

R E V I E W

New findings on the pharmacodynamic actions of olive oil: our contribution to better evidence about its remedial properties

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Summary. *Objective:* Olive oil (OO) is a typical Mediterranean product which influences positively the human health, that has been well-recognized since ancient times. Numerous substances isolated from OO, in particular from extra virgin olive oil (EVOO), are still the subjects of extensive scientific researches. The objective of this review was to bring together rather scattered information on the remedial constituents of OO, as well as available data on their pharmacodynamic actions. *Material and Methods:* For this analysis, we searched for studies on the remedial substances of OO published in English on the PubMed in 2005-2015, in which the pharmacodynamic actions of OO were investigated. We considered studies which were conducted on cell lines and experimental animals, as well as clinical trials. We excluded studies where pharmacodynamic actions of the mentioned substances could not be precisely distinguished and described. *Results:* Oleic acid decreases the concentration and oxidation of LDL, reduces the risk of developing cardiovascular diseases, as well as possesses some anti-tumor activities. Oleuropein exhibits neuro-, hepato- and cardioprotective activities, possesses hypotensive and anti-inflammatory effects, as well as strong potential in prevention of colon and breast cancer. Hydroxytyrosol possesses pronounced anti-oxidant properties, as well as anti-inflammatory, anti-platelet and anti-tumor activities. Tyrosol exhibits anti-oxidant properties that are less pronounced than those of hydroxytyrosol. *Conclusion:* OO has multiple beneficial effects of protecting the humans' health and even of preventing and/or treating different diseases. Additional measures should be taken to encourage the production and consumption of OO globally, as well as to inform and educate the professional and general public about its favorable pharmacodynamic actions on human health.

Key words: olive oil, pharmacodynamic actions, remedial properties

Introduction

The traditional Mediterranean diet (MD) is characterized by a high intake of olive oil (OO) as the principal source of fat, fruit, nuts, vegetables, and cereals; a moderate intake of fish and poultry; a low intake of dairy products, red meat, processed meats, and sweets; and wine in low to moderate amounts, consumed during the meals (1). The nutritional and medicinal effects of olive fruit and OO have been well-recognized since ancient times. Recent multicenter, parallel and

randomized PREDIMED trial (2) demonstrated that MD, supplemented with extra-virgin olive oil, resulted in a substantial reduction in the risk of major cardiovascular events among high-risk persons.

Montenegrins, as well as other Mediterranean peoples, have cultivated olive trees and produced OO for centuries. According to the International Olive Oil Council (IOC)(3), depending on the year, the total production of OO in Montenegro is about 400-500 tons, and the estimated OO consumption is about 0.5l/citizen per year, that is significantly less than the average

consumption in the EU and in many non-European countries. According to the same source, the annual production and consumption of OO in Italy, Greece and Spain is significantly higher compared the one in Montenegro.

Virgin olive oil (VOO) is produced from the fruit of the olive tree solely by mechanical or other physical means under conditions, with no chemical treatments. VOO fit for consumption include the *extra virgin olive oil (EVOO)*- which has free acidity, expressed as OA, of no more than 0.8 g/100 g, *virgin olive oil* - which has free acidity, expressed as OA, of no more than 2 g/100 g, and *ordinary virgin olive oil (OVOO)* - which has a free acidity, expressed as OA, of not more than 3.3 g/100g (3). EVOO is considered to have the highest quality because of the lowest free acidity, the highest content of so-called "minor components", as well as for superior physico-chemical and organoleptic characteristics. In that sense, the remedial properties of OO are mostly attributed to the EVOO, so it was suggested that EVOO should be specifically recommended as part of a MD, rather than a more general recommendation of "OO" (4).

EVOO primarily consists in triacylglycerols (~99%), mono- and diacylglycerols, free fatty acids and a variety of lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols and pigments (5). According to the IOC standards (3), allowable oleic acid (OA) range in EVOO is 55.0%-83.0% of the total fatty acid content. That is why the beneficial effects of OO on humans' health have been attributed to this substance for long. However, EVOO also contains a large number of so-called "minor substances" with very useful biological properties. The most important among them are the phenolic compounds - oleuropein, oleuropein aglycone, oleocanthal, hydroxytyrosol and tyrosol. These compounds show anti-oxidant and anti-inflammatory properties, prevent lipoperoxidation, induce favorable modifications in a lipid profile, improve endothelial functions and exhibit antithrombotic properties (6). Many studies confirm strong anti-tumor effects of phenolic compounds of EVOO (7).

Our recent studies on experimental animals (8-10) confirmed the fact that, from the pharmacodynamic point of view, OO cannot be regarded as an indifferent substance. This conclusion led us to more

detailed analysis of the latest information on its pharmacodynamic actions.

The objective of this review was to put together the new and rather scattered information on the pharmacodynamic actions of different OO constituents.

Material and Methods

Studies

The substances of interest, their biological activities and pharmacodynamic actions were selected and described on the basis of studies published on the "PubMed" database using the following keywords (the number of studies containing the relevant keywords is indicated in parentheses): "olive oil review" (503), "olive oil phenols" (1001) and "olive oil phenolic compounds" (508). From all the studies found, we used the most recent ones (7 studies in total) with multiple citations and a large number of used literature sources.

Information on the pharmacodynamic properties of each selected substance and its mechanism of action were found by reviewing the studies published on the "PubMed" database in English mainly for the period of 2005-2015. Studies that we took into account were carried out on cell lines and experimental animals. We also took into account different studies containing the results of clinical trials. We excluded those studies, in which pharmacodynamic properties of the selected substances could not be accurately distinguished and described.

Substances

For selected substances, we used the following keywords to search the database (the number of studies containing relevant keywords is indicated in parentheses):

- a) Oleic acid*: "oleic acid LDL" (683/120), "oleic acid cardiovascular" (1421/214), "oleic acid antitumor" (90/42) and "oleic acid lipoprotein" (1594/218); We used 7 studies of those found with the indicated keywords. (*For the OA: the first number in parentheses indicates the total number of studies containing relevant keywords found in the database, the second number indicates only those studies published in the years of 2005-2015. This is due

to the fact that OA has long been studied, and the majority of its pharmacodynamic properties were discovered in the previous decades).

- b) Oleuropein: “oleuropein neuroprotective” (6), “oleuropein hepatoprotective” (2), “oleuropein cardioprotective” (8), “oleuropein hypertension” (9) and “oleuropein antitumor” (5);

We used 16 studies of those found with the indicated keywords.

- c) Oleuropein aglycon: “oleuropein aglycone antioxidant” (24), “oleuropein aglycone neuroprotective” (2) and “oleuropein aglycone antitumor” (2);

We used 8 studies of those found with the indicated keywords.

- d) Oleocanthal: “oleocanthal inflammation” (6), “oleocanthal antitumor” (3) and “oleocanthal neuroprotective” (6);

We used 8 studies of those found with the indicated keywords.

- e) Hydroxytyrosol: “hydroxytyrosol antioxidant” (158), “hydroxytyrosol inflammation” (30), “hydroxytyrosol aggregation” (18) and “hydroxytyrosol antitumor” (5);

We used 10 studies of those found with the indicated keywords.

- f) Tyrosol: “tyrosol antioxidant” (74) i “tyrosol bioavailability” (23);

We used 2 studies of those found with the indicated keywords.

Oleic acid

Unlike other plant oils, which mainly contain polyunsaturated fatty acids, OO contains monounsaturated OA, which is one of its main constituents (11) (Fig. 1). This substance has already been intensively studied for several decades, but even today data on its pharmacodynamic actions and mechanisms of these effects remain controversial. In various studies, it was found that OA reduces concentration (12) and oxidation (13-14) of low density lipoproteins (LDL) and decreases the risk of cardiovascular diseases (CVD) (12-15), as well as has antitumor activity (16). Nagyova A et al. (14) concluded that two table spoons (approx. 20 g) of EVOO consumed daily for 6 weeks

in elderly lipidemic patients favourably affected serum lipoprotein spectrum and fatty acid composition which probably contributed to the increased resistance of serum lipids to oxidation.

The mechanism by which OA reduces the risk of CVD is not only in reduction of the LDL concentration in the blood (12), but also in reduction of its oxidative modification (13) and atherogenic properties. According to Cho et al. (2010) (12), the main mechanism of reduction of LDL concentration is the increase of its clearance via LDL receptors in the liver. The mechanism of reduction of LDL oxidative modification is related to the chemical characteristics of OA. OA is a monounsaturated fatty acid (MUFA), which is less susceptible to oxidative modification than other fatty acids. Thus, the larger amounts of OA are incorporated into LDL, the less susceptible to oxidative modification they become (13-14). It means that the quantitative and qualitative composition of fatty acids in a diet can strongly affect the rate of oxidative modification of LDL (13).

In addition, OA could reduce the risk of CVD favorably influencing the coagulation activity of the blood, primarily the factor VII (FVII), which is considered as the potential factor in development of ischemic stroke (15-16). It is known that 2-3 hours after a meal rich in fat, there is a significant increase in the coagulation activity of FVIIc, which is maintaining for several hours. If a diet is rich in MUFA, there is less postprandial increase in the FVIIc activity.

Nowadays, the anti-tumor effect of OA is actively investigated, but still the mechanisms by which this effect is achieved are not fully understood. According to Carrillo et al. (2012), all modern theories come down to the inhibition of proliferation and the induction of apoptosis of tumor cells. In the first case, they say about inhibition of over-expression of HER2 (erbB-2) and intracellular calcium signaling pathway that promotes the cell proliferation. In the second case, they

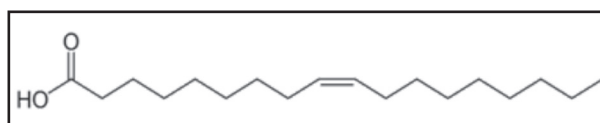


Figure 1. Structural formula of oleic acid (<https://commons.wikimedia.org>)

note a production of reactive oxygen species (ROS) and spending of calcium reserves of the cellular endoplasmic reticulum that directly induces their apoptosis (17).

Phenolic compounds

The so-called "minor fraction" that makes up to 2% of EVOO consists of more than 230 chemical compounds (aliphatic alcohols and triterpenes, sterols, hydrocarbons, volatile compounds and antioxidants) (17). Recent articles showed that the properties of EVOO favorable for human health may be linked to lipophilic and hydrophilic phenols. The hydrophilic phenols are unique for EVOO, and cannot be found in other oils and fats (18). They include various groups of phenolic compounds, though in this review we focused on phenolic alcohols (hydroxytyrosol and tyrosol) and secoiridoids (oleuropein, oleuropein aglycone and oleocanthal) since these substances are undoubtedly of the most scientific and clinical interest.

Oleuropein

Oleuropein (OL) exhibits neuro-(19-21), hepato- (22-23) and cardioprotective effects (24-28), has hypotensive activity (29) and strong potential in preventing the development of colon and breast cancer (30-34) (Fig. 2).

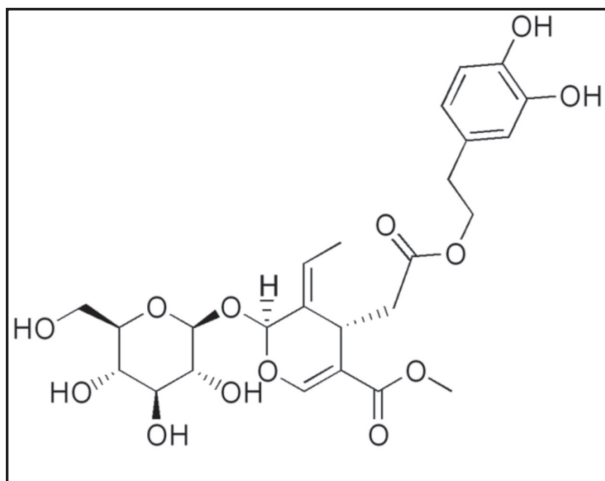


Figure 2. Structural formula of oleuropein (<https://commons.wikimedia.org>)

Research of the mechanisms of neuroprotective activity is still in the initial phase. An investigation carried out on rats showed that OL in single daily dose of 50 mg/kg p.o. for 6 months reduced oxidative damage of the substantia nigra by increasing the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) (19). *In vitro* studies showed that OL can form a non-covalent connections with A β -peptide (20) and prevent the aggregation of Tau proteins (21), which are typical findings in different neurodegenerative disorders.

It is assumed the multifactorial mechanism of hepatoprotective activity of OL (22, 23). In the study carried out on rats, Kim Y et al. (2010) showed that 0.03% oleuropein-supplemented high-fat diet for 10 weeks influenced the expression of genes encoding some key regulatory molecules responsible for fatty acid uptake and transport into hepatocytes, as well as genes involved in response to oxidative stress and synthesis of pro-inflammatory cytokines (22). In another study, it was observed that OL in doses of 100 and 200 mg/kg administered intraperitoneally once daily for 3 consecutive days, prior to CCl₄ administration, or once daily for 2 consecutive days 6h after CCl₄ intoxication, significantly reduced oxidative stress and inflammatory response in male mice liver by preventing activation of hepatic stellate (Ito) cells through TNFB1 and activation of caspase-3 (23).

Several mechanisms that underlie cardioprotective effects of OL are described: reduction of oxidative stress and release of pro-inflammatory cytokines (24), anti-aggregation (25-26), anti-atherogenic (27-28) and hypotensive effects (29). If it comes to the mechanism underlying the anti-aggregation effect, we did not find any precise data related to OL. Still, it is assumed that OL, like other OO phenols, can inhibit cAMP phosphodiesterase (26). The mechanisms of anti-atherogenic effect include the ability of OL to inhibit LDL oxidation (27) and to reduce adhesion of monocytes to stimulated endothelium (28). The anti-hypertensive effect of OL in rats (29) in doses of 20, 40, and 60 mg/kg/day, was probably related to stimulation of release of vasodilatory nitric oxide, as well as antioxidant and sympatholytic activity.

Several possible mechanisms of anti-tumor activity of OL are described in different studies. OL

induced apoptosis of breast cancer cells via the mitochondrial way, prevented their proliferation by extending the S-phase of the cell cycle, and inhibited the anti-apoptotic and pro-inflammatory protein NF- κ B and its main oncogene target cyclin D1 (30). In the study on human colon adenocarcinoma cells, Carden et al. (2013) showed that OL reduces their growth by inducing apoptosis via activation of the p53 way (31). OL can also neutralize free radicals that are formed in the faecal masses and thus protect the mucous membrane of the colon (32). When administered orally to mice who developed spontaneous tumors, OL completely regressed tumors in 9-12 days (33). In addition, an anti-angiogenic effect of OL was found in some studies (34), that means the potential to prevent the growth of the other types of tumors.

Oleuropein aglycone

Oleuropein aglycone (OLA) is an aglycone form of OL which appears during olive fruit crushing, within the process of hydrolysis by endogenous β -glucosidases (35).

This substance has a less pronounced antioxidant effect than that of other olive phenols (36), but, according to the available data, it drew the attention of scientists because it has potent neuroprotective (37-41) and antitumor activities (42).

It was published that both *in vitro* and *in vivo* OLA prevents the formation of toxic peptide aggregates and toxic effects of previously formed aggregates on neurons (37-40). Luccarini et al. (37) found the beneficial properties of 450 μ M OLA injected into the nucleus basalis magnocellularis of adult male Wistar rats against neurodegeneration. Previous findings led other authors to an assumption that OLA could possess beneficial effects in the prevention/treatment of Alzheimer's and Parkinson's diseases (41).

In the investigation on breast cancer cells, Menendez JA et al. (2007) found a significant increase of the trastuzumab efficacy in the presence of OLA in different concentrations from 6.25 μ M to 100 μ M (42). In addition, in the same study it was shown that cells resistant to trastuzumab regained their sensitivity to drug when it was co-administered with OLA.

Oleocanthal

Oleocanthal (OC) has appeared relatively recently in surveys, but it has already proved to be the molecule with useful pharmacological properties in various pathological conditions, including inflammation (43), tumors (44-48) and neurodegenerative diseases (49-50) (Fig. 3).

Beauchamp GK et al. (2005) showed that OC exerts an anti-inflammatory effect similar to that of ibuprofen (43), thus it was called a "natural anti-inflammatory agent". It is believed that daily use of OC as an integral part of the MD can reduce inflammation and the risk of tumor development and thrombosis due to the inhibition of cyclooxygenase (COX). According to the same authors, OC inhibits COX 1 and COX 2 enzymes significantly more at equimolar concentrations in comparison to ibuprofen - OC (25 μ M) inhibits 41%-57% and ibuprofen (25 μ M) inhibits 13%-18% of COX activity.

In several studies on different tumor cell lines, OC showed anti-proliferative and anti-migratory effects (44-47). In the study on multiple myeloma cells, OC inhibited MIP-1 α , which is thought to play a key role in bone marrow damaging (44). By various mechanisms, OC also induced apoptosis of human colon adenocarcinoma cells (45) and, at the same time, showed anti-proliferative/migratory/invasive effects on human prostate and breast cancer cells by, probably, the inhibition of c-Met phosphorylation (46-47). Margarucci L et al. (2013) found that OC inhibits Hsp90 (so-called "chaperone"), which is essential for a variety of tumors to grow (48). In this respect, there is an opinion that this substance can become a parent compound for a new class of Hsp90 inhibitors (48).

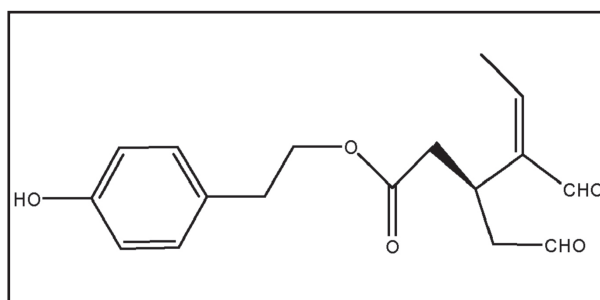


Figure 3. Structural formula of oleocanthal (<https://commons.wikimedia.org>)

The mechanisms by which OC exerts neuroprotective effect are not well understood yet. Intraperitoneal administration of OC to wild type of mice (10 mg/kg/day, twice daily over a duration of 2 weeks) increased the clearance rate of A β -amyloid from the brain by up-regulation of P-gp and LRP1 (which are the main transport proteins of A β -amyloid in the blood-brain barrier) and by up-regulation of the enzymes responsible for degradation of A β -amyloid (49). In another *in vitro* study, it was stated the ability of OC to react with Tau protein amino groups and thus to prevent its fibrillation (49).

Hydroxytyrosol

By its chemical structure, hydroxytyrosol (HT) belongs to the group of phenolic alcohols (Fig. 4). In different sources, it is stated that HT has pronounced antioxidant properties (51-52), as well as anti-inflammatory (53-55), anti-platelet (56) and anti-tumor activity (57-60).

Owing to the hydroxyl groups in its chemical structure, HT is known as one of the most potent antioxidants of EVOO (36). Due to this property, HT can block the oxidation of LDL (51) and protect cells from the oxidative damage by binding of various endoge-

nous and exogenous free radicals (52). It is believed that the majority of beneficial effects of HT for human health are mostly related to its antioxidant properties.

HT exerts an anti-inflammatory effect by inhibiting COX-1 and COX-2 (53) and by reducing the secretion of inflammatory mediators (IL-1 α , IL-1 β , IL-6, IL-12, TNF- α), as well as by reducing the expression of genes encoding the enzymes involved in the inflammatory response (54). Sindona G et al. (2012) found that HT reduces the inflammation by inhibition the production of pro-inflammatory cytokine CCL2 (55) in endothelial cells, that could be used for the therapeutic purpose.

HT exerts an anti-platelet effect by decreasing the production of thromboxane B2 in platelets, and by increasing the production of NO in leukocytes. VOO also contains HT acetate in similar concentrations as those of HT (160-479 μ M/kg). Quantitatively, HT acetate possesses a stronger anti-platelet activity compared to HT, which can be comparable with aspirin (56).

If it comes to the anti-tumor activity, as an antioxidant, HT can prevent DNA damage (57), block the growth of cells at the G2/M-phase of the cell cycle and induce apoptosis, which was found both *in vitro* and *in vivo* (58-60). Similarly to OL, HT promotes the expression of p53 protein (31).

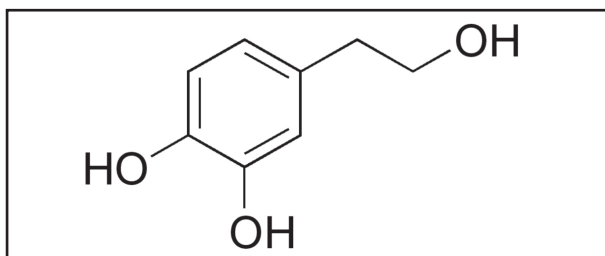


Figure 4. Structural formula of hydroxytyrosol (www.sigmaaldrich.com)

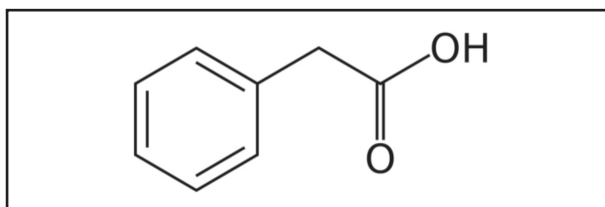


Figure 5. Structural formula of tyrosol (<https://commons.wikimedia.org>)

Tyrosol

Tyrosol (T) is a phenolic alcohol (Fig. 5) with a very good bioavailability which could be used as a biomarker of OO consumption (61). It has antioxidant properties which are, according to the relevant literature, less pronounced than those of HT, but, at the same time, it possess as the specific mechanism to restore the intracellular antioxidant defence (62).

Conclusion

The consumption of EVOO has multiple beneficial effects or maintain a good health and or preventing or even treating different diseases. Due to its antioxidant, cardioprotective, neuroprotective, hepatoprotective, anti-inflammatory and anti-tumor activi-

ties, EVOO exerts beneficial effects in prevention and treatment of different cardiovascular, inflammatory, immunological, neurological, hematological and gastrointestinal diseases, as well as malignancies. Considering the fact that Montenegro has a great potential for the olives growing, additional measures should be taken as soon as possible to encourage the production and the consumption of OO, as well as to educate the professional and general public about its favorable pharmacodynamic actions on human health.

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