanisms of amyloid inhibition, particularly the molecular interactions between inhibitors and their protein targets, will be critical for the rational design of next-generation therapeutics.11

When investigating amyloid proteins like α-syn or Aβ peptides, a primary challenge lies in their intrinsically disordered and highly flexible structures. This inherent variability limits the effectiveness of conventional drug design approaches typically applied to well-folded globular proteins. Consequently, identifying suitable drug candidates is complex and may benefit from an integrated strategy that combines biophysical methods with computational modeling to elucidate viable molecular scaffolds for treating PD and AD.

This review compiles and highlights MD simulation studies conducted to clarify the mechanisms by which olive-derived polyphenols interact with key amyloidogenic proteins implicated in the pathogenic aggregation processes of AD and PD. Longtime MD simulations have emerged as powerful tools for characterizing ligand-protein interactions at the molecular level, allowing for the reconstruction of binding pathways and the observation of slow conformational changes often missed in standard MD.83 These simulations have revealed how compounds such as olive polyphenols may exert their fibril-disrupting effects, offering detailed interaction models that support their role as modulators of amyloid aggregation.

Among the compounds investigated, OA and HT have emerged as the most extensively studied and effective inhibitors of toxic aggregation, acting at multiple stages of the aggregation

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process. Understanding their molecular mechanisms of action provides a foundation for identifying key structural determinants critical for amyloid-polyphenol interactions. These pharmacophores are now being used to guide the search for novel compounds with enhanced affinity for amyloidogenic targets such as α-syn and Aβ.

5.1. Translational and clinical evidence: towards human application

Research consistently supports that the MD and MIND diets may protect against dementia, largely due to the beneficial effects of their components on AD-related processes.146 EVOO, in particular, has demonstrated strong neuroprotective effects in experimental models.147-149 Human studies also reveal that individuals with consistent EVOO consumption show markedly less cognitive decline over time, with a 50% lower risk of developing dementia.150 151, 152 Nevertheless, attributing these neuroprotective effects to individual polyphenols remains challenging, as there is a lack of clinical trials in humans evaluating the isolated impact of compounds such as oleuropein or HT. A recent randomized cross-over clinical trial investigated the combination of oleuropein and S-acetyl glutathione for 6 months on cognitive and behavioral functions in patients with mild AD. Notable enhancements were seen in cognitive deterioration, memory, visuospatial abilities, attention, language, executive functions and behavioral disorders, emphasizing the potential efficacy of dietary supplementation with olive polyphenols and bioavailable glutathione in mild AD patients supporting that dietary supplementation with oleuropein and S-acetyl glutathione can significantly improve cognitive and behavioral functions in mild AD patients.153

Regarding the influence of individual variability on the effectiveness of olive polyphenols, such as HT and oleuropein, it has been shown that their biological activity is strongly modulated by inter-individual factors. Thus, genetic polymorphisms in metabolic enzymes (e.g. CYP2A6, CYP2D6, COMT) could influence biotransformation and circulating levels of active metabolites, while gut microbiota composition determines conversion of precursors and generates additional bioactive compounds.67, 154-157 The dietary matrix also affects absorption, with fat-rich carriers like EVOO enhancing bioavailability compared to aqueous extracts. 49 Also, lifestyle and metabolic status, including smoking, obesity, insulin resistance, age and sex, could modify both metabolism and the biological response, contributing to the substantial heterogeneity observed in clinical outcomes.158 Together, these factors suggest that the health benefits of olive polyphenols are context-dependent and highlight the need for personalized approaches in nutraceutical applications, as well as further genotype- and microbiome-stratified clinical trials. In this context, it is also worth noting the lack of studies investigating interventions in advanced disease stages, particularly in PD models with established α-syn pathology. Most available research focuses on early or preventive settings, which poorly reflect the clinical reality of diagnosis after significant neurodegeneration. Moreover, reports of null or negative findings remain limited, likely due to underreporting or publication bias, thereby hindering a balanced understanding of therapeutic limitations.159

Future evaluation of olive polyphenols should rely on randomized, double-blind, placebo-controlled trials specifically designed to detect disease-modifying effects. A 24-month study in early AD or PD patients could test a standardized extract containing defined doses of oleuropein, HT and OC against placebo. Primary outcomes would include validated measures of cognitive or motor progression, while secondary endpoints should assess biomarkers of neurodegeneration (CSF/plasma Aβ, tau, α-syn, neurofilament light, MRI or dopaminergic PET) and functional outcomes. Safety monitoring should address known systemic effects such as hypotension, hypoglycemia and bleeding risk. Exploratory analyses of inflammation, oxidative stress, metabolism and microbiome profiles could clarify mechanisms of action. Such a design would isolate polyphenol-specific effects from broader dietary influences, provide robust safety data at pharmacological doses and determine whether these compounds can slow disease progression rather than merely provide symptomatic relief.

5.2. Limitations and off-target effects in amyloid inhibition

Although preclinical studies suggest that olive polyphenols such as oleuropein, HT and OC can interfere with aggregation of amyloidogenic proteins implicated in AD and PD, their therapeutic development is limited by potential off-target effects arising from their pleiotropic bioactivity. These compounds can interact with hydrophobic protein regions, raising the risk of disrupting physiological oligomers essential for cellular functions, and their modulation of proteostasis pathways (autophagy, proteasome) may cause unintended proteotoxic stress.160 OC also displays COX inhibitory activity similar to Nonsteroidal anti-inflammatory drugs (NSAIDs)161, while other polyphenols affect kinases and cytochrome P450 enzymes, potentially leading to drug-drug interactions and organ-specific side effects. Their antiplatelet activity further raises bleeding concerns in patients on anticoagulant therapy.162 At higher doses, polyphenols may act as pro-oxidants or interfere with essential metalloproteins through metal chelation.51, 163 Additional risks include effects on BBB permeability, drug transporters and microbiome-drug interactions, as well as non-specific plasma protein binding that could alter pharmacokinetics, issues particularly relevant for elderly AD/PD patients on polypharmacy.164

Regarding availability, a key limitation of *in vitro* studies with olive polyphenols is that they frequently use concentrations in the µmol/L-mmol/L range, whereas *in vivo* plasma levels after dietary intake are typically in the low nmol/L range. To bridge this

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discrepancy and enhance physiological relevance, various delivery strategies have been explored to improve the bioavailability and bio accessibility of these compounds. Approaches include esterification or lipophilisation, as well as encapsulation techniques employing liposomes, nanoparticles or other carrier systems designed to protect polyphenols from degradation and facilitate their absorption in humans.165, 166

Preclinical models are invaluable for studying amyloid aggregation and testing olive polyphenols, but important limitations affect their translational relevance. *C. elegans* provides genetic simplicity yet differs greatly from humans in metabolism and physiology, while rodent models, though closer, still show differences in BBB function, polyphenol metabolism, and gut microbiota.167, 168 Amyloid pathology in transgenic mice also progresses more rapidly and uniformly than in humans and animal cognitive tests only partly capture human decline.169 These species-specific differences may overestimate efficacy, underscoring the need for cautious interpretation and human validation.

Taken together, these considerations highlight the double-edged nature of polyphenol promiscuity: while broad molecular interactions may exert their neuroprotective potential, they also create multiple avenues for off-target toxicity or drug interactions. Current clinical data, largely derived from short- to medium-term dietary or supplement studies, support good tolerability at nutritional levels, but rigorous, long-term studies are lacking for pharmacological doses aimed at modifying amyloid pathology. A systematic evaluation of these limitations, including dedicated toxicological profiling, selectivity assays and carefully monitored clinical trials, will be essential before olive polyphenols can be advanced as disease-modifying therapeutics in neurodegenerative disorders.

Conclusions

A growing body of evidence supports the therapeutic potential of olive-derived polyphenols, particularly OA, HT and OC, as modulators of pathogenic protein aggregation in AD and PD. In AD models, OA and HT inhibit Aβ and tau fibrillization, stabilize soluble monomeric species, and reduce the seeding potential of aggregates. These compounds also attenuate membrane interactions and neurotoxicity, with *in vivo* studies showing preserved cognitive function and reduced plaque burden. OC complements these actions by enhancing autophagic clearance, modulating APP processing and reducing neuroinflammatory responses that exacerbate aggregation. In PD, both OA and HT disrupt α-syn oligomerization and fibril formation by stabilizing non-toxic conformers and altering interdomain contacts within the protein. Mechanistic insights from MD simulations and structural studies reveal specific interaction motifs, such as aromatic and hydrophilic residues within amyloidogenic cores, targeted by these polyphenols. These interactions underpin their ability to modulate early nucleation events, redirect aggregation pathways and destabilize mature fibrils across both diseases. Taken together, olive polyphenols act as multifunctional modulators of protein aggregation in AD and PD, targeting both shared and disease-specific mechanisms. Their low toxicity, dietary accessibility and capacity to act on both Aβ/tau and α-syn make them attractive candidates for disease-modifying interventions, especially useful when given early, suggesting value as preventive strategies in at-risk groups (e.g., genetic predisposition, mild cognitive impairment, prodromal PD). With a favorable safety profile and presence in EVOO-rich diets such as MD and MIND, they offer a low-risk option to delay amyloid-related neurodegeneration. However, clinical validation remains a key challenge. Future research should prioritize pharmacokinetic optimization, structure-activity analyses and translational studies to fully harness their therapeutic potential in age-related neurodegenerative diseases.

Conflicts of interest

There are no conflicts to declare

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Table 1. Modulation of Protein Aggregation by Olive Polyphenols in AD

Compound	Activity	Mechanism	Model	References
OA	Inhibition of the formation of Aβ toxic oligomers and growth into mature fibrils *in vitro*	Binding to the N-terminus of the monomeric and/or oligomeric peptide. Reducing the exposure of the hydrophobic regions in Aβ (1-42)	*In vitro* assays	18 78
	Induction of preformed Aβ fibrils disaggregation	Targeting 17-28 hydrophobic α helix region of Aβ peptide (relevant for stabilizing assembled fibrils)	Long-time molecular dynamics	83
	Reduction of Aβ oligomers binding to the membrane and cytotoxicity *in vitro*	Preventing Aβ (1-42) binding to the cell membrane at the GM1 ganglioside level	RA-SH-SY5Y cells	18
	Reduction in Aβ plaque accumulation and toxic oligomer formation *in vivo* Attenuation of Aβ-induced locomotor deficits	Antioxidant-independent mechanism	*C. elegans* (CL2006) (CL4176)	88 46, 90
	Reduction in Aβ plaque load (size and density) and disruption of preformed fibrils *in vivo*. Reduction of Aβ toxic species	Enhancing autophagy and activating microglia to migrate to Aβ deposits for plaque disassembly Reducing the amount of soluble amyloid oligomers	Mice: CRND8 APPswe/PS1d E9 5xFAD Wistar rats injected with aggregated Aβ (1-42)	91 84, 92 93
	Reduction of tau protein aggregation	Interacting with the small nucleating segment PHF6	*In vitro* assays *In silico*: MD simulations	94, 96
	Amelioration of proteotoxicity related to tau aggregation *in vivo*		*C. elegans* (BR5706)	89, 90.
HT	Promotion of off-pathway non-toxic fibrils with antiparallel β-sheet conformation *in vitro*	Interacting with the central region of Aβ (1-42) peptides (16-19) via alternative hydrogen bonding facilitated by hydroxyl groups	*In vitro* assays *In silico:* MD simulations	18 99
	Reduction of Aβ oligomers binding to the membrane and derived cytotoxicity *in vitro*	Preventing Aβ1–42 binding to the cell membrane at the GM1 ganglioside level	RA-SH-SY5Y cells	18

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	Reduction in Aβ plaque accumulation *in vivo* Neuroprotection and attenuation of Aβ-induced locomotor deficits		*C. elegans* (CL2331) (CL2355) (CL4176)	46, 100.
	Reduction in Aβ (1-42) and pyroglutamate-modified Aβ (pE3-Aβ) plaques in the cortex and hippocampus		Mice: TgCRND8	98
	Reduction of tau aggregation *in vitro*		*In vitro* assays P301L tau mutant	94
OC	Induction of structural modifications of Aβ oligomers in cell cultures		*In vitro*: Primary hippocampal cell cultures	101
	Inhibition of tau fibrillization *in vitro*	Stabilizing the protein in its unfolded state by forming an adduct with lysine residues in PHF6 region Inducing stable conformational changes in tau's secondary structure	*In vitro* assays	102 103
	Reduction of Aβ plaque load in brain	Aβ clearance through upregulation of BBB transport systems Inducing shift in APP processing	Mice: 5xFAD TgSwDI	68 105 106 107

Table 1. Summary of phenolic compounds reported to modulate amyloid protein aggregation associated with AD, particularly Aβ and tau. The table includes the mechanisms of action by which each compound interferes with protein misfolding and aggregation pathways, as well as the experimental systems used to evaluate these effects. Many compounds act at multiple stages of aggregation and via more than one mechanism. (AD: Alzheimer´s disease; OA: Oleuropein aglycone; HT: Hydroxytyrosol; OC: Oleocanthal; Aβ: Amyloid-β; MD: Molecular dynamics; PHFs: Paired helical filaments; GM1: Monosialotetrahexosylganglioside 1; BBB: Blood Brain Barrier).

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Table 2. Modulation of Protein Aggregation by Olive Polyphenols in PD

Compound	Activity	Mechanism	Model	References
OA	- Inhibition of α-syn fibrillation *in vitro and* promotion of harmless off-pathway oligomers in cell cultures. -Interruption of α-syn fibrillation *in vitro*. -Inhibition of aggregation and toxicity of administered preformed fibrils in cell cultures.	-Interacting with monomeric and oligomeric species. -Promoting the formation of small, low-toxicity oligomers.	-*In vitro* assays -SH-SY5Y cells	19 127 20
	-Prevention of long-range and hydrophobic interactions that favor amyloid aggregation -Stabilization of the α-syn monomer structure and promoting non-toxic aggregates. - α-syn trimer destabilization	-Stabilizing the NAC and C-terminal regions of α-syn.	MD simulations	128 129 20
	- Decrease α-syn accumulation and restoring of motor function *in vivo*		*C. elegans:* (OW13) (NL5901)	132 20
HT	-Inhibition of α-syn aggregation destabilization of pre-formed fibrils *in vitro.* -Inhibition of α-syn-induced cytotoxicity.	-Reducing interaction of aggregates with the cell membrane. -Stabilizing specific regions of the protein	*In vitro* assays	134 79
	-Destabilization and disruption of α-syn oligomers	-Forming hydrogen bonds with residues in the N-terminal and NAC regions of the α-syn trimer. - Disrupting long-range electrostatic interactions by catechol groups.	MD simulations	135 139
	-Decrease of α-syn levels and improvement of swim performance *in vivo*.		*C. elegans* (OW13)	141 132
TYR	- Mild inhibition of fibril formation *in vitro*		*In vitro* assays	134 142

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	-Reduction of α-syn inclusions and neurotoxicity *in vivo*. -Delay of α-syn-dependent loss of dopaminergic neurons		*C. elegans* (NL5901) (UA44)	145

Table 2. This table summarizes the reviewed phenolic compounds that have been reported to have an effect on α-syn aggregation, the mechanisms of action by which each polyphenol is able to modulate α-syn´s misfolding/aggregation and the experimental system used to assess these effects. In most of the cases one single polyphenol interferes with several aggregation steps and by more than one mechanism. (PD: Parkinson´s disease; OA: Oleuropein aglycone; HT: Hydroxytyrosol; TYR: Tyrosol; α-syn: α-synuclein; MD: Molecular dynamics).

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Figure 1. Chemical structure of olive phenolic compounds able to inhibit amyloid misfolding and aggregation. A) Oleuropein, Oleuropein aglycone Oleocanthal, Hydroxytyrosol, Tyrosol. B) Relative composition of the above polyphenols reported in EVOO and olive leaves. Concentration ranges and specific values shown in the graphs are based on data from [170] and [171]. Created in BioRender. Cañuelo, A. (2025) https://BioRender.com/lcsqe82

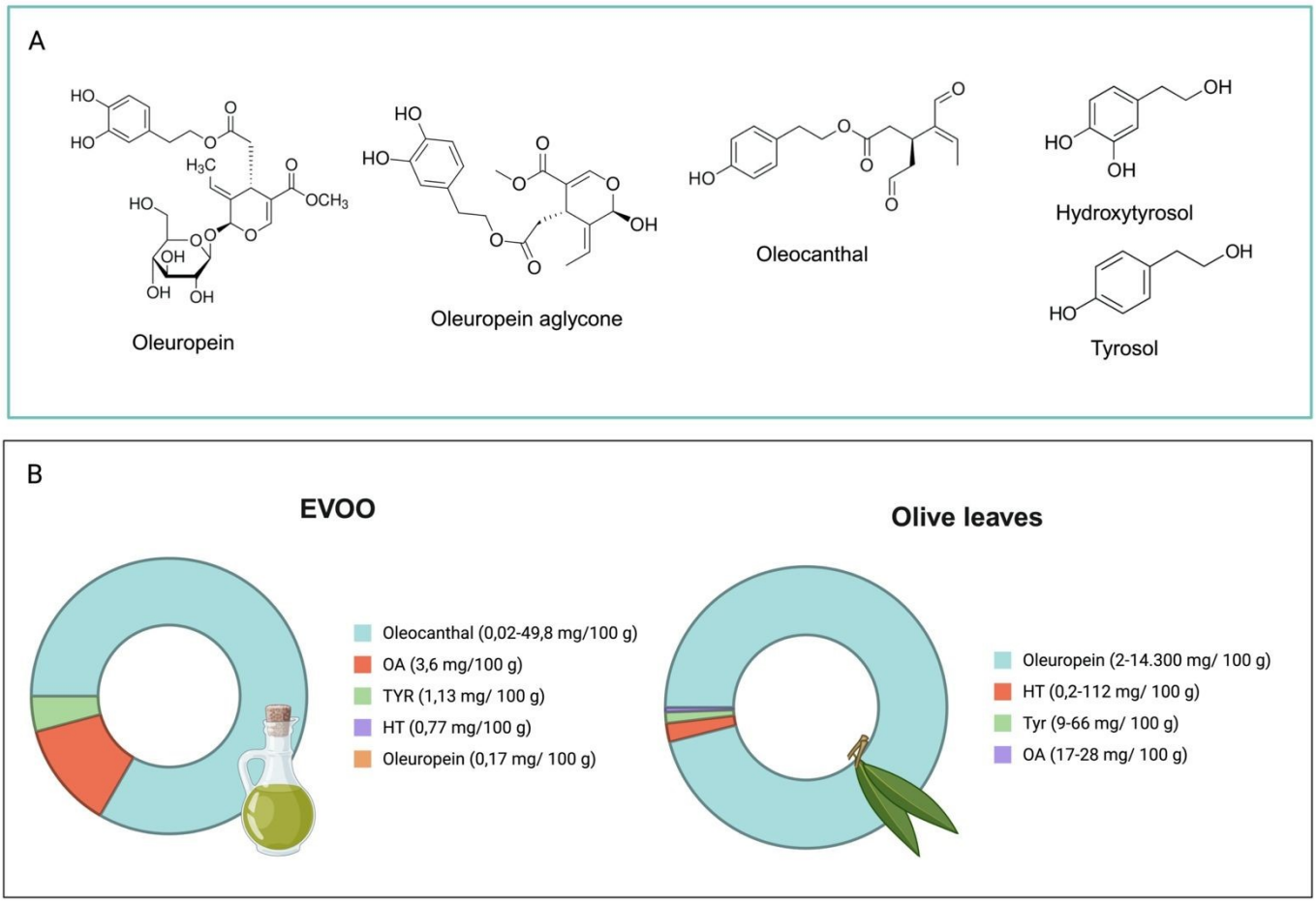

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Figure 2. Olive polyphenols modulatory effects on amyloid aggregation processes implicated in AD. Schematic illustration of the primary aggregation pathways of amyloid-β (Aβ) and tau proteins. Aβ species can exist as monomers, dimers, oligomers, protofibrils, fibrils and ultimately as insoluble amyloid plaques. These forms are in dynamic equilibrium, allowing bidirectional conversion between species depending on conditions. Aggregates differ in size, conformation, and solubility, with fibrils and plaques representing the most insoluble forms. Key olive polyphenols, Oleuropein Aglycone (OA), Hydroxytyrosol (HT) and Oleocanthal (OC) have demonstrated inhibitory effects on Aβ and tau aggregation. OA can disaggregate preformed Aβ fibrils; HT promotes the formation of non-toxic, off-pathway oligomers and fibrils; OC induces structural changes in Aβ oligomers, reducing their toxicity. Created in BioRender. Cañuelo, A. (2025) https://BioRender.com/6smboyh

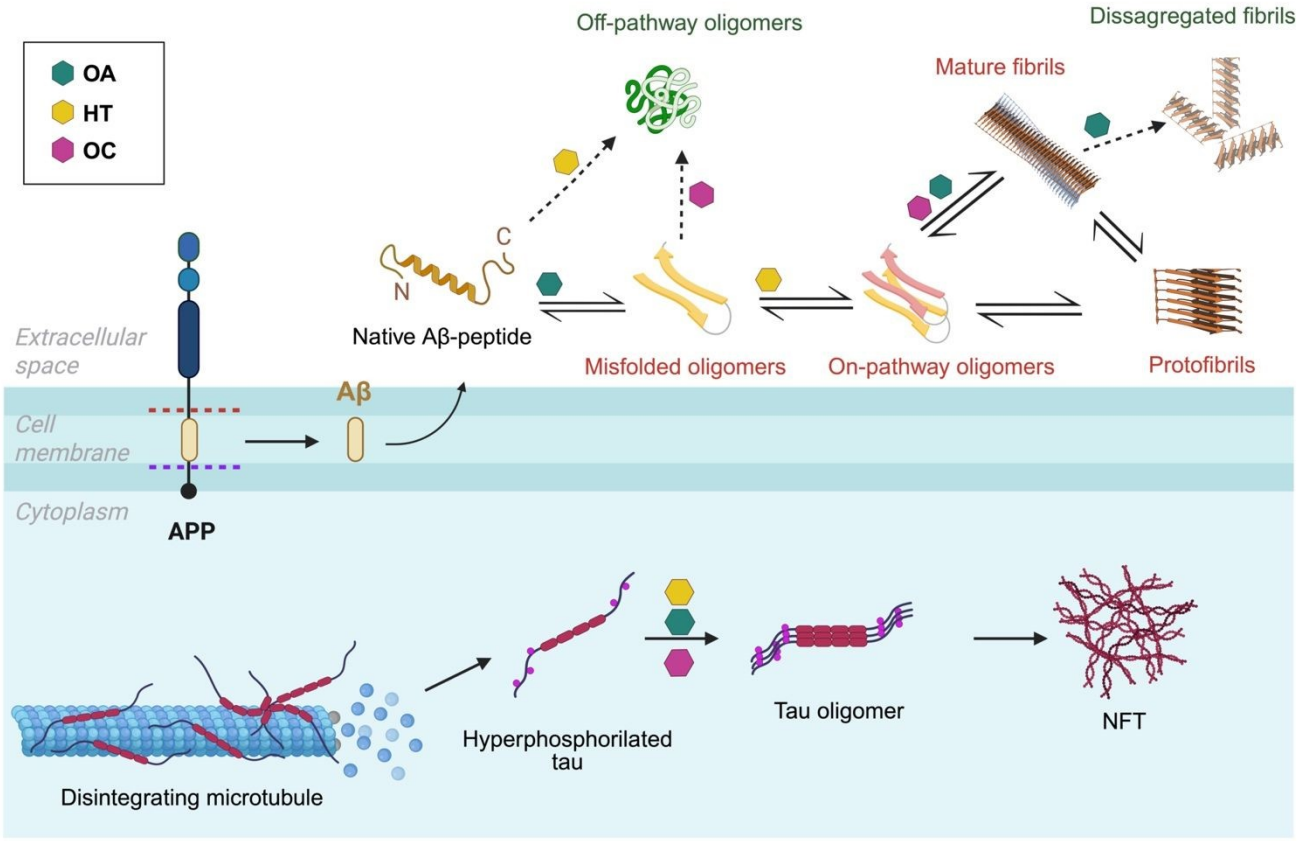

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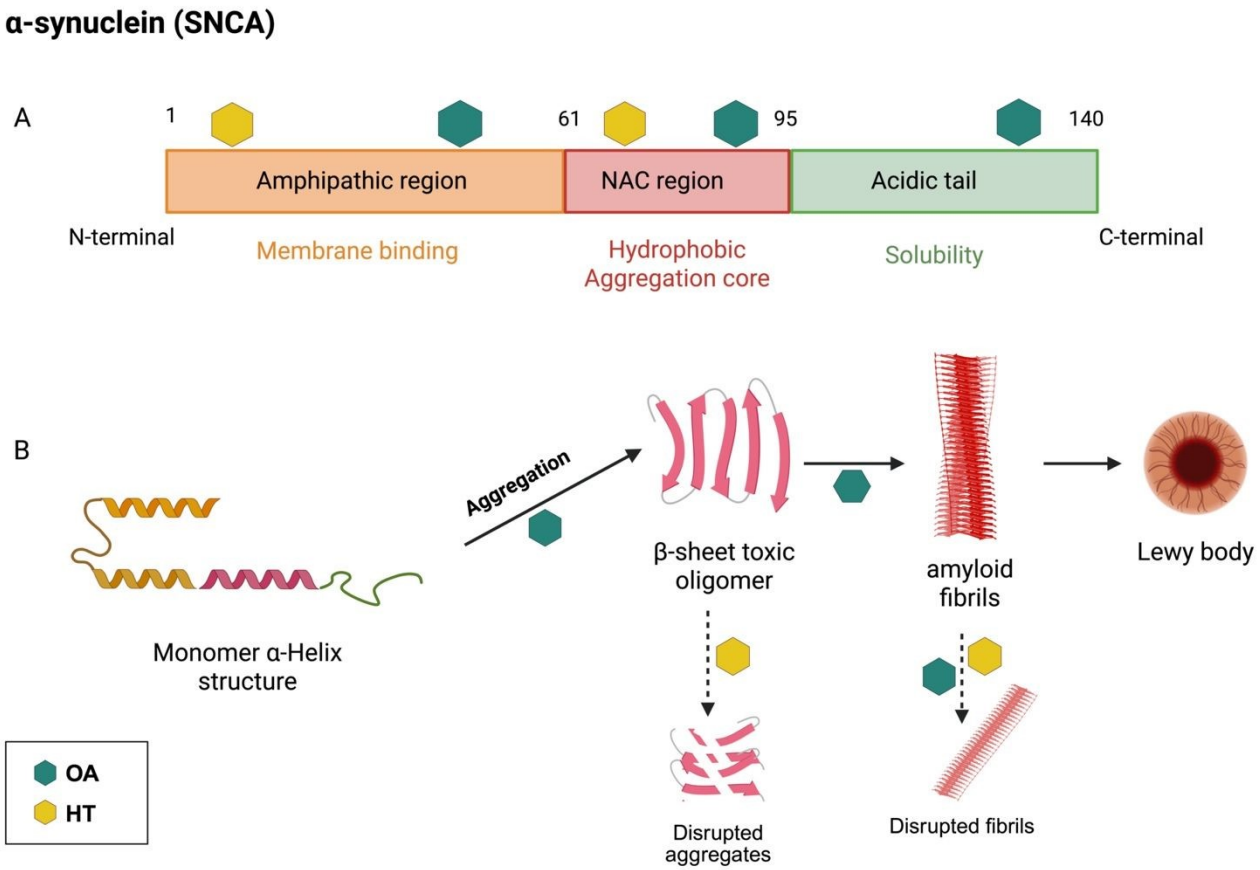
Figure 3. Olive polyphenols modulatory effects on α-syn aggregation processes implicated in PD. A) Domain structure of the human α-Syn protein (SNCA gene). α-Syn consists of three major domains: an N-terminal amphipathic region, a central non-Aβ component (NAC) domain, and a C-terminal acidic region. All known pathogenic mutations in α-Syn are located within the N-terminal region preceding the NAC domain. Amino acid positions are indicated above the schematic. Approximate binding or interacting regions for Oleuropein Aglycone (OA) and Hydroxytyrosol (HT) are highlighted within the peptide sequence. (B) Schematic representation of α-Syn secondary structure and its aggregation pathway. α-Syn aggregation follows a nucleation-dependent polymerization process, beginning with monomeric proteins that self-assemble into β-sheet-rich amyloid fibrils. Mature fibrils serve as a reservoir for toxic oligomers and small fibrillar intermediates. These oligomeric species, primarily generated via secondary nucleation, are considered highly cytotoxic and contribute to cellular dysfunction by disrupting membrane integrity and triggering cell death. OA has been shown to stabilize α-syn monomers, promoting the formation of non-toxic aggregates while also destabilizing α-syn trimers and fibrils. HT contributes to the disruption of α-syn oligomers and disassembly of preformed fibrils. Created in BioRender. Cañuelo, A. (2025) https://BioRender.com/clrihbp



Data availability statement

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No primary research results, software or code have been included and no new data were generated or analysed as part of this review.