# Health-promoting properties of oleocanthal and oleacein: two secoiridoids from extra-virgin olive oil

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**Abstract**

Extra virgin olive oil (EVOO) polyphenols, including the secoiridoids oleocanthal (OLC) and oleacein (OLE), are attracting attention because of their beneficial effects on health. Data on OLC and OLE bioavailability are scarce, as most research on EVOO polyphenols has concentrated on hydroxytyrosol, tyrosol, and oleuropein. Consequently, relevant goals for future research are the elucidation of OLC and OLE bioavailability and finding evidence for their beneficial effects through pre-clinical and clinical studies. The aim of this review is to shed light on OLC and OLE, focusing on their precursors in the olive fruit and the impact of agronomic and processing factors on their presence in EVOO. Also discussed are their bioavailability and absorption, and finally, their bioactivity and health-promoting properties.

**Keywords:** Mediterranean diet, food processing, metabolism, bioavailability, bioactivity. polyphenols, synergism.

## 1. Introduction

Extra virgin olive oil (EVOO) is a staple of the Mediterranean diet and is highly appreciated for its unique nutritional and organoleptic attributes (Polari et al. 2018). A Mediterranean diet rich in EVOO may prevent type 2 diabetes, cancer, neurodegenerative and cardiovascular diseases (Anna Tresserra-Rimbau et al. 2011; A. Tresserra-Rimbau et al. 2014; Martínez-González et al. 2015). The nutritional and health-promoting properties of EVOO are mainly attributed to a high content of monounsaturated acids (C18:1, between 55-83%), its minor components (alcohols, sterols and hydrocarbons), and phenolic compounds (Leporini et al. 2018), particularly phenolic alcohols and secoiridoid derivatives (Soler et al. 2010). The phenolic composition of EVOO depends on a very complex multivariate interaction between the genotype and agronomic, environmental and technological factors (Chiacchierini et al. 2007), the processing steps critically affecting yield, quality and nutritional attributes (Fregapane and Salvador 2013).

During the mechanical extraction process, mainly the crushing and malaxing steps, hydrolysis reactions take place catalyzed by the endogenous $\beta$-glucosidase and oxidoreductase enzymes of the olive fruits acting on phenolic glycosides. Subsequently, the aglycone derivatives known as secoiridoids (SEC) are released (López de las Hazas et al. 2016; Servili et al. 2004; Velázquez-Palmero et al. 2017; Clodoveo et al. 2014; Alessandra Bendini, Lorenzo Cerretani, Alegria Carrasco-Pancorbo, Ana Maria Gómez-Caravaca, Antonio Segura-Carretero 2007; Hachicha Hbaieb et al. 2015).

Among the different types of SEC, two compounds have recently attracted attention: oleocanthal (OLC) and oleacein (OLE). These SEC were identified for the first time in olive oil by Montedoro and co-workers in 1993 (Montedoro et al. 1993), but it was not until 2005 that Beauchamp and co-workers discovered the anti-inflammatory activity of OLC (Beauchamp et al. 2005). Since then, other biological properties have been attributed to this

compound, including anti-cancer (LeGendre, Breslin, and Foster 2015) and anti-Alzheimer effects, both *in vitro* and *in vivo* (Qosa et al. 2015; Monti et al. 2012), and a protective role against arthropathy *in vitro* (Morena Scotece et al. 2012) and against cardiovascular diseases *in vivo* and in human trials (Agrawal et al. 2017). OLE, like OLC, decreases cyclooxygenase (COX) 2 activity, thereby reducing inflammation (Rosignoli et al. 2013), and is also thought to be the main component responsible for the anti-sclerotic effect of EVOO (Naruszewicz, Czerwiska, and Kiss 2015). OLE has also shown *in vitro* activity against cancer (Roberto Fabiani et al. 2006) and an anti-estrogenic effect (Keiler et al. 2015).

Although SEC are the most abundant and complex family of phenolic compounds in EVOO, their bioavailability has been little studied (Silva et al. 2017b), either in *in vitro* or in preclinical and clinical studies. The processes of OLC and OLE absorption and metabolism are important for understanding their biological properties *in vivo* (Corona et al. 2006; Deiana, Serra, and Corona 2018). New research is required to investigate if intestinal absorption and/or metabolism are the main factors that determine OLC and OLE bioavailability and mechanisms of action (Pinto et al. 2011).

The aim of this work is to review all the known factors involved in the beneficial effects of OLC and OLE, ranging from their precursors in the olive fruit and their production in EVOO to their absorption and bioavailability, and finally, their bioactivity and health-promoting properties.

## 2. Biosynthesis and biotransformation of secoiridoids in olive oil

### *2.1 Biosynthesis of secoiridoids in Olea europaea L.*

*Olea europaea* L. has an economic importance in the Mediterranean area and provides different commercial products, including food, lumber, cosmetics, and above all, olive oil (Obied et al. 2008). The beneficial effects of a Mediterranean diet on cardiovascular diseases

have been partially attributed to a high EVOO consumption (Ramon Estruch, M.D., Ph.D., Emilio Ros, M.D., Ph.D.Jordi Salas-Salvado, M.D. et al. 2013), and the identification of anti-inflammatory properties of OLC (Beauchamp et al. 2005) has made SEC a hot research topic. New oleoside conjugates and metabolites in *Olea europaea* L. have been identified, and the behavior of these compounds during processing and storage of olive products has been described, but to date scarce attention has been directed at OLC and OLC.

The diverse range of SEC in the Oleaceae family is of chemotaxonomic interest (Obied et al. 2008). In their review of iridoid biosynthesis in the Oleaceae family, Jensen, Franzyk, and Wallander (2002) described that SEC are derived from iridoids by cleavage of the cyclopentane ring and from the formation of deoxyloganic acid *via* iridodial and iridotrial (Jensen, Franzyk, and Wallander 2002). They reported at least five different routes, deoxyloganic acid being the common intermediate. Many SEC are produced in route 1 *via* loganin and secologanin (Jensen, Franzyk, and Wallander 2002) (**Fig. 1**). The most interesting SEC in *Olea europaea* L., due to their bioactive properties, are oleosides or oleosidic secoiridoids, which are characterized by an exocyclic 8,9-olefinic functionality.

The major oleosides in *Olea europaea* L. are oleuropein and ligstroside, characterized as esters of elenolic acid linked to hydroxytyrosol (OH-TY) or tyrosol (TY), respectively. SEC conjugates bearing an esterified phenolic moiety, such as oleuropein, are produced by a branching in the mevalonic acid pathway, where it merges with an oleoside moiety from the phenylpropanoid metabolism. The biosynthesis of oleuropein, the most extensively studied oleoside, is affected by the olive fruit ripening process, and whether the season is low or high fruiting (Obied et al. 2008).

*2.2 Agronomic and processing factors in the biosynthesis and biotransformation of SEC*

Olive oil is obtained from the fruit of olive trees (*Olea europaea*, L.) using mechanical techniques (Leone et al. 2015) with three main steps: preparation of the paste (crushing and

malaxation), and solid-liquid and liquid-liquid separations. The composition and concentration of polyphenols in EVOO and virgin olive oil (VOO) are strongly affected by agronomic, biochemical and technological factors (Clodoveo et al. 2014).

The olive fruit composition, mainly phenolic compounds content, can be strongly affected by *agronomic factor*s as genetics, cultivar, ripening stage and environmental growth conditions including biotic and abiotic stresses (Peres, Martins, and Ferreira-Dias 2017; S. Cicerale 2012). The content of phenolic glycosides, initially present in the olive tissues, and the activity of various endogenous enzymes also play a role in the olive fruit composition. The OLC and OLE concentration has been positively correlated with an early harvest of olives (Gómez-Rico, Fregapane, and Salvador 2008; Karkoula et al. 2012; de Torres et al. 2016), because in the green stage, the level of $\beta$-glucosidase activity increases proportionally with the oleuropein and ligstroside content, whereas in the black stage, when the phenolic glycoside concentration declines, the glucosidase activity is low (Clodoveo et al. 2014).

The main ***biochemical factors*** affecting the phenolic compound composition are the endogenous enzymes of the olive fruits, such as $\beta$-glycosidase (which hydrolyzes phenolic glycosides) and oxidoreductases like polyphenoloxidases and peroxidases (which oxidase phenolic compounds) (Servili et al. 2004; Peres, Martins, and Ferreira-Dias 2017).

The crushing process is the key ***technological factor*** in the release of endogenous enzymes and the commencement of their activities, which depends on the temperature, particle size of olive fruit fragments, exposure to atmospheric oxygen and the differential crushing of the olive tissues (Maurizio Servili, Agnese Taticchi, Sonia Esposto 2012; Clodoveo et al. 2014). The endogenous enzymatic activity can be modulated during malaxation by controlling its duration and the atmospheric conditions inside the malaxer (Clodoveo 2012). The concentration of OLC and OLE was enhanced by increasing the temperature during malaxation of the olive paste up to 30 and 35 °C (Lukić et al. 2018;

Taticchi et al. 2013; de Torres et al. 2016). The enzymatic oxidation of the SEC aglycones by polyphenoloxidases and peroxidases (Clodoveo 2012; Taticchi et al. 2013) may be reduced at higher malaxation temperatures, boosting the release of phenols from the cell wall polysaccharides and other olive tissues catalyzed by the endogenous hemicellulases and polygalacturonases (Clodoveo 2012; Taticchi et al. 2013; Esposto et al. 2013; Vierhuis et al. 2001). Moreover, a higher temperature can increase the partition coefficient between oil and water phases in olive paste (Gómez-Rico, Fregapane, and Salvador 2008), enhancing the solubility of these compounds in the oil phase (de Torres et al. 2016).

## 3 Bioavailability, absorption and metabolism of SEC

### *3.1 Bioavailability*

The bioavailability of OLC and OLE has been scarcely studied, either in *in vitro* studies or in preclinical and clinical trials. Most research in this field has been focused on the phenolics OH-TY, TY and oleuropein. Although the biological activities of the phenolic compounds present in VOO have been clearly demonstrated (Sara Cicerale, Lucas, and Keast 2010), it is difficult to find evidence for the specific role of each component in the beneficial effects of the oil, or for the synergistic activity of a combination of compounds. To achieve any effect in a specific tissue or organ, the bioactive compounds must be bioavailable. Thus, data on the bioavailability of OLC and OLE in humans (and even animals) would be of great interest to assess their potential health benefits.

Bioavailability is the amount of a substance from an administered matrix that appears in the systemic circulation. Oral bioavailability depends on the degree of absorption, but also on the extent of the first-pass metabolism, which can occur either in the intestine or the liver, before the substance reaches the systemic circulation. It is therefore essential to study how OLC and OLE are absorbed and biotransformed/excreted, which will be discussed below. Other factors such as diet, genomic profile, enzymatic activity and colonic microflora can also

influence the bioavailability of the ingested phenolic compounds. It is thought that OLE may be absorbed in the small intestine by passive diffusion through the membrane due to its favorable partition coefficient (log $p$ = 1.02). In addition, OLE was found to be stable at gastric acid pH, 67% remaining unchanged after 4 h of incubation (Naruszewicz, Czerwiska, and Kiss 2015).

*3.2 Absorption*

Absorption is a complex kinetic process that depends on numerous physiological, physicochemical, and dosage form factors (Griffin and Driscoll 2007). The absorption and metabolism of phenolic compounds are determined primarily by their physicochemical characteristics (Guo et al. 2017), including molecular size (López de las Hazas et al. 2016), basic structural properties, polarity (Vissers et al. 2002; Sara Cicerale, Lucas, and Keast 2010), degree of polymerization or glycosylation (Carbonell-Capella et al. 2014), solubility, lipophilicity and conjugation with other phenols (Guo et al. 2017). The chemical structure of polyphenols, more than the concentration, determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma (D'Archivio et al. 2007).

The mechanism by which absorption of olive oil phenolic compounds occurs remains unclear. Once olive oil has been ingested, it produces a micellar solution composed of a lipid and an aqueous phase (Singh, Ye, and Horne 2009). Polyphenol glycosides can be modified in the oral cavity by the hydrolytic activity of saliva, although most pass through the stomach to reach the small intestine and colon. Before absorption in the small intestine, these compounds must be hydrolyzed by intestinal enzymes (Vissers et al. 2002), and similarly, when they reach the colon, they are usually metabolized by the microbiota (D'Archivio et al. 2010).

Chemical hydrolysis of SEC can take place in the acidic medium of the stomach (Lopez et al. 2014; Corona et al. 2006) or in the more alkaline conditions of the small intestine (Soler et al. 2010; Pinto et al. 2011). This leads to an increase of free phenolic

alcohols (Muriana et al. 2017) released into the aqueous phase and becoming available for absorption. SEC remain highly stable during digestion in the mouth, whereas in the gastric, duodenal and colonic regions they undergo important losses; their recovery index in the duodenal step was found to oscillate between 7 and 34% (Quintero-Flórez et al. 2017). Studies indicate that SEC, which are apparently not absorbed in the small intestine, are likely to reach the large intestine to be degraded by the colonic microflora (Corona et al. 2006). Some authors suggest that the breakdown of the ester bond of OLC is relatively probable, either in acidic or alkaline conditions or by esterases located in the small intestine or the liver (Rubió et al. 2012) (**Fig 2**).

The absorption of SEC is not well elucidated, a possible mechanism being passive diffusion (Scalbert and Williamson 2000). SEC are a group of coumarin-like compounds, which are usually glycosidically bound (Corona et al. 2006). The absorption of SEC through passive diffusion would therefore require the removal of the attached glycosyl moiety (Vissers et al. 2002) by enzymes (glycosidases), which can be present in the gastrointestinal mucosa or secreted by the colon microflora (Scalbert and Williamson 2000).

Another pathway of polyphenol absorption is the one supported by Hollman *et al.* (1999), who described how glucosides can promote polyphenol absorption across the intestinal epithelium. They suggested this occurs by interaction with the sodium-dependent glucose transporter SGLT1 or, as mentioned above, by the action of glycosidases (Hollman et al. 1999).

*3.3 Metabolism*

Biotransformation is the chemical alteration of a foreign molecule in the body (Chan 1959) to facilitate its elimination, and is fundamental to the understanding and evaluation of the health benefits associated with phenolic compounds (S Cicerale, Lucas, and Keast 2012). The metabolism of SEC can be carried out by phase I (hydrogenation, hydroxylation,

hydration, etc.) or phase II reactions (glucuronidation, methylation, sulphation, etc.). The OLC metabolites found in plasma and urine are the result of hydrogenation, hydration (Silva et al. 2017a; García-Villalba et al. 2010), hydroxylation and glucuronidation (García-Villalba et al. 2010) and are formed mainly in the small intestine and liver.

Although the liver appears to be the major organ involved in glucuronidation, high levels of some UGT isoforms are found in the kidney and intestine, suggesting that extrahepatic glucuronidation may be significant (Fisher et al. 2001; Scalbert and Williamson 2000).

Catechol-*O*-methyl (COMT) transferase, present in a wide range of tissues, is responsible for the methyl conjugation process. It catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to phenolic compounds with an *O*-diphenolic (catechol) structure (Manach et al. 2004). Its activity is highest in the liver and kidneys, although significant methylation has been reported for catechin in the small intestine of rats (Manach et al. 2004). Methylated forms of OLC have not been detected (Silva et al. 2017b; García-Villalba et al. 2010), due to the absence of the ortho-diphenolic structure needed for the COMT enzyme to methylate (Mateos, Goya, and Bravo 2005). Thus, only OH-TY and deacetoxy-oleuropein-aglycone derivatives undergo methylation. However, TY methylated conjugates have been identified in one study (Suárez et al. 2011) in plasma samples, suggesting the presence of enzymatic activity able to methylate tyrosol-like molecules such as OLC.

Sulfotransferases catalyze the transfer of a sulfate moiety from 3'-phosphoadenosine-5'-phosphosulfate to a hydroxyl group on various substrates (steroids, bile acids, polyphenols, etc.), and the reaction occurs mainly in the liver rather than in the small intestine (64). These enzymes could sulfate OLC and OLE, but no sulfate metabolites of OLC have been identified in any study in humans to date (Silva et al. 2017a; García-Villalba et al. 2010). A possible

reason for the absence of such metabolites is that OLC inhibits the sulfotransferases by mimicking the activity of other polyphenols (Gee et al. 1998; Burchell and Coughtrie 1997). Another plausible explanation is that sulfation is generally a higher-affinity, lower-capacity pathway than glucuronidation in the same substrate, which if ingested at higher doses results in a shift from sulfation toward glucuronidation (Koster et al. 1981). In studies this could be overcome by administering very low doses of the substrate. On the contrary, in a recent study OH-TY-sulphate-3′ was the major metabolite detected in urine after a high dose administration of OH-TY to healthy volunteers (Khymenets et al. 2016). OH-TY-sulfate and OH-TY-acetate-sulfate were the main circulating metabolites detected in both urine and human plasma (López de las Hazas et al. 2018; Rubió et al. 2014). In a study on human HepG2 cells, no sulphate conjugates of any of the assayed phenols (OH-TY and TY) were detected (Mateos, Goya, and Bravo 2005), so human trials are necessary to confirm this pathway.

Phase 2 metabolites excreted in the bile can be deconjugated by colonic microbiota, and either degraded to more simple compounds such as phenolic acids (Corona et al. 2006; Scalbert and Williamson 2000), or reabsorbed as aglycones through intestinal membranes, completing an enterohepatic recycling (Manach et al. 2004). There is a lack of research on the specific location of the steps by which polyphenols such as SEC are metabolized, and thus only tentative metabolic pathways can be given (**Fig. 3**).

## 4. Health effects

SEC, as well as other EVOO polyphenols, have been targeted by numerous studies aiming to understand the health effects of EVOO consumption. However, OLC and OLE have not been extensively studied, so their full potential as health-promoting compounds remains unknown. Most research on OLC and OLE bioactivity has been performed *in vitro*, whereas

their effects in the human body might be different, because these molecules may undergo modifications such as glucuronidation or hydrolyzation during absorption and metabolism (Silva et al. 2017b). Studies are also limited if experiments are not performed with physiological concentrations (Espín, García-Conesa, and Tomás-Barberán 2007). As mentioned before, OLC and OLE absorption and metabolism are still poorly understood, so to improve the design of future *in vitro* research, their biotransformation and bioavailability need further study. In the next sections, the health effects of these polyphenols are discussed, being broadly shown in **Fig. 4** and fully summarized in **Tables 1** and **2**.

*4.1 Oleocanthal*

In 2005, Beauchamp and co-workers (Beauchamp et al. 2005) correlated the anti-inflammatory activity of OLC with its inhibitory effect on COX-1 and COX-2, enzymes responsible for producing inflammatory mediators such as prostaglandins and thromboxane (Rosignoli et al. 2013). The anti-inflammatory activity of OLC was higher than that of ibuprofen, the typical drug prescribed for inflammatory processes. Many diseases have been attributed to chronic inflammatory processes, aggravated by aging, including atherosclerosis, arthritis, cancer, diabetes and Alzheimer's disease (AD). The following sections describe the effect of OLC on inflammatory-mediated diseases and its function in health.

*4.1.1. Arthropathy*

OLC ameliorates osteoarthritis and rheumatoid arthritis *in vitro*. Osteoarthritis is characterized by mechanical stress in the joints, although inflammation contributes to its symptoms and progression (Bonnet and Walsh 2005). In contrast, rheumatoid arthritis is caused mainly by inflammation, specifically an auto-immune process. In both cases, pro-inflammatory cytokines and other mediators create an inflammatory state that leads to the up-regulation of cartilage-degrading factors (Goldring and Otero 2011). The down-regulating

effect of OLC on these cytokines and mediators has been determined (Iacono et al. 2010; Morena Scotece et al. 2012).

In a study carried out by Iacono and co-workers (Iacono et al. 2010), a chondrogenic cell line was stimulated with lipopolysaccharide (LPS) to induce the production of nitric oxide (NO), a mediator in the pathogenesis of osteoarthritis, in the presence and absence of OLC. The OLC-treated cells produced less NO than the non-treated control, which was attributed to the phosphorylation of the p38 kinase that promotes the inhibition of the inducible NO synthase (iNOS), the enzyme responsible for NO production (Iacono et al. 2010). In further experiments the same research group investigated how OLC in chondrogenic and macrophage cell lines (Morena Scotece et al. 2012), also stimulated with LPS, affected the production of the pro-inflammatory cytokines, macrophage inflammatory protein 1α (MIP-1α), interleukin (IL) 6, and NO (Morena Scotece et al. 2012). The results showed that OLC inhibited the expression and production of these pro-inflammatory cytokines in chondrogenic cells and decreased their expression and production in macrophages. It was also able to reduce the iNOS expression and production of NO and pro-inflammatory cytokines, IL-1β, tumor necrosis factor α (TNF-α) and the granulocyte-macrophage colony-stimulating factor in these macrophages (Morena Scotece et al. 2012). Further *in vivo* studies and human trials are needed to assess all the effects of these SEC on arthropathies.

*4.1.2 Cancer*

Cancer is a multifactorial disease characterized by uncontrolled cell proliferation with the potential to invade or spread to other parts of the body. To control this abnormal cell growth, anti-cancer drugs are designed to reduce cell proliferation and to promote cell death (Thurston 2006). Studies have shown that OLC exhibits anti-cancer activity in both processes using different mechanisms of action (R. Fabiani 2016).

Cancer proliferation can be controlled by tyrosine-protein kinase Met (c-Met) phosphorylation. *In vitro* studies have shown that OLC is able to reduce the expression of the c-Met receptor, which seems to be involved in tumor growth, survival and angiogenesis (Akl et al. 2014; Elnagar, Sylvester, and El Sayed 2011). OLC also inhibits the heat shock protein (Hsp90), which leads to an improper folding of the tumor cell proteins and finally to a decrease in tumor growth (Margarucci et al. 2013). Another target of OLC is the transcription factor STAT3, whose downregulation blocks its products, resulting in an inhibition of hepatocellular carcinoma cell growth and metastasis, both *in vitro* and *in vivo* (Pei et al. 2016; Gu, Wang, and Peng 2017). OLC also has the ability to downregulate the extracellular signal–regulated kinases (ERK1/2) and the protein kinase B (AKT) cell signaling pathways, reducing the ERK1/2, P90 ribosomal s6 kinase and AKT phosphorylation and inhibiting cell proliferation in melanoma (Fogli et al. 2016), myeloma (M. Scotece et al. 2013), and non-melanoma skin cancer (Polini et al. 2018), as well as colon and breast cancers (Khanal et al. 2011).

Another mechanism of action that can halt or eradicate cancer is apoptosis. LeGendre and co-workers (LeGendre, Breslin, and Foster 2015) showed that in the absence of caspase-3 or poly (ADP-ribose) polymerase (PARP), enzymes involved in cell apoptosis, OLC increased the phosphorylation of ERK1/2 of cancer cells, which rapidly causes cell death through necrosis. In the same study, OLC showed an ability to selectively change the lysosomal membrane permeabilization, which helps to liberate pro-apoptotic enzymes in tumor cells. Furthermore, OLC inhibits the mammalian target of rapamycin (mTOR), which blocks mitotic cells in the G1 phase and results in apoptotic cell death (Khanfar et al. 2015). Recent discoveries show that OLC has an antitumor effect through increasing intracellular reactive oxygen species (ROS) in liver and colon cancer cells, which brings about cell death (Antonella Cusimano et al. 2017). OLC also induces the activation of apoptosis mechanisms

such as the cleavage of PARP and caspase-3, which causes DNA fragmentation in tumor cells (Khanal et al. 2011; M. Scotece et al. 2013; Akl et al. 2014; Gu, Wang, and Peng 2017). Other apoptosis-promoting effects of OLC are a decrease in the expression of antiapoptotic protein Bcl2 (Fogli et al. 2016), and the inhibition of COX-2, resulting in the activation of AMP-activated protein kinase and ultimately apoptosis of the tumor cell (Khanal et al. 2011).

Khanal and co-workers (Khanal et al. 2011) showed that OLC inhibits the activity of activator protein 1, a transcription factor that controls cell differentiation, proliferation and apoptosis. OLC inhibits MIP-1α in multiple myeloma cells, which promotes apoptosis and curtails cell proliferation (M. Scotece et al. 2013). This polyphenol was found to inhibit migration and invasion *in vitro*, preventing tube formation in human endothelial cells and thus impeding metastasis (Gu, Wang, and Peng 2017).

Finally, OLC, which modulates the estrogen receptor (ER) α (Keiler et al. 2015), has proved to be effective against breast cancer in *in vitro* assays (Ayoub et al. 2017). The inhibition of ER-α impedes 17β-estradiol-induced proliferation (Ayoub et al. 2017). In the same work, Ayoub and co-workers showed that OLC and tamoxifen (an antitumor drug) work synergically against breast cancer (Ayoub et al. 2017).

*4.1.3 Neurological diseases*

The OLC neuroprotective effect has been mainly studied in Alzheimer's disease (AD), due to the latter's prevalence in current society, but it has also been found useful for treating traumatic brain injury. The major effect of OLC on neurological diseases is linked to a capacity to reduce oxidative stress and prevent apoptosis in neuronal cells (Mete et al. 2017).

AD is a slow-progressing neurodegenerative disorder characterized by the misfolding, aggregation and increased toxicity of the *β*-amyloid peptide and tau protein in the brain. The misfolded protein and peptide act as a prion inside the brain, causing aggregation and

362 inducing neuronal apoptosis and inflammatory signals (Nussbaum, Seward, and Bloom 2013).
363 OLC reduces AD symptoms by acting on both *β*-amyloid and tau, and lessening their toxicity.
364 Firstly, OLC inhibits mTOR, which is involved in the synthesis of *β*-amyloid and tau
365 (Khanfar et al. 2015). Secondly, it is able to change the *β*-amyloid structure, resulting in a
366 protein that is easier to eliminate, less reactive and less toxic (Qosa et al. 2015; Abuznait et al.
367 2013; Pitt et al. 2009; Batarseh et al. 2017). Thirdly, OLC can inhibit the fibrillation of the tau
368 protein, modifying it to a conformationally more stable secondary structure, hence preventing
369 its abnormal functionality (Monti et al. 2012; Li et al. 2009). OLC induces P-glycoprotein
370 expression and functionality, which is responsible for *β*-amyloid clearance (Shinde et al.
371 2015; Abuznait et al. 2011; Qosa et al. 2015). OLC also protects neurological cells from
372 apoptosis, reducing ROS levels and upregulating Hsp90 and AKT, two proteins in charge of
373 cell viability (Giusti et al. 2018). OLC could be used as a complement in AD care, enhancing
374 the effect of donepezil, a drug for AD treatment that helps to eliminate *β*-amyloid through the
375 blood brain (Batarseh and Kaddoumi 2018).

376 Parkinson's disease, another neurological disorder highly prevalent in elderly people,
377 also features an abnormal protein aggregation. OLC could be a candidate drug against this
378 disease, ameliorating its symptoms as it does with AD (Dauer and Przedborski 2003;
379 Angeloni et al. 2017).

380

381 *4.1.4 Cardiovascular diseases*

382 Olive oil rich in OLC has shown several effects against cardiovascular diseases, such
383 as improvement in endothelial function in patients with early atherosclerosis (Widmer et al.
384 2014) and an anti-platelet effect in healthy men (Agrawal et al. 2017). It has also exhibited
385 nuclear factor κB inhibition (Brunelleschi et al. 2007), which leads to a reduced expression of

vascular cell adhesion molecule 1 (VCAM-1), thus decreasing leukocyte adherence in the endothelium and promoting a normal endothelial function (Libby 2006).

Despite the considerable research accomplished in this field, more data are required to fully understand the properties and health potential of this olive oil polyphenol. In particular, studies are needed to determine the effect of OLC on the sirtuin family of proteins, which regulate genome maintenance, longevity, and metabolism (Milne and Denu 2008), and are responsible for cellular mechanisms like aging, transcription, apoptosis, and inflammation (Preyat and Leo 2012). Another potential therapeutic target of OLC is the prevention and treatment of type 2 diabetes. This disease is characterized by insulin resistance caused by the non-phosphorylation of the insulin receptor of the cell, which is blocked by pro-inflammatory molecules such as TNF-α (Wellen and Hotamisligil 2005). Hence, OLC may have the ability to reduce insulin resistance by the inhibition or reduction of these pro-inflammatory molecules. A recent study proposed a link between OLC/OLE and diabetes after discovering that a leaf extract rich in OLC and OLE diminished hyperalgesia in diabetic rats. This neuropathic disorder produced by damage in the peripheral nervous system is a typical complication of chronic diabetes (M. E. Czerwińska et al. 2018).

*4.2 Oleacein*

OLE also displays beneficial properties for human health, with *in vitro* protective effects against atherosclerosis and oxidation, and anti-inflammatory activity (Rosignoli et al. 2013; Paiva-Martins and Gordon 2005; Angelino et al. 2011). Its full therapeutic potential remains to be elucidated, but every year new data point to OLE as the main phenol responsible for the positive effect of olive oil on cardiovascular disease. To date, it is known to prevent oxidation, inhibit neutrophil adhesion, and reduce blood pressure (Naruszewicz, Czerwiska, and Kiss 2015). Other health-promoting activities of OLE in the organism include

metal ion chelation (Paiva-Martins and Gordon 2005) and anti-inflammatory activity through COX-2 inhibition (Rosignoli et al. 2013).

The protective effect of OLE against cardiovascular diseases, mainly atherosclerosis, is due to different actions: reduction of oxidation, demonstrated by *in vitro* free radical scavenging (Angelino et al. 2011; Paiva-Martins and Pinto 2008; M. Czerwińska, Kiss, and Naruszewicz 2012); lowering of hypertension through the inhibition of the angiotensin-converting enzyme (Hansen et al. 1996), and suppressing the *in vitro* production of superoxide (Rosignoli et al. 2013; M. Czerwińska, Kiss, and Naruszewicz 2012) and LPS-induced NO (M. Czerwińska, Kiss, and Naruszewicz 2012; Sindona et al. 2012). Also, OLE decreases inflammation by the inhibition of TNF-α-induced production of the pro-inflammatory gene CCL2 and inhibition of CCL2 transcription (Sindona et al. 2012).

Czerwińska and co-workers (M. Czerwińska, Kiss, and Naruszewicz 2012) discovered that OLE reduced neutrophil release by myeloperoxidase, which is responsible for lipid peroxidation and generates reactive nitrogen species (RNS). Thus, OLE reduced the level of low-density lipoprotein (LDL) in the atherogenic form. In additional experiments, Czerwińska and co-workers (M. E. Czerwińska, Kiss, and Naruszewicz 2014) found that OLE induced a decrease in CD11b and CD18 expression and increased CD62L expression, which prevents neutrophil adhesion and enables them to roll along the vascular wall. OLE also inhibited neutrophil endopeptidase activity, protecting natriuretic peptides from degradation and impeding the release of neutrophil elastase.

In addition, OLE exhibited a downregulatory effect on the expression of adhesion molecules VCAM-1, intercellular adhesion molecule 1 (ICAM-1) and E-selectin (Sindona et al. 2012). In other *in vitro* experiments, OLE produced a decrease in the high-mobility group protein B1, a cell ischemia and cell damage biomarker (Filipek et al. 2017), whose stimulation increased the expression of ICAM-1 and VCAM-1 on the surface of endothelial

cells (Klune et al. 2008). It also reduced tissue factor secretion, which is a potent activator of the coagulation cascade (Filipek et al. 2017). OLE enhanced anti-inflammatory activity by stimulating the expression of CD163, an anti-inflammatory gene, increasing the secretion of two anti-inflammatory factors, IL-10 and heme oxygenase (HO) 1 (Filipek et al. 2015). OLE also inhibited arachidonate 5-lipoxygenase, which is responsible for the first steps in the biosynthesis of pro-inflammatory leukotrienes (Vougogiannopoulou et al. 2014). Moreover, it has recently been found to exert a protective effect in humans against atherosclerosis, and OLE-rich olive oil had an anti-platelet effect in healthy men (Agrawal et al. 2017).

The endothelial restoring capacity of OLE was tested in endothelial progenitor cells, which are responsible for neovascularization of ischemic tissue and participate in the re-endothelization of an injured arterial wall. Cells from healthy patients were treated with angiotensin II and were in contact or not with OLE or oleuropein. When the cells were treated with the polyphenols, proliferation and telomerase activity increased, and the percentage of senescent cells and intracellular ROS formation decreased. The polyphenols restored the migration, adhesion and tube formation of the endothelial progenitor cells reduced by angiotensin II. The beneficial effect was attributed to an activation of nuclear factor (erythroid-derived 2)-like 2 and an increase of HO-1 expression, OLE being more efficient than oleuropein (Parzonko et al. 2013).

*In vitro* studies demonstrated that OLE has a protective role in human blood cells against oxidative-induced hemolysis (Paiva-Martins et al. 2010). It reduced $H_2O_2$-induced DNA damage in human blood mononuclear cells (R Fabiani et al. 2008) and also protected LDL from oxidation (Visioli et al. 1995). OLE showed an anti-cancer effect, promoting apoptosis in leukemia cells (Roberto Fabiani et al. 2006) and in non-melanoma skin cancer cells (Polini et al. 2018). Finally, OLE had an antiestrogenic effect, binding both ER-α and ER-β (Keiler et al. 2015).

460 In summary, OLE provided protection against atherosclerosis by reducing
461 hypertension (Hansen et al. 1996), preventing neutrophil adhesion, reducing oxidative
462 damage (M. E. Czerwińska, Kiss, and Naruszewicz 2014), improving injured cell wall
463 recovery (Parzonko et al. 2013), promoting anti-inflammatory chemokines (Filipek et al.
464 2015), and inhibiting pro-inflammatory chemokines (Sindona et al. 2012). OLE has also
465 shown an anti-cancer (Roberto Fabiani et al. 2006) and antiestrogenic effect (Keiler et al.
466 2015).
467

## 5. Conclusion

In the last 15 years, the secoiridoids OLC and OLE have been a focus of research on EVOO. Their biosynthesis and biotransformation during the growth of the olive crop and in the oil extraction process have been studied with the aim of increasing their final concentration in EVOO. Further experiments are required to assess the stability of these compounds in the oil.

The mechanisms of OLC and OLE absorption are unclear, but possibly include passive diffusion. In human studies, the OLC metabolites found in plasma and urine have been mainly attributed to processes of hydrogenation, hydration, hydroxylation and glucuronidation. However, more research is required on the extent of absorption and metabolism processes, which will shed light on the bioavailability of these SEC and their plasma concentration levels.

The protective effects of OLC and OLE have been widely investigated *in vitro* and some studies have been conducted *in vivo*. OLC shows therapeutic promise against cancer, arthropathy, AD and cardiovascular diseases, but its full health-promoting capacity remains undetermined. More studies, *in vitro* with cells and tissues and *in vivo* with animals, and clinical trials are required to draw better conclusions. Like OLC, OLE is still under-explored by the scientific community, but results to date show protective effects against atherosclerosis and cancer. Finally, additional efforts are needed to discover and characterize new properties of these compounds.

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**Table 1**. Health effects of OLC

| | Ref | Cell model | OLC concentration/ treatment | Mechanism of action | Biological effect |
|---|---|---|---|---|---|
| **Inflammation** | | | | | |
| | (Beauchamp et al. 2005) | COX-1, COX-2, LOX inhibition assay kit | 7, 25, 100 µM. | Inhibition of COX-1 and COX-2 | Reduced inflammation |
| | (Rosignoli et al. 2013) | Human monocytes stimulated with Lps | 100 µM | Inhibition of COX-2 | Reduced inflammation |
| **Cancer** | | | | | |
| Adeno-carcinoma | (Khanfar et al. 2015) | HeLa and Caco-2 | 10 µM | Inhibition of mTOR | Inhibition of mTOR arrests apoptosis-causing mitotic cells in the G1 phase |
| Breast | (Elnagar, Sylvester, and El Sayed 2011) | MDA-MB-231 | 5-20 µM $IC_{50}$= 15 µM | Inhibition of C-met phosphorylation | Decrease of tumor growth, survival and angiogenesis |
| | (Akl et al. 2014) | MDA-MB-231, MCF-7 and BT-474 MDA-MB-231 in athymic nude mice | 5-15 µM (Significant effect from 5 µM) | Inhibition of C-met phosphorylation<br>Reduction of ERK1/2 and AKT phosphorylation<br>Cleavage of PARP and caspase-3 | Decrease of tumor growth, survival and angiogenesis<br>Activation of apoptosis |
| | (LeGendre, Breslin, and Foster 2015) | MDA-MB-231 | 20 µM | During serum starvation condition, activation of ERK1/2 phosphorylation without cleavage of caspase-3 or PARP<br>Inhibition of acid sphingomyelinase | Cell necrosis<br>Change in lysosomal membrane permeabilization, and release of necrosis enzymes |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (Khanfar et al. 2015) | MCF-7, MDA-MB-231, T47D | 10 µM | Inhibition of Mtor | Inhibition of mTOR arrests apoptosis-causing mitotic cells in the G1 phase |
| | | (Ayoub et al. 2017) | BT-474, MCF-7, T-47D<br>BT-474 in mice | 1-80 µM (Significant effect from 20 µM) | Inhibition of 17β-estradiol | Inhibition of proliferation |
| | Colon | (Khan al et al. 2011) | HCT-116 and HT-29<br>JB6 Cl41 murine | 1-10 $\mu g \cdot mL^{-1}$<br>(Significant effect from 1 $\mu g \cdot mL^{-1}$) | Reduction of ERK1/2 and p90RSK phosphorylation<br>Inhibition of ap-1 activity<br>Activation of AMPK<br>Inhibition of COX-2<br>Cleavage of PARP and caspase-3. | Reduction of cancer proliferation<br>Activation of apoptosis |
| | | (ACusimano et al. 2017) | SW480, HT29 | 50 µM | Increase of ROS concentration | DNA damage |
| | Hepato-carcinoma | (Pei et al. 2016) | Huh-7, HepG2 and HCCLM3<br>Male BALB/c athymic nude mice | 10-80 µM<br>$IC_{50}$(Huh-7)= 30.08,<br>$IC_{50}$(HepG2)= 29.92,<br>$IC_{50}$(HCCLM3)= 31.37 | Blocking of STAT 3 activation | Inhibition of tumor growth and metastasis |
| | | (ACusimano et al. 2017) | HepG2, Hep3B, Huh7, PLC/PRF/5 | 50 µM | Increase of ROS concentration | DNA damage |
| | Leukemia | (Roberto Fabiani et al. 2006) | HL60 | 7.5-120 µM | | Less proliferation<br>Increase of apoptosis |
| | Multiple Myeloma | (M. Scotece et al. 2013) | ARH-77 (human) and MOPC-31C (murine) | 10-50 µM $IC_{50}$= 10 µM | Reduction of ERK1/2, p90RSK and AKT phosphorylation<br>Inhibition of MIP-1α<br>Cleavage of PARP and caspase-3 | Reduction of cancer proliferation<br><br>Activation of apoptosis |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Pancreas | (LeGendre, Breslin, and Foster 2015) | BxPC3 | 20 µM | During serum starvation condition, activation of ERK1/2 phosphorylation without cleavage of caspase-3 or PARP<br>Inhibition of acid sphingomyelinase | Cell necrosis<br>Change in lysosomal membrane permeabilization and release of necrosis enzymes |
| | Prostate | (Elnagar, Sylvester, and El Sayed 2011) | PC3 | 5-20 µM $IC_{50}$=20µM | Inhibition of Cmet phosphorylation | Decrease of tumor growth, survival and angiogenesis |
| | | (LeGendre, Breslin, and Foster 2015) | PC3 | 20 µM | During serum starvation condition, activation of ERK1/2 phosphorylation without cleavage of caspase-3 or PARP<br>Inhibition of acid sphingomyelinase | Cell necrosis<br>Change in lysosomal membrane permeabilization and release of necrosis enzymes |
| | Skin | (Fogli et al. 2016) | A375 and 501Mel | 10 µM<br>$IC_{50}$ (A375)= 13.6 µM,<br>$IC_{50}$ (501Mel)=20 µM | Reduction of ERK1/2, p90RSK and AKT phosphorylation<br>Decrease expression of Bc12 | Reduction of cancer proliferation<br>Inhibition of anti-apoptosis |
| | | (Gu, Wang, and Peng 2017) | A375 or A2058<br><br>HUVEC | 20-40 µM (Significant effect from 20 µM) | Caspase-3, caspase-9 and PARP cleavage<br>Inhibition of tube formation | Induction of apoptosis<br>Inhibition of migration and invasion |
| | | (Polini et al. 2018) | A431 (non-melanoma skin cancer) | 1-100, $IC_{50}$= 30 µM | Less phosphorylation of Akt and ERK1/2 | Reduction of cancer proliferation |
| **Arthopathy** | | | | | | |
| | | (Iacono et al. 2010) | ATDC5 | 1-25 µm (Significant effect from 5 µM) | Reduction of LPS-induced NO<br>Phosphorylation of p38 kinase | Less joint inflammation |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (Morena Scotece et al. 2012) | ATDC5 and J774 | 50 μM | Inhibition of MIP-1α and IL-6<br>Reduction of NO production and iNOS<br>Reduction of IL-1β, TNF-α and GM-CSF | Less joint inflammation |
| | | (Rosignoli et al. 2013) | Human monocytes | 100 μM | Reduction of LPS-induced NO | Less joint inflammation |
| **Alzheimer's disease** | | | | | | |
| | $\beta$-Amyloid | (Pitt et al. 2009) | Hippocampal cells | 0-100 μM (Significant effect from 0.01 μM) | Change in oligomeric structure of Aβ<br>Reduction of Aβ binding | Increased immunoreactivity and less deterioration of dendritic spines.<br>Enhanced clearance of Aβ |
| | | (Abuznait et al. 2013) | bEnd3 | 0.5-50 μM (Significant effect from 0.5 (P-gp) and 1 μM (LRP1)) | Induction of P-gp and LRP1 | Enhanced clearance of Aβ |
| | | (Khanfar et al. 2015) | MDA-MB-231 (breast cancer) | 25 μM | Inhibition of mTOR | Less synthesis of amyloid-β and tau protein |
| | | (Qosa et al. 2015) | TgSwDI mice<br>hCMEC/D3, SH-SY5Y-APP | 5 mg.kg·day$^{-1}$ OLC for 4 weeks.<br>0,1,5,10 μM (Significant effect from 5 μM) | Up-regulation of P-gp, LRP1 and ApoE-dependent pathway<br>Reduction of astrocyte activation and IL-1β<br>Increase in P-gp and LRP1 expression | Reduction of brain inflammation caused by AD<br>Enhanced clearance of Aβ |
| | | (Batarseh et al. 2017) | CCF-STTG1 and SH-SY5Y | 5 μM | Reduction of IL-6 and GFAP levels after 7 days<br>Reduction of the expression of GLT1 and PSD-95 | Less AD-associated inflammation<br>Reduction of Aβo-induced down-regulation of synaptic protein |
| | | (Giusti et al. 2018) | SH-SY5Y | 1-10 μM (Significant effect from 1 μM) | Less ROS, upregulation of Hsp90 and Akt | Inhibition of apoptosis |
| | | (Batar | 5xFAD mice | 476 μg | $\beta$-Amyloid easier to clear | Enhanced donopezil effect |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | seh and Kaddoumi 2018) | | OLC/kgmice/day | | |
| | Tau protein | (Li et al. 2009) | Chemical assay | 1-100 µM (Significant effect from 1 µM) | Inhibition of tau transformation from random coil to β-sheet | Tau more stable |
| | | (Monti et al. 2012) | Chemical assay | 60-100 µM | Schiff base between aldehyde and NH2 of lys residue of tau | Tau more stable |
| **CVD** | | | | | | |
| | | (Brunellesc hi et al. 2007) | Human monocytes | Olive oil extract rich in OLC (1.855 $g \cdot L^{-1}$) | Nf-κB inhibition | Less adherence of leukocytes |
| | | (Widmer et al. 2014) | Double-blind, randomized trial with 82 patients with early atherosclerosis | 30 $mL \cdot day^{-1}$ of EVOO rich in OLC (70 $mg \cdot L^{-1}$) | Reduction in ICAM, WBC, lymphocytes, monocytes and platelet count | Improvement of endothelial function |
| | | (Agrawal et al. 2017) | Double-blind, randomized, controlled crossover study, with 27 healthy men | 40 mL of EVOO rich in OLC (310 $mg \cdot L^{-1}$) | Anti-platelet | Protection against atherosclerosis |

Abbreviations: COX-1: Cyclooxygenase 1, COX-2: Cyclooxygenase 2, LOX: Lipoxygenase, Lps: lipopolysaccharide, mTOR: mammalian target of rapamycin, c-Met: tyrosine-protein kinase Met, ERK1/2: extracellular signal–regulated kinases, AKT: protein kinase B, PARP: Poly (ADP-ribose) polymerase, P90RSK: P90 ribosomal s6 kinase, Ap-1: Activator protein 1, AMPK: AMP-activated protein kinase, ROS: Reactive oxygen species, STAT3: Signal transducer and activator of transcription 3, MIP-1α: Macrophage Inflammatory Protein 1α, Bcl2: B-cell lymphoma 2, IL-6: Interleukin 6, iNOS inducible nitric oxide synthase, Il-1β: Interleukin 1β, TNF-α: Tumor necrosis factor α, GM-CFS: Granulocyte-macrophage colony-stimulating factor, Aβ: Amyloid-β, P-gp: P-glycoprotein, LRP1: Low density lipoprotein receptor-related protein 1, ApoE: Apolipoprotein E, IL-1β: Interleukin 1β, GFAP: Glial fibrillary acidic protein, GLT1: glutamate transporter 1, PSD-95: Post synaptic density protein 95, Hsp90: heat shock protein 90, Nf-κB: Nuclear factor κB, ICAM-1: Intercellular Adhesion Molecule 1, VCAM-1: Vascular cell adhesion molecule 1, WBC: White blood cells.

**Table 2**: Health effects of OLE.

| | | Ref | Cell model | OLE concentration/ treatment | Mechanism of action | Biological effect |
|---|---|---|---|---|---|---|
| **CVD (atherosclerosis)** | | | | | | |
| | Biochemical assay | (Visioli et al. 1995) | Healthy human LDL | 10 µM | Protects LDL from oxidation | Protection against atherosclerosis |
| | | (Hansen et al. 1996) | Angiotensin converting enzyme from rabbit lung | $IC_{50}$ = 26 µM | Ace inhibition | Hypertension |
| | | (Paiva-Martins and Gordon 2005) | Buffer solutions 3.5, 5.5, 7 | 400 µM | Fe chelator | Metal chelator |
| | | (Paiva-Martins and Pinto 2008) | Water/methanol solution | $IC_{50}$= 0.3mol OLC/mol radical compound | Radical scavenging | Reduction of cell oxidative damage |
| | | (M. Czerwińska, Kiss, and Naruszewicz 2012) | Chemical assay | 1-50 µM (Significant effect from 10 µM) | Radical scavenging | Reduction of cell oxidative damage |
| | | (Vougogiannopoulou et al. 2014) | Isolated human recombinant 5-LO | $IC_{50}$= 2 µM | 5-LO inhibition | No formation of pro-inflammatory leukotrienes |
| | White blood cell | (R Fabiani et al. 2008) | Human blood mononuclear cells | 10 µM | Reduction of H2O2-induced DNA damage | DNA protection |
| | | (M. Czerwińska, Kiss, and Naruszewicz 2012) | Human neutrophils | 10 µM | Reduction of Lps-induced NO-production | Reduced inflammation |
| | | | | | Reduction of lipid peroxidation release<br>Reduction of RNS | Reduction of atherogenic form of LDL |
| | | (Rosignoli et al. 2013) | Human monocytes | 100 µM | Cox-2 inhibition<br>Reduction of superoxide production | Antiinflammatory<br>Reduction of cell oxidative damage |
| | | (M. E. Czerwińska, Kiss, and Naruszewicz 2014) | Human isolated neutrophils | 20,50,100 µM (Significant effect from 20 µM) | Less CD11b and CD18 expression, more CD62L expression<br>Inhibition of neutrophil elastase, MMP-9 and IL-8 release<br>Inhibition of neutrophil endopeptidase activity | Less neutrophil adhesion and easier to roll along the vascular wall<br>Less inflammation<br>Protection of natriuretic peptides from degradation |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (Filipek et al. 2015) | Monocyte/macrophage cells | 10-20 µM and Hemoglobin-haptoglobin complex (Significant effect from 10 µM) | Stimulation of CD163 gene expression<br>Increase in secretion of IL-10 and HO-1 | Reduced inflammation |
| | Other blood cells | (Paiva-Martins et al. 2010) | Human red blood cells | 5-10 µM (Significant effect from 5 µM) | Interaction between oleacein and RBC membrane proteins | Protection of RBCs from oxidative-induced hemolysis |
| | | (Angelino et al. 2011) | Human red blood cells | 0.7 $mg \cdot g^{-1}$ | Radical scavenging | Reduction of cell oxidative damage |
| | | (Sindona et al. 2012) | HUVEC human umbilical vein endothelial cells | 10 $\mu g \cdot mL^{-1}$ | Reduction of lps-induced no-production<br>Inhibition of TNF-α induced CCL2 transcription<br>Less VCAM-1 and ICAM-1 expression<br>Less e-selectin expression | Less inflammation<br>Less neutrophil adhesion |
| | | (Parzonko et al. 2013) | Human endothelial progenitor cells | 1-10 µM (Significant effect from 1 µM) | Activation of nrf2<br>Increase of HO-1 | Restored migration, adhesion and tube formation of endothelial progenitor cells |
| | | (Filipek et al. 2017) | Human isolated carotid plaques | 5-20 µM (Significant effect from 5 µM) | Decreased HMG1<br>Less TF secretion | Less cell damage<br>Less adhesion of neutrophils<br>No activation of coagulation cascade |
| | Human studies | (Widmer et al. 2014) | Double-blind, randomized trial with 82 patients with early atherosclerosis | 30 $mL \cdot day^{-1}$ of EVOO rich in OLE (73 $mg \cdot L^{-1}$) | Reduction in ICAM, WBC, lymphocytes, monocytes and platelet count | Improvement of endothelial function |
| | | (Agrawal et al. 2017) | Double-blind, randomized controlled crossover study with 27 healthy men | 40 mL of EVOO rich in OLE (312 $mg \cdot L^{-1}$) | Anti-platelet effect | Protection against atherosclerosis |
| **Others** | | | | | | |
| | Cancer | (Roberto Fabiani et al. 2006) | HL60 (leukemia) | 7.5-120µM (Significant effect from 17.5 µM) | | Less proliferation<br>Increase of apoptosis |
| | | (Polini et al. 2018) | A431(non-elanoma skin cancer) | 1–100 µM, $IC_{50}$= 10 µM | Less phosphorylation of Akt and ERK1/2 | Reduction of cancer proliferation |
| | Antiestrogenic effect | (Keiler et al. 2015) | MVLN cells | 10 nM-10 µM (Significant effect from 10 µM) | Binds ER-α and ER-β | Antiestrogenic effect |

Abbreviations: LDL: Low density lipoprotein, ACE: Angiotensin-converting enzyme, 5-LO: Arachidonate 5-lipoxygenase, Lps: lipopolysaccharide, COX-2 Cyclooxygenase 2, RNS: Reactive nitrogen species, MMP-9: Matrix metallopeptidase 9, IL-8: Interleukin 8, IL-10: Interleukin 10, HO-1: Heme oxygenase 1, RBC: Red blood cells, TNF-α: Tumor necrosis factor α, CCL2: C-C Motif Chemokine Ligand 2, ICAM-1: Intercellular Adhesion Molecule 1, VCAM-1: Vascular cell adhesion molecule 1, Nrf2: Nuclear factor (erythroid-derived 2)-like 2, HMG1: high-mobility group protein 1, TF: tissue factor, WBC: White blood cells, AKT: protein kinase B, ERK1/2: extracellular signal–regulated kinases, ER-α: Estrogen receptor α, ER-β Estrogen receptor β.

## Figure Captions

**Figure 1** Biosynthesis of secoiridoids in *Olea europaea* L

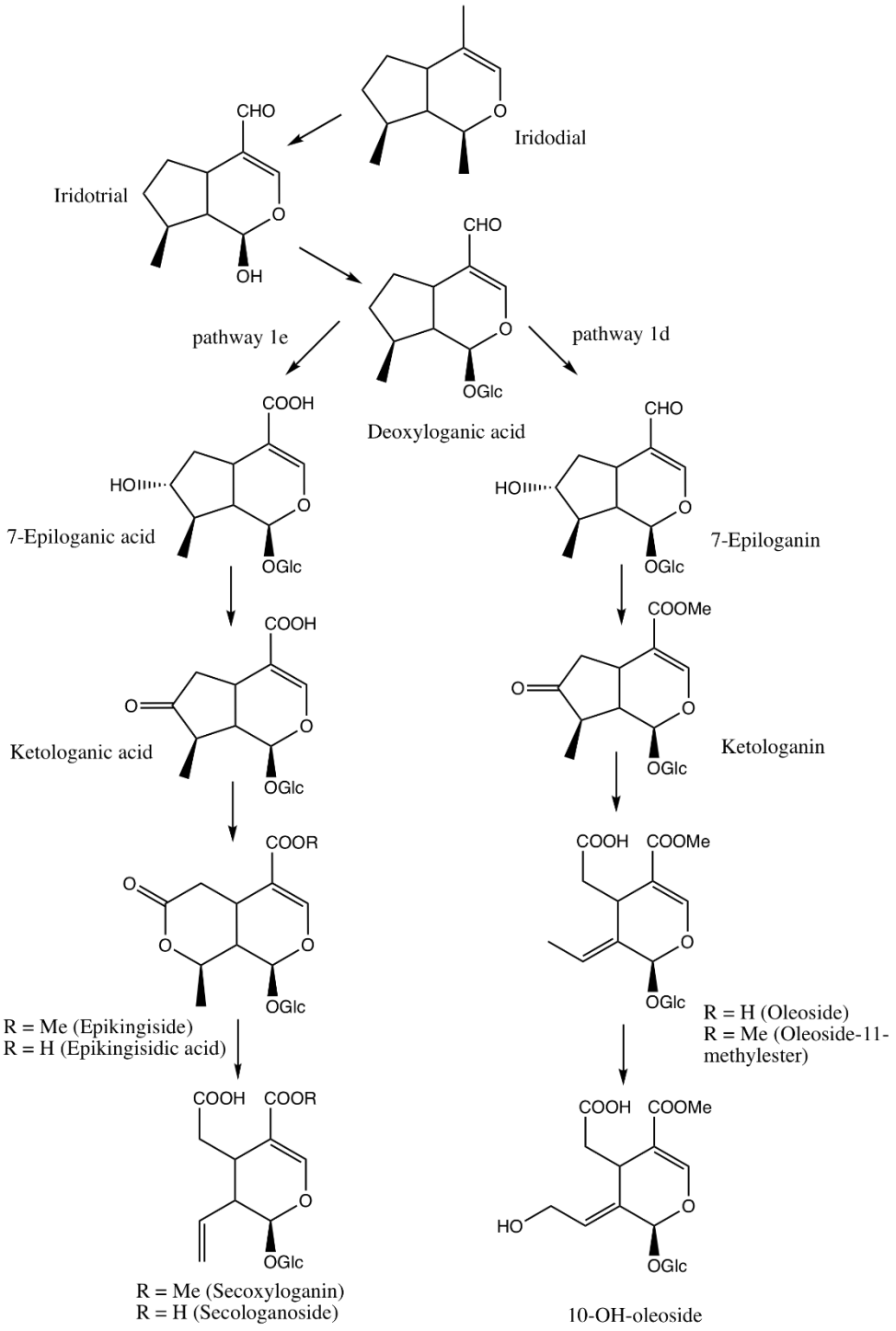
**Figure 2.** First gastrointestinal steps encountered by the OLC molecule after oral administration. According to several studies, the ester bond is partially affected, but the intact molecule of OLC will ultimately reach the blood stream.

**Figure 3**. Plausible metabolic pathways OLC according to the literature.

ALK (stands for NADPH-dependent aldoketoreductase, produces hydrogenation), CYP450 (produces hydroxylation, and oxidation changes), UGT (stands for UDP-glucuronosyl transferase), COMT (stands for cathecol-O-methyl-transferase, and has apparent specificity for ortho-diphenolic structures, but according to Rubió et al 2012. tyrosol-like structures could suffer methylation), SULT (stands for sulfotransferase), difficult to identify one enzyme responsible for hydration.

**Figure 4**: Benefficial effect of oleocanthal in AD

**Figure 1**


Iridodial
CHO
Iridotrial
OH
CHO
OGlc
Deoxyloganic acid
pathway 1e
pathway 1d
COOH
HO
7-Epiloganic acid
OGlc
CHO
HO
7-Epiloganin
OGlc
COOH
Ketologanic acid
OGlc
COOMe
Ketologanin
OGlc
COOR
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COOH COOMe
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R = Me (Oleoside-11-methylester)
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R = H (Secologanoside)
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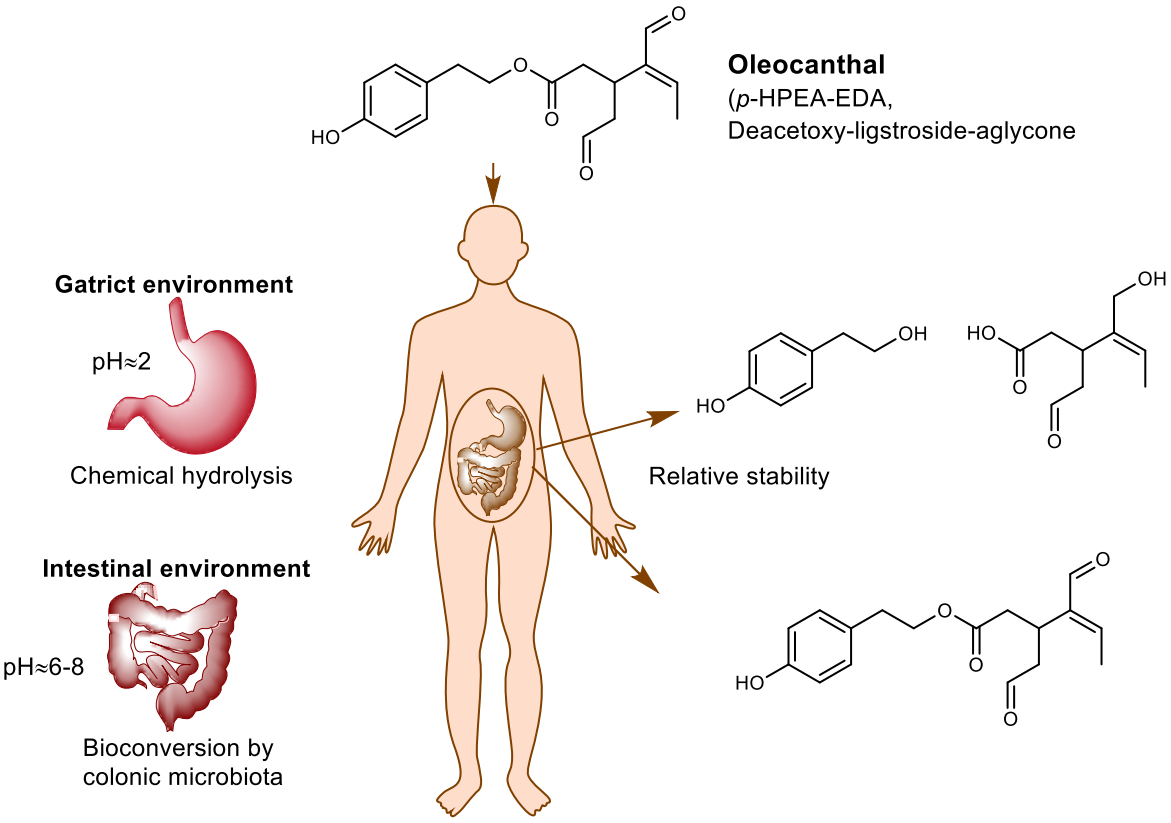
COOH COOMe
HO
10-OH-oleoside
OGlc

**Figure 2**


Oleocanthal
(p-HPEA-EDA,
Deacetoxy-ligstroside-aglycone
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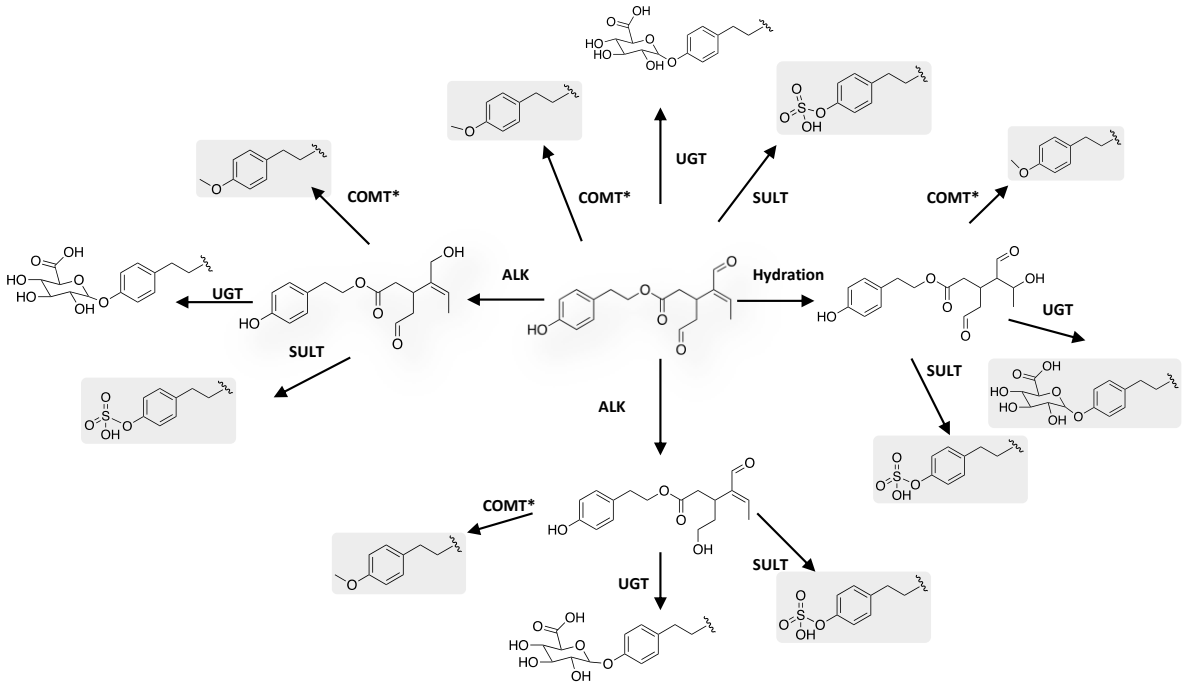
pH≈2
Chemical hydrolysis
Intestinal environment
pH≈6-8
Bioconversion by
colonic microbiota
Relative stability

# Figure 3


UGT
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