REVIEW ARTICLE

Olive leaf and its various health-benefitting effects: a review study

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ABSTRACT

Olive tree (*Olea europaea* L.) is an evergreen plant, cultivated ever since ancient times and has widely been used in traditional medicine. Mediterranean diet, rich in olive and its derivatives has been associated with a lower incidence of cardiovascular diseases, cancer, inflammation and stroke. In addition to olive and its oil, olive leaf and its various beneficial properties has lately been drawing the attention of many researchers. It has been postulated that many of the beneficial effects of OLE root from the strong antioxidant effects, exerted majorly by components such as oleuropein and hydroxytyrosol. Many considerable health-benefits of olive leaf extract (OLE) have been described in the literature, such as the effects on cardiovascular system, anticancer effects, antimicrobial effects, hypoglycemic effects and so on. In this study the antioxidant effects and some of the other health-benefitting effects of OLE are reviewed.

Keywords: Olive leaf, oleuropein, biological activities, metabolism

INTRODUCTION

Ever since ancient times, olive tree has been a symbol of peace, glory and abundance, its leafy branches rewarded to the victorious side in games or wars as a crown[1]. It is among the oldest agricultural tree crops, and has been a main source of oil since many years ago. It seems to have been cultivated since prehistoric times, and has been in close association with human religion, culture, medicine and nutrition[2].

Olive is a member of the family *Oleaceae*, consisting of about 30 genres and 600 species. The genus *Olea* L. includes more than 30 species, that are distributed across Europe, Asia, Oceania and Africa, among which *Olea europaea* L. is the only one that's cultivated. It is divided into five subspecies including: *laperrinei* in Saharan massifis, *cuspidata* in South Africa to south of Egypt and from Arabia to northern India and south west of China, *guanchica* in the Canary Islands, *maroccana* in south-western Morocco and *cerasiformis* in Maderia[3].

Olive tree is a hardy, perennial evergreen plant that can survive in many environments, but is mostly distributed in tropical and subtropical areas, accommodated to a semi-aired environment including calcareous and sandy, sunny slopes. It is 3-12meters or even more in height, and can bear fruit for over 1000 years in some instances. It has numerous branches, with oppositely aligned leaves that are leathery and lanceolate, dark green on the above and silvery beneath. the olive fruit is a globular, oblong or sometimes crescent-shaped stone fruit, consisting of : a) the mesocarp, the fleshy portion, which is edible after processing, and from which edible oil is extracted, b) the exocarp, the hairless skin, containing stomata, which is also edible after processing, and c) the endocarp enclosing the seed[2,4,5].

The term "olive leaves" refers to a combination of both leaves and branches produced at the end of the pruning of olive trees and the harvesting and cleaning of olives. They are a by-product of olive orchards, found in high amounts in the olive oil industries. They comprise almost 25kg per olive tree and 5% of the weight of harvested olives at the oil factories[1,6].

Olive and olive oil are a main portion of the Mediterranean diet, which is associated with a lower incidence of cardiovascular diseases, cancer, inflammation and stroke[7,8]. Besides, the olive leaf is also rich in various bioactive com-pounds such as polyphenols. For example, oleanolic acid, maslinic acid,ursolic acid, erythrodiol, and uvaol have been identified from oliveleaves[9]

Olive leaf is used as a traditional herbal tea, with many curing effects, among Mediterranean people[1]. It also has been used as a traditional treatment for arteriosclerosis, rheumatism, gout, diabetes mellitus and fever[7]. In addition, olive leaves have traditionally been used to treat neurologic and rheumatic diseases in Lebanon, and also to relief muscle and joint pain in some regions of Iran[10].

In the recent years, the potential health benefits of olive leaf, has raised increasing interest among scientists, with antioxidant, antiatherosclerotic, antihypertensive, antimicrobial and anti-mutagenic effects reported in various studies.In this study the different components of olive leaf extract, their metabolism and bioavailability, and the different healing properties of this magical -yet underrated- gift of nature are reviewed.

Components and structure: Olive leaf extract, also known as OLE, is a bitter-tasting, brown colored liquid, derived from the leaves of the olive tree (*Olea europaea* L.,Oleaceae)[11]. Olive leaves have been the subject of many studies recently, due to their many beneficial effects on human health. Most of these effects have been attributed to the phenolic compounds in olive leaves. Olive polyphenols are produced as a result of response to pathogen attack and insect injuries in olive trees[1].

The polyphenol compounds found in olive leaf are divided into five principal groups[1]:

a) oleuropeosides consisting of oleuropein and verbascoside; **b)** flavones consisting of luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin, and diosmetin; **c)**flavonols (rutin) **d)** flavan-3-ols (catechin); **e)** substituted phenols consisting of tyrosol, hydroxytyrosol, vanillin, vanillic acid, and caffeic acid.

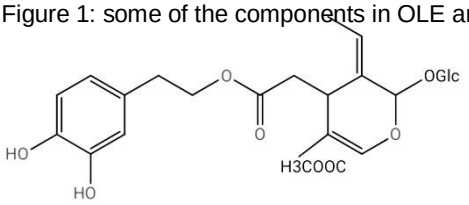
In addition, olive leaf contains triterpenes (oleanolic and maslinic acid) and chalcones (olivin, olivin-diglucoside)[7].

Oleuropein is the most abundant component in OLE, followed by hydroxytyrosol, a precoursor of oleuropein, and verbascoside, a conjugated glucoside of hydroxytyrosol and caffeic acid[1]. Hydroxytyrosol and tyrosol are the components responsible for the bitter taste, astringency, and resistance to axidation in olive fruit[12]. The chemical structure of some of these compounds (Fig. 1).

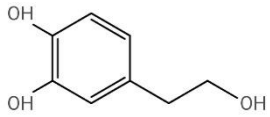
It should be noted that similar to many natural products, the composition of components in OLE may vary depending on developmental phase, season of the year, climatic conditions, genetic diversity of tree lineage, geographical location, plant nutrition and cultiva[11,13]r The results of the study by Hashemi *et al.* indicate that the amounts of oleuropein in OLE may vary in different seasons and also depending on the climate of the cultivation area, hot seasons and temporal regions decreasing the amounts of oleuropein substantially[14].

In several studies regarding the toxic effects of oleuropein and its metabolites, these compounds have proved completely non-toxic in different animal species. No lethality or adverse effects for oleuropein were observed in mice, even when administered in doses as high as 1000 mg/kg. Also, when injected into fertilized eggs, oleuropein showed no interference with normal embryonic development. More systematic efforts are required to assess the safety of oleuropein and its metabolites in humans[15].

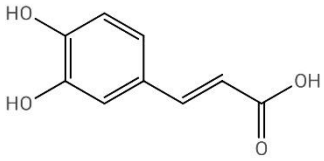
Figure 1: some of the components in OLE and their chemical structure.



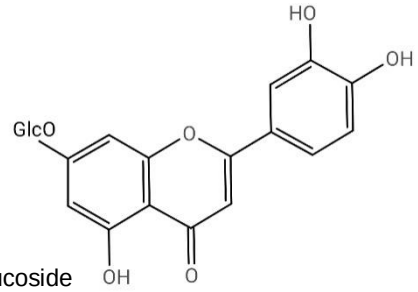
Oleuropein



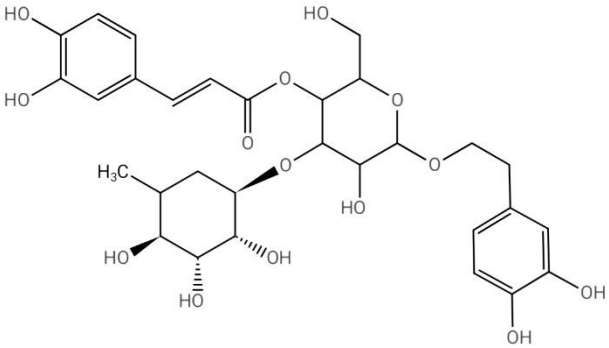
Hydroxytyrosol



Caffeic acid



Luteolin-7-glucoside



Verbascoside

Bioavailability and metabolism: The various properties of olive leaf and its components, and their health benefitting features have been widely studied in vitro, in animal studies, or in few cases, in humans. But many a feature of a component might prove mostly useless in clinical setting, if it is poorly absorbed or has a low bioavailability. Therefore in order to apply these features to practical and clinical conditions, and to be able to further improve these effects, an understanding of the mechanism of absorption and bioavailability of these components is required.

Phenolic compounds and their derivatives, tyrosol and hydroxytyrosol for instance, are absorbed in a dose-

dependent manner. Even with moderate doses (25 mL/d), which are lower than the average daily intake in the Mediterranean diet, almost 98% of the phenolic compounds can be detected in plasma and urine in conjugated forms, mostly glucuronidated and sulfated. In the process of passing the epithelial cells of the GI tract, polyphenols are subject to a classic phase II/III biotransformation, and an important first pass metabolism. This process takes place to the extent that the polyphenols, in their free-form are deemed undetectable in plasma and urine. Therefore, it is not surprising that some authors note that the attained concentrations of these compounds after absorption, are too low to explain the effects observed in *in vitro* and in *in vivo* studies with much higher concentrations. There are still, hypotheses explaining the findings of nutritional interventional studies, where the effects of olive derivatives are related to their phenolic content in a dose-dependent manner. In one hypothesis it is postulated that the conjugated forms of polyphenols are active, exerting the effects observed following the ingestion of the compounds. In another hypothesis it is proposed that the conjugated forms of polyphenols might act as a depot, and that the glucuronide and/or the sulfate moieties are eventually removed intracellularly. None of these hypotheses have yet been fully corroborated experimentally[16].

Edgecombe *et al.* studied the in situ intestinal absorption of oleuropein in rats, under both iso-osmotic and hypotonic luminal conditions. Although the mechanism of absorption was mostly unclear, the permeability of oleuropein was significantly greater in hypotonic conditions. Given the fact that oleuropein contains a glucose moiety, it was thought in this study, that it could be absorbed through the glucose transporters present in the GI tract epithelial cells. There are two glucose transporters in the GI tract that might be involved in the absorption of oleuropein, one is a facilitated-diffusion glucose transporters (GLUT2), and the other is a sodium-dependent glucose transporter (SGLT1), using active transport to move glucose across a concentration gradient. GLUT2 is present on the basolaterl surface of epithelial cells, and SGLT1 on the apical surface, therefore, it is feasible for oleropein to enter the epithelial cell through SGLT1, and eventually the circulation through GLUT2. However, this theory is not supported by the results of the preliminary work in this study, using phlorizin, an inhibitor of SGLT1, which did not alter the absorption of oleuropein, suggesting little role for SGLT1 in this process. Although, this finding might be due to the higher affinity of oleuropein for SGLT1, compared to phlorizin, resulting in phlorizin not exerting its inhibitory effect on SGLT1 in the process of the absoption of oleuropein. Another theory suggests a paracellular basis for the absoption of oleuropein. Although the fact that the average diameter of the paracellular pores between the epithelial cells in GI tract is smaller compared to the large molecule of oleuropein, might contradict this theory, it might be incorrect to consider the paracellular pores as cylindrical and one-dimensional. It might rather be more correct to view them as three dimensional gaps, in which, only one dimension might be restrictive for the oleuropein molecule, whereas the other two might be able to let the oleuropein molecule through, if it is in the right orientation. Another explanation for the paracellular theory of absorption, suggests that there is a small subpopulation of paracellular junctions that are larger than the average size of the normal paracellular pores. These larger paracellular junctions, might be in charge of the intestinal permeability of oleuropein via the paracellular routes. It should be of note that the role of lymphatic absorption and bile is excluded in this study. It is possible that oleuropein is absorbed through the lymphatic system as well, if presented in an oily matrix, and bile has been shown to open paracellular junctions in the intestine, which could possibly increase the paracellular absorption of oleuropein. Therefore, it might be that the absorption of oleuropein in humans, when taken orally, is actually higher than what is predicted in this study, using an in-situ intestinal perfusion mode[17].

It has also been reported that oleuropein is rapidly absorbed, reaching maximal plasma concentration two hours after oral administration[12].

In the study by de Bock *et al.* in 2013, the bioavailability and metabolism of oleuropein and hydroxytyrosol, when taken as OLE, was evaluated. In this study nine volunteers (five men) aged 42.8±7.4, randomly received either capsulated or liquid OLE, and plasma and urine samples were collected at fixed intervals for 24h after ingestion, and were analyzed for their phenolic content. The liver function parameters including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, and international normalized ratio were unaltered following the ingestion of OLE. Conjugated hydroxytyrosol metabolites (glucuronidated and sulfated) were the most abundant OLE phenolic metabolites detected in plasma (96-99%), with oleuropein amounts much lower. Conjugated metabolites of hydroxytyrosol were also the primary metabolites detected in urine, unaffected by preparation, dose or gender. The peak plasma concentration of metabolites was reached earlier in liquid preparation compared to capsulated type, and the liquid type also lead to greater oleuropein peak levels and AUC (area under curve) in plasma. There was a significant gender effect as well, in which the peak plasma concentration of conjugated metabolites of hydroxytyrosol was higher in men. Men also had 4.5 times higher AUC for plasma conjugated hydroxytyrosol, but three times lower AUC for plasma oleuropein, compared to women. According to the findings in this study, absorption and metabolism of OLE phenolic compounds, and their renal clearance are relatively rapid as well. The bioavailability and metabolism of these compounds and their dependence on factors like type of preparation and gender need further verification through larger studies[18].

HEALTH-BENEFITTING PROPERTIES

Many health benefitting properties for OLE have been reported in the literature, including antioxidant effects, cardioprotective effects, anticancer effect, antimicrobial effect, anti-inflammatory effect and many other. Some of these properties and their mechanisms are described below.

Antioxidant effects: The certain disadvantages to synthetic antioxidants, possible toxic effects for instance, has given rise to increasing interest in finding natural

antioxidants from herbal origins, and in recent studies such compounds have been widely studied[19].

Olive phenolics have exhibited powerful antioxidant activities, both in vitro and in vivo.[20] The beneficial effects of diets rich in vegetables, like olive and its products, have widely been attributed to antioxidant effects exerted by these compounds. Due to a very high oleuropein content, olive leaves are of the highest antioxidant and scavenging capacity among the different parts of olive tree (oleuropein makes up for up to 264mg/g of dry leaf when expressed as tyrosol equivalent)[1].

It is known that the structure of phenolic compounds is an important determinant of their antioxidant capacity; therefore, it is suggested that the strong antioxidant and scavenging capacity of OLE might be due to the high content of several phenolic compounds and hydroxyl groups in their structure[7].

Olive biphenols and secobiphenols are shown to react stochiometrically with free radicals forming more stable variants.[21] Oleuropein, the major component in olive leaf extract, and its metabolite hydroxytyrosol, both possess a cathecol group in their structure, which is required for optimum antioxidant/scavenging activity. Both oleuropein and hydroxytyrosol have been reported to scavenge superoxide anion as well as inhibiting the respiratory burst of neutrophils and hypochlorous acid-derived radicals. Also, with oleuropein showing greater activity, they both scavenged hydroxyl radicals. The antioxidant activity of oleuropein could also be associated to its ability to chelate metal ions such as Fe and Cu (iron and copper), which have a catalyzing role in reactions generating free radicals, along with its ability to inhibit several inflammatory enzymes such as lipoxygenases, without affecting the cyclo-oxigenase pathway[1].

The study by Kontogianni *et al.* showed different components extracted from olive leaves, such as oleuropein, luteolin glucosides, flavonoids and hydroxytyrosol, to have similar antioxidant potency.[22] There are studies suggesting that the flavonoids, oleuropeosides, and substituted phenols in olive leaf, exhibit a synergistic effect in their scavenging ability in mixed form, as occurred in olive leaf extract.[1]

The study by Salta *et al.* on the antioxidant capacity of olive leaf extract on commercially available oils such as olive oil, sunflower oil and palm oil, showed that both antioxidant capacity and antioxidant stability were substantially improved for all oils studied, with OLE showing higher antioxidant activity compared to butylated hydroxyl toluene (BHT).[23] De Leonardis *et al.* also showed that the addition of a hydroxytyrosol-rich extract to food lipids such as butter, lard, and cod liver oil increased the oxidative stability of these lipids.[24] In the study by Bouaziz *et al.* enrichment of refined olive and husk oils with olive leaf extract resulted in increased resistance to oxidative deterioration, and it was suggested in this study that OLE could be used as a competent substituent for synthetic antioxidants[25].

Many common diseases such as cancer, atherosclerosis, rheumatoid arthritis and degenerative processes associated with aging, are suggested to involve uncontrolled lipid oxidation and inflammation. A strategy for preventing and treatment of such conditions, could be decreasing this excess oxidation process by exogenous intake of antioxidant agents, olive products for instance[21].

A large body of studies have demonstrated the protective effect of antioxidant agents against DNA damage, a fundamental process in carcinogenesis. It is speculated, that this might suggest the attribution of anti-cancer effects exhibited by OLE, to its highly significant antioxidant activity[15].

The involvement of low density lipoproteins (LDL) in oxidative processes, plays a major role in the onset of atherosclerotic lesions. Several biphenols such as oleuropein, hydroxytyrosol, and luteolin have shown significant protective effect against copper sulphate-induce oxidation of LDLs in a dose-dependent manner, therefore reducing LDL atherogenic potential[21].

The complications occurred in diabetes mellitus such as nephropathy and retinopathy, are often a result of a state of increased oxidative stress. In the study by Al-Azzawie *et al.* it was demonstrated that OLE treatment in diabetic rabbits resulted in decreased levels of malondialdehyde, as a parameter for increased oxidative stress, along with a significant decrease in the activities and levels of most enzymatic and non-enzymatic antioxidant agents, suggesting strong antioxidant activity for OLE in diabetic state. It was suggested in this study that treatment with OLE in diabetic patients could reduce the complications resulting from oxidative stress in diabetic patients[26].

The protective effect of Oleuropein in myocardial ischemia and cerebral ischemic injury have also been suggested to be in attribution to its antioxidant effect, protecting the tissue against oxidative damage induced in such events[7].

Cardiovascular effects: The lower incidence of heart disease in the Mediterranean region has been associated to the high content of phenolic compounds in olive and its product, making up a large portion of the diet in this area. The cardiovascular-benefitting effects of OLE have been widely studied, and attributed to the main components, oleuropein and oleacein[1].

The cardiovascular effects of OLE can be explained in separate parts, as described below:

Anti-hypertensive effects: In 1990, Zarzouelo *et al.* studied the hypotensive and vasodilatory effects of the decoction of olive leaf in rats. In this study, following intravenous administration of different doses of lyophilized decoction of olive leaf, a marked hypotensive effect was observed in normotensive rats. Also the decoction was able to induce smooth muscle relaxation in isolated rat aorta, both in presence and absence of endothelium, indicating that the vasodilatory effect was independent of vascular endothelium integrity.[27]

In another study by Khayyal *et al.* [28]the hypertensive activity of OLE was demonstrated. In this study which took place in two phases, both the prophylactic hypotensive effect and the hypotensive effect of OLE were assessed. In the first phase of the study on the prophylactic effects of OLE, male Wistar rats were divided into three groups; a control group receiving physiological saline, the second group treated with a daily dose of 50mg/kg of L-NAME for eight weeks, and the third group receiving the same treatment of L-NAME along with concomitant administration

of either 25, 50 or 100mg/kg dose of OLE. By the end of the eighth week, the rats in L-NAME group had developed 85% increased blood pressure over initial values. In the group treated with OLE, concomitant treatment with a 25mg/kg dose of OLE was not effective on preventing the rise in blood pressure, while 50mg/kg dose of OLE had reduced the increase in blood pressure to only 51%, and 100mg/kg dose of OLE treatment had completely prevented the rise in blood pressure induced by L-NAME. This study proceeded to assess the hypotensive effect of OLE in rats with established hypertension. The animals were rendered hypertensive by daily treatment with L-NAME for six weeks, and then were divided into two subgroups; one receiving treatment with OLE along with continued treatment of L-NAME, and the other continued to receive L-NAME alone for another six weeks period. By the end of week 12, animals in L-NAME group had developed 87% rise in blood pressure; whereas the animals in L-NAME+OLE group, who had developed 46% increase in blood pressure after six weeks of treatment with L-NAME, had only 12% higher blood pressure compared to normal values after receiving concomitant treatment with OLE for six weeks. It has been established that the effects of L-NAME on increasing blood pressure are not only due to inactivation of nitric oxide, but are also associated with generation of reactive oxygen radicals, and the administration of antioxidant scavenging agents might block such effect from taking place. Therefore, given the strong scavenging capacity of OLE, it is possible that the observed hypotensive effect might be due to such actions.

In addition to mechanisms described above, other mechanisms of action for the hypotensive effect of OLE have been suggested. Inhibiting effect of oleacein on angiotensin converting enzyme (ACE), and calcium channel blocking activity of β-(3,4-dihydroxy-phenyl)ethanol, an active constituent of *Olea europaea,* are examples of such mechanisms. It would therefore seem that the hypotensive activity of OLE is a result of simultaneous action of various mechanisms[28].

Anti-atherosclerotic and anti-hyperlipidemic effects: Jemai *et al.* studied the effect of OLE on cholesterol-fed rats. Hyperlipidemia induced by 16weeks of cholesterol-rich diet resulted in elevated levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). The simultaneous administration of oleuropein, oleuropein aglycone and hydroxytyrosol-rich OLE resulted in significant decrease in the levels of TC, TG and LDL-C compared to cholesterol-fed rats. In addition OLE was able to increase the levels of high density lipoprotein cholesterol (HDL-C) by 60% in rats treated with OLE. It was also shown in this study that, through its antioxidant action, oleuropein-, oleuropein aglycone- and hydroxytyrosol-rich extract exhibited protective effect against experimentally induced atherogenesis[6]. A dose-dependent reduction in lipid oxidative damage, inversely related to the phenolic content, has also been reported for olive oil[29].

In a similar study the low-dose administration of hydroxytyrosol (25mg/kg) has proved to significantly lower the serum levels of TC and LDL-C, while increasing the levels of HDL-C, in cholesterol-fed rats[30]. The same effects on TC, LDL-C and HDL-C have been reported for black and green olive extracts as well[31].

An anti-hyperlipidemic activity, along with anti-hypertensive, dieuretic/natrieuretic and hypoglycemic activities have also been reported for oleanolic acid[1].

It has been suggested that this hypocholesterolemic effect might take place via the intestinal inhibition of dietary cholesterol absorption, or by reduced hepatic production of cholesterol, or by stimulating its biliary secretion and excretion in feces[6].

Cardioprotective effects: Poudyal *et al.* studied the metabolic effects of OLE in rats fed a high carbohydrate-, high fat- (HCHF) diet. In this study animals were divided into two groups; one group fed either normal corn starch diet (CS) or HCHF diet for 16 weeks, and the other group who received eight weeks of treatment with OLE after eight weeks of being on similar diets as the first group. After 16 weeks, rats in the HCHF group had developed signs of metabolic syndrome including collagen deposition in heart and liver, cardiac stiffness, diminished aortic ring reactivity, and hypertension. OLE supplementation was able to reverse the increased cardiac stiffness and prevent any further decrease in thoracic aorta vasorelaxation in response to nitroprusside and acetylcholine. OLE treatment also normalized the cardiac inflammatory state and collagen deposition in HCHF+OLE group compared to HCHF group. In this study treatment with OLE did not affect the increased blood pressure in HDHF-fed rats[32].

In the study by Esmailidehaj *et al.* 56 male Wistar rats were divided into seven groups, and apart from the one control group the other six groups received a single 100mg/kg dose of oleuropein 1, 3, 6, 12, 24, and 48 hours before the excision of the heart, respectively. After excision, the hearts underwent 30 minutes of regional ischemia and 120 minutes of reperfusion, while being monitored for electrocardiogram and intraventricular pressures. Compared to the control group, treatment with OLE, 1 and 3 hours before excision of the heart, significantly reduced the size of infarcted area and the reperfusion-induced cardiac dysfunction. Both ischemic and reperfusion arrhythmia were also attenuated in these groups, compared to the control group. However, oleuropein had no preconditioning effect in this study, since it showed no cardioprotective effects 24 hours after administration[33].

The clinical use of Doxorubicin (DXR), an anti-neoplastic drug that is highly effective against many malignancies, is often limited, due to its undesirable cardiac toxicity side effects, which often leads to congestive heart failure. The effect of oleuropein on DXR-induced cardiac toxicity has been investigated. It has been reported that oleuropein can protect against this side effect by inhibiting lipid peroxidation products, decreasing oxidative stress, and reducing nitric oxide species in cardiomyocytes. Therefore oleuropein can be used for the successful treatment of acute DXR cardiotoxicity[34].

Anticancer effect: Cancer has been a main cause of mortality in the past century, and finding a definite cure for different types of it, a challenge for medicine. To fight cancer, medicine has relied mostly on toxic compounds, most of which do not discriminate between malignant and normal cells, leading to undesirable side effects and toxicity[15].

Through large epidemiological studies, the overall incidence of cancer in the Mediterranean area is thought to be the lowest among other parts of the world, which is mostly due to the lower incidence of breast, endometrium, prostate and colon cancer and leukemia[8].

Herbal compounds were first utilized for their anticancer effects by Hartwell in 1967, who used Podophyllotoxin and its derivatives as anticancer agents.[35] Polyphenols have also proven to have cancer-preventive properties, evidenced on a large body of epidemiological data, and animal and in vitro studies[8].

Oleuropein, as one of the major components in olive and its derivatives, despite having long been studied for its various health benefiting features, has only recently started to be investigated for its anticancer effects.[13] The antioxidant effects of olive leaf extract, explained earlier, could be considered as one of the contributing factors in its anticancer effects.

The study by Goulas *et al.* has proved OLE to have profound antioxidant activity and potent inhibitory effect on cancer and endothelial cells proliferation. In this study where the effect of OLE on human breast adenocarcinoma (MCF-7), human urinary bladder carcinoma (T-24) and bovine brain capillary endothelial (BBCE) was evaluated, luteolin and its glucosides, followed by oleuropein, hydroxytyrosol and its acetate derivative, were the most active compounds, inhibiting cancer and endothelial cells proliferation at low micromolar concentrations[13].

Mutations are one of the important factors in carcinogenesis, and decreasing the rate of mutations may reduce the incidence of malignancies. In the study by Issazadeh *et al.* the anti- mutagenic activity of olive leaf aqueous extract on *Salmonella typhimurium* against sodium azide and 2- nitrofluorene were evaluated. The anti-mutagenic activity was screened using Ames test, in which the existence of a growth inhibitory zone around a mutagen agent disk indicates the occurrence of mutation. In this study, the aqueous extract of olive leaf inhibited the mutagenic effects of sodium azide and 2- nitrofluorene by 54.21% and 51.62% respectively[35].

Bouallagui *et al.* evaluated the in vitro inhibitory effect of hydroxytyrosol rich OLE on the human breast adenocarcinoma cells (MCF-7) proliferation. The findings of this study showed a dose-dependent inhibition of growth in MCF-7 cells induced by OLE components. The accumulation of cells arrested in G1 phase of the cell cycle, and the decreased amount of cells in G2/M phase, indicated that this antiproliferative effect was due to arrest of the cell cycle in the G1 phase. As a consequence of the cell cycle arrest, the number of cells were decreased, resulting in cytotoxic effects eventually. Also, in other studies, hydroxytyrosol rich extracts were shown to inhibit human colon adenocarcinoma cells proliferation by arresting the cell cycle in G2/M phase, and induce apoptosis in HL60 (human leukemia cells) following arrest in G0/G1 phase[8].

The extensive study by Hamdi *et al.,* assessing oleuropein as an anti-tumor agent, provided a lot of valuable findings in this field. In this study which was done both in vitro and in vivo, oleuropein showed very significant anti-tumoral activities. Firstly, the effect of oleuropein was assessed in vitro, on the cell proliferation of normal human fibroblasts and cell lines derived from advanced high-grade human tumors consisting of: human myeloid progenitor cells (TF-1a), renal adenocarcinoma cells (768-a), human breast ductal carcinoma cells (T-47D), human malignant melanoma cells (RPMI-751) and human colon adenocarcinoma cells (LoVo). Cells were incubated with increasing doses of oeluropein (0.005 to 0.025%), and after five days, all cell lines were growth-inhibited in a dose-dependent manner, although 786-a and normal human fibroblasts were inhibited only with the highest concentration of oleuropein. In addition to inhibiting growth, oleuropein also inhibited cell motility in all cell lines, and cell invasion in LoVo. Oleuropein also induced cell rounding, a phenomenon contributed to the disruption of actin cytoskeleton. In this study oleuropein was able to disrupt purified actin filaments in a cell-free assay, as well as disrupting the actin cytoskeleton in living cells. These effects were reversible on normal cells, whereas they were irreversible on tumor cells, indicating a selective action of oleuropein against normal and tumor cells. Co-incubation of the cells with β-glucosidase and removing the glucose moiety of oleuropein, resulted in reduced cell rounding and growth inhibition activity of oleuropein, suggesting a glucose-based mechanism of entry into the cell. It has previously been reported that some human cancer cells exhibit over-expression of certain GLUTs, thyroid, prostate, cervix, breast, and colon cancer, for instance. Therefore cancer cells that over-express GLUTs might possibly be more prone to the effect of oleuropein, which might explain the differential sensitivity to oleuropein in different cell lines in this study (leukemia > melanoma > colon and breast > kidney). Furthermore, the fact that normal cells have no or very slight expression of certain GLUTs may explain the reversibility of effects in normal cells but not in cancer cells, after treatment with oleuropein. This research went on to determine the oleuropein anti-tumor activity in vivo as well. Using Swiss albino mice that spontaneously develop soft tissue sarcomas, the oral intake of 1% oleuropein in the drinking water, induced dramatic tumor regression. Tumors >2 cm in diameter showed complete regression in 10/11 animals and partial regression in one animal. Oleuropein seemed to have a similar effect on mice bearing both, single or multiple lesions, with multiple lesions taking only slightly longer to regress completely (9 VS 12 days). It was also noted in this study that the oleuropein treated mice had tumors of a non- cohesive, crumbly consistency, unlike those of the untreated mice which were more fibrous and solid[15].

In conclusion oleuropein can represent a new class of anti-cancer agents, with its antioxidant activity preventing mutations and genetic damage, its anti-angiogenic activity preventing tumor progression, and finally inhibiting of cancer cells directly by tumor regression. Therefore, given the unique combination of such effects in these compounds, olive products and their derivatives should be improved from just dietary products into active anticancer drugs worthy of human studies[15].

Antimicrobial effects: The antimicrobial properties of olive leaves have traditionally been used to treat fever and different infections[36]. The antimicrobial effects can be classified in three categories:

Antibacterial effects: Despite the great revolutionary influence of antibiotics in the field of treating infectious diseases, the increasing resistance among strains and the challenge to find wide-spectrum antibiotics, remain as important issues still.[36] This has risen the motivation to find and develop new, non-antibiotic antibacterial agents.

The phenolic compounds extracted from the olive fruit, have shown an ability to inhibit the growth of *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus.* Oleuropein has been shown to be effective against the sporulation of *Bacillus cereus*, and hydroxyltyrosol has been reported effective against *Heamophilus influenza, Moraxella catarrhalis, Salmonella typhi, Vibrio paraheamolyticus* and *S. aureus*[36]

In the study by Sudjana *et al.* OLE was tested for its antibacterial activity in vitro. The most susceptible organism to OLE was *Campylobacter jejuni* with minimum inhibitory concentrations (MICs) of 0.31%, followed by *Helicobacter pylori* with MICs of 0.62% and *S. aureus* with MICs of 0.78%, and the least susceptible organisms with MICs > 50% were *Bacillus subtilis*, *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*[11].

In another study describing the activity of olive leaf extract, in accordance with the previous study, *B. subtilis* was the least susceptible, where *E. coli, P. aeruginosa, K. pneumoniaea* and *S. aureus* were the most susceptible organisms to OLE[36]

Ahmed *et al.* evaluated the antimicrobial effect of OLE on samples of raw peeled undeveined shrimp. This study reported *E. coli, P. aeruginosa, K. pneumoniae* and *S. aureus* susceptible to OLE. Possibly due to the spore forming ability of the species, *B. subtilis* was inhibited at a higher concentration of OLE (0.6% VS 20%)[37].

In accordance with the previous study, in the study by Markin *et al.* on the in vitro antimicrobial activity of OLE, *E. coli, P. aeruginosa, K. pneumoniaea* and *S. aureus* proved sensitive to OLE, where *B. subtilis* was inhibited only at a higher concentration of OLE. In this study, olive leaves were reported especially effective against *Pseudomonas* and *Klebsiella,* two bacterial genera with major resistance problems[36].

The in vitro anti- *H. pylori* activity of essential oils and herbal extracts has widely been demonstrated in the literature.[38] In the study by Romero *et al.* on the anti- *H. pylori* activity of olive oil, very low concentrations of olive oil extract proved strongly effective against *H. pylori,* even against some antibiotic resistant strains. In this study, it was demonstrated that it was the phenolic compound in olive oil that was responsible for this effect. These results propose the possible effect of olive oil and its phenolic compounds in preventing peptic ulcer and gastric cancer, but these effects still need to be confirmed by in vivo evaluations.[38] The same effect for the phenolic compounds in olive leaf extract, remains to be confirmed by laboratory and clinical data.

In conclusion, despite OLE having no significant broad-spectrum antibacterial activity, it has considerable effect against *H. pylori* and *C. jejuni*[11] and it can exert antibacterial acivity against several microorganisms including *E. coli, S. aureus, K. pneumoniae, Bacillus cereus, Salmonella Typhi and Vibrio parahaemolyticus*[37].

Antiviral effects: Plants have long been used as a source of remedies for viral diseases. In the last decades, as an alternative to conventional antiviral drugs, a lot of phytochemicals have been utilized for their antiviral effects in this field[39]. The antiviral effects of phytochemicals on vesicular stomatitis virus (VSV) have been described[39]. Recently in a US patent, OLE has been claimed to have potential antiviral activities against herpes mononucleosis, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukaemia virus[40].

In the study by Ma *et al.* on the antiviral activities of six isolated components of OLE, oleuropein showed significant effect against respiratory syncytial virus and parainfluenza type 3 virus, with a larger therapeutic index compared to ribavirin, an approved drug for the treatment of RSV infections in human. Also, Ligustroside showed potent antiviral activity against parainfluenza type 3 virus, with Lucidumoside C and oleoside dimethylester showing moderate effects against it. In this study it was also claimed that the antiviral activity of OLE was unlikely to be in direct contribution to its antioxidant properties[41].

The study by Micol *et al.* proved OLE to be effective against viral haemorrhagic septicaemia virus (VHSV). In this study, where OLE antiviral activity was measured by the number of VHSV foci formation, OLE was able to inhibit foci formation in VHSV in a dose- dependent manner. The findings in this study, also indicate that OLE interacts with the viral envelop, creating virus particles with reduced membrane- fusion capacity.[39] The effect of OLE on the surface of phospholipid bilayers has previously been described.[42] In the previous study it was observed that the cell- to- cell fusion, induced by VHSV in uninfected cells, when incubated with OLE, has decreased to 25-39%. Therefore, this effect by OLE can possibly be contributed to its interaction with the lipid/protein components of VHSV envelope, involved in its membrane-fusion capacity[39].

Lee-Huang *et al.* studied the anti-HIV activity of OLE. In this in vitro study it was shown that OLE can inhibit acute infection, cell-to-cell transmission and replication of HIV-1 in a dose-dependent manner. HIV infection can alter the expression patterns of cellular genes involved in apoptosis, stress, cytokine, and protein kinase C signaling pathways. It was shown in this study, that OLE treatment can also reverse many of these HIV infection-induced changes. In the effective dose range, no OLE toxicity on uninfected target cells were reported in this study[43].

OLE has been shown to exhibit antiviral activities against other DNA and RNA viruses as well[40].

Antifungal effects: In the study by Markin *et al.* OLE showed antifungal activity against *Candida albicans* with minimal fungicidal concentration (MFC) of 15% (w/v), and the three dermatophytes, *M. canis, T. mentagrophytes* and *T. rubrum* with MFC of 1.25% (w/v). In this study, where OLE showed antimicrobial activity against both bacteria and fungi, it is suggested that it can come of good use in cases where prolonged treatment with of antibiotics can increase the risk of fungal opportunistic infections[36]. In another study Nasrollahi et al investigated the antifungal activity of olive leaf extract against Candida albicans PTCC-5027. In their study the antifungal activity of the extract was analyzed by measuring the minimum inhibitory

concentration (MIC) and minimum fungicidal concentration (MFC), using the microdilution test and disc diffusion assay. The olive leaf aqueous extracts exhibited antifungal effects against the yeast with an MIC of 24 mg/ml, MFC of 48 mg/ml, and inhibition zone diameter of 21 mm. The results of their study indicated the sensitivity of *Candida albicans* PTCC-5027 to olive leaf aqueous extracts[44].

Despite OLE having shown multiple antimicrobial activities in various studies, in vivo studies are very scarce among them, and these features still need to be evaluated in vivo, in order to be clinically reliable.

Hypoglycemic effect: Olive leaves have long been used as a treatment for diabetes and its complications in traditional medicine, especially in Europe where they are widely used as a traditional remedy for diabetes and hypertension[1].

In the study by Pudyal *et al.* on the effect of OLE on metabolic syndrome in high carbohydrate-, high fat (HCHF) - fed rats, 16 weeks treatment with OLE lowered blood glucose levels and improved glucose tolerance in HCHF+OLE rats compared with control group[32].

Various mechanisms of action have been reported for the hypoglycemic activity of olive leaves. In the study by Gonzalez *et al.* it was reported that oleuropien accelerated the uptake of glucose by cells[1].

De Bock *et al.* reported a significantly improved insulin sensitivity and pancreatic β-cell secretory function. In this study 46 middle-aged over-weight men (aged 46.4±5.5 years and BMI 28.0±2.0 kg/m2) randomly took capsulated OLE or placebo for 12 weeks and were crossed over to the other treatment after a six week period of wash out. The participants in OLE group were instructed to take four capsules of OLE in a single dose daily, containing 51.1 mg oleuropein and 9.7 mg hydroxytyrosol. The subjects were assessed at the end of each interventional phase by collecting and evaluating blood samples. The results in this study showed that OLE was associated with 15% increase in insulin sensitivity and 28% pancreatic β-cell function improvement in OLE group compared to placebo group. The subjects in OLE group had 32% increase in interleukin-6 as well. Mildly elevated interleukin-6 levels can result in increased insulin-regulated glucose metabolism in the muscle acutely, and a pro-inflammatory insulin resistant state in the liver chronically. Therefore it can be postulated that OLE improves insulin sensitivity and glucose uptake via increased interleukin-6[45].

Diabetes type1 and 2 are both associated with impaired antioxidant status, with increased oxidative stress leading to tissue damage and eventually contributing to important complications of diabetes including retinopathy, nephropathy and coronary heart disease. It has previously been postulated that antioxidant agents such as ascorbic acid, vitamin E, flavonoid and polyphenol compounds might prevent these complications in diabetic patients. With respect to the widely acknowledged antioxidant activity of polyphenol compounds in OLE, Al-Azzawie *et al.* suggested an antioxidant basis for anti-diabetes activity of oleuropein derived from olive leaves. This study aimed to evaluate the hypoglycemic effect of oleuropein and its effect on oxidative stress and enzymatic and non-enzymatic antioxidant agents in alloxan-induced diabetic rabbits. In this study the animals were divided into three groups, a control group of healthy rabbits, a group of diabetic rabbits with no treatment, and a group of diabetic rabbits who received a daily dose of 20 mg/kg OLE as treatment for 16 weeks. Diabetic rabbits in OLE group showed a significant decrease in blood glucose after initiation of treatment which was strictly apparent after eight weeks of treatment. All diabetic rabbits showed increase in plasma and erythrocyte malondialdehyde (MDA) as a sign of increased oxidative stress, but rabbits treated with OLE showed a gradual decrease in plasma and erythrocyte MDA, and eventually a significant decrease at week 10 of treatment with OLE. OLE treated rabbits also showed significant decrease in the activities and levels of most enzymatic and non-enzymatic antioxidant agents by the end of the 16 week period of treatment, to values that were similar to those of healthy control rabbits[26].

In the study by Komaki *et al.* it was suggested that the hypoglycemic effect of olive leaf compounds root from their inhibitory effect on salivary and pancreatic amylase. In this study which took place in several parts, the in vitro effect of olive leaf extract on α-amylase from human saliva and pancreas were measured. The ethanol extract of olive leaves were able to inhibit human salivary and pancreatic α-amylase with IC50 values of 4.0 and 0.02 mg/mL respectively. The study proceeded to evaluate the effect of olive leaves on post prandial blood glucose in diabetic rats. Animals were divide in four groups, receiving starch, olive leaf powder, oleanolic acid and luteolin respectively. The elevation of glucose level was significantly inhibited by olive leaves, oleanolic acid and luteolin after 0.5h compared to starch group. And lastly, the post prandial hypoglycemic effect of olive leaves in human subjects was evaluated. the subjects who according to their blood glucose level were divided into two groups, normal and borderline for diabetes, were fed 300g of cooked rice in the first phase of the study, and the same amount of cooked rice in addition to 1g of olive leaf in the second phase. The blood glucose level of the borderline group was decreased significantly 0.5h after loading of olive leaf along with cooked rice in comparison to the control phase of the experiment, while no such effect was observed in the normal group who had similar blood glucose levels after loading of cooked rice, whether it was with or without the addition of olive leaves[46].

Further investigation on the hypoglycemic effect of olive leaf is required to determine the more exact mechanism for this effect.

Anti-inflammatory effects: Olive leaves have long been utilized in traditional medicine for their anti-inflammatory properties to treat fever, joint pain and gout[7,10]. The Mediterranean diet, rich in olive and its derivatives has been associated with lower cardiovascular incidents, in which the anti-inflammatory role of these compounds in decreasing atheroma formation and preventing atherosclerosis might be a contributing factor[47].

The study by Richard *et al.* reported hydroxytyrosol, one of the main compounds in OLE, to be of major anti-inflammatory properties. In this study where the in vitro anti-inflammatory effect of hydroxytyrosol was evaluated, Murine macrophage cells were stimulated using lipopolysaccharide with and without addition of hydroxytyrosol, and the release of inflammatory cytokines (nitric oxide (NO), prostaglandin E2 (PGE2), cytokines,

interleukins, chemokines) and expression of their genes was determined in both phases. Hydroxytyrosol showed significant inhibitory effect against NO and PGE2 with an IC50 of 11.4 ± 1.9 and 19.5 ± 2.6 μM, respectively. Hydroxytyrosol also inhibited the secretion of TNF-α, IL-1α, IL-1β, IL-6, IL-12, and GM-CSF. In addition, hydroxytyrosol was able to inhibit expression of the genes of inducible nitric oxide synthase (iNOS), IL-1α, CXCL10/IP-10, MIP-1β, matrix metalloproteinase-9 (MMP-9), and prostaglandin E2 synthase (PGES), judging by the reduced mRNA levels of these agents. The reduced secretion of MMP-9 gene can result in thrombus stability and therefore reduced risk of cardio vascular accidents. It was noted in this study, that the inhibition in secretion of TNF-α and COX-2, with no inhibition observed in their gene expression, can indicate a posttrancriptional level of regulation for hydroxytyrosol also[47].

Rosillo *et al.* evaluated the anti-inflammatory effect of olive oil polyphenol compounds on collagen-induced arthritis (CIA) in mice. In this study animals were divided into four groups: healthy naïve group with no arthritis induction, CIA control group, and two CIA groups receiving 100 and 200 mg/kg daily dose of polyphenol as treatment. By the end of the treatment course, mice in both treatment groups had developed arthritis with lower severity compared to those in CIA control group. In addition treatment with olive polyphenols caused arthritis-induced X-ray changes such as joint edema and bone erosions, and histological changes such as extensive infiltration of inflammatory cells into articular tissues, exudation into the synovial space, synovial hyperplasia and cartilage erosion to be less apparent in the treatment group. The treatment group receiving a daily 200 mg/kg dose of polyphenol had bone integrity similar to that of naïve group with no arthritis. Treatment with polyphenols could also reduce the joint levels of IL-1β, TNFα, IL-6, and PGE2 compared to those in control group. In addition the expression of cyclooxygenase-2 and microsomal prostaglandin E synthase-1 genes were reduced under the effect of polyphenol treatment[48].

The effect of Olive leaf extract on suppression of cytokine production and NLRP3 inflammasomes in human placenta was also examined by Kaneko et al. They stated that Olive leaf treatment inhibited the secretion of inflammatory cytokines and NF-κB p65 protein expression in human placental tissue culture. Olive leaf also suppressed toll-like receptor ligands-induced IL-1β secretion in human placental tissues. Since IL-1β are involved in the regulation of NLRP3 inflammasomes, they showed that Olive leaf significantly decreased NLRP3 and pro-IL-1β protein expression and suggested that it has an inhibitory effect on activation of NLRP3 inflammasome and may be used as a supplement for the treatment and prevention of inflammatory[49].

The effect of olive leaf extract on the reduction of inflammatory activation and DNA damage in human arterial endothelial cells was also evaluated by Bursi et al. The extent of the anti-inflammatory effects of olive leaf components and their exact mechanisms are still unclear, and in order to utilize such effects in the clinical field, further investigations on this topic are required.

Other effects:

Analgesic effect: Several studies have shown that Ca^{2+} is involved in regulation of pain sensitivity, and blocking the movement of calcium ion can contribute to antinocioception[10]. It has previously been reported that the extracts from olive leaf, can have calcium channel blocking activity[50,51]. Therefore an analgesic activity for OLE should be considered.

In the study by Esmaeili-Mahani *et al.* the analgesic effect of OLE on male Wistar rats was evaluated through tail-flick, hot-plate and formalin tests. The results showed a significant analgesic effect for OLE in tail-flick and hot-plate tests in a dose-dependent manner, and also a significant decrease in the pain response in the first and the second phase of formalin test following the i.p. administration of 200 mg/kg OLE. The study proceeded to determine the effect of OLE on analgesic and hyperalgesic effects of morphine by concomitant injection of non-analgesic doses of OLE with morphine (administered in 5mg/kg and 1μg/kg i.p. doses, to induce analgesic and hyperalgesic effects respectively.) the findings indicated that OLE could improve the antinocioceptive effect of 5mg/kg morphine, and block the low-dose induced hyperalgesia. These effects have been attributed to various components in OLE including the main component, oleuropein, and also to hydroxytyrosol and luteolin which have shown antinocioceptive and anti-inflammatory effects in previous studies. Vanilloid compounds of OLE have also shown to be effective against nocioception and inflammatory responses. In addition cafeic acid and all of its derivatives with aliphatic chain have induced significant antinocioceptive effects in experimental models of pain[10].

Neuroprotective effect: The delayed neuronal damage caused by cerebral ischemic injury, is the result of several factors, including enhanced nitric oxide production and attenuated redox regulatory system. Enhanced nitric oxide production leads to increased apoptosis, and attenuated redox regulatory system leads to increased oxidative stress. Therefore antioxidant agents can prevent delayed neuronal damage following ischemic injuries[52].

Dekanski *et al.* evaluated the neuroprotective effect of OLE in transient global ischemia in Mongolian gerbils. In this study the different parameters of oxidative stress and neuronal damage in the animals' hippocampus were investigated and the effcts of OLE were compared to those of quercetin, a known neuroprotective herbal flavonoid. The animals were divided into four groups; three treatment groups who were exposed to ten minutes of ischemia by occlusion of common carotid arteries and one control group who went under sham operation without occlusion of common carotid arteries, in order to rule out any effects caused by the operation itself, and not the ischemic injury. One treatment group was treated with PEG 400, and the other two were given a combination of quercetin and PEG 400, and OLE and PEG 400 respectively. The brains were removed immediately after 80min, 120min, 4h and 24h following exposure to 10min ischemia, and the samples were used to evaluate the biochemical and histological changes. Pretreatment with OLE significantly reduced the production of superoxide and nitric oxide, inhibited lipid peroxidation, and increased superoxide dismutase activity in all time points evaluated. These effects were significantly higher compared to those of quercetin in the group

pretreated with this agent. Several morphological changes were observed in the pyramidal neurons of hippocampal CA1 region in PEG treated rats following ischemia, including the shrinkage of perikarions, increased density of the cytoplasm, dark and irregular shaped nuclei and signs of extracellular edema. These changes were far less in samples from the quercetin and OLE treated groups, with quercetin decreasing morphological changes to 11.3±2.7%, and OLE being significantly more effective by decreasing morphological changes to 5.2±1.5%. It is noted in this study that the neuroprotective effect of OLE is probably far more complicated and is worthy of further investigations[7].

Platelet aggregation effects: As it was noted earlier, the diet in Mediterranean era has been associated with a lower incidence of cardiovascular accidents. Also OLE has previously been reported to possess anti-arteriosclerotic and cardioprotective effects[6,32,33,34]. These effects could be associated with the possible effect of OLE on platelet function.

In the study by Singh *et al.* the in vitro effect of OLE on platelet aggregation was investigated. 11 healthy young men between the ages of 18 to 54 were recruited in this study. All volunteers were non- smokers, reported no history of cardiovascular diseases or diabetes and were screened through a questionnaire for the level of their physical activity, diet, medical history and use of aspirin type-drugs, non-steroid anti-inflammatory drugs, blood pressure and other drugs. Subjects were requested not to alter their habitual food intake during the study period. Participants were required to complete a food record over a period of seven days, which was analyzed to monitor their intake of polyphenol rich food such as omega-3 polyunsaturated fatty acid rich foods, alcohol and cocoa products and to confirm that they were not consuming any antioxidant supplements, and subjects who were on medication, used antioxidant supplements, or had high dietary intakes of alcohol, seafood and cocoa products were excluded from the study. A total of 22mL of venous blood was collected from each volunteer and samples were screened for baseline platelet count (PLT) and mean platelet volume (MPV) before addition of OLE. Five increasing concentrations of OLE were then added to the samples, containing 5.4µg/mL, 16.2µg/mL, 27µg/mL, 37.8µg/mL, and 54.0 µg/mL of oleuropein respectively. Whole blood samples with no additives were used as a control and baseline measurement. Compared to control group, in samples with additive OLE, platelet aggregation fell in a dose-dependent manner in respond to OLE, with a steep fall between 37.8 µg/mL and 54.0 µg/mL concentrations of oleuropein, the latter making a significant difference compared to baseline. OLE had the same inhibitory effect on platelet ATP release, 54.0 µg/mL being the most effective concentration of oleuropein. The findings of this study indicate an inhibitory effect for the phenolic compounds in OLE, on the in vitro platelet activation in healthy, non-smoking male individuals[12].

Kontogianni et al also examined the effect of the dual antiplatelet activity of OLE. A thorough evaluation of the antiplatelet activity profile of hexane olive leaf extract in human platelets indicated a potent activity accomplished through a two axis inhibition of platelet activation triggered both by ADP and thrombin. In their study, to delineate the extract components responsible for this dual activity, an NMR based method was established to determine and quantify the triterpenoid content leading to the characterization of uvaol, erythrodiol, and oleanolic acid. The antiplatelet profile of the total extract and of the 3 determined triterpenoids was evaluated against in vitro platelet aggregation induced by several platelet agonists as also on PAC-1 binding and P-selectin membrane expression both in healthy volunteers and in platelets from patients with an acute coronary syndrome receiving dual antiplatelet therapy with aspirin and ticagrelor. Eventually, they concluded that the OLE was effective to inhibit ADP-induced platelet activation due to its erythrodiol content and TRAP-induced platelet activation due to the activity of uvaol and oleanolic acid[53].

Hepatoprotective effects: In the study by Poudyal *et al.* where the effect of OLE on metabolic syndrome in rats induced by high carbohydrate- , high fat-diet (HCHF) was evaluated, the effects of OLE on the liver changes following metabolic syndrome was assessed as part of the study. The liver function markers which were elevated in HCHF and CS (corn starch) groups by week 8 and 16 respectively, were normalized in the groups treated with OLE, in the same time period. In addition the wet liver weight, equally high in both CS and HCHF groups, was significantly lower in OLE treated rats which, as explained shortly later, is probably due to reduced portal collagen deposition and fat deposition in liver. After 16 weeks on HCHF diet, the rats developed enlarged fat vacuoles within the hepatocytes, increased portal inflammatory cell infiltration, and portal fibrosis; whereas these changes were all normalized in HCHF+OLE group. Nonalcoholic steatohepatitis (NASH), which is one of the hepatic presentations of metabolic syndrome is proposed as a fatty liver disease associated with diffuse fatty infiltration, inflammation, pericellular fibrosis, and extremely elevated transaminase activity. In this study it was demonstrated, that OLE was able to improve the pathological changes of NASH in HCHF fed rats[32].

Skin-protective effects: The topical administration of antioxidant compounds including the biphenols found in olive products, such as extra virgin olive oil and table olives, has long been recognized to protect the skin against UV radiation-induce photo-oxidative stress. Reducing the UV radiation-induced peroxidation and scavenging free oxygen and NO radicals, which are responsible for erythema following UV exposure, have been reported as the mechanism of action for this effect. Among biphenols present in olive leaf extract, caffeic acid has been shown to have similar skin protective effects. In order for topical agents to exert potential skin-protective effects, efficient percutaneous absorption is required. Caffeic acid has been shown to be able to penetrate through the stratum corneum, the main barrier in the way of absorption of exogenous agents through the skin. Caffeic acid, dissolved in a saturated, buffered aqueous solution, pH 7.2, topically applied immediately after exposure to UVB radiation in healthy volunteers, showed efficient protection against UVB radiation-induced skin erythema[21].

CONCLUSION

Olive leaves have long been utilized for their various beneficial effects in traditional medicine. Recent studies have also demonstrated many positive effects for OLE on human health. Despite the great body of studies done on OLE and its beneficial effects, human studies on this subject are still very scarce. Among the studies cited above, only the skin-protective effects, hypoglycemic effects and the effects on platelet aggregation have been tested through human studies, and even though the metabolism and bioavailability of the compounds in OLE have been investigated through some human researches, the data are still insufficient and are unable to provide a thorough understanding of this subject.

The increasing rate of malignancies and chronic conditions such as diabetes mellitus and cardiovascular diseases, and the growing need to find new remedies for them, bring special attention to the anticancer, hypoglycemic, anti-inflammatory and cardiovascular effects of herbal compounds such as OLE. The cardiovascular, anti-inflammatory and anticancer effects of OLE have already shown remarkable results in vitro and in animal studies, and it seems about time that we begin investigating these effects in human studies. The antioxidant effects, as a fundamental base for other effects, still lacks a lot of information on the exact mechanism of action, and the extent to which these effects can apply to humans remains as a point of question still. As for the antimicrobial effects of OLE, which have only been studied in vitro, perhaps more investigations through animal studies could take us one step closer to understanding these effects and utilizing them in future human studies.

In conclusion, although there is still a long way ahead of olive leaf and its compounds to find their place among drugs actively used in modern medicine, their remarkable health-benefitting effects should not be overlooked, which are very much worthy of further investments and investigations.

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