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Olive and Its Phenolic Compound as the Promising Neuroprotective Agent

(Sebatian Fenolik di dalam Buah Zaitun Berpotensi sebagai Agen Perlindungan Saraf)

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ABSTRACT

Recent progress in alternative medicine has highlighted the benefits of olive as an integral part of therapeutic diet to promote healthy living. Among the thirty different phenolic compounds of olive known to date; oleocanthal, oleuropein, tyrosol and hydroxytyrosol are being increasingly investigated for their potential in prevention and healing of several major forms of neurological dysfunctions and disorders. A considerable amount of literature suggests the neuroprotective effects of olive and its phenolic compounds are owing to their roles as anti-oxidant, anti-inflammatory and anti-apoptotic agents. At preclinical level, olive attenuated cognitive dysfunctions and the functional outcomes in spinal cord injury, delayed the progression of amyloid beta pathology, improved motor and mitochondrial dysfunctions in Parkinson's disease, reversed diabetic-related neurological complications and also ameliorated cerebral pathologies in stroke. In this paper, we aim to review the neuroprotective role of olive and its phenolic derivatives in the following diseases or deficits of the nervous system that include cognitive dysfunction, neurodegenerative diseases, stroke, peripheral neuropathy and spinal cord injury.

Keywords: Neuroprotection; neurodegeneration; neuropathy; nerve; olive; Olea europaea

ABSTRAK

Perkembangan terkini dalam perubatan alternatif telah membuktikan khasiat buah zaitun sebagai bahan penting dalam diet yang menggalakkan gaya hidup sihat. Antara tiga puluh sebatian fenolik yang terdapat di dalam buah zaitun; oleocantal, oleuropein, tirosol dan hidroksitirosol telah dikaji dengan kerap akan potensinya dalam mencegah dan merawat beberapa penyakit berkaitan saraf. Beberapa kajian telah mengesyorkan keupayaannya dalam memberi perlindungan adalah disebabkan oleh fungsinya sebagai anti-oksidan, anti-radang dan anti-apoptosis. Kesan zaitun ke atas perlindungan sistem saraf masih belum diterokai sepenuhnya pada peringkat ujian klinikal setakat ini. Manakala pada peringkat pra-klinikal pula, buah zaitun mampu memperbaiki fungsi kognitif otak, mengurangkan komplikasi akibat daripada kecederaan saraf tunjang, melambatkan kesan patologi oleh protein amiloid beta, memperbaiki fungsi motor dan mitokondria dalam penyakit Parkinson, merawat komplikasi penyakit diabetes dan mengurangkan kerosakan tisu kepada otak semasa strok. Dalam kertas ini, kesan perlindungan buah zaitun dalam kategori penyakit seperti berikut: kerosakan fungsi kognitif, penyakit neurodegeneratif, strok, periferal neuropati dan kecederaan saraf tunjang akan didalami.

Kata kunci: Buah zaitun; neurodegeneratif; neuropati; Olea europaea*; perlindungan saraf; saraf*

OLIVE AND ITS PHENOLIC COMPOUND: ANTI-OXIDANT POTENTIAL

Oxidative stress is a well known contributing factor in development of various disease (Kumar et al. 2017; Matough et al. 2012). Recent developments in phytomedicine have reported various health-improving effects of numerous plant extracts (Budin et al. 2009; Esmaeili et al. 2016; Ruszymah et al. 2012). Olive, or also known as *Olea europaea* is a native tree commonly found in Mediterranean region (El & Karakaya 2009). Generally, olive trees are cultivated for oil, leaf extracts and fruits. Olive oil consists primarily of triacylglycerols (~99%), free fatty acids and lipids such as hydrocarbons, sterols, aliphatic alcohol, tocopherols and pigments (Boskou et al. 2006). At least 30 different phenolic compounds have been found to originate from the olive that include oleuropein, tyrosol and hydroxytyrosol (Parkinson & Cicerale 2016). Most of these phenolic compounds were reported to have anti-oxidant potential (Aghagolzadeh et al. 2017; De La Cruz et al. 2010; Pourkhodadad et al. 2016). More specifically, oleuropein, one of the aforementioned phenolic compounds, prevents free radical formation through chelation of metal ions such as copper and iron, which catalyse the generation of free radicals (Assimopoulou et al. 2002). Hydroxytyrosol (HT) nullified free radicals by forming intramolecular bond with radicals through donation of a hydrogen ion (Visioli & Galli 1998). Recent reports from the European Food Safety Authority have backed the anti-oxidant potential of HT against LDL-induced oxidative damage with specific recommendation of dietary dosage as supplemental in diet (EFSA 2011). In addition, tyrosol reduced reactive oxygen and nitrogen

species such as nitric oxide in Parkinson's disease (PD) model of rats (Dewapriya et al. 2013).

The neuroprotective effects of olive oil or its components have been proven in various neurological disorders. Considering the widespread etiology and pathophysiology of these disorders, we have restricted our focus on cognitive dysfunction, neurodegenerative diseases, ischaemic reperfusion brain injury (stroke), peripheral neuropathy and spinal cord injury in this review to elucidate the neuroprotective roles of olive and its phenolic compounds.

NEUROPROTECTIVE EFFECTS OF OLIVE IN COGNITIVE DYSFUNCTION

Cognition is a higher mental process that enable humans to 'acquire knowledge and understand through thought, experience and senses' (Oxford Dictionaries 2018). Such high order process involves thinking, memory, judgement, evaluation, perception of surroundings, attention, reasoning, planning, decision making and comprehension (Bisson et al. 2015). Cognitive dysfunction is precipitated through aging and various pathologies such as Alzheimer's disease (AD), brain trauma and post-traumatic stress. To date, several studies have suggested the olive to prevent and even delay the progression of various cognitive deficits.

In the PREDIMED study, 578 participants that was undergoing Mediterranean diet with olive oil for 5 years, were exposed to various neuropsychological tests to assess their cognitive functions. To the authors' surprise, elderly participants with high cardiovascular risk performed well in the assigned cognitive tests which led the authors to surmise that consumption of foods rich in polyphenols such as olive oil in particular, can improve cognitive functions even among the older population (Valls-Pedret et al. 2012). Laboratory experiments showed the olive oil to modify gene and microRNA (miRNA) expression profiles associated with aging (Luceri et al. 2017). In addition, phenolic-rich extra virgin olive oil (H-EVOO) treatment upregulated the expression of genes associated with synaptic plasticity such as *Notch1*, *BMP*, *NGFR*, *GLP1R, Myst3* and *CRCTC3* in aged mice. Intriguingly, the aged mice treated with H-EVOO displayed a similar miRNA expression profiles at the cortex of that young mice. Conversely, the aged mice treated with phenolic-deficient extra virgin olive (L-EVOO) showed increased miRNA expressions of miR-29 and miR-34 (associated with aging and AD). Furthermore, when tested in mnemonic and motor coordination paradigms, the aged mice treated with H-EVOO performed better than the aged L-EVOO treated group, suggesting the centrality of phenolic compounds in mediating the effects of olive oil in improving the cognitive and motor functions (Luceri et al. 2017).

In line with these findings, one of the phenolic compounds of olive, oleuropein was shown to be neuroprotective in a rat model of post-traumatic stress disorder (PTSD). In this study, the rats were exposed to a single prolonged stress (SPS) (series of validated tests done to induce traumatic experience in rats to mimic human PTSD condition) and were given oleuropein 20, 50 and 100 mg/kg intraperitoneally for 21 consecutive days. Oleuropein reversed the SPS-induced cognitive impairment and also the downregulation of brain-derived neurotrophic factor (BDNF) (a growth factor that is important in neuronal growth and survival) (Lee et al. 2017). In addition to PTSD model, oleuropein also dramatically improved the learning and memory retention of pharmacological rat model of cognitive dysfunction, where the cognitive decline was induced by colchicine infusion at the hippocampal CA1 area (Pourkhodadad et al. 2016).

NEUROPROTECTIVE EFFECTS OF OLIVE IN NEURODEGENERATIVE DISEASES

Neurodegenerative disease is a spectrum of disorders that involves progressive destruction of structure, integrity and function of neurons that ultimately lead to neuronal death (Brown et al. 2005). Alzheimer's disease (AD) and Parkinson's disease (PD) are the most commonest neurodegenerative diseases to plague millions of older population around the globe. As these diseases are incurable to date, recent researches have explored a new set of potential therapies involving natural products to manage these diseases.

Amyloid plaques and neurofibrillary tangles are two brain abnormalities that define AD. The primary component of amyloid plaques is amyloid beta (Aβ). Accumulation of Aβ leads to neurodegeneration and the mental deterioration notably seen among the AD patients. In the pathogenesis of AD, Aβ clearance usually involves apolipoprotein E (ApoE)-dependent pathway that comprises of ATP-binding cassette transporter ABCA1 protein in regards to their role in regulating cellular cholesterol (promote endocytosis and lysosomal degradation of Aβ) and stabilising toxic Aβ oligomers (Cerf et al. 2011; Lee et al. 2012). Administration of extra-virgin olive oil (EVOO) has been shown to enhance Aβ clearance in the AD mice model. EVOO increased the expression of ApoE and ABCA1 proteins through modulation of their regulatory receptors, peroxisome proliferator-activated receptor gamma and liver X-receptors. Hyperphosphorylation of tau (proteins that stabilises microtubules) can result in the formation of neurofibrillary tangles and subsequent neurodegeneration. EVOO also reduced the hyperphosphorylation of tau proteins in the mice AD model (Qosa et al. 2015).

The neuroprotective effects of olive was further corroborated through oleocanthal, another phenolic compound of olive. Oleocanthal attenuated toxic amyloid beta oligomers (Aβo)-induced neuroinflammatory response by reducing IL-6 and reversing the Aβo-induced down-regulation of glutamate transporter-1, a neurosupportive protein in astrocytes) (Batarseh et al. 2017). Moreover, in a separate study, microinjections of oleuropein and Aβ42 aggregate into the rat nucleus basalis magnocellularis (NBM) formed a completely non-toxic growth aggregates, which was unlike the injection of toxic

Aβ42 aggregate alone. Further laboratory investigations showed Aβ aggregates grown in the presence of OLP not to show any tendency to disassemble and undergo toxic oligomerisation, suggesting the potential role of OLP in hampering the formation of toxic Aβ aggregates (Luccarini et al. 2014).

The findings on neuroprotective effects of olive were extended to various animal models of Parkinson's disease (PD) as well (Aghagolzadeh et al. 2017). Olive leaf extract (OLE) improved the motor deficits induced by intrastriatal injections of 6-OHDA to rats (chemically induced PD in rats) in various behavioural paradigms such as rotational test, narrow beam test and grip strength test. OLE also attenuated the subcutaneuous rotenone-induced PD-like motor dysfunction and weakness in rats which appears to be mediated by increased in anti-oxidant activities and reduced free radicals generation (Sarbishegi et al. 2018).

Several *in vitro* models have elucidated the mechanistic effects of phenolic compounds of olive oil in PD. Parkinsonian toxin, 1-methyl-4-78 phenylpyridinium (MPP+), is a commonly used drug to cause neuronal death through induction of mitochondrial dysfunction in dopaminergic cells, particularly in the substantia nigra (Lotharius & Malley 2000). Tyrosol was shown to reverse the MPP+-induced mitochondrial dysfunction and decrease in intracellular ATP generation in CATH.a cells. Furthermore, tyrosol also up-regulated the expression of anti-apoptotic proteins, Bcl-2 ad Bcl-xl to counteract against the pro-apoptotic proteins, Bax and BID (Dewapriya et al. 2013). Conversely, hydroxytyrosol, another phenolic component of olive had no apparent effect on MPP+ induced cytotoxicity in SH-SY5Y cells. However, HT was able to express its neuroprotection against 6-OHDA-induced cell death in the same cell line (Yu et al. 2016). It is likely that different cell lines may have varied response to cytotoxicity induced by MPP+ and 6-OHDA. Therefore, more experiments should be carried out to elucidate this discrepancy.

NEUROPROTECTIVE EFFECTS OF OLIVE IN ISCHEMIA-REPERFUSION (STROKE) MODEL

A number of investigators have reported the neuroprotective effects of olive oil in rodent models of ischaemic reperfusion brain injury (Hassanshahi et al. 2013; Mardookhi et al. 2016). Olive oil reduced cellular apoptosis in ischaemic mice by increasing the expression of anti-apoptotic protein, bcl2 in the hippocampus (Hassanshahi et al. 2013). In a follow up study, mice pretreated with olive oil (0.75 mL/kg/day) for a month prior to middle cerebral artery occlusion, showed reduced infarct volume in total brain hemisphere, cortex and striatum and also exhibited better neurologic defect score compared to the ischaemic group. Molecular findings showed the neuroprotective effects of the olive oil could be owing to its interaction with NF-κβ and tumor necrosis factor receptor 1 (TNFR1) (Mardookhi et al. 2016).

Apart from damaging the brain cells, occlusion of cerebral vessels also could deteriorate the integrity of the blood vessels. Loss of vascular structural integrity will result in destruction of blood brain barrier (BBB) and would further aggravate the pathology by causing cerebral edema. Evans blue (EB) dye is a good tool for assessing the permeability of BBB due to its macrostructure (Banks et al. 2000) and high affinity for albumin (Wolman et al. 1981). A number of studies have assessed the effects of olive treatment on the integrity of BBB using EB. Significant reduction in extravasation of EB in brain tissue and also brain water content was observed in ischaemic rats following the administration of OLE (Mohagheghi et al. 2011). In a separate study, oleuropein reversed the bilateral common carotid artery occlusion and reperfusion-induced decrease in arteriolar diameter, increase in microvascular leakage and leukocyte adhesion to venules and reduction in capillary perfusion (Mastantuono et al. 2015). Thus, oleuropein is potentially a promising candidate to reduce secondary injury to the brain as a result of stroke.

In stroke, development of occlusion in the cerebral vessel was mostly caused by the formation of atherosclerotic plaque. High low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) levels are the leading causes for increased risk of atherosclerotic plaque formation. In the EUROLIVE randomized trial done in 2006, olive increased the levels of HDL, decreased total cholesterol-HDL ratio and LDL-HDL ratio (Poulsen et al. 2006). The severity of tissue damage from ischaemic insult can also be measured through the levels of lactate dehydrogenase (LDH) efflux *in vitro* (González-Correa et al. 2008). During the hypoxic stage, brain LDH level increases, whereas during the reoxygenation process, the LDH level drops. In the study conducted by González-Correa et al. (2008), the rats treated with virgin olive oil (VOO) for 7 days were subjected to hypoxia-reoxygenation and followed by assessment of LDH efflux. VOO which contains high levels of HT and HT-acetate, were found to reduce the LDH level during the hypoxic condition in a dose-dependent manner.

NEUROPROTECTIVE EFFECT OF OLIVE IN PERIPHERAL NEUROPATHY

Peripheral neuropathy is characterised by manifestations of physical symptoms such as tingling sensations, numbness, weakness and pain that are commonly attributed to type-2 diabetes mellitus (Chawla et al. 2016). Streptozotocin (STZ)-induced diabetes is one of the consensually accepted models for diabetic neuropathy in rats. STZ-induced diabetic rats are validated through their significant impairment of nerve conduction velocity (NCV), sciatic Na^+, K^+-ATPase activity (to measure structure or function of large myelinated fibers), thermal nociceptive threshold and mechanical nociceptive tolerance (as indicators of neuropathic pain), plasma thiobarbituric acid-reactive substances (TBARS, as indicator of oxidative stress). HT reversed most of these impairments, with significant improvements in thermal response latency, suggesting its protective role in diabetic neuropathy (Ristagno et al. 2012). In 2012, Ristagno et al. reported HT to significantly reduce

TABLE 1. Studies reporting on neuroprotective effects of olive and its phenolic compound in different disease etiology

Etiology	References	Substance	Type, Dose & Duration	Model	Findings		Conclusion
					Biochemical	Behavioral	
Cognition	Valls-Pedret et al. (2012)	Mediterranean diet + olive oil	Cross-sectional study from PREDIMED (RCT) 5 years	-	-	Cognitive performance	Consumption of olive oil resulted better cognitive performance in elderly cohort at high cardiovascular risk
	Pourkhodadad et al. (2016)	Oleuropein	*In vivo, Ex vivo*, 10, 15, 20 mg/kg p.o for 10 days	Colchine-induced intracerebral injection (0.5 µL) at hippocampal CA1 area in rats	*GPx, CAT **NO, MDA †Caspase 3	• Morris water maze - Spatial memory	• OLP improved learning and memory retention • OLP restored GPx and CAT activity • OLP reduced NO and MDA production
	Luceri et al. (2017)	H-EVOO L-EVOO	*In vivo*, 6mg/kg p.o for 6 months	Young mice (4-6 months old) Aged mice (16 months old)	• Gene expression eg: Notch1, Crctc-3, Bmp 7, FOXO, Insrr, Glp1r, NGFR, Myst3, nav1 • miRNA expression eg: miR-101a, miR-124, miR-30c-b/5p	• Step-down inhibitory avoidance test - contextual memory • Morris water maze - Spatial memory • Light-dark preference test - Anxiety • Grip strength test - Neuromuscular function	• H-EVOO up-regulate genes related to synaptic plasticity and motor & cognitive behaviour • H-EVOO treated rats performed better behavioral assessment compared to L-EVOO rats
	Lee et al. (2018)	Oleuropein	*In vivo*, 20, 50, 100 mg/kg i.p for 21 days	PTSD model: Single Prolonged Stress in rats	• Serum corticosteroid §TNF-α, IL-1β • hippocampal BDNF • CREB	• Object recognition task • Morris water maze - Spatial memory • Open field test	• OLP reversed SPS-induced cognitive and memory impairment • OLP up-regulated BDNF and CREB expression • OLP reduced expression of pro-inflammatory cytokines
Neurodege nerative disease: Alzheimer's disease	St-Laurent-Thibault et al. (2011)	Hydroxytyrosol Tyrosol	*In vitro* 50, 100 µM	AD model: Aβ-induced (100 µg/mL) in N2a cells	* GSH • Cell death: LDH assay • Cell apoptosis: NF-κβ	-	• HT & Tyr regulate NF-kB activity and protect against Aβ-induced cellular apoptosis • HT & Tyr significantly reduced LDH level at dose-dependent manner
	Luccarini et al. (2014)	Oleuropein aglycone	*In vivo* 450 µM injection to NBM, single dose and assessed after 30 days	AD model: Aβ42 (50 µM) injections at NBM in rats	• ChAT-positive neuron • Glial reaction • Aβ peptide level	-	OLP aglycone protect against formation of toxic species of Aβ42 aggregates
	Qosa et al. (2015)	Extra-virgin olive oil	*In vivo* 700 mg/day for 3 & 6 months	AD model: TgSwDI mice	• $A\beta_{40}$ and $A\beta_{42}$ peptide level • Total Aβ plaque level • ^{125}I-$A\beta_{40}$ clearance	• Burrowing test • Nest construction test	EVOO slowed progression of Aβ pathology in mice brain, enhance Aβ clearance and reduced tau protein phosphorylation

Continues TABLE 1.

Etiology	References	Substance	Type, Dose & Duration	Model	Findings		Conclusion
					Biochemical	Behavioral	
					• ApoE-dependent pathway: PPARγ, LXRs, RXR, sAPPα and sAPPβ level • Tau expression and phosphorylation		
	Batarseh et al. (2017)	Oleocanthal	*In vitro* 5 μM	AD model: CCF-STTG1 human astrocytoma cell line, SH-SY5Y cell line transfected with APP695 (SH-SY5Y-APP), non-transfected SH-SY5Y cells	IL-6, GFAP • Aβ monomer clearance: LRP1, IDE, ABCA1 • Astrocyte protein GLUT1, GLT1, NEP, GAPDH • Neuronal protein Synaptic markers: PSD-95, SNAP-25 LRP1, Soluble APPα, Soluble APPβ	-	Oleocanthal attenuated Aβo-induced inflammation, restored astrocyte neuro-supportive function by preventing Abo down-regulation effects on GLT1 and GLUT1 transporters in astrocytes, and attenuated Abo- induced synaptic protein down-regulation in SH-SY5Y-APP neurons
Parkinson's disease	Dewapriya et al. (2013)	Tyrosol	*In vitro* 50, 100, 200 μM	PD model: MPP+-induced (1 mM) CATH.a neuron cell death	*SOD-1, SOD-2, DJ-1 **ROS, NO †Bax, caspase 3, cytochrome c ‡Bcl-2, Bcl-$_{XL}$ • Cell viability of Tyr & MPP+ • phospho-Tyrosine hydroxylase • MMP • Intracellular ATP level • Akt, ERK pathways	-	Tyr reverse MPP+ induced mitochondrial dysfunction
	Yu et al. (2016)	Hydroxytyrosol	*In vitro* 20, 90 μM	PD model: 6-OHDA in SH-SY5Y cells	*NQO1, GST, GCL, HO-1 • Cell viability of 6-OHDA & HT	-	HT reverse 6-OHDA induced cell death and increased expression of phase II enzymes in dose-dependent mannner
	Aghagolzadeh et al. (2017)	Olive leaf extract	*In vivo* 50, 100, 150 mg/kg/day p.o for 7 weeks	PD model: Intrastriatal injection of 6-OHDA (10 μg) to rats	*SOD, GPx, CAT, GR, GSH **MDA	• Apomorphine-induced circling behavior • Narrow beam tes • Grip strength test	OLE improve motor coordination, increased anti-oxidant enzymes, reduced lipid peroxidation in PD rats
	Sarbishegi et al. (2018)	Olive leaf extract	*In vivo* 75, 150, 300 mg/kg/day p.o for 30 days	PD model: Subcutenoues injection of ROT(2.5 mg/kg/48h) to rats	*SOD, Gpx, CAT **MDA	• Rotarod test • Hanging test	• OLE (150 and 300 mg/kg) ameliorates ROT-induced motor dysfunction and weakness in rats

Continues TABLE 1.

Etiology	References	Substance	Type, Dose & Duration	Model	Findings		Conclusion
					Biochemical	Behavioral	
Ischaemic-Reperfusion Injury (Stroke)					Tyrosine hydroxylase-positive neurons		• OLE (150 and 300 mg/kg) increased anti-oxidant enzymes activity, reduced free radicals formation and prevent TH-positive neuron death
	Mohagheghi et al. (2011)	Oleuropein	*In vivo* 50, 75, 100 mg/kg/day p.o for 30 days	I-R model: Middle cerebral artery occlusion in rats	• Infarct volume • Brain water content (edema) • BBB permeability • Serum LDL, HDL	• Neurologic deficit scores	OLE improve BBB integrity and cerebral edema after MCAO and reduced neurologic deficit scores
	Hassanshahi et al. (2013)	Olive oil	*In vivo* 180 μL/day p.o for 7 days	I-R model: Common carotid artery ligation in mice	†Bax ‡Bcl-2	• Memory test: y-maze, shuttle box	Olive oil increase anti-apoptotic proteins in ischaemic hippocampus and decreased memory loss in mice
	Mastantuono et al. (2015)	Oleuropein	*In vivo* 10, 20 mg/kg i.v for 3 min, 10 min before ischemia	I-R model: Bilateral Common carotid artery ligation in rats	§§eNOS **ROS through DCF fluorescence intensity • Arteriolar diameter • Microvascular leakage • Leukocyte adhesion in venules • Capillary perfusion • Tissue damage evaluation via TTC staining	-	OLP increase arteriolar diameter, decrease microvascular leakage, increase capillary perfusion
	Mardookhi et al. (2016)	Olive oil	*In vivo* 0.25, 0.50, 0.75 mL/kg p.o for 30 days	I-R model: Middle cerebral artery occlusion in rats	• Cell apoptosis: NF-$\varkappa\beta$ §TNFR1 • Infarct volume	• Neurologic deficit scores	Olive oil (0.75 ml/kg) reduced infarct volume, improve neurologic deficits and attenuate expression of inflammatory mediators, TNFR1 and NF-$\varkappa\beta$
Peripheral Neuropathy	Kaeidi et al. (2011)	Olive leaf extract	*In vitro, In vivo* *In vitro*: 100, 200, 400, 600 μg/mL *In vivo*: 100, 300, 500 mg/kg for 3 weeks	Experimental diabetes: High-glucose medium in naïve and NGF-treated PC12 cells STZ-induced i.p injection (55 mg/kg) in rats	*In vitro* & *In vivo*: †Caspase 3, Bax ‡Bcl-2 • Serum glucose level • Body weight	• Tail-flick test: Nociceptive threshold	OLE (300, 500 mg/kg) attenuate hyperalgesia in diabetic rats
	Ristagno et al. (2012)	Hydroxytyr-osol	*In vivo* 10, 100 mg/kg/day p.o for 6 weeks	Experimental diabetes: STZ-induced injection (60 mg/kg) i.p in rats	**MDA • Nerve conduction velocity • Na+, K+-ATPase activity	• Hot plate paw withdrawal test: Thermal nociceptive threshold	HT improve NCV, reduced thermal nociceptive threshold impairment and MDA level

Continues TABLE 1.

Etiology	References	Substance	Type, Dose & Duration	Model	Findings		Conclusion
					Biochemical	Behavioral	
					• Plasma glucose, creatinine, cardiac troponin T, AST/GOT	• Randall-Selitto paw withdrawal test: Mechanical nociceptive tolerance	
Spinal Cord Injury	Khalatbary & Zarrinjoei (2012)	Oleuropein	*In vivo* 20 mg/kg i.p, single dose immediately or 1 h after trauma	Experimental SCI: Weight dropping technique in rats	§§iNOS, COX-2, PARP **Nitrotyrosine §TNF-α, IL-1β	-	OLP reduced SCI-induced inflammatory reactions through reduction of TNF-α and IL-1β
	Impellizzeri et al. (2012)	Oleuropein aglycone	*In vivo* 20, 40, 100 μg/kg i.p 1 and 6 h after trauma	Experimental SCI: Aneurysm clips to the dura of rats at T5-T8	*MPO §§PAR, iNOS **TBARS, Nitrotyrosine †Bax, NF-κβ p65, ‡Iκβ-α, caspase 3 Bcl-2 • Cell apoptosis via TUNEL-like staining §TNF-α, IL-1β • Histological damage • GDNF level • PKA activity	• Basso Mouse Scale - Motor recovery	• OLP aglycone reduced neutrophil infiltrations and improved motor recovery • OLP aglycone improve expression of GDNF
	Khalatbary & Ahmadvand (2012)	Oleuropein	*In vivo* 20 mg/kg i.p, single dose immediately or 1 h after trauma	Experimental SCI: Weight dropping technique in rats	*GSH **MDA †Bax ‡Bcl-2 • Cell apoptosis via TUNEL staining • Demyelination - Luxol fast blue staining	• Basso-Beattie-Bresnahan behavioral score test - hind limb motor function	OLP improve functional outcome of post-trauma SCI rats and attenuated myelin degradation

Abbreviations: 6-OHDA - 6-Hydroxydopamine, ABCA1 - ATP-binding cassette transporter, AD – Alzheimer's disease, APP – amyloid precursor protein, AST/GOT - aspartate aminotransferase/glutamic-oxaloacetic transaminase, Aβ – amyloid beta, BBB – blood brain barrier, BDNF - brain-derived neurotrophic factor, Bmp 7 - bone morphogenetic protein 7, CAT – Catalase, Crctc-3 - CREB- regulated transcription coactivator 3, CREB - cAMP response element-binding protein, DCF – 2'-7'-dichlorofluorescein, DJ-1 - Protein deglycase DJ-1, eNOS – endothelial nitric oxide synthase, FOXO - fork- head box transcription factors, GAPDH - glyceraldehyde-3-phosphate dehydrogenase, GCL - Glutamate cysteine ligase, GDNF - glial cell-derived neurotrophic factor, GFAP - Glial fibrillary acidic protein, Glp1r - glucagon-like peptide 1 receptor, GLT1- astrocyte glutamate transporter-1, GLUT1 - Glucose transporter 1, GPx – Glutathione Peroxidase, GR – glutathione reductase, GSH – reduced glutathione, GST - Glutathione S-transferase, H-EVOO – phenolic rich extra virgin olive oil, HDL - High-density lipoproteins, HO-1 - Heme oxygnase-1, HT – hydroxytyrosol, I-R – Ischemia-Reperfusion, IDE – insulin degrading enzyme, IL-6 – interleukin 6, IL-β – interleukin-β, iNOS - Inducible nitric oxide synthase, Insrr - insulin receptor, L-EVOO – phenolic deficient extra virgin olive oil, LDH – lactate dehydrogenase, LDL - Low-density lipoproteins, LRP1 – LDL receptor-related protein 1, LXRs - liver-X receptors, MCAO – middle cerebral artery occlusion, MDA – malondialdehyde, MMP – Mitochondrial membrane potential, MPP+ - 1-methyl-4-phenylpyridinium, Myst3 - histone acetyltransferase, nav 1 - neuronal navigator, NBM – nucleus basalis magnocellularis, NCV – nerve conduction velocity, NEP - nuclear export protein, NF-κβ – nuclear factor-kappa beta, NGF – nerve growth factor, NGFR - nerve growth factor receptor, NO – nitric oxide, NQO1 - NAD(P)H quinone oxidoreductase, OLE – olive leaf extract, OLP – oleuropein, oxLDL – oxidized LDL, PAR - poly-ADP-ribose, PD – Parkinson's disease, PKA – protein kinase A, PPARγ - peroxisome proliferator–activated receptor gamma, PREDIMED - Prevención con Dieta Mediterránea, PSD-95 - postsynaptic density protein 95, PTSD – post traumatic stress disorder, RCT - randomised controlled trial, ROS – reactive oxygen species, ROT – rotenone, RXR - retinoid-X receptor, sAPPα – soluble amyloid precursor protein α, sAPPβ – soluble amyloid precursor protein β, SNAP-25 - Synaptosomal-associated protein 25, SOD -1 – superoxide dismutase 1, SOD-2 - superoxide dismutase 2, STZ – streptozotocin, TBARS - Thiobarbituric acid reactive substances, TNF-α – tumor necrosis factor-α, TNFR1 - Tumor necrosis factor receptor 1, TTS - 2,3,5- triphenyl- tetrazolium chloride staining, TUNEL - Terminal deoxynucleotidyltransferase-mediated UTP end labeling, Tyr – tyrosol *Anti-oxidant enzymes, **Radicals, †Pro-apoptotic molecules, ‡Anti-apoptotic molecules, §Pro-inflammatory cytokines, §§Pro-inflammatory enzymes

diabetic-induced thermal hypoalgesia by approximately 34% in diabetic rats. The authors speculated that the attenuating effect towards thermal hyperalgesia seen was probably due to HT protection against degeneration of skin nerve fibers (Leonelli et al. 2007; Ristagno et al. 2012). In addition, administration of olive leaf extract (OLE) (300 and 500 mg/kg) to STZ-induced diabetic rats also significantly improved the tail flick latency (test to indicate pain response in rats). In the same study, OLE also profoundly decreased the hyperglycemia-induced activation of caspase 3 (denotes pro-apoptotic state) and other pro-apoptotic markers such as Bax, which propose that the amelioration of diabetic neuropathy in STZ-induced rats by OLE may be mediated by its anti-apoptotic potential (Kaeidi et al. 2011).

NEUROPROTECTIVE EFFECT OF OLIVE IN SPINAL CORD INJURY

Mechanical injury is often followed by series of inflammatory reactions, which in case of spinal injury could damage the non-regeneratable spinal tissues. Intraperitoneal administration of oleuropein (20 mg/kg) to rats following contusive spinal injury significantly reduced the inflammation-induced rise in expression of TNF-α and IL-1β (Khalatbary & Zarrinjoei 2012) via inhibition of NF-κβ and MAPK signaling-associated pathways (Feng et al. 2017). Follow up study on the injured rats showed oleuropein (20 mg/kg) to significantly improve the motor recovery of the rats (Khalatbary & Zarrinjoei 2012). Similar findings were also reported by Impellizzeri et al. (2012) where the researchers showed intraperitoneal injection of oleuropein aglycone (20, 40, 100 μg/kg) to improve the spinal cord injury-induced motor deficits.

CONCLUSION

Myriad of studies have reported neuroprotective potential of olive oil and its phenolic compounds in various *in vivo* and *in vitro* models of neurological disorders. The neuroprotective effects of olive and its derivatives are seem to be attributed to its anti-inflammatory, anti-oxidant and anti-apoptotic properties. Existing literature has thrown up numerous questions in need of further investigations such as the influence of olive on NF-κβ and TNFR1 in ischaemic injury, the mechanism of interaction between the phenolic compounds of olive and various pro- and anti-apoptotic proteins in neuroinflammation and the exact role of olives in innate anti-oxidant system. Therefore, more research is needed to elucidate the neuroprotective mechanism of olive and its components. There are no conflicting interests among the authors.

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