Biological Activities of the Olive Tree's Polyphenolic Components

Subjects: Nutrition & Dietetics

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The main polyphenolic components present in the fruit and in the wax that covers the olive leaves are represented by hydroxytyrosol, luteolin, oleuropein, verbascoside, gallic acid, vanillic acid, caffeic acid, and the aldehyde derivative oleocanthal. Among all of them, the main and most studied representative is hydroxytyrosol (HT), (2-(3,4-dihydroxyphenyl) ethanol, 3,4-dihydroxyphenethyl alcohol, DOPET, C8H10O3, and presents a molecular mass of 154.16. It is a natural compound, whose structure corresponds to a type of polyphenol, widely distributed throughout the plant kingdom, being especially abundant in the Oleaceae family. It can be found in high concentrations in extra olive oil, fruits and leaves of Olea europaea, and in other products such as fruits, vegetables, and tea. HT in oil is found in free form, as an acetate, or as part of more complex compounds such as oleuropein (a secoiridoide glycoside esterified with a phenylpropanoid alcohol, the major phenolic component of green olive pulp), the flavonoid luteolin (a 5,7-dihidroxi-4-cromenona with hydroxytyrosol), and verbascoside (a phenylethanoid with hydroxytyrosol and a phenylpropanoid sugar ester with caffeic acid).

Keywords: animal nutrition ; diet ; feeding ; hydroxytyrosol ; maslinic acid

1. Pharmacokinetics and Toxicity

In relation to pharmacokinetics, bioavailability studies have shown that HT is absorbed in animals and humans in a dose-dependent manner but following non-lineal kinetics, after ingestion of olive oil, exerts its biological effects and is excreted in the urine mainly as glucuronide conjugates [1][2]. Absorption of HT takes place in the small intestine and colon [3]. It has been suggested that transport across the intestinal epithelium may occur by passive bidirectional diffusion [4]. Absorption of this compound is rapid, reaching peak plasma concentrations 5–10 min after ingestion, followed by a rapid decline [5]. This absorption differs according to the vehicle in which it is transported, with several authors demonstrating that rats absorbed 75% of HT when administered in an aqueous solution and 90% when an oily vehicle was used [6].

Different authors [7] have studied the tissue distribution of intravenously administered ^{14}C-labeled HT in rats. At 5 min after injection, less than 8% was still present in the blood, approximately 6% in the plasma, and 1.9% in the cellular fraction of the blood; therefore, it was estimated that the half-life of the compound in blood was very small, below 2 min. Similar levels of ^{14}C were found in the cells of skeletal muscle, liver, lungs, and heart, while the kidney cells accumulated 10 times more than the other organs. ^{14}C was also detected in the brain, indicating that HT was able to cross the blood–brain barrier, although it has been described that HT can also be generated endogenously in the brain from dopamine [8] and from dihydroxyphenylacetic acid (DHPA) via dihydroxyphenylacetic reductase present in the brain [9].

HT metabolites were found in blood 5 min after intravenous injection of ^{14}C-HT, indicating that the compound was rapidly metabolized in cells, especially in the liver and enterocytes, proposing three metabolic pathways for HT: oxidation, through the enzymes alcohol-dehydrogenase and aldehyde-dehydrogenase giving rise to dihydroxyphenylacetic acid; methylation, through the enzyme catechol-O-methyltransferase (COMT) giving rise to homovanillic alcohol (HVOL); and methylation plus oxidation, to form homovanillic acid (HVA) [7].

Regarding excretion, it was found that 90% of the radioactivity was detected in the urine 5 h after the intravenous injection of ^{14}C-HT and a small proportion was excreted in the feces [7]. These results coincide with the results obtained in humans where most of the HT and tyrosol were found in the urine collected during the first 4 h after administration. ingestion of 50 mL of virgin olive oil [10].

Serra et al. [11], using Wistar rats as an in vivo model, showed that just one hour after ingestion of a phenolic extract from olive pomace, these phenolic compounds and a large part of their metabolic derivatives were absorbed, metabolized, and

distributed throughout the body. Most tissues such as the liver, heart, spleen, thymus, testicles, and even the brain after crossing the blood–brain barrier. In addition, they revealed a clear renal route of detoxification [11].

The safety profile of HT appears to be excellent; no adverse effects have been demonstrated even at very high doses [7][12][13]. To study acute toxicity, a single dose of 2 g/kg body weight was administered to rats and no toxic effects or macroscopic alterations were found in organs; only the appearance of piloerection was reported 2 h after administration, and this disappeared in less than 48 h [7]. In addition to this phenomenon, several toxicity studies have been conducted using aqueous extracts of olive pulp in which the HT content ranged from 50–70% of the total amount of phenols [13].

In another study, oral administration to Sprague-Dawley rats of a single gavage dose of solid olive pulp extract at levels between 0 and 2 g/kg caused no adverse effects, except in soft or liquid feces [12]. At a dose of 2 g/kg/day of this extract, no acute toxicity was found, with no teratogenic or mutagenic effects. As part of a micronucleus assay, Sprague-Dawley rats were given a single dose of 5 g olive pulp extract/kg by gavage and after 6 days were given the same daily dose for the next 29 days with no mortality or clinical signs of toxicity. This study showed that the LD50 of the solid olive pulp extract was greater than 5 g/kg (equivalent to 3 g/kg HT), suggesting that the extract is practically nontoxic [12]. Given its excellent safety profile, future availability as a health food supplement could be envisaged.

2. Antioxidant and Anti-Ageing Effects

Defense against reactive oxygen species (ROS) is essential to protect cellular molecules such as lipids, proteins, or DNA and to prevent the development of damage that can lead to major diseases. When defensive mechanisms are overcome by the action of free radicals, subsequent cellular damage can lead to diseases such as atherosclerosis, cardiovascular, skin, and neurodegenerative diseases, diabetes mellitus, metabolic syndrome, and cancer, among others. Finally, physiological processes such as aging have been associated with an imbalance between the action of ROS and antioxidants. Some antioxidant agents can be found in various types of food.

Hydroxytyrosol (HT), compared to other olive oil phenolic compounds, shows much more effective antioxidant characteristics, such as scavenging free radicals, breaking peroxidative chain reactions, preventing lipid peroxidation, inhibiting hypochlorous acid-derived radicals, etc. [14]. In addition, a decrease in ROS production, derived from iron- or copper-induced oxidation of low-density lipoprotein (LDL), has been reported after treatment with HT in an in vitro model, suggesting a chelating action on these metals [15]. The ability to scavenge or reduce ROS generation was further confirmed in both 12-myristate 13-phorbol acetate (PMA)-treated leukocytes and in the hypoxanthine/xanthine oxidase cell-free system by chemiluminescence methods [16][17].

In a recent study, Hybertson et al. [18] demonstrated the antioxidant effects of a mixture of phenols formed carnosol, withaferin A, and luteolin via the nuclear factor erythroid 2 (Nrf2)-mediated pathway. Separately, each of these polyphenols exhibited significant antioxidant activity based on the ability to activate Nrf2 by binding to different antioxidant response elements (ARE), facilitating the regulation of the expression of a wide variety of cytoprotective genes [18].

In addition, this pathway is synergistically activated by the combined presence of the three polyphenols [17]. Their activity on canonical genes, non-canonical genes, as well as genes that appear to be regulated by mechanisms other than Nrf2 in cultured HepG2 cells, was able to protect these cells from oxidative stress challenge caused by the presence of different oxygen-free radicals such as peroxides [18].

3. Anticancer and Antiproliferaty Effects

Nowadays, cancer is among the deadliest diseases. Research has shown that the incidence of several cancers is much lower in the Mediterranean area compared to other countries. Thus, the consumption of extra virgin olive oil may be associated with a reduced risk of certain cancers, a fact that has been supported by several epidemiological studies in humans [19]. These health benefits may be attributed more to the phenolic compounds than to their fatty acid profile, as several studies have suggested that polyphenols may have a protective effect against cancer [20][21].

The HT-rich diet has recently received special attention due to its antioxidant, anti-proliferative, pro-apoptotic, and anti-inflammatory activities, capable of specifically counteracting all the hallmarks of cancer. Thus, mounting experimental evidence has suggested that, in addition to antioxidant and anti-inflammatory capacities, HT exerts anticancer effects through the activation of molecular signaling pathways, leading to cell apoptosis and growth arrest in several tumor cell lines [22][23][24][25].

It is well known that H2O2 plays a role in cell signaling, and this fact seems to be related to proapoptotic effects [26]. Furthermore, proapoptotic effects were observed in HL60 cells incubated with HT even under conditions that do not support H2O2 accumulation (between 23.8 and 38.0% depending on the medium) suggesting that mechanisms other than H2O2-releasing activity could be involved in proapoptotic activity, probably due a mitochondrial apoptotic way [27]. Polyphenols such as HT could therefore be beneficial for their contribution to redox homeostasis. On the other hand, several studies have shown that HT is able to arrest the cell cycle, reduce growth and proliferation and induce apoptosis in HL60 and HT29 cells [28].

Other authors also studied the behavior of HT at concentrations of 50–100 µM on the cell cycle in the HL60 cell line, demonstrating inhibition of cell proliferation, arresting cells in the G0/G1 phase with a concomitant decrease in the S and G2/M phases [29]. Subsequently, the same authors showed that HT (100 µM) causes an increase in the expression of $p21^{WAF/Cip1}$ (cyclin-dependent kinase inhibitor 1A) and $p27^{Kip1}$ (cyclin-dependent kinase inhibitor 1B) and inhibits cyclin-dependent kinase-6 in the same cell type, arresting the G2/M and G0/G1 phases of the cell cycle; they also found that HT promotes apoptosis in cells that are in S phase [30].

At the same time, different authors showed in MCF-7 breast cancer cells that HT and olive leaf extract at different doses exhibited a blockade of the G1 to S phase transition, manifested by an increase in the number of cells in the G0/G1 phase [24][29]. Similarly, both HT and olive leaf extract rich in HT and oleuropein decreased the number of cells in the G2/M phase of the cell cycle [24][31].

Recently, the molecules of luteolin have been described as a potential agent in triple-negative breast cancer (MDA-MB-231 cell line) treatments by suppressing the protein expressions of nuclear erythroid factor 2 (Nrf2), heme oxygenase 1 (HO-1), and Crypto-1, which contribute significantly and critically to the functioning of cancer stem cells [32]. Luteolin is capable of inhibiting the expressions of stem-related transcription factors, the ATP-binding cassette transporter G2 (ABCG2), CD44, the activity of aldehyde dehydrogenase 1 (ALDH1), as well as the spherical-forming properties of breast cancer stem cells [32].

In this regard, other authors studied the anticancer and antiangiogenic activity of another of the polyphenols present in olive extracts, oleocanthal, in hepatocellular carcinoma, demonstrating not only inhibition of proliferation but also inhibition of cell cycle progression and cell death, cell cycle progression, and induction of apoptosis by affecting the cell cycle protein cyclin D1 and the anti-apoptotic proteins Bcl-2 and survivin, but was also able to inhibit epithelial–mesenchymal transition (EMT) through down-regulation of Twist, which is a direct target of STAT3 (signal transducer and activator of transcription 3) together with down-regulation of MMP2 [33].

Overall, the data provided in these studies are positive in terms of the anti-tumor and anti-angiogenic capacity of the different olive polyphenols in different cancer cells, supported by studies of the inhibition of cell proliferation, anti-metastatic potential, and decreased expression of certain angiogenic factors. Although not conclusive, they indicate that polyphenols have properties and potential therapeutic effects that could be taken into account for the description of the possible beneficial effects of olive oil and the development of supplements based on polyphenolic extracts and nutraceuticals.

4. Immunomodulatory and Anti-Inflammatory Effects

HT has been described as the most powerful anti-inflammatory compound among the polyphenols present in olive oil, showing an effective inhibition of NO and prostaglandin E2 (PGE2) production, decreasing the secretion of cytokines (IL-1α, IL-1β, IL-6, IL-12, TNF-α) and chemokines (CXCL10/IP-10, CCL2/MPC-1), and reducing gene expression of iNOS, IL-1α, CXCL10/IP-10, MIP-1β, matrix metalloproteinase-9 (MMP-9), and prostaglandin E2 synthase (PGES) [34][35][36][37].

In vitro studies in human monocytic THP-1 cells treated with LPS to induce inflammatory response have shown that HT showed inhibition of proinflammatory cytokines (TNF-α) and reduced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression by more than 60% [34]. Other studies showed that HT decreased thromboxane B2 (TXB2) synthesis in an in vitro platelet-rich plasma model, probably as a result of reduced production of arachidonic acid-derived eicosanoids [38]. In this regard, these authors have found a reduction in thromboxane A2 (TXA2) synthesis as measured by the reduction of its metabolite, thromboxane B2. This decrease was mainly due to the inhibition of the activity of the enzyme cyclooxygenase [39][40].

In addition, HT prevents prostaglandin E2 synthesis by indirectly blocking iNOS and COX-2 enzymes. This effect arises from the prevention of transcriptional activation of NF-κB, interferon regulatory factor-1, and transducer and activator of transcription 1a, which prevent activation of J774 mouse macrophages [41].

HT is known to be capable of producing arylating/alkylating adducts on NF-κB cysteine residues. The action of HT on this factor blocks the transcription of COX-2 and 5-lipoxygenase enzymes, reducing prostaglandin E2 synthesis and thus the chronic influence associated with diseases such as cancer [42].

In vivo studies in rats with acute inflammation induced by the treatment through interplantar injection of different doses of carrageenan showed that rodents that received, by gavage feeding, a preparation in which the main ingredients included HT (22%), showed significant inhibition of both acute inflammation and pain associated with carrageenan administration [43]. They also found beneficial effects in patients with stabilized coronary heart disease [44].

Intake of virgin olive oil, rich in HT, was shown to be more effective than refined olive oil in reducing levels of interleukin-6 (IL-6) and C-reactive protein (CRP), recognized inflammatory markers in cardiovascular disease [45].

Antiviral and Antimicrobial Effects

Different studies have shown that olive oil and olive leaf extracts act as antimicrobial agents with activity against Escherichia coli, Candida albicans, Kluyveromyces marxianus, Clostridium perfringens, Streptococcus mutans, Shigella sonnei, Salmonella enterica, and others. It appears that the main components of olives and olive leaves responsible for the antimicrobial effect are HT and the dialdehyde and decarboxymethyl forms of elenolic acid [46][47][48].

In addition, it has been shown in vitro that HT at low concentrations also possesses antimicrobial properties against infectious agents of the respiratory and gastrointestinal tract such as Vibrio parahaemolyticus, Vibrio cholerae, Salmonella typhi, Haemophilus influenzae, Staphylococcus aureus, Moraxella catarrhalis, and are also effective against mycoplasmas such as Mycoplasma pneumoniae [48][49][50]. These concentrations are even lower than those used with certain antibiotics, such as ampicillin.

Yamada et al. demonstrated that hydroxytyrosol (HT) was able to inactivate different influenza A viruses, including H1N1, H3N2, H5N1, and H9N2, and also the Newcastle disease virus, suggesting that the mechanism of the antiviral effect of this polyphenol may require the presence of a viral envelope for its action [51]. At the same time, a pre-treatment of MDCK (Madin-Darby Canine Kidney cells) with HT did not affect the spread of H9N2 when subsequently inoculated; although, the H9N2 virus inactivated with hydroxytyrosol maintained its hemagglutinating activity unaltered and bound to MDCK cells in a similar way to untreated virus, implying that HT targets the virus and not the host cell [51].

Neuraminidase activity in the HT-treated virus also remained unchanged. However, in cells inoculated with HT-inactivated H9N2 virus, neither the protein nor the mRNA was detected. Electron microscopy analysis showed morphological abnormalities in the HT-treated H9N2 virus, with most of the structures found in the virions being atypical. These results suggest that the viral structure is altered by HT [51].

On the other hand, a very important current opportunistic infection in humans is acquired immunodeficiency syndrome (AIDS), caused by the immunodeficiency virus (HIV). Despite the existence of compounds and combinations against the HIV virus to reduce morbidity and mortality in patients, they do not cure the infection, and the necessary studies aimed at destroying this virus are being carried out.

In addition, anti-HIV therapies must be maintained over the long term with significant problems of drug resistance and chronic toxicity. They observed that olive leaf extract containing HT inhibits acute infection and cell-to-cell transmission of HIV-1 between uninfected MT2 cells cultured with HIV-1-infected Th9 cells at a dose-dependent concentration, with an IC50 of about 0.2 μg/mL [52]. In addition, this extract also inhibits HIV-1 replication as assayed by p24 expression in infected Th9 cells, with no cytotoxicity detected in uninfected target cells [53][54].

During the last five years, different papers have been published demonstrating the antiviral activity of polyphenols, proposing that many polyphenols are able to inhibit the replication of different types of SARS-CoV [55][56][57][58][59][60]. Several of these works revealed that polyphenols were able to inhibit HIV DNA polymerase α and reverse transcriptase and thus able to block DNA elongation by competing with incoming nucleoside triphosphates (NTPs) [56], as well as type 3C L-protease activity (3CL-Pro) [57][58][59].

Following this approach, Wu et al. [60] have demonstrated the antiviral capacity of polyphenols to significantly inhibit RNA-dependent RNA polymerase (RdRp) in SARS-CoV-2, with even greater inhibitory capacity than that found in one of the most effective inhibitors, the FDA-approved drug remdesivir, thus confirming the antiviral activity of some polyphenols.

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