Olive Leaf (*Olea europaea* L. *folium*): Potential Effects on Glycemia and Lipidemia

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Keywords

Olive leaf · Diabetes · Glycemia · Lipidemia · Cardiovascular effects

Abstract

Background: Olive tree (*Olea europaea*, Oleaceae) leaves have been widely used in traditional herbal medicine to prevent and treat various diseases especially in Mediterranean countries. They contain several potentially bioactive compounds that may have hypoglycemic and hypolipidemic properties. **Summary:** The literature has recently been attempting to define the relationship between olive leaf (*Olea europaea* L. *folium*) polyphenols and a number of health problems. Oleuropein, the basic phenolic compound of olive leaf and its extract, is responsible for the characteristic bitter taste and unique aroma of olive fruits. Furthermore, it is shown that oleuropein and its hydrolyzed products have many beneficial effects on human health because of its antioxidant characters. A number of studies report that olive leaf has potentially positive effects on the parameters related to diabetes and cardiovascular diseases by various mechanisms. Besides, toxicity studies suggest that olive leaf is generally safe even at high doses. **Key messages:** Although current results obtained until today seem promising, the studies in this subject are usually on cell culture and animal trials. Moreover, mostly the extract forms of olive leaves are used

in the studies. More randomized controlled human clinical trials with extensive toxicity studies are needed to evaluate potential health effects and safety.

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Introduction

Olive tree (*Olea europaea*, Oleaceae) leaves have an extensive use in traditional herbal medicine with the aim of preventing and treating several diseases particularly in Mediterranean region. The related literature aims to identify the relationship between olive cultivars and diabetes, cardiovascular disease, cancer, and a number of health problems [1, 2].

Nowadays, there has been a considerable interest in natural antioxidants from plant materials to replace synthetic ones [3]. Among natural antioxidants, the olive tree has been widely accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves [4]. It is well known that the activity of the olive tree by product extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative damage [5]. The olive tree has long been recognized as having antioxidant molecules, such as oleuropein, hydroxytyrosol, and tyrosol; caffeic acid; and ligstroside associated with the prevention of certain diseases [6, 7].

The main active component in olive leaf and its extract is oleuropein, a natural product of the secoiridoid group. Several studies have shown that oleuropein possesses a wide range of pharmacologic and health-promoting properties including antiarrhythmic, spasmolytic, immune-stimulant, cardioprotective, hypotensive, anti-inflammatory, antioxidant, and anti-thrombotic effects [2, 8]. Many of these properties have been described as resulting from the antioxidant characters of oleuropein [2]. Previously, oleuropein was reported to have an antihyperglycemic, lipid-regulating, and cardioprotective effects especially in cell culture and animal models [9–13]. Furthermore, with regard to the antioxidant properties of oleuropein, its mechanism in attenuating hyperglycemia and cardiovascular risk parameters is still not well recognized. In this review, the effects of olive leaf and its extract on glycemia, lipidemia, and cardiovascular parameters were examined.

Methods

Literature in the PubMed, ScienceDirect, Google Scholar, Cochrane Library, SciELO, and MEDLINE databases for publications in English with the descriptors “*Olea europaea*”, “olive leaves”, “olive leaf”, “olive leaves extracts”, “olive leaf extracts”, “phenolic compounds”, “polyphenols”, “oleuropein”, “hydroxyl-tyrosol”, “chemical composition”, “health”, “glycemia”, “diabetes”, “hypolipidemic effects”, “cardiovascular effects”, “pharmacological effects”, “safety”, and “toxicology” was searched. Publications in languages other than English (which at least have an abstract in English or other languages available) were also included in the review.

Olive Leaf and its Chemical Structure

Literature on olive polyphenols principally has focused on olive oil consumption as a main dietary source. However, phenolic compounds are found more in olive leaves. For this reason, olive leaf (*Olea europaea* L. *folium*) is used in many fields including pharmaceuticals, cosmetics, and food industry [14].

Olive tree includes secoiridoids, carbohydrates, sugar alcohols, and terpenoids as biochemicals [15]. Basic components in olive leaf are secoiridoids such as oleuropein, ligstroside, I methyloleuropein, and oleoside; flavanoids such as apigenin, kaempferol, luteolin, and chrysoeriol; and phenolic compounds such as caffeic acid, tyrosol, and hydroxytyrosol [7, 16, 17].

Secoiridoids, chemical components of leaf, are glycosidically bound and produced by secondary metabolisms of terpenes as the pioneers of various indole alkaloids. Secoiridoids are generally derived from an oleoside type of glucoside oleosides that are characterized with the combination of elenolic acid and glucoside residues [18].

Oleuropein (Oleuropein 1), one of the secoiridoids, is a basic phenolic compound found in olive leaf and the reason of characteristic bitter taste of olive cultivars [18]. Oleuropein is an ester of 2-(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) and has the oleosidic skeleton that is common to the secoiridoid glucosides of

Oleaceae, mainly in its aglycone form, which makes the sugar moiety insoluble in oil [18, 19]. Upon hydrolysis, oleuropein can produce elenolic acid, hydroxytyrosol, tyrosol, and glucose [2]. Oleuropein and hydrolysis products found in olive leaf have important biological characteristics. It is considered that hydroxytyrosol is particularly correlated with health benefits of olive products [20].

Oleuropein content (6–14%) is very high in dry matter of olive leaves [8]. Oleuropein amount in olive leaves may vary depending on harvest season and rise up to 17–23% [21]. However, this complex phenol is found less in olive oil types except extra virgin olive oil, while it is highly available in olive trees and leaves [16, 18]. Table olive production processes increase the transformation of oleuropeins into hydrolysis products such as hydroxytyrosol and tyrosol, and as a result, decrease non-hydrolyzed oleuropein forms [22].

Mannitol, one of the other components of olive leaf, consists of 3% of dry weight of olive leaf and is used as an additive in food and drug industry due to its sweetness ratio and low energy content. The main triterpene from the olive leaf is oleanolic acid, and it consists of 3% of olive leaf's dry weight and has very important pharmacological features [6].

Olive Leaf Extract

There are olive leaf extracts dissolved in dry and various organic solvents. Extracts are produced from olive leaves [16]. Ethanol extract of olive leaves contains high amount (approximately 20%) of oleuropein. The oleuropein amount in extracts sold in liquid, capsule, or tablet form differs depending on the brand.

This amount varies according to extraction method and solvent types. In a study, high amount of oleuropein (37.8 mg/g dried leaf) was extracted through Soxhlet method using methanol, and the value was found to be 14.2 mg/g dried leaf after CO₂ modification supercritical fluid extraction method [23]. The study examined the effect of extraction solvent (type, composition, pH, and temperature) and method (maceration and Soxhlet) on oleuropein content of olive leaf extracts. According to study results, extraction with Soxhlet method produced more oleuropein compared to maceration carried out with 80% ethanol and 20% acetonitrile at high temperatures and low pH values (max. = 3) [24]. It is important to note that all olive leaf extracts must comply with Pharmacopoeia monographs and local/national legislations independently of extraction method and solvent type.

Metabolism and Bioavailability of Olive Leaf and its Extracts Components

Knowledge on the absorption and disposition of olive oil or olive leaf phenolic components were quite limited [25]. However, it has been reported recently that olive leaf extracts increase in plasma oleuropein and hydroxytyrosol [14]. Studies on the bioavailability of olive leaf extracts show that bioavailability changes individually and depends on gender and dose [14, 25]. In a study, 9 volunteers, including 5 men, were given oleuropein and hydroxytyrosol olive leaf extracts in capsule or liquid form in order to examine their bioavailability amount and mechanisms [14]. Conjugated metabolites of hydroxytyrosol were found as primary metabolites in plasma and urine after extract intake. All conjugated hydroxytyrosol metabolites were found in urine within 8 h after oleuropein. Plasma oleuropein peak concentration was higher after liquid extract intake compared to capsule extract. Moreover, there was a gender effect on the bioavailability of phenolic

compounds, with males showing greater plasma area under the curve for conjugated hydroxytyrosol [14]. Another study noted that tyrosol and hydroxytyrosol, phenolic compounds of olive oil, are absorbed in humans dose-dependently and excreted as glucuronide conjugates. Additionally, it was reported that increase in the dose of phenolic compounds also increased glucuronide conjugation rate [25].

Effects of Olive Leaf on Health Glycemia

Infusion and/or decoction of olive leaves have long been used in the treatment of diabetes [26]. Antidiabetic effect of oleuropein, main phenolic component of olive leaf, is shown in cell culture or animal models and limited number of studies conducted on humans [9–11]. Mechanisms correlated with the effects on glycemia and diabetes are shown in Figure 1.

The first mechanism suggested on the antidiabetic effect of olive leaf and extract is that they cause hypoglycemia. In a study with diabetic rabbits, it was found that ethanol extract of olive leaf decreased blood glucose [10]. Potential mechanism in this result is the increase in peripheral intake of glucose and insulin secretion induced by glucose [9, 10]. It was reported that olive leaf extract may increase glucagon-like peptide-1 secretion in *in vivo* and *in vitro* environment and thus can be used for nutrition treatment in Type 2 diabetes [11]. Another study examined the effect of 500 mg olive leaf extract on both diabetic patients and streptozotocin-induced diabetic rats for 1 week [27]. Results of the study showed that diabetic patients had lower HbA1c and fasting plasma insulin level, while there was no difference in postprandial plasma insulin level. In addition, there was a decrease in digestion and absorption of starch in intestines of animal model. According to these results, it is recommended that olive leaf extract can be used as an adjunct treatment for the normalization of glucose homeostasis in diabetic patients [27]. In another study, Wistar male rats were given olive leaf powder (6.25%) with standard diet orally for 6 weeks, and it was found at the end of this period that serum glucose level decreased significantly [28]. A systematic review and meta-analysis examined 8 clinical studies including 162 rats, and it was found that olive leaf extract increased insulin level significantly (4.83 μ IU/mL) and decreased blood glucose level (4.21 mg/dL) in diabetic rats [29].

Hydrolysis products of olive leaf polyphenols can also affect glycemia separately. It was reported that although there are limited number of studies about the effect of hydroxytyrosol, which is one of the most important hydrolysis products, on carbohydrate metabolism, it may have positive effects. Studies that conducted hydroxytyrosol application (50 mg/kg/day $\times$ 17 weeks, 20 mg/kg $\times$ 8 weeks, and 0.04% $\times$ 8 weeks) decreased plasma glucose concentrations and treated insulin resistance [30–32]. In another study, application of hydroxytyrosol (10 mg/kg/day) for 5 weeks decreased homeostatic model assessment-insulin resistance [33].

The effect of olive leaf and its extracts on body weight, which is one of the important parameters of glycemia and diabetes, is controversial. However, it is considered that limited number of studies share a common result that olive leaf extracts do not affect body weight [12, 34, 35]. A systematic review and meta-analysis study reported that there was not a significant change in body weight of rats after extract application [29]. Only one clinical study indicated that in relation to olive leaf extract (51.1 mg/day), hydroxytyrosol (9.67 mg/day) did not affect body weight of overweight men [36].

Fig. 1. Potential mechanisms and benefits of olive leaves on glycemia. AGE, advanced glycation end; GLP-1, glucagon-like peptide-1.

It is also suggested that olive leaf and its extracts may prevent some complications related to Type 2 diabetes. A study reported that methanolic extract of olive leaf inhibited protein glycation and decreased advanced glycation end products formation [37]. Another study demonstrated that olive leaf extract was able to cure glucose metabolism in liver and kidneys of rats by minimizing oxidative stress in rats [38]. Similarly, in a study conducted on rats, it was reported that 8 and 16 mg/kg doses of olive leaf extract rich in oleuropein and hydroxytyrosol decreased serum glucose level, cured antioxidant perturbations, and may, by this means, restrain oxidative stress correlated with diabetes pathology and its complications [34]. It was found in a study conducted with overweight men that supplementation of olive leaf (51.1 mg oleuropein, 9.7 mg hydroxytyrosol per day) in capsule form for 12 weeks increased fasting interleukin (IL)-6 and insulin-like growth factor-binding protein-1 and -2 concentrations. However, it did not change IL-8 and tumor necrosis factor- α levels. Moreover, 12-week supplementation increased insulin sensitivity by 15% and pancreatic β -cell capacity by 28% [36]. The study examining the effect of olive leaf and fruit extracts and oleuropein on β -cell toxicity induced by cytokine concluded that both extracts and oleuropein significantly decreased reactive oxygen species (ROS) induced by cytokine, enhanced abnormal antioxidant defense, and provided redox homeostasis [39]. In addition to this, in a study examining the effect of olive leaf extract on neuropathic pain in streptozotocin-induced diabetic rats and glucose-induced cells, it was found that 200, 400, and 600 μ g/mL of extract decreased cell damage [40]. Application of the extract at the doses of 300 and 500 mg/kg/day cured hyperalgesia, inhibited Caspase-3 activation, and decreased Bax (an apoptosis promoter)/Bcl₂ (an apoptosis inhibitory) ratio. These results showed that the extract inhibited neural damage induced by high glucose level/diabetes, suppressed thermal hyperalgesia, decreased neuronal apoptosis, and cured diabetic neuropathic pain [40]. It was also found that

Fig. 2. Potential mechanisms and benefits of olive leaves on lipidemia and cardiovascular disease. ACO, acyl-CoA oxidase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PPAR α , peroxisome proliferator activated receptor alpha.

oleanolic acid, another component of olive leaf, also increased Na⁽⁺⁾ excretion rate without affecting K⁽⁺⁾ and Cl⁽⁻⁾ rates, decreased plasma creatinine concentration, and enhanced renal profile in streptozotocin-induced diabetic rats [41]. These results imply that olive leaf extract may have a potential effect in the treatment of Type 2 diabetes and prevention of its complications.

Besides its positive effects on diabetes, there is very limited information on olive leaf and gut microbiota. The effects of dietary polyphenols on health depend on their bioavailability and systemic plasma concentrations change at nanomolar levels [35]. Determination of changes in olive leaf polyphenols before their absorption or their effects on gut microbiota is very important in terms of their local or systemic effects. In a previous study, rats were given olive leaf extract 20 mg/kg/day by intragastric gavage for 30 days. It was found that olive leaf extract could reduce the total aerobic bacterial count in rat caecal flora [42]. Another study demonstrated that olive oil bioactive extract attenuated the lipopolysaccharide-induced subclinical chronic inflammation on animal growth by improvements in intestinal integrity unrelated to alterations in gut microbial ecology and function [43].

Lipidemia and Cardiovascular Effects

Lipid-regulating and cardioprotective effects of oleuropein and olive leaf extracts were examined in cell culture, animals, and limited number of human studies/clinical trials [2, 12, 44, 45]. Potential effects and effect mechanisms of olive leaf extracts on lipid profile and cardiovascular parameters are shown in Figure 2.

Hypolipidemic effects of olive leaf extracts have been proved in a number of studies [12, 44, 45]. It was found that 50 and 100 mg/kg/day doses of olive leaf extract may positively affect atherosclerosis by decreasing total cholesterol and low-density lipoprotein (LDL) cholesterol levels in rats similar to atorvastatin (at a dose of

20 mg/kg). Another study demonstrated that orally taken hydroxytyrosol and triacetylated derivatives (3 mg/kg body weight) decreased serum total cholesterol, triglyceride, and LDL cholesterol levels and inhibited lipid peroxidation by increasing catalase and superoxide dismutase activity in rats fed with a diet rich in cholesterol for 16 weeks [12]. In rats fed with a high-fat diet, supplementation of olive leaf extract reversed chronic inflammation and oxidative stress correlated with plasma malondialdehyde [46]. Similar to previous in vivo and animal studies, olive leaf extract (136 mg oleuropein; 6 mg hydroxytyrosol) significantly decreased plasma total cholesterol (−0.32 mmol/L), LDL cholesterol (−0.19 mmol/L), and triglyceride (0.18) levels in 60 pre-hypertensive men after 6 weeks. In addition, IL-8 level also decreased [44].

Potential effects of olive leaf on lipid metabolism and inflammation emerge by means of metabolic pathways and inflammation-related genes [13, 47–50]. In a study supporting these results, it was found that daily 20 mL olive leaf extract supplementation given to 29 healthy male participants changed expression level of early growth response protein 1, cyclooxygenase-2, and inhibitor of DNA binding 3 genes in peripheral blood mononuclear cells [47]. A study conducted with ovariectomized rats showed that olive leaf extract supplementation at the doses of 200 or 400 mg/kg body weight decreased serum triglyceride level particularly at high doses [48]. Furthermore, it was determined that 1–10 μ g/mL concentration of oleuropein and hydroxytyrosol extracts inhibited ROS and protected cardiomyocytes against toxicity and carbonyl stress induced by 4-hydroxynonenal [13]. Another study found that oleuropein increased inducible nitric oxide synthase expression and nitric oxide production according to dose in mouse macrophages [50].

There are studies in the literature proving that olive leaf extract decreases endoplasmic reticulum stress, and thus it may also decrease myocardial infarction [51, 52]. A study demonstrated that hydroxytyrosol and oleuropein decreased apoptosis and endoplasmic reticulum stress, induced by acrolein, in cardiomyocyte cells by reducing 78-kDa glucose-regulated protein, the C/EBP homologous protein, and Bax expression levels in rats [52]. Another study supported the claim that hydroxytyrosol and olive leaf extract reversed the increase in C/EBP homologous protein and 78-kDa glucose-regulated protein expression levels induced by isoproterenol, and it may show cardioprotective effect by means of endoplasmic reticulum stress [51].

Safety of Olive Leaf and its Extract

Although there are strong evidence regarding biological activity of olive leaf extract and its components, there is limited knowledge on their systemic toxicity and reliability [53–55]. Toxicity studies show that olive leaf extracts are generally reliable and do not show toxic effect even at high doses [53, 54]. Results of a study showed that supplementation of olive leaf extract in male and female rats at single dose of 2,000 mg/kg (acute toxicity) and 100, 200, and 400 mg/kg doses (subacute toxicity) given for 28 days did not result in any toxicity [54]. Water soluble extract of olive leaf was given at the doses of 360, 600, and 1,000 mg/kg/day for 90 days and did not cause any mortality and toxicity [53]. Only one study reported some histological changes such as fatty cytoplasmic vacuolation, necrosis of the hepatocytes, and a slight hemorrhage in both liver and kidneys of rats after the supplementation of olive leaf extract (0.9%) for 6 weeks [56]. In addition, there is no adequate evidence about its genotoxic effects in the literature [16]. Thus, more studies are needed to fully understand the safety of olive leaf extracts for humans.

Conclusion

One of the natural antioxidant sources, olive leaf has been used in order to prevent and treat some diseases in traditional medicine for ages. Furthermore, olive leaf and its products such as olive oil are important component of Mediterranean diet. A large number of studies in the literature report that olive leaf has positive effects on the parameters related with diabetes and cardiovascular diseases through many mechanisms. It is possible to say that even the current results obtained until today seem promising. However, there are still numerous key points that need to be answered, and these will require further research. According to the literature, the studies in this subject are generally on cell culture and animal trials. Therefore, more randomized controlled human clinical trials are needed. Additionally, mostly the extract forms of olive leaf are used in the studies. So, potential effects of powder or dried olive leaf consumption on health should be taken into consideration in future studies. It should also be considered whether the individuals with diabetes and cardiovascular disease or those who take a routine medication have herb-drug interaction or not. In addition, potential health effects of olive

leaf should be evaluated with extensive toxicity studies, and further clinical studies should be carried out in this subject.

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Author Contributions

N.A.-T.: design and drafting the work or revising it critically for important intellectual content, final approval of the version to be published. D.A.: drafting the work or revising it critically for important intellectual content, final approval of the version to be published.

References

- 1 Boss A, Bishop KS, Marlow G, Barnett MP, Ferguson LR. Evidence to Support the Anti-Cancer Effect of Olive Leaf Extract and Future Directions. *Nutrients*. 2016 Aug;8(8):E513.
- 2 Hassen I, Casabianca H, Hosni K. Biological activities of the natural antioxidant oleuropein: exceeding the expectation – A mini-review. *J Funct Foods*. 2015;18:926–40.
- 3 Salah MB, Hafedh A, Manef A. Anti-diabetic activity and oxidative stress improvement of Tunisian Gerboui olive leaves extract on alloxan induced diabetic rats. *J Mater*. 2017;8:1359–64.
- 4 Servili M, Baldioli M, Selvaggini R, Macchioni A, Montedoro G. Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of Nüzhenide and its distribution in the constitutive parts of fruit. *J Agric Food Chem*. 1999 Jan;47(1):12–8.
- 5 Lins PG, Marina Piccoli Pugine S, Scatolini AM, de Melo MP. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. *Heliyon*. 2018 Sep;4(9):e00805.
- 6 Castellano JM, Delgado Hervás T, Guinda Garín MÁ, Gutiérrez-Adánéz P, Rada M, Santos-Lozano JM. Determination of major bioactive compounds from olive leaf. *Food Science and Technology – Zurich*. 2015;64(1):431–8.
- 7 Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, et al. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. *Inflammopharmacology*. 2009 Apr;17(2):76–84.
- 8 El SN, Karakaya S. Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. *Nutr Rev*. 2009 Nov;67(11):632–8.
- 9 Khan Y, Panchal S, Vyas N, Butani A, Kumar V. *Olea europaea*: a phyto-pharmacological review. *Pharmacogn Rev*. 2007;1(1):114–8.
- 10 Al-Azzawie HF, Alhamdani MS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sci*. 2006 Feb;78(12):1371–7.
- 11 Rafferty EP, Wylie AR, Elliott CT, Chevallier OP, Grieve DJ, Green BD. In vitro and in vivo effects of natural putative secretagogues of glucagon-like peptide-1 (GLP-1). *Sci Pharm*. 2011 Jul-Sep;79(3):615–21.
- 12 Jemai H, Fki I, Bouaziz M, Bouallagui Z, El Feki A, Isoda H, et al. Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. *J Agric Food Chem*. 2008 Apr;56(8):2630–6.
- 13 Bali EB, Ergin V, Rackova L, Bayraktar O, Küçükboyacı N, Karasu Ç. Olive leaf extracts protect cardiomyocytes against 4-hydroxynonenal-induced toxicity in vitro: comparison with oleuropein, hydroxytyrosol, and quercetin. *Planta Med*. 2014 Aug;80(12):984–92.
- 14 de Bock M, Thorstensen EB, Derraik JG, Henderson HV, Hofman PL, Cutfield WS. Human absorption and metabolism of oleuropein and hydroxytyrosol ingested as olive (*Olea europaea* L.) leaf extract. *Mol Nutr Food Res*. 2013 Nov;57(11):2079–85.
- 15 Guinda Á, Castellano JM, Santos-Lozano JM, Delgado-Hervás T, Gutiérrez-Adánéz P, Rada M. Determination of major bioactive compounds from olive leaf. *Lebensm Wiss Technol*. 2015;64(1):431–8.
- 16 EMA. Assessment report on *Olea europaea* L., folium. United Kingdom: Committee on Herbal Medicinal Products (HMPC); 2012. Report No.: EMA/HMPC/430506/2009.
- 17 Gariboldi P, Jommi G, Verotta L. Secoiridoids from *Olea europaea*. *Phytochemistry*. 1986;25(4):865–9.
- 18 Soler-Rivas C, Espín JC, Wichers HJ. Oleuropein and related compounds. *J Sci Food Agric*. 2000;80(7):1013–23.

- 19 Omar SH. Oleuropein in olive and its pharmacological effects. *Sci Pharm*. 2010 Apr-Jun; 78(2):133–54.
- 20 Echeverría F, Ortiz M, Valenzuela R, Videla LA. Hydroxytyrosol and Cytoprotection: A Projection for Clinical Interventions. *Int J Mol Sci*. 2017 Apr;18(5):930.
- 21 Le Tutour B, Guedon D. Antioxidative activities of *Olea europaea* leaves and related phenolic compounds. *Phytochemistry*. 1992; 31(4):1173–8.
- 22 Charoenprasert S, Mitchell A. Factors influencing phenolic compounds in table olives (*Olea europaea*). *J Agric Food Chem*. 2012 Jul;60(29):7081–95.
- 23 Şahin S, Bilgin M, Dramur MU. Investigation of Oleuropein Content in Olive Leaf Extract Obtained by Supercritical Fluid Extraction and Soxhlet Methods. *Sep Sci Technol*. 2011; 46(11):1829–37.
- 24 Yateem HA, Al-Rimawi F. Optimum Conditions for Oleuropein Extraction from Olive Leaves. *Int J Appl Sci Technol*. 2014;4(5):153–7.
- 25 Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, et al. Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Lett*. 2000 Feb;468(2–3):159–60.
- 26 Hashmi MA, Khan A, Hanif M, Farooq U, Perveen S. Traditional Uses, Phytochemistry, and Pharmacology of *Olea europaea* (Olive). *Evid Based Complement Alternat Med*. 2015; 2015:541591.
- 27 Wainstein J, Ganz T, Boaz M, Bar Dayan Y, Dolev E, Kerem Z, et al. Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. *J Med Food*. 2012 Jul; 15(7):605–10.
- 28 Ghosian Moghaddam M, Masomi Y, Razavian M. The effect of oral consumption of olive leaves on serum glucose level and lipid profile of diabetic rats. *J Basic Clin Pathophysiol*. 2013;1(2):39–44.
- 29 Abunab H, Dator WL, Hawamdeh S. Effect of olive leaf extract on glucose levels in diabetes-induced rats: A systematic review and meta-analysis. *J Diabetes*. 2017 Oct;9(10): 947–57.
- 30 Cao K, Xu J, Zou X, Li Y, Chen C, Zheng A, et al. Hydroxytyrosol prevents diet-induced metabolic syndrome and attenuates mitochondrial abnormalities in obese mice. *Free Radic Biol Med*. 2014 Feb;67:396–407.
- 31 Hamden K, Allouche N, Damak M, Elfeki A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. *Chem Biol Interact*. 2009 Aug;180(3):421–32.
- 32 Tabernero M, Sarriá B, Largo C, Martínez-López S, Madrona A, Espartero JL, et al. Comparative evaluation of the metabolic effects of hydroxytyrosol and its lipophilic derivatives (hydroxytyrosyl acetate and ethyl hydroxytyrosyl ether) in hypercholesterolemic rats. *Food Funct*. 2014 Jul;5(7):1556–63.
- 33 Pirozzi C, Lama A, Simeoli R, Paciello O, Pagano TB, Mollica MP, et al. Hydroxytyrosol prevents metabolic impairment reducing hepatic inflammation and restoring duodenal integrity in a rat model of NAFLD. *J Nutr Biochem*. 2016 Apr;30:108–15.
- 34 Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. *J Agric Food Chem*. 2009 Oct; 57(19):8798–804.
- 35 Peyrol J, Riva C, Amiot MJ. Hydroxytyrosol in the Prevention of the Metabolic Syndrome and Related Disorders. *Nutrients*. 2017 Mar; 9(3):306.
- 36 de Bock M, Derraik JG, Brennan CM, Biggs JB, Morgan PE, Hodgkinson SC, et al. Olive (*Olea europaea* L.) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: a randomized, placebo-controlled, crossover trial. *PLoS One*. 2013;8(3):e57622.
- 37 Kontogianni VG, Charisiadis P, Margianni E, Lamari FN, Gerothanassis IP, Tzakos AG. Olive leaf extracts are a natural source of advanced glycation end product inhibitors. *J Med Food*. 2013 Sep;16(9):817–22.
- 38 Salah MB, Abdelmelek H, Abderraba M. Effects of olive leaf extract on metabolic disorders and oxidative stress induced by 2.45 GHz WIFI signals. *Environ Toxicol Pharmacol*. 2013 Nov;36(3):826–34.
- 39 Cumaoglu A, Ari N, Kartal M, Karasu Ç. Polyphenolic extracts from *Olea europea* L. protect against cytokine-induced β -cell damage through maintenance of redox homeostasis. *Rejuvenation Res*. 2011 Jun;14(3):325–34.
- 40 Kaeidi A, Esmacili-Mahani S, Sheibani V, Abbasnejad M, Rasouljan B, Hajializadeh Z, et al. Olive (*Olea europaea* L.) leaf extract attenuates early diabetic neuropathic pain through prevention of high glucose-induced apoptosis: in vitro and in vivo studies. *J Ethnopharmacol*. 2011 Jun;136(1):188–96.
- 41 Mapanga RF, Tufts MA, Shode FO, Musabayane CT. Renal effects of plant-derived oleoanolic acid in streptozotocin-induced diabetic rats. *Ren Fail*. 2009;31(6):481–91.
- 42 Kiraz A, Simsek T, Tekin SZ, Elmas S, Tekin M, Sahin H, et al. Investigation of the effects of oleuropein rich diet on rat enteric bacterial flora. *Bratisl Lek Listy*. 2016;117(12):734–7.
- 43 Liehr M, Mereu A, Pastor JJ, Quintela JC, Statts S, Rimbach G, et al. Olive oil bioactives protect pigs against experimentally-induced chronic inflammation independently of alterations in gut microbiota. *PLoS One*. 2017 Mar;12(3):e0174239.
- 44 Lockyer S, Rowland I, Spencer JP, Yaqoob P, Stonehouse W. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. *Eur J Nutr*. 2017 Jun;56(4): 1421–32.
- 45 Olmez E, Vural K, Gok S, Ozturk Z, Kayalar H, Ayhan S, et al. Olive Leaf Extract Improves the Atherogenic Lipid Profile in Rats Fed a High Cholesterol Diet. *Phytother Res*. 2015 Oct;29(10):1652–7.
- 46 Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. *J Nutr*. 2010 May;140(5):946–53.
- 47 Boss A, Kao CH, Murray PM, Marlow G, Barnett MP, Ferguson LR. Human intervention study to assess the effects of supplementation with olive leaf extract on peripheral blood mononuclear cell gene expression. *Int J Mol Sci*. 2016 Dec;17(12):2019.
- 48 Yoon L, Liu YN, Park H, Kim HS. Olive Leaf Extract Elevates Hepatic PPAR α mRNA Expression and Improves Serum Lipid Profiles in Ovariectomized Rats. *J Med Food*. 2015 Jul; 18(7):738–44.
- 49 Efentakis P, Iliodromitis EK, Mikros E, Papa-christodoulou A, Dagnes N, Skaltsounis AL, et al. Effects of the olive tree leaf constituents on myocardial oxidative damage and atherosclerosis. *Planta Med*. 2015 Jun;81(8):648–54.
- 50 Visioli F, Bellosta S, Galli C. Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. *Life Sci*. 1998;62(6):541–6.
- 51 Wu LX, Xu YY, Yang ZJ, Feng Q. Hydroxytyrosol and olive leaf extract exert cardioprotective effects by inhibiting GRP78 and CHOP expression. *J Biomed Res*. 2018 Sep;32(5):371–9.
- 52 Xu Y, Wu L, Chen A, Xu C, Feng Q. Protective effects of olive leaf extract on acrolein-exacerbated myocardial infarction via an endoplasmic reticulum stress pathway. *Int J Mol Sci*. 2018 Feb;19(2):493.
- 53 Clewell AE, Béres E, Vértési A, Glávits R, Hirka G, Endres JR, et al. A comprehensive toxicological safety assessment of an extract of *Olea Europaea* L. leaves (Bonolive™). *Int J Toxicol*. 2016 Mar-Apr;35(2):208–21.
- 54 Guex CG, Reginato FZ, Figueredo KC, da Silva AR, Pires FB, Jesus RD, et al. Safety assessment of ethanolic extract of *Olea europaea* L. leaves after acute and subacute administration to Wistar rats. *Regul Toxicol Pharmacol*. 2018 Jun;95:395–9.
- 55 Kendall M, Batterham M, Obied H, Prenzler PD, Ryan D, Robards K. Zero effect of multiple dosage of olive leaf supplements on urinary biomarkers of oxidative stress in healthy humans. *Nutrition*. 2009 Mar;25(3):270–80.
- 56 Omer SA, Elobeid MA, Elamin MH, Hassan ZK, Virk P, Daghestani MH, et al. Toxicity of olive leaves (*Olea europaea* L.) in Wistar albino rats. *Asian J Anim Vet Adv*. 2012;7(11): 1175–82.