



(REVIEW ARTICLE)



The multifunctional therapeutic potentiality of extra virgin olive oil administration through the intervention in pathophysiological mechanisms: Focus on Alzheimer's disease

Tzekaki E^{1,4}, Prodromou S^{3,4}, Tsolaki M^{2,3,4,*} and Pantazaki A A^{1,4,*}

¹ Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Makedonia, Greece.

² Department of Neurology, Medical School, "AHEPA" General Hospital Medical School, Aristotle University of Thessaloniki, Faculty of Health Sciences 54124 Thessaloniki, Makedonia, Greece.

³ Greek Association of Alzheimer's Disease and Related Disorders – GAADR.

⁴ Center for Interdisciplinary Research and Innovation, Laboratory of Neurodegenerative Diseases (LND), Aristotle University of Thessaloniki.

GSC Advanced Research and Reviews, 2021, 07(01), 101–113

Publication history: Received on 13 March 2021; revised on 16 April 2021; accepted on 19 April 2021

Article DOI: <https://doi.org/10.30574/gscarr.2021.7.1.0081>

Abstract

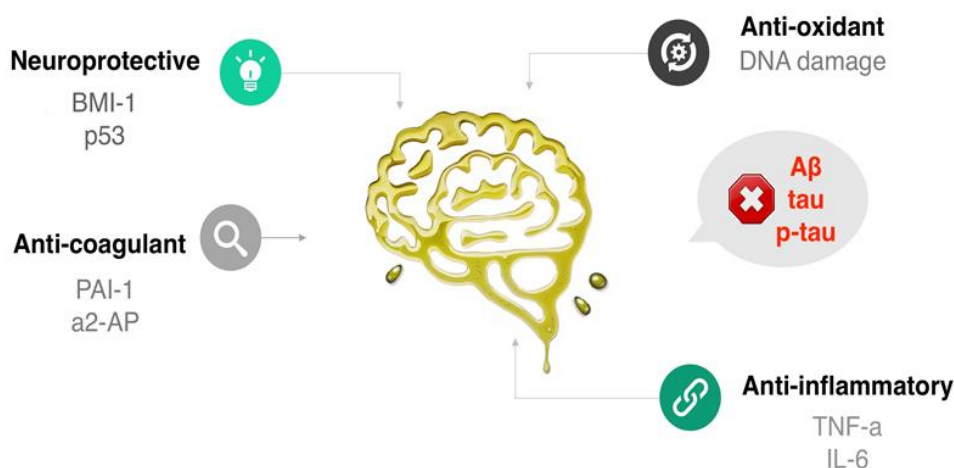
Proper dietary habits pave the way for a good and healthy life in order to maintain and prolong the quality of life. It is well known that quality of life in the elderly can be achieved by non-pharmacological approaches such as performing physical activity, cognitive training, or adhering to a Mediterranean Diet (MedDiet). The MedDiet is suggested as the prevalent dietary regimen and is strongly correlated with prevention of degenerative diseases and longevity. The most distinguished and beneficial ingredient of MedDiet is extra virgin olive oil (EVOO). Indeed, numerous epidemiological studies have proved that the consumption of olive oil was associated with better overall health. The foremost component of EVOO is polyphenolic compounds which are under investigation for its biological and pharma-nutritional properties. In this review we recorded several representative *in vitro* and *in vivo* studies performed in culture cell lines, in animal or clinical trials, indicating that the regular intake of EVOO is associated with enhanced neuroprotective, antioxidant, anti-inflammatory, anti-atherosclerotic, anti-cancer and anti-microbial properties. Furthermore, it is emerged the demand of more randomized controlled or longitudinal observational studies to be performed to confirm the efficacy of the beneficial health effect of EVOO.

Keywords: Extra virgin olive oil; Anticancer properties; Antioxidant properties; Anti-inflammatory properties; Neuroprotective properties

* Corresponding author: Pantazaki A

Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Makedonia, Greece.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.

Graphical abstract**1. Introduction**

The term “Mediterranean Diet” (MedDiet) was introduced for the first time by Ancel Keys in the early 1960s in order to signify an exceptional dietary practice followed by the countries around the Mediterranean basin [1]. The positive effects of the Mediterranean diet were first proposed in the early 1970s [2]. Fifty (50) years later, numerous studies confirmed the initial findings and associated with reduced risk of many diseases such as cardiovascular disease (CVD), stroke, certain types of cancer, diabetes, non-alcoholic liver disease and finally Mild Cognitive Impairment (MCI) and Alzheimer’s disease (AD). Currently, there is not an effective and approved pharmacological agent specific for MCI, and for this reason it is recommended the use of the non-pharmacological personalized interventions (e.g., cognitive stimulation, training, etc.) which are also not approved yet.

2. Intrinsic beneficial EVOO properties due to composition

The composition of EVOO is 98% fatty acids (glycerol tri-esters with fatty acids), among which is oleic acid (55-83%) and 2% other minor ingredients, in lower concentrations such as free fatty acids, phospholipids, polar phenols, tocopherols, pigments, squalene along with other hydrocarbons, tri-terpenic acids and others [1]. Among functional foods, one finds the EVOO, an essential food of the MedDiet in countries such as Spain, Italy, and Greece which represent the most important producers in the world. As documented by numerous studies published in recent decades, most of the beneficial effects of the MedDiet on promoting human health can be attributed to EVOO [2-4].

EVOO is regarded as a functional food since epidemiological studies and multidisciplinary research have reported convincing evidence that its intake beneficially affects one or more targeted functions in the body, improves health, and reduces the risk of disease [4], [6-9]. Its properties on health have been related to the major and minor component fractions of EVOO. The beneficial properties of EVOO have been attributed to its high content of monounsaturated fatty acids (MUFA), which represent up to 80% of its total lipid composition. However, recent evidence has shown that the minor components of EVOO, such as phenolic and other compounds with antioxidant actions, determine an increase in the health characteristics of the oil itself [9, 10].

Among the EVOO chemical components, the phenolic fraction has received considerable attention due to its bioactivity in different chronic diseases. Polar phenolic compounds are a family of compounds found in fruits, vegetables, wine, tea, and in a variety of oils including virgin olive oil. EVOO contain different classes of phenolic compounds such as phenyl alcohols, hydroxytyrosol (HT) and tyrosol, quinic/hydroxycinnamic acids (caffeic and p-coumaric acid) and benzoic acid (vanillic acid), flavones (apigenin and luteolin), secoiridoids oleuropein (OLE), and ligstroside derivatives [11]. This class of compounds is inextricably linked to the stability of oils as they exhibit strong antioxidant activity by binding various reactive oxygen species (ROS). Additionally, apart from the MUFA and the phenolic compounds the nutritional and antioxidant properties of EVOO are related to the presence and content of tocopherols and carotenoids which are of grave importance for human health [12]. Table 1 showed the content in minor components of virgin olive oil and refined one.

Table 1 Content in minor components of extra virgin olive oil and refined one.

Minor components	Extra Virgin Olive Oil (mg/kg)	Refined Extra Virgin Olive Oil (mg/kg)
Hydrocarbons	3800	390
Tocopherols	150	100
Phenols	350	80
Sterols	500	1500
Volatile Esters	100	30
Volatile carbonyl compounds	40	10
Tri-terpene Alcohols	3500	2500
Aliphatic Alcohols	200	100

EVOO contains oleic acid approximately 0.8 grams/100 grams and peroxides less than 20 milli-equivalents of oxygen which are referred as free acidity and they formed by extraction of olive fruits. The production of olive oil must take place by mechanical means without solvents and under 30°C for avoiding the degradation. The MUFA which are contained in EVOO- principal role have oleic acid- are known for their potential to reduce low-density lipoprotein cholesterol (LDL-C) levels. The bioactivity of the phenolic compounds could be related to different properties such as anti-inflammatory and antioxidant ones, although the molecular action mechanism of these compounds in relation to many diseases might have different cellular targets or may be multimodal /or pleotropic [1]. Diet rich in fruits and vegetables contributes to a better life-span, to the longevity and to the diminution of the inflammation and oxidative stress risk associated with chronic diseases (e.g., CVD, arteriosclerosis, cancer, diabetes, cataract, disorders of the cognitive function, and neurological diseases) [13]. The high concentration of EVOO in polyphenols seems to have anti-inflammatory and antioxidant properties [14] and oleocanthal (OC) in particular exerts an anti-inflammatory action similar to ibuprofen [15, 16].

EVOO itself is rich in vitamins D, A, E and helps the absorption of the whole group of fat - soluble vitamins. EVOO, rich in polyphenols, is able to reduce heterocyclic amines and plasma levels of C-reactive protein [17]. Therefore, the positive impact of EVOO on human health could be attributed to a synergistic effect of polyphenolic compounds, the high content of oleic acid, and the content in vitamins D, A, E. The components contained in olive oil are summarized in Fig.1.

**Figure 1** Components contained in olive oil

3. Biological activities of EVOO

The favorable outcomes of EVOO consumption is broadly known and accepted. For this reason, the European Food Safety Authority (EFSA) [Commission Regulation (EU) 432/2012] has adopted two health approvals [18]. First, EVOO is highly recommended to be used to supersede saturated fats for maintenance of normal blood cholesterol levels [5]. Second, EFSA declares that EVOO's antioxidant protection of blood lipids from oxidative stress is due thanks to the polyphenols which are dominant in a human daily intake of 20 g of EVOO. This claim is only referred to EVOO containing at least 5 mg of HT and its derivatives per 20 g of OO [5].

An increasing tendency of meta-analyses results prove a refinement on morbidity and mortality's levels, cardiovascular problems (e.g. stroke, hypertension), neurodegenerative disorders, types of cancer and metabolic diseases such as type II diabetes thanks to olive oil consumption [3,19–21]. One of the greatest public health challenges worldwide is the obesity pandemic. Studies support that the use of virgin olive oil as the sole culinary fat lessened the body mass index but long-term trials are needed for confirmation [3]. Studies focusing on CVD suggest that virgin olive oil reduce blood pressure and also has anti-atherosclerotic potential [3].

The above-mentioned ingredients contained in olive oil exert an anti-oxidant potential against the conglomeration of reactive oxygen and nitrogen species steering to changes in redox signaling and molecular damage [22]. Free radicals formed by an unpaired electron on oxidants or on nitric oxide (NO) exert destructive effects on lipids, DNA molecules, and proteins. Moreover, the excessive oxidant compounds are inextricably linked to the onset of CVD, neurodegenerative diseases, and cancer undoubtedly [23].

Specifically, tau protein which is a microtubule-associated protein that promotes microtubule assembly and stability is fibrillated and aggregated into neurofibrillary tangles in AD and related tauopathies. OC abolished fibrillization of tau by locking tau into the naturally unfolded state [24, 25].

OC has also a non-steroidal anti-inflammatory potential as ibuprofen has [24]. OLE, another dominant constituent of olive oil is proved to have anti-inflammatory activity [26] and antioxidant activity [5]. Studies showed beneficial outcomes in decrease of coronary atherosclerosis and control blood pressure [27] and it was also explored for its antitumor activity [28].

3.1. Experiments in cells culture

EVOO or OLE treatment alone may act as a natural PAI-1 (Plasminogen activator inhibitor 1) inhibitor by incrementally destabilizing PAI-1 protein levels selectively in estrogen receptor (ER) and progesterone receptor (PR) negative [ER-/PR-] breast cancer cells, accompanied by cell growth inhibition. In contrast, ER+/PR+ breast cancer cells where PAI-1 expression is absent or low, do not adequately respond to this treatment [7].

It is proposed that HT, a major polyphenol found in olive oil, completely scavenges free radicals *in vitro* and exerting cytoprotection potential against oxidative stress-induced damage in PC12 cells. HT completely protects the cells from hydrogen peroxide-induced death [29].

Moreover, OLE significantly increased the cleaved PARP [Poly (ADP-Ribose) Polymerase 1] levels in MCF-7 human breast cancer cells in a dose-dependent mechanism, increasing apoptotic cascade by preventing the activation of PARP [30], and further promote the expression of a significant level of cleaved PARP1 after 48 h treatment of melanoma cells with OLE [31]. DNA damage is the primary activator of the enzyme PARP-1 that catalyzes the reaction of poly (ADP-ribosylation), a post-translational modification of proteins involved in many physiological processes, such as gene expression, maintenance of genomic stability, and cell death [32].

The significance of these outcomes may become understanding taken into consideration that a PARP-1 inhibitor, olaparib, is the first-in-class PARP-1 inhibitor marketed in 2014 as monotherapy for advanced BRCA-deficient ovarian cancer. Breast cancer is a social burden and the most frequent cancer in women and the first cause of death by cancer in women. Approximately 2–3% of all breast cancer cases are due to germline mutations in either the BRCA1 or BRCA2 (BRCA1/2) gene. These kinds of mutations have the highest identifiable life-time risk of developing breast cancer. BRCA1/2 mutation carries lack of expression and/or function of the corresponding protein, which induces genomic instability [33].

In addition, a series of new olaparib derivatives were designed and synthesized as multifunctional PARP-1 and cholinesterase inhibitors acting as neuroprotectors [34–37], to treat AD, an upshot that may be assured with the

consumption of a natural product as EVOO. Moreover, OLE induces apoptosis in SKBR3 breast cancer cells activating the mitochondrial apoptotic pathway, which leads to cleavage of PARP-1 and caspase-9, and caspase-3, also [38, 39]. HT decreases PARP-1 protein levels and its activity in MCF-7 breast cancer cells [34]. Moreover, OC inhibited colony formation and induced apoptosis, as confirmed by PARP cleavage, activation of caspases 3/7, and chromatin condensation in colon cancer cells [40].

An olive secoiridoid, possesses powerful antioxidant and anti-inflammatory activities, which suggests its potential application to treat neuroinflammatory disorders. Oleacein-treated multiple myeloma cell line induced the increase in cleaved PARP1, caspase-3 and caspase-8 leading to apoptosis induction [36].

Increased levels of the enzyme iNOS generate augmented levels of NO in conjunction with ROS and other neurotoxic factors that steer to neuronal death [37, 41]. Pretreatment of ATDC-5 cells with OC significantly inhibited the bacterial lipopolysaccharide-induced NO production in a dose-dependent manner in a murine chondrocyte cell line [42]. In lipopolysaccharide-stimulated murine peritoneal macrophage isolated cells, OC was able to downregulate iNOS inhibiting its protein expression [43]. The *in vitro* study by Palmieri et al. (2012) clearly revealed that the incubation of human endothelial cells (AEhy926) with OLE (10, 20 and 50 µg/mL) under anoxia stress resulted in significant decrease in the level of NO and the expression of iNOS [44].

The effects of OO phenolic extract and individual compounds were investigated on MMP-9 in THP-1 cells, a human monocyte-like cell line [45], due to impaired nuclear factor-κB signaling providing further evidence on the mechanisms by which olive oil reduces the inflammatory burden associated with disorders, such as atherosclerosis. Table 2 shows representative significant biological effects of bioactive phenolic compounds of virgin olive oil.

Table 2 Significant biological effects of bioactive phenolic compounds of virgin olive oil.

Activity	Result	References
Neuroprotective	Effectiveness against Alzheimer's disease	[29]–[34]
	Reduction of oxidative stress	[5], [8], [35]
Antioxidant	Improving root stability ROS commitment Inhibition of LDL oxidation Inhibition of DNA oxidation Reduction of GSSG Increase GSH	[36], [37]
Anti-inflammatory	Reduction of cytokines' levels Decrease of myocardial injury	[6] [58-59]
Anticoagulant	ROS commitment Antiproliferative action Induction of apoptosis Anti-immigration action Inhibition of angiogenesis	[43]
Hepatoprotective	Reduction of steatosis	[44]
Antimicrobial	Antimicrobial action of cell membrane	[9], [45]

3.2. Experiments in animals

Studies carried out in rats proved that EVOO administration lessened the inflammatory levels with a concomitant refinement of serum antioxidant potential [46]. *In vitro* screening tests of OO containing biophenols at 800 mg/kg of OO was supplemented for 14 days to male Wistar rats at a dose corresponding to 20 g OO/per day to humans. The results reinforced the antioxidant capacity which OO exerts at blood, brain, muscle and intestines' levels [5].

OLE is characterized by antibacterial [47] and antiviral activity [48]. OLE induced reduction of Poly(ADP-Ribose) Polymerase 1 (PARP-1) activation, concluding that OLE treatment counteracts neuronal damage through modulation of the PARP1-SIRT1 interplay in the TgCRND8 mouse model [49]. OLE was also found to inhibit the inducible nitric oxide synthase (iNOS) expression in rats [50], reviewed about [51], in mice [52], having a neuroprotective role and anti-inflammatory effect in mice [53].

Cerebral infarction is a common cerebrovascular disease throughout the world having high rates of mortality and disability, and it overrunning society [54]. Having born in mind that intravenous thrombolysis therapy is effective but many patients fail to receive the treatment in time [55]. Yu et al. 2016 proved the neuroprotective effect exert from OLE on cerebral ischemia and reperfusion injury in a middle cerebral artery occlusion model in mice in a dose-dependent manner. OLE is a lipophilic molecule with small molecular weight and can cross the blood-brain barrier (BBB) when the BBB is broken during cerebral ischemic injury [56]. Treatment with OLE is proposed to reduce cerebral infarct volume and provides neuroprotection in focal cerebral ischemia/reperfusion injury in mice [56]. Researchers declare for its safety and indeed there is yet to find the lethal dose when used an elevated dose (1 g/kg body weight) of OLE in albino mice [57]. The use of OLE lessened myocardial injury in rats caused by an acute myocardial infarction through the resistance of oxidative stress and anti-inflammatory activity, as anti-inflammation is a major cause of apoptosis [58, 59]. Neuroprotective effect in spinal cord injury in rats proposed by the use of OLE [60]. OLE supplementation in rats ameliorates oxidative tissue damage by scavenging free radicals [61]. Beneficial results and robust anti-oxidant activity showed in short-term feeding rats with olive oil [5]. Treatment of elderly rats with OLE through oral gavage reduced oxidative damage in the substantia nigra pars compacta molded by increasing antioxidant enzyme activity [62].

Additionally, OLE ameliorates the oxidative/nitrosative stress and suppresses inflammation and improved histological and plasma markers of liver damage in carbon tetrachloride (CCl₄)-induced liver injury in male BALB/cN mice [53]. In a rat model of TBI (trauma brain injury) it was proved that the production of eNOS and iNOS were significantly decreased with OC treatment in a dose-dependent manner [63] indicating that OC has a protective potential on neural cells after TBI by decreasing the oxidative stress in plasma, and apoptosis [63].

TgSwDI mice fed with EVOO demonstrated a great benefit in hallmarks of AD- the tau protein, and amyloid- β . EVOO-enriched diet for a duration of 6 months restored the levels of A β and tau protein in the brain, accompanied by improved cognitive behavior; suggesting that the long-term consumption of EVOO, starting at an early age, provides a protective effect to fight AD [64].

As is also the case for short term transgenic tau mice (hTau) models [65] and long-term feeding with EVOO aged rats and C57B1/6J mice which made headway in biochemical parameters, improved memory, and motor coordination thanks to antioxidant phenols of olive oil which reduce the unbalanced levels of oxidative stress in ageing rats and in mice respectively [66,67]. Transgenic SwDI mice, expressing human amyloid β precursor protein (APP) after enriched EVOO diet, showed decreased total brain A β accompanied by cognitive improvement. Mice, which were diagnosed with A β aggregation, after three-month EVOO consumption, diminished this symptom. It is possible that EVOO reduced the brain's A β by enforcing the A β clearance across the BBB and by lowering A β production via a modulated process of APP in the brains of TgSwDI mice [64,68].

3.3. Experiment in humans

Studies carried out in humans proposed that the EVOO consumption containing high and/or moderate bio-phenolic ingredients protects LDL from oxidation and increases the levels of HDL cholesterol entailing a dose-dependent attenuation of oxidative stress [8, 69]. It is also known the protection of DNA strands from oxidative damage and the decrease of cancer development [70]. In the frame of a clinical trial which deals with EVOO administration in MCI patients it was demonstrated restoration of DNA damage and PARP-1 attenuation which correlates with reduction of oxidative stress (unpublished data).

A decreased risk of dementia has been strongly related to the direct effect of MedDiet components on the metabolism of AD hallmarks [71, 72]; a substantial part of those positive effects has been attributed to EVOO consumption [4, 73]. In this field studies in elderly shed light to the risk of the onset of dementia thanks to the high intake of EVOO and polyphenols [74–76], exhibiting a significantly positive impact on cognitive function.

Cognitive performance has been associated with dietary habits and, in parallel, cognitive decline and neurodegenerative disorders have been related to oxidative stress. We could hypothesize that reducing oxidative stress through the consumption of antioxidant-rich foods could protect people from neurodegenerative diseases. Previous research has

confirmed that participants who intensely consumed EVOO exhibited a decrease in cognitive decline in a 4-year timeline, in contrast to those whose intake of EVOO was rare or zero [77].

The clinical trial MICOIL [78] very recently demonstrated significant improvement in ADAS-cog ($p=0.001$) and MMSE ($p=0.05$) in groups of patients after one year intervention received EVOO and mainly independent of the presence of APOE $\epsilon 4$, whereas the non-consuming group displayed worse or similar to baseline performance in almost all domains. In detail, consuming groups had better outcomes in respect with ADAS-cog ($p=0.003$), Digit Span ($p=0.006$), and Letter fluency ($p=0.003$).

As a continuation of the clinical trial MICOIL [78], the biochemical study of Tzekaki et al. [79] was to investigate one of the possible different pathways involved in the AD onset and on disease progression and evaluate the beneficial effect of the annual EVOO administration in MCI patients, not only in neuropsychological tests but in AD biomarkers and biochemical pioneers actors of biochemical pathways in order to support, strengthen and confirm the clinical trial, and prove consistency. Cascades of events correlated with inflammatory responses and oxidative stress may activate a mechanism of some pathological events. Synaptic dysfunction and neurodegeneration in the brain is a result of events happening in the intra-cellular level. Biomarkers for Amyloid β ($A\beta$) plaque, neurofibrillary tangles, and brain atrophy serve as the limited window on biology available to the clinician. The biomarkers field is already vast, there is a growing demand to predict, diagnose, and to targeted fight the generative causes of AD for reversing them, and especially through natural approaches.

Taken into consideration the already known beneficial properties of EVOO including neuroprotective, antioxidant, anti-inflammatory, anticoagulant, anti-microbial [65,68,71,72,77,78,80–86] we toured and gleaned determinant factors of various biochemical pathways which may be altered by the EVOO intervention, and accessible to be measured in serum of MCI patients.

In this frame, we focused on the effect of the annual EVOO consumption on factors of the fibrinolytic system, and especially on the principal one PAI-1, (which already has been reported as an AD biomarker), on $\alpha 2$ -antiplasmin, and moreover on the tPA protease. Really, one of the main mechanisms in which EVOO is implicated was in the annulation of PAI-1 activity. As a result, we found increased tissue plasminogen activator (tPA) and $\alpha 2$ -antiplasmin activity which reflected augmented $A\beta$ fragments' degradation [79]. A malfunction of the tPA-plasmin system causes defective proteolytic degradation of $A\beta$ plaques in advanced stages of AD [87]. Soluble $A\beta$ -induced synaptic dysfunction is an untimely event in the pathogenesis of AD that precedes the deposition of insoluble $A\beta$ and correlates with the evolution of cognitive deficits rather than with the number of plaques [87]. The already known EVOO's antioxidant properties were demonstrated to intervene in AD prevention in this trial by implication in the oxidative stress quenching by measurement of the lipid peroxidation characteristic biomarker, the malondialdehyde [6].

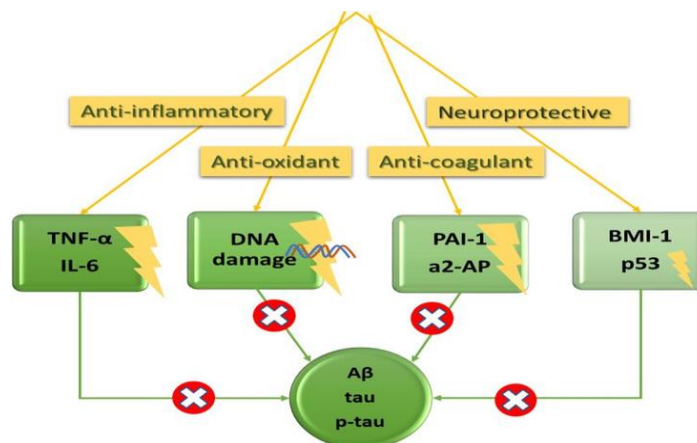


Figure 2 Model depicting the properties of EVOO administration on different pathways exerting its neuroprotective, anti-coagulant, anti-oxidant and anti-inflammatory effect on health defence.

A second scientific query was directed towards the effect of EVOO on neuroprotective proteins and focused on the B lymphoma Mo-MLV insertion region 1 (Bmi1) levels. BMI-1 is mechanistically required to repress Microtubule Associated Protein Tau (MAPT) transcription and hinder p53 stabilization, thereby preventing neurodegeneration. Increased BMI-1 levels activate the neuroprotective cascade which correlated with diminution of p53 levels. Moreover, p53-mediated activity moderates the induced neuronal apoptosis through the quantified decreased levels of caspase-8

neurological disorders have been extensively investigated from the perspective of different cell pathways. The protective effect of EVOO as a whole and of its components separately is proved against LDL oxidation and oxidative stress, stemming from brain to lessen the inflammatory levels after spinal cord injury (in animal model), and to overcome the hurdle of amyloid deposition (*in vitro* and in animal models) and to delay the cognitive decline among non-demented older individuals. In conclusion, further investigation in a bigger cohort of human patients is demanded for acquiring warranties certifying this treasure as a natural and alternative, less toxic therapy for numerous disorders worldwide.

Abbreviations

MedDiet, Mediterranean Diet; EVOO, Extra Virgin Olive Oil; CVD, cardiovascular disease; MCI, Mild Cognitive Impairment; AD, Alzheimer's disease; MUFA, monounsaturated fatty acids; HT, hydroxytyrosol; ROS, reactive oxygen species; NO, nitric oxide; OC, oleocanthal; OLE, Oleuropein; PAI-1, Plasminogen activator inhibitor 1; ER, Estrogen receptor; PR, progesterone receptor; PARP1, Poly(ADP-Ribose) Polymerase 1; BCA1, Breast cancer type 1 susceptibility protein; iNOS, Inducible nitric oxide synthase, intracellular nitric oxide synthase; MMP-9, metalloproteinase 9; BBB, blood-brain barrier; TBI, traumatic brain injury; tPA, tissue plasminogen activator, BMI1, B lymphoma Mo-MLV insertion region 1 homolog; MAPT, Microtubule-associated protein tau.

Compliance with ethical standards

Acknowledgments

This work was funded by Alzheimer Hellas, Thessaloniki, Greece and Yanni's Olive Grove Company providing the Early Harvest EVOO and EVOO, Potidea Chalkidiki, Greece.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

- [1] Kalogeropoulos N, Tsimidou MZ. Antioxidants in greek virgin olive oils. *Antioxidants*. 2014; 3: 387–413.
- [2] Trichopoulou A, Martínez-González MA, Tong TYN, Forouhi NG, Khandelwal S, Prabhakaran D, Mozaffarian D, de Lorgeril M. Definitions and potential health benefits of the Mediterranean diet: Views from experts around the world. *BMC Med*. 2014; 12: 112.
- [3] Gaforio JJ, Visioli F, Alarcón-De-la-lastra C, Castañer O, Delgado-Rodríguez M, Fitó M, et al. Virgin olive oil and health: Summary of the iii international conference on virgin olive oil and health consensus report, JAEN (Spain) 2018. *Nutrients*. 2019; 11: 2039.
- [4] Klimova B, Novotný M, Kuca K, Valis M. Effect of an extra-virgin olive oil intake on the delay of cognitive decline: Role of secoiridoid oleuropein? *Neuropsychiatr Dis Treat*. 2019; 15: 3033–40.
- [5] Kouka P, Tekos F, Papoutsaki Z, Stathopoulos P, Halabalaki M, Tsantarliotou M, et al. Olive oil with high polyphenolic content induces both beneficial and harmful alterations on rat redox status depending on the tissue. *Toxicol Reports*. 2020; 7: 421–32.
- [6] Tzekaki EE, Papaspyropoulos A, Tsolaki M, Lazarou E, Kozori M, Pantazaki AA. Restoration of BMI1 levels after the administration of early harvest extra virgin olive oil as a therapeutic strategy against Alzheimer's disease. *Exp Gerontol*. 2021; 144: 111-178.
- [7] Tzekaki EE, Geromichalos G, Lavrentiadou SN, Tsantarliotou MP, Pantazaki AA, Papaspyropoulos A. Oleuropein is a natural inhibitor of PAI-1-mediated proliferation in human ER-/PR- breast cancer cells. *Breast Cancer Res Treat*. 2021; 186(2):305-316.
- [8] Tsartsou E, Proutsos N, Castanas E, Kampa M. Network meta-analysis of metabolic effects of olive-oil in humans shows the importance of olive oil consumption with moderate polyphenol levels as part of the mediterranean diet. *Front Nutr*. 2019; 12(6): 6.
- [9] Tripoli E, Giammanco M, Tabacchi G, Di Majò D, Giammanco S, La Guardia M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr Res Rev*. 2005; 18: 98-112.

- [10] Román GC, Jackson RE, Reis J, Román AN, Toledo JB, Toledo E. Extra-virgin olive oil for potential prevention of Alzheimer disease. *Rev Neurol (Paris)*. 2019; 175: 705-723.
- [11] Gorzynik-Debicka M, Przychodzen P, Cappello F, Kuban-Jankowska A, Gammazza AM, Knap N, et al. Potential health benefits of olive oil and plant polyphenols. *Int J Mol Sci*. 2018; 19: 686.
- [12] Ranalli F, Ranalli A, Contento S, Casanovas M, Antonucci M, Di Simone G. Bioactives and nutraceutical phytochemicals naturally occurring in virgin olive oil. The case study of the Nocellara del Belice Italian olive cultivar. *Nat Prod Res*. 2013; 27: 1686-90.
- [13] Minatel IO, Borges CV, Ferreira MI, Gomez HAG, Chen C-YO, Lima GPP. Phenolic Compounds: Functional Properties, Impact of Processing and Bioavailability. *Phenolic Compd. - Biol. Act*. 2017; 1: 2-4.
- [14] Virruso C, Accardi G, Colonna-Romano G, Candore G, Vasto S, Caruso C. Nutraceutical properties of extra-virgin olive oil: A natural remedy for age-related disease? *Rejuvenation Res*. 2014; 17: 217-20.
- [15] Beauchamp GK, Keast RSJ, Morel D, Lin J, Pika J, Han Q, et al. Ibuprofen-like activity in extra-virgin olive oil. *Nature*. 2005; 437: 45-6.
- [16] Yadav TC, Kumar N, Raj U, Goel N, Vardawaj PK, Prasad R, et al. Exploration of interaction mechanism of tyrosol as a potent anti-inflammatory agent. *J Biomol Struct Dyn*. 2020; 38: 382-397.
- [17] Monti SM, Ritieni A, Sacchi R, Skog K, Borgen E, Fogliano V. Characterization of phenolic compounds in virgin olive oil and their effect on the formation of carcinogenic/mutagenic heterocyclic amines in a model system. *J Agric Food Chem*. 2001; 49: 3969-75.
- [18] EFSA Panel on Dietetic Products. 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). 2011; 9: 20-33.
- [19] Solfrizzi V, Panza F, Frisardi V, Seripa D, Logroscino G, Imbimbo BP, Pilotto A. Diet and Alzheimer's disease risk factors or prevention: The current evidence. *Expert Rev Neurother*. 2011; 11: 677-708.
- [20] Trichopoulou A. Mediterranean diet, traditional foods, and health: Evidence from the Greek EPIC cohort. *Food Nutr Bull*. 2007; 28: 236-40.
- [21] Yubero-Serrano EM, Lopez-Moreno J, Gomez-Delgado F, Lopez-Miranda J. Extra virgin olive oil: More than a healthy fat. *Eur J Clin Nutr*. 2019; 72: 8-17.
- [22] Sies H, Jones D. Oxidative Stress. *Encycl. Stress*. 2007; 4: 180–183.
- [23] Fitó M, De La Torre R, Covas MI. Olive oil and oxidative stress. *Mol Nutr Food Res*. 2007; 51: 1215-1224.
- [24] Li W, Sperry JB, Crowe A, Trojanowski JQ, Smith AB, Lee VMY. Inhibition of tau fibrillization by oleocanthal via reaction with the amino groups of tau. *J Neurochem*. 2009; 110: 1339-51.
- [25] Monti MC, Margarucci L, Tosco A, Riccio R, Casapullo A. New insights on the interaction mechanism between tau protein and oleocanthal, an extra-virgin olive-oil bioactive component. *Food Funct*. 2011; 2: 423-8.
- [26] Barbaro B, Toietta G, Maggio R, Arciello M, Tarocchi M, Galli A, et al. Effects of the olive-derived polyphenol oleuropein on human health. *Int J Mol Sci*. 2014; 15: 18508–18524.
- [27] Bulotta S, Celano M, Lepore SM, Montalcini T, Pujia A, Russo D. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: Focus on protection against cardiovascular and metabolic diseases. *J Transl Med*. 2014; 12: 219.
- [28] Lemonakis N, Gikas E, Halabalaki M, Skaltsounis AL. Development and Validation of a Combined Methodology for Assessing the Total Quality Control of Herbal Medicinal Products - Application to Oleuropein Preparations. *PLoS One*. 2013; 8: 78277.
- [29] Peng S, Zhang B, Yao J, Duan D, Fang J. Dual protection of hydroxytyrosol, an olive oil polyphenol, against oxidative damage in PC12 cells. *Food Funct*. 2015; 6: 2091-2100.
- [30] Liman R, Çoban F, Cığerci I, Bulduk İ, Bozkurt S. Antiangiogenic and Apoptotic Effects of Oleuropein on Breast Cancer Cells. *Br J Pharm Res*. 2017; 16: 1-10.
- [31] Ruzzolini J, Peppicelli S, Andreucci E, Bianchini F, Scardigli A, Romani A, et al. Oleuropein, the main polyphenol of *Olea europaea* leaf extract, has an anti-cancer effect on human BRAF melanoma cells and potentiates the cytotoxicity of current chemotherapies. *Nutrients*. 2018; 10: 19-50.

- [32] Martire S, Mosca L, d'Erme M. PARP-1 involvement in neurodegeneration: A focus on Alzheimer's and Parkinson's diseases. *Mech Ageing Dev.* 2015; 146–148: 53–64.
- [33] Nicolas E, Bertucci F, Sabatier R, Gonçalves A. Targeting BRCA deficiency in breast cancer: What are the clinical evidences and the next perspectives? *Cancers (Basel).* 2018; 10: 506.
- [34] Calahorra J, Martínez-Lara E, Granadino-Roldán JM, Martí JM, Cañuelo A, Blanco S, et al. Crosstalk between hydroxytyrosol, a major olive oil phenol, and HIF-1 in MCF-7 breast cancer cells. *Sci Rep.* 2020; 10: 6361.
- [35] Nichols E, Szoeki CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2019; 18: 88–106.
- [36] Juli G, Oliverio M, Bellizzi D, Cantafio MEG, Grillone K, Passarino G, et al. Anti-tumor activity and epigenetic impact of the polyphenol oleacein in multiple myeloma. *Cancers (Basel).* 2019; 11(7): 990.
- [37] Santos RM, Lourenço CF, Ledo A, Barbosa RM, Laranjinha J. Nitric oxide inactivation mechanisms in the brain: Role in bioenergetics and neurodegeneration. *Int J Cell Biol.* 2012; 1: 1-13.
- [38] Shamshoum H, Vlavcheski F, Tsiani E. Anticancer effects of oleuropein. *BioFactors.* 2017; 43: 517-528.
- [39] Chimento A, Casaburi I, Rosano C, Avena P, De Luca A, Campana C, et al. Oleuropein and hydroxytyrosol activate GPER/ GPR30-dependent pathways leading to apoptosis of ER-negative SKBR3 breast cancer cells. *Mol Nutr Food Res.* 2014; 58: 478–89.
- [40] Cusimano A, Balasus D, Azzolina A, Augello G, Emma MR, Di Sano C, et al. Oleocanthal exerts antitumor effects on human liver and colon cancer cells through ROS generation. *Int J Oncol.* 2017; 51: 533-544.
- [41] Wallace MN, Geddes JG, Farquhar DA, Masson MR. Nitric oxide synthase in reactive astrocytes adjacent to β -amyloid plaques. *Exp Neurol.* 1997; 275: 7918-7924.
- [42] Iacono A, Gómez R, Sperry J, Conde J, Bianco G, Meli R, et al. Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. *Arthritis Rheum* 2010; 62: 1675-82.
- [43] Montoya T, Castejón ML, Sánchez-Hidalgo M, González-Benjumea A, Fernández-Bolaños JG, Alarcón De-La-Lastra C. Oleocanthal Modulates LPS-Induced Murine Peritoneal Macrophages Activation via Regulation of Inflammasome, Nrf-2/HO-1, and MAPKs Signaling Pathways. *J Agric Food Chem.* 2019; 67: 5552–9.
- [44] Palmieri D, Aliakbarian B, Casazza AA, Ferrari N, Spinella G, Pane B, et al. Effects of polyphenol extract from olive pomace on anoxia-induced endothelial dysfunction. *Microvasc Res.* 2012; 83: 281-9.
- [45] Dell'Agli M, Fagnani R, Galli G V., Maschi O, Gilardi F, Bellosta S, et al. Olive oil phenols modulate the expression of metalloproteinase 9 in THP-1 cells by acting on nuclear factor-kB Signaling. *J Agric Food Chem.* 2010; 58: 2246-52.
- [46] Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis.* 2007; 190: 181-6.
- [47] Campolo M, Di Paola R, Impellizzeri D, Crupi R, Morittu VM, Procopio A, et al. Effects of a polyphenol present in olive oil, oleuropein aglycone, in a murine model of intestinal ischemia/reperfusion injury. *J Leukoc Biol.* 2013; 93: 277-87.
- [48] Hasegawa Y, Hamada JI, Morioka M, Yano S, Kawano T, Kai Y, et al. Neuroprotective effect of postischemic administration of sodium orthovanadate in rats with transient middle cerebral artery occlusion. *J Cereb Blood Flow Metab.* 2003; 23: 1040-51.
- [49] Luccarini I, Pantano D, Nardiello P, Cavone L, Lapucci A, Miceli C, et al. The Polyphenol Oleuropein Aglycone Modulates the PARP1-SIRT1 Interplay: An *in Vitro* and *in Vivo* Study. *J Alzheimer's Dis.* 2016; 54: 1-14.
- [50] Andreadou I, Sigala F, Iliodromitis EK, Papaefthimiou M, Sigalas C, Aligiannis N, et al. Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. *J Mol Cell Cardiol.* 2007; 42: 549-58.
- [51] Omar SH. Oleuropein in olive and its pharmacological effects. *Sci Pharm.* 2010; 78: 133-154.
- [52] Sánchez-Fidalgo S, Cárdeno A, Sánchez-Hidalgo M, Aparicio-Soto M, De la Lastra CA. Dietary extra virgin olive oil polyphenols supplementation modulates DSS-induced chronic colitis in mice. *J Nutr Biochem.* 2013; 26: 513-520.

- [53] Domitrović R, Jakovac H, Marchesi VV, Šain I, Romić Ž, Rahelić D. Preventive and therapeutic effects of oleuropein against carbon tetrachloride-induced liver damage in mice. *Pharmacol Res.* 2012; 65: 451-64.
- [54] Kikuchi K, Tanaka E, Murai Y, Tancharoen S. Clinical Trials in Acute Ischemic Stroke. *CNS Drugs.* 2014; 28: 929–938.
- [55] Virmani R, Ladich ER, Burke AP, Kolodgie FD. Histopathology of carotid atherosclerotic disease. *Neurosurgery.* 2006; 59: 219-227.
- [56] Yu H, Liu P, Tang H, Jing J, Lv X, Chen L, et al. Oleuropein, a natural extract from plants, offers neuroprotection in focal cerebral ischemia/reperfusion injury in mice. *Eur J Pharmacol.* 2016; 775: 113–9.
- [57] Omagari K, Kato S, Tsuneyama K, Hatta H, Sato M, Hamasaki M, et al. Olive leaf extract prevents spontaneous occurrence of non-alcoholic steatohepatitis in SHR/NDmcr-cp rats. *Pathology.* 2010; 42: 66–72.
- [58] Janahmadi Z, Nekooeian AA, Moaref AR, Emamghoreishi M. Oleuropein Offers Cardioprotection in Rats with Acute Myocardial Infarction. *Cardiovasc Toxicol.* 2015; 15: 61–68.
- [59] Ahmad M, Dar N, Bhat Z, Hussain A, Shah A, Liu H, et al. Inflammation in Ischemic Stroke: Mechanisms, Consequences and Possible Drug Targets. *CNS Neurol Disord - Drug Targets.* 2014; 13: 1378-96.
- [60] Khalatbary AR, Ahmadvand H. Neuroprotective effect of oleuropein following spinal cord injury in rats. *Neurol Res.* 2012; 34: 44-51.
- [61] Ogun M, Ozcan A, Karaman M, Merhan O, Ozen H, Kukurt A, et al. Oleuropein ameliorates arsenic induced oxidative stress in mice. *J Trace Elem Med Biol.* 2016; 36: 1-6.
- [62] Sarbishegi M, Mehraein F, Soleimani M. Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. *Iran Biomed J.* 2014; 18: 16-22.
- [63] Mete M, Aydemir I, Unsal UU, Collu F, Vatandas G, Gurcu B, et al. Neuroprotective effects of oleocanthal, a compound in virgin olive oil, in a rat model of traumatic brain injury. *Turk Neurosurg.* 2018; 28: 1-8.
- [64] Qosa H, Mohamed LA, Batarseh YS, Alqahtani S, Ibrahim B, LeVine H, et al. Extra-virgin olive oil attenuates amyloid- β and tau pathologies in the brains of TgSwDI mice. *J Nutr Biochem.* 2015; 26: 1479-90.
- [65] Lauretti E, Nenov M, Dincer O, Iuliano L, Praticò D. Extra virgin olive oil improves synaptic activity, short-term plasticity, memory, and neuropathology in a tauopathy model. *Aging Cell.* 2020; 19: 1–11.
- [66] Pitozzi V, Jacomelli M, Zaid M, Luceri C, Bigagli E, Lodovici M, et al. Effects of dietary extra-virgin olive oil on behaviour and brain biochemical parameters in ageing rats. *Br J Nutr.* 2010; 103: 1674-1683.
- [67] Pitozzi V, Jacomelli M, Catelan D, Servili M, Taticchi A, Biggeri A, et al. Long-term dietary extra-virgin olive oil rich in polyphenols reverses age-related dysfunctions in motor coordination and contextual memory in mice: Role of oxidative stress. *Rejuvenation Res.* 2012; 15: 601-12.
- [68] Qosa H, Batarseh YS, Mohyeldin MM, El Sayed KA, Keller JN, Kaddoumi A. Oleocanthal Enhances Amyloid- β Clearance from the Brains of TgSwDI Mice and in Vitro across a Human Blood-Brain Barrier Model. *ACS Chem Neurosci.* 2015; 4: 973-982.
- [69] Marrugat J, Covas MI, Fitó M, Schröder H, Miró-Casas E, Gimeno E, et al. Effects of differing phenolic content in dietary olive oil on lipids and LDL oxidation: A randomized controlled trial. *Eur J Nutr.* 2004; 4: 973-982.
- [70] Machowetz A, Poulsen HE, Gruendel S, Weimann A, Fitó M, Marrugat J, et al. Effect of olive oils on biomarkers of oxidative DNA stress in Northern and Southern Europeans. *FASEB J.* 2007; 21: 45-52.
- [71] Knopman DS. Mediterranean diet and late-life cognitive impairment: A taste of benefit. *JAMA - J Am Med Assoc.* 2009; 302: 686-687.
- [72] Tsolaki MN, Koutsouraki ES, Katsipis GK, Myserlis PG, Chatzithoma MA, Pantazaki AA. Ch. 1 Alternative Anti-Infective / Anti-Inflammatory Therapeutic Options for Fighting Alzheimer ' s Disease. 2017; 6: 1–153.
- [73] Foscolou A, Critselis E, Panagiotakos D. Olive oil consumption and human health: A narrative review. *Maturitas.* 2018; 118: 60-66.
- [74] Lefèvre-Arbogast S, Gaudout D, Bensalem J, Letenneur L, Dartigues JF, Hejblum BP, et al. Pattern of polyphenol intake and the long-term risk of dementia in older persons. *Neurology.* 2018; 90: 1979-1988.
- [75] Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, Martínez-González MÁ, et al. Mediterranean Diet and Age-Related Cognitive Decline. *JAMA Intern Med.* 2015; 175: 1094-1103.

- [76] Martinez-Lapiscina EH, Clavero P, Toledo E, San Julian B, Sanchez-Tainta A, Corella D, et al. Virgin olive oil supplementation and long-term cognition: The Predimed-Navarra randomized, trial. *J Nutr Heal Aging*. 2013; 17: 544–52.
- [77] Berr C, Portet F, Carriere I, Akbaraly TN, Feart C, Gourlet V, et al. Olive oil and cognition: Results from the three-city study. *Dement Geriatr Cogn Disord*. 2009; 28: 357-64.
- [78] Tsolaki M, Lazarou E, Kozori M, Petridou N, Tabakis I, Lazarou I, et al. A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study. *J Alzheimer's Dis*. 2020; 78: 801-817.
- [79] Tzekaki EE, Tsolaki M, Pantazaki AA, Geromichalos G, Lazarou E, Kozori M, Sinakos Z. The pleiotropic beneficial intervention of olive oil intake on the Alzheimer's disease onset via fibrinolytic system. *Exp Gerontol*. 2021; 6: 111344.
- [80] Cicicale S, Lucas LJ, Keast RSJ. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Curr Opin Biotechnol*. 2012; 23: 129–35.
- [81] Venkat S. A review on hepatoprotective activity. *Int J Curr Res*. 2017; 9(6): 51876-51881
- [82] Nour-Eldin F. Phenols and blood coagulation. *J Biomed Mater Res*. 1968; 2: 23-42.
- [83] Abuznait AH, Qosa H, Busnena BA, El Sayed KA, Kaddoumi A. Olive-oil-derived oleocanthal enhances β -amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: *In vitro* and *in vivo* studies. *ACS Chem Neurosci*. 2013; 4: 973–82.
- [84] Boskou G, Salta FN, Chrysostomou S, Mylona A, Chiou A, Andrikopoulos NK. Antioxidant capacity and phenolic profile of table olives from the Greek market. *Food Chem*. 2006; 94: 558-564.
- [85] Lozano-Castellón J, López-Yerena A, Rinaldi de Alvarenga JF, Romero del Castillo-Alba J, Vallverdú-Queralt A, Escribano-Ferrer E, et al. Health-promoting properties of oleocanthal and oleacein: Two secoiridoids from extra-virgin olive oil. *Crit Rev Food Sci Nutr*. 2020; 60: 2532–48.
- [86] Piroddi M, Albini A, Fabiani R, Giovannelli L, Luceri C, Natella F, et al. Nutrigenomics of extra-virgin olive oil: A review. *BioFactors*. 2017; 43: 17–41.
- [87] Diaz A, Merino P, Guo JD, Yepes MA, McCann P, Katta T, et al. Urokinase-type plasminogen activator protects cerebral cortical neurons from soluble Ab-induced synaptic damage. *J Neurosci*. 2020; 40: 4251-4263.
- [88] Flamier A, El Hajjar J, Adjaye J, Fernandes KJ, Abdouh M, Bernier G. Modeling Late-Onset Sporadic Alzheimer's Disease through BMI1 Deficiency. *Cell Rep*. 2018; 23: 2653-2666.
- [89] Su P, Zhang J, Wang D, Zhao F, Cao Z, Aschner M, et al. Review the role of autophagy in modulation of neuroinflammation in microglia. 2016; 319: 155–67.
- [90] Abdouh M, Chatoo W, El Hajjar J, David J, Ferreira J, Bernier G. Bmi1 is down-regulated in the aging brain and displays antioxidant and protective activities in neurons. *PLoS One*. 2012; 7: 31870.
- [91] Janelidze S, Stomrud E, Palmqvist S, Zetterberg H. Plasma β -amyloid in Alzheimer's disease and vascular disease. *Nat Publ Gr*. 2016; 6: 1-11.