

**Review**

Natural Polyphenols Oleuropein and Hydroxytyrosol in Therapeutics: Potentials Against Cancer, Inflammation, Oxidative Stress, and Diabetes

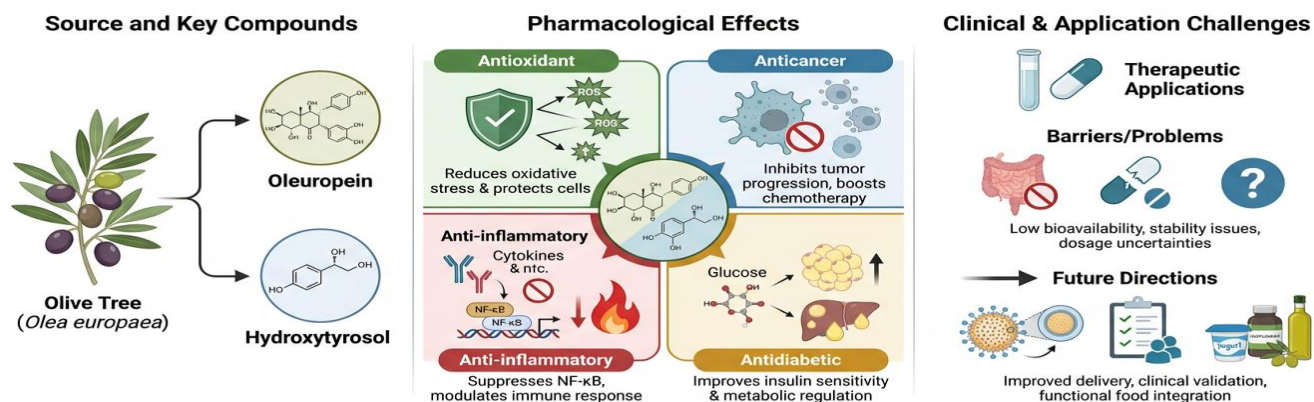
Faryal Shams^{1,2,†}, Muhammad Ibrahim^{1,3,†}, Shana Perrella³, Sumaira Saeed^{*3}, Fabiana Monfrecola³, Carmen Lenti³, Immad Khan², Faiq Firdous⁴¹Faculty of Natural Sciences, Islamia College University Peshawar, 25120, Pakistan.²Institute of Chemical Sciences, University of Peshawar, 25120, Pakistan.³Department of Precision Medicine, Università degli Studi della Campania, 80138, Italy.⁴Center for Omics Sciences, Islamia College Peshawar University, 25120 Pakistan.Correspondence: (Sumaira Saeed) sumiara.saeed@unicampania.it[†]These authors contributed equally to this work**Abstract**

Oleuropein and hydroxytyrosol, two major polyphenolic compounds extracted from the olive tree (*Olea europaea*), have attracted significant scientific interest due to their strong pharmacological properties, including anticancer, antioxidant, anti-inflammatory, and antidiabetic effects. These compounds play a crucial role in modulating oxidative stress, neutralizing free radicals, and protecting against cellular damage, thereby contributing to neuroprotection, cardiovascular health, and metabolic regulation. Their anticancer potential has been widely demonstrated through their ability to inhibit tumor progression, induce apoptosis, and demonstrate the ability to enhance the efficacy of chemotherapy in various tumor models, including breast and hepatocellular carcinoma. Additionally, their anti-inflammatory activity is attributed to the regulation of cytokines, suppression of NF- κ B signaling, and modulation of immune responses, which could be beneficial in managing chronic inflammatory and autoimmune diseases. Moreover, their role in diabetes management has been extensively studied, demonstrating their ability to improve insulin sensitivity and regulate glucose metabolism, and reduce lipid accumulation in hepatic and adipose tissues, thus preventing metabolic complications. Despite extensive preclinical evidence supporting the therapeutic benefits of these compounds, their clinical translation remains challenging due to issues related to bioavailability, stability, and optimal dosage. Future research should focus on improving their pharmacokinetic properties, developing advanced drug delivery systems, and carrying out extensive clinical trials to confirm their safety and effectiveness in humans. The integration of these compounds into functional foods, nutraceuticals, and pharmaceutical formulations holds immense promise for preventive and therapeutic applications, potentially offering natural alternatives to conventional therapeutic agents for managing metabolic, inflammatory, and degenerative diseases.

Keywords: Oleuropein, Hydroxytyrosol, Antioxidant, Anticancer, Anti-inflammatory, Antidiabetic.

Graphical abstract

Therapeutic Potential of Oleuropein & Hydroxytyrosol from Olive (*Olea europaea*)



1. Introduction

There are 30 species of the genus *Olea* in the world, and there are about 80 distinct names for it. The most well-known species, *Olea europaea*, is found in the Mediterranean areas, is the only species with global commercial significance. In the coastal parts of the eastern Mediterranean basin, the olive plants are mainly found, the neighboring seaside regions of South Europe, North Iran, North Africa and Western Asia [1]. The hierarchy is thought to have originated in southwest Asia and higher Mesopotamia [2]. Some refer to it as Hatay, Kahramanmaraş, and Gaziantep. Olive cultivation is predominantly distributed across Mediterranean countries such as Spain, Italy, Greece, and nearby islands, while additional cultivation regions include Iran, China, and Pakistan [3].

Typically, *Olea europaea* grows to a height of 10m and is a short, dense tree or shrub. The blooms might be functionally unisexual, bisexual, or many. In this tree, the most important part is its fruit, an oval-shaped fruit that is green in color before ripening and then turns blackish-violet as it ripens. In wild plants, it is usually smaller than in cultivated ones, measuring between 1 and 2.5 cm in length [1]. These trees have a thousand-year lifespan. Despite the fact that fruit is produced when the trees are three to four years old, plants that are twelve to twenty years old yield the most. It can continue to produce fruit for up to 100 years after it is pruned, and it can continue to do so for many more [2]. The extremely sturdy wood of these trees is typically used to make long-lasting furniture and various handicrafts, but in some locations it is also valued as firewood because even when wet, it burns efficiently [3]. A monoecious plant is the olive tree that depends on the wind for cross-pollination or self-pollination. It has both perfect and imperfect blossoms. Typically, nearby cultivars or the wild type, such as oleaster, pollinate cultivated plants [1]. The stomatal density varies among cultivars, and the leaves exhibit hypostomatic characteristics [4].

The most prevalent bioactive ingredients in goods made from olive trees are oleuropein. The principal bioactive ingredient in olive trees, this secoiridoid, gives them their distinctively bitter flavor and protects them from becoming rotten [5]. According to reports, in olive leaves the dry source concentration of oleuropein ranges from 6 to 9% (60 to 90 mg/g in olive plants) [6]. But, based on the cultivar and planting circumstances, this could change [7]. In the olive leaves, Oleuropein is a secoiridoid glycoside belonging to the coumarin class of compounds. It is distinguished by an ester bond between 2-(3',4'-dihydroxyphenyl) ethanol (hydroxytyrosol) and the elenolic acid glucoside [8, 9]. The chemical structure of oleuropein is presented in figure 1. An ortho-diphenolic group in oleuropein's chemical structure has the ability to scavenge ROS by the donation of hydrogen and to stabilize the ROS by the intramolecular hydrogen bonding. The tyrosol and supplementary single hydroxyl substitutions don't have any antioxidant qualities, whereas o-diOH substitutions ensure [10] and the antioxidant activity of oleuropein is increased due to its capacity which enables binding to metal ions such as iron [11]. The primary health

benefits of oleuropein are typically linked to this antioxidant capability. According to a current study, the oleuropein has antimicrobial, cardioprotective, antiviral, antioxidative, anti-inflammatory and antihypertensive properties. It can also act as a natural prooxidant and anticancer agent and has hypocholesterolemic and hypoglycemic effects, as well as the ability to enhance lipid metabolism [12]. Although individual components show certain effects, consuming olive leaf extracts in their whole form, without isolating the main element, may provide greater health benefits, because all of the extract's bioactive chemicals work in concert, which probably influences how well they are absorbed and how bioavailable they are [13].

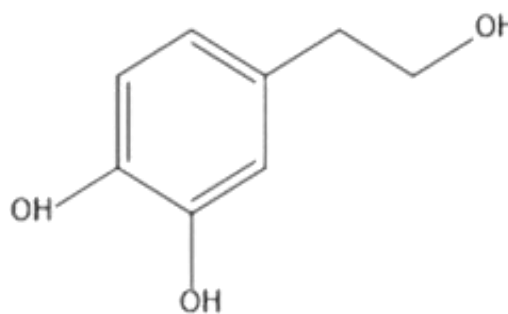


Figure 1. Chemical structure of oleuropein.

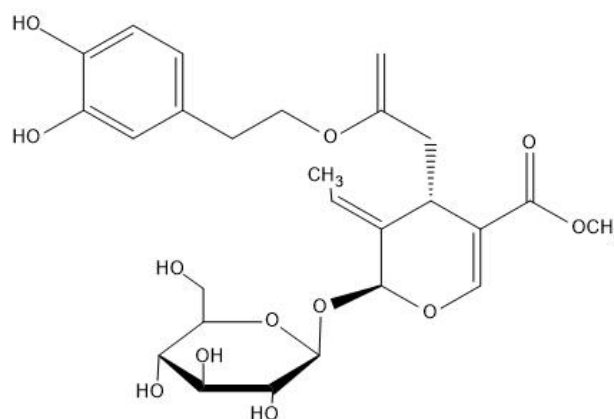


Figure 2. Chemical structure of hydroxytyrosol.

Leaf extract has been discovered to contain a phenolic alcohol known as hydroxytyrosol (3,4-dihydroxyphenylethanol) at a concentration around 2.28 mg per 100 g. The chemical structure of hydroxytyrosol is illustrated in figure 2. This strong antioxidant is produced when oleuropein is hydrolyzed [14]. Because of its straightforward chemical structure, hydroxytyrosol is highly bioavailable and easy for the body to absorb. It does not accumulate or cause toxicity, as it reaches plasma levels within 15–20 minutes and removed by the digestive or renal systems 6–8 hours later. Since it contains both a lipophilic and a hydrophilic end, it can cross cellular membranes more readily, this amphipathic molecule that is soluble in both water and fats facilitates molecular transport throughout the

body. Consuming hydroxytyrosol has several advantages for the body due to its structural and molecular characteristics [15]. Because of its great antioxidant potential, hydroxytyrosol shows promise in protecting against chronic diseases. The *o*-dihydroxyphenyl moiety is principally responsible for this efficiency. Its primary functions include breaking chains, giving hydrogen atoms to peroxy radicals (ROO*), scavenging free radicals, and chelating metals. Additionally, it strengthens the body's defenses against activates specific cellular signaling pathways and oxidative stress to improve antioxidant protection [16].

2. Anticancer properties of the olive leaves compounds

According to published research, oleuropein and hydroxytyrosol, the two primary phenolic compounds present in olive leaves, have anticancer effects when taken alone and as pharmacological adjuvants. The anti-tumor properties of these substances have been studied *in vitro* by using different varieties of cellular models that include hepatocellular carcinoma and breast cancer. A summary of the major *in vitro* studies investigating the anticancer effects of olive-derived compounds is presented in [table 1](#).

2.1. In Vitro Antitumor Effects of Oleuropein

Previous research conducted a follow-up study to investigate the properties of different doses of the oleuropein compound and its derivative on the survival of MCF-7 cells. The peracetylated aglycone had the strongest effect, and at 100 μM , the only derivatives of oleuropein showed the growth inhibition. Cell viability was extensively decreased as a result of the peracetylated chemical noticeably greater cytotoxic effect compared to the control. These results showed during the G2/M phase, the peracetylated substances (peracetylated hydroxytyrosol and peracetylated aglycone) stop the development of MCF-7 cells, hence causing an antiproliferative impact [8]. When the tumor cells of breast cancer (MCF-7) were treated with the concentration of 200 μM oleuropein, the Bax-expression, Bcl-2-expression, and TP53 gene expression were subsequently examined. According to the results, the Bax gene was significantly upregulated by 0.6 times when compared to cells that were left untreated and cells that were exposed to 100 μM oleuropein. Furthermore, it was discovered that oleuropein causes MCF-7 cells to undergo apoptosis via a p53-dependent mechanism, and that treatment significantly increases expression of the p53 gene (by 2.5 and 3.5 times in comparison to the cells that were not treated) [17].

Treatment of breast cancer cells with oleuropein, a concentration of 0–100 μM for a period of 24h, 48h, and 72h produced similar trends. The viability of breast tumor cells (ER-negative breast tumor) was significantly reduced by oleuropein in a dose- and time-dependent manner, whereas the MCF-7 cells showed higher resistance against the treatment. According to the wound healing assay, oleuropein therapy markedly reduced MDA-MB-231 cell migration. After 72 hours of intervention, the MDA-MB-231 cells showed a marked increase in sub-G1 phase accumulation with

increasing the concentration of oleuropein. Additionally, in a dose-related manner, the apoptotic percentage in MDA-MB-231 cells increased dramatically. Furthermore, by significantly the study demonstrated that oleuropein inhibited the signaling pathway of NF- κ B by dropping NF- κ B activity at 36 and 48 hours ([Table 1](#)) [18].

The cell lines for triple-negative breast tumor, such as MDA-MB-231 and MDA-MB-468 have been shown to have a lower cell survival rate when exposed to Oleuropein. The antiproliferative properties of compound in both cell lines, were demonstrated to be quantity and time-dependent. The MDA-MB-231 cells lines were analyzed with a concentration of 100 to 700 μM , and the MDA-MB-468 cells lines with a concentration of 100 to 400 μM were shown to have cytotoxic effects that were statistically significant ($p < 0.0001$). According to data, the expected main mechanism of cell death in MDA-MB-468 cells lines treated to oleuropein was apoptosis. MDA-MB-231, on the other hand, tended to necrotize and showed more resistance to apoptosis. Additionally, oleuropein altered the expression of several genes implicated in apoptosis. In the MDA-MB-468 cells lines, the indication of pro-apoptotic proteins such as BNIP2, GADD45A, BNIP3, BCL10 and BID, caspase family adherents (CASP1 and CASP14), the TNF receptor superfamily members (FADD and TNFRSF21), as well as CYCS and CFLAR, was significantly increased ([Table 1](#)). These proteins promote programmed cell death by starting intrinsic, extrinsic, or both pathways. The antiapoptotic genes TNFRSF11B and surviving (BIRC5) were downregulated in MDA-MB-231 cell lines, although CASP4 remained marginally enhanced, despite alterations in genes that inhibit apoptosis induction [19]. When used as a treatment alone, oleuropein has little effect on the viability of hepatocellular carcinoma cells. In HepG2 cell lines, oleuropein at doses between 20 and 100 μM caused morphological changes, decreased cell growth, and cell death. These cells showed increased caspase activity and modulation of Bcl-2 family proteins, along with inhibition of the PI3K/AKT pathway, a crucial regulator of apoptosis [20].

For a period of 24 hours, HepG2 cells were exposed with different quantities of isolated oleuropein showed no change in MTT decrease, indicating that cell viability was unaffected. However, under the condition of oxidative stress, oleuropein partially restores the viability of HepG2 cells. The cell viability of HepG2 and cytoskeletal stability are partially restored when oleuropein is used in conjunction with paraquat, a poisonous weedkiller known to induce the oxidative stress and promote cell death. The Oleuropein's protective effects on cellular viability include a marked decrease increased necrotic cell death and suppression of caspase-3 cleavage. In the multimodal therapy group such as (PQ + OP), PQ-induced necrosis seems to occur after an apoptotic event, suggesting a transition from the necrotic route to apoptosis. Instead of experiencing immediate necrosis as observed in cells treated with oxidative stress, oleuropein helps HepG2 cells showed increased resistance to PQ-induced damage, surviving longer before undergoing a more regulated cell death process [21]. Research has focused on concurrent use of phytochemicals and chemotherapeutic medications.

OLEUROPEIN AND HYDROXYTYROSOL: MECHANISMS OF ACTION AND THERAPEUTIC POTENTIALS

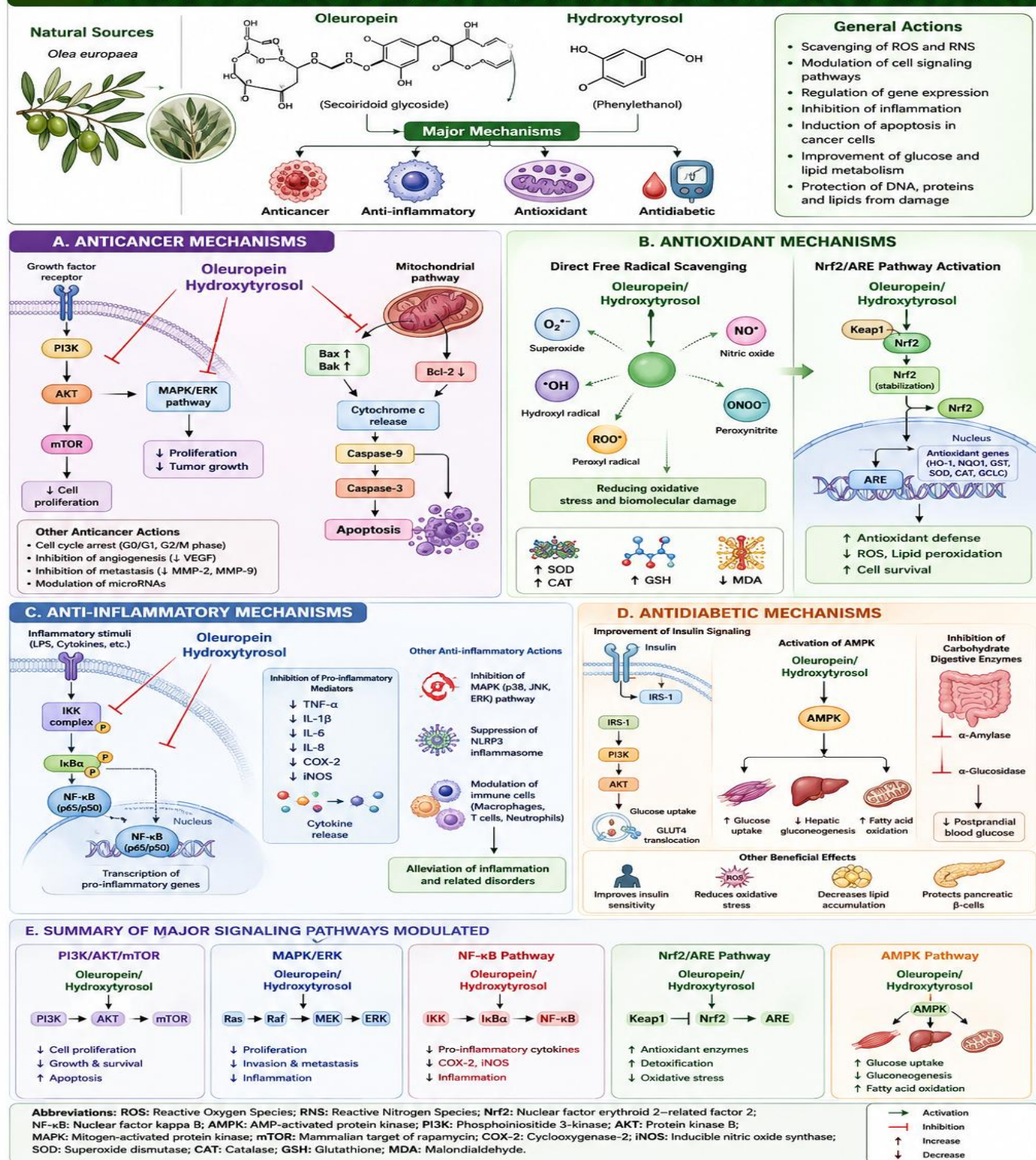


Figure 3. Integrated schematic of the signaling pathways and therapeutic processes influenced by hydroxytyrosol and oleuropein. (Uppermost) *Olea europaea*, the main natural source and chemical structures. (A) Anticancer strategies that include both promotion of the intrinsic apoptotic pathway (Bax/Bak overexpression and caspase activation) and inhibition of the PI3K/AKT/mTOR and MAPK/ERK pathways. (B) Enhancing endogenous antioxidant enzymes (SOD, CAT, GSH) by direct ROS/RNS scavenging and activation of the Nrf2/ARE signaling pathway. (C) Anti-inflammatory actions via inhibiting pro-inflammatory cytokines and mediators (iNOS, COX-2) and suppressing the NF- κ B signaling cascade. (D) Improved insulin signaling, AMPK activation, and inhibition of digestive enzymes that break down carbohydrates are examples of antidiabetic processes. (E) A summary table of the main signaling pathways that these polyphenols affect. ROS stands for reactive oxygen species, NF- κ B for nuclear factor kappa B, and AMPK for AMP-activated protein kinase.

It has been demonstrated that oleuropein (200 μM) enhances cisplatin's (50 μM) anti-tumor effect on HepG2 cells. The major molecular pathways and therapeutic mechanisms influenced by oleuropein and hydroxytyrosol are summarized in figure 3. The activation of gene for pattern metalloproteinase-7 and nerve growth factor (NGF) was measured. MMP-7 activity is necessary promoting the alteration of pro-NGF, that induces cell death, into NGF, that supports cell persistence and variation. Growth factors signaling dysregulation has a key effect on the onset and spread of tumor, including angiogenesis and tumor metastasis. Concurrent therapy the cisplatin-oleuropein synergistic action may offer a more effective chemotherapeutic strategy against HCC than either agent alone because oleuropein alters MMP-7 action without varying NGF gene regulation [22].

2.2. In Vitro antitumor effect of hydroxytyrosol

Hepatocellular carcinoma (HCC) cells have also been used to study hydroxytyrosol anticancer effect. When hydroxytyrosol (10–40 μM) was first applied to the HepG2 cell line, no alterations in the antioxidant status or cell integrity were observed [23]. However, Martin (2010) showed that when the HepG2 cell lines were exposed with HT (0.5, 1.0, 5.0, and 10.0 μM) exhibited a significant rise in both the regulation and activity of glutathione-associated enzymes, including glutathione reductase, glutathione peroxidase and glutathione S-transferase), which are crucial for neutralizing ROS. Moreover, the Nrf2 transcription factor underwent nuclear translocation as a result of HT.

Table 1. An overview of research using cell lines to examine the anticancer properties of the primary chemicals inside the olive plants.

Complex	Range of Concentration	Cancer Types	Cell based Models	Examined	References
Oleuropein	1, 10, 100 μM	Breast	MCF-7 and T-47D	Decline in cell viability with cell cycle arrest at G2/M phase.	[8]
Oleuropein	100, 200 μM	Breast	MCF-7	Bax Gene Upregulation and activation of p53-dependent apoptotic pathways through increased expression of p53 gene.	[17]
Oleuropein	0 to 100 μM	Breast	MDA-MB-231 and MCF-7	Viability of cell and migration were decreased, with cell cycle arrest at the sub-G1 phase was observed. Apoptosis was increased, indicated by elevated PARP and caspase-3/7 cleavage, along with reduced the activation of NF- κ B.	[18]
Oleuropein	0 to 700 μM	Breast	MDA-MB-231 and MDA-MB-468	Reduced the viability of cell, with apoptosis primarily driven by downregulation of the anti-apoptotic genes TNFRSF11B, BIRC5, and CASP4.	[19]
Oleuropein	20 to 100 μM	HCC	HepG2	Cells showed morphological changes and reduced proliferation, with increased caspase activity and the involvement of family Bcl-2, reduced signaling of PI3K/AKT, but was no change in feasibility.	[20]
Oleuropein	10 to 100 $\mu\text{mol/L}$	HCC	HepG2	Cell viability was maintained, with lowered caspase-3 activation.	[21]
Oleuropein	200 μM and 50 μM	HCC	HepG2	Modulation of the Pro-NGF/NGF ratio through regulation of MMP-7 activity.	[22]
Hydroxytyrosol	10 to 40 μM	HCC	HepG2	No variations in cell integrity or antioxidant levels were observed.	[23]
Hydroxytyrosol	0.5, 1.0, 5.0 and 10.0 μM	HCC	HepG2	Increased expression of antioxidant enzymes, enhanced the activation of ERK and AKT pathways, and promoted nuclear translocation of Nrf2 transcription aspect.	[24]
Hydroxytyrosol	30 to 200 μM	HCC	Hep3B e HepG2	Cytostatic effects through increased FAS expression, enhancement of the endogenous antioxidant system, increased the IL-6 reduction.	[25]
Hydroxytyrosol	1 μM and 5 μM	HCC	HepG2	Reduced ER stress.	[26]
Hydroxytyrosol	100 to 400 μM	HCC	HepG2, Hep3B, SK-HEP-1 and Huh-7	Reduced cell proliferation with G2-M arrest, increased cleavage of PARP, suppression of the NF- κ B and PI3K/AKT pathways	[27]
Hydroxytyrosol	0 to 100 μM	HCC	HepG2	Reduced cell viability accompanied by increased cytosolic calcium.	[28]

The Nrf2 is essential because it supports the body's defense against oxidative stress by regulating the expression of many antioxidant and detoxifying enzymes. Two signaling proteins, extracellular regulated kinases (ERK) and protein kinase B (AKT), which belong to the ERK and PI3K/AKT pathways, respectively, were activated to promote this translocation (Table 1) [24].

The antiproliferative effect of hydroxytyrosol (30 to 200 μM) was observed in Hep3B and HepG2 cell lines through suppression of the lipogenic enzyme fatty acid synthase (FAS), activation of the cellular antioxidant structure, and decrease of IL-6 levels at dosage concentrations up to 80 μM . But, IL-6 levels increased with higher levels [25]. Furthermore, hydroxytyrosol with a concentration of (1 μM and 5 μM) treatment was demonstrated to lessen the endoplasmic reticulum stress that tunicamycin induced in HepG2 cells [26]. In various hepatocellular carcinoma cell models (HepG2, Hep3B, SK-HEP-1, and Huh-7), the hydroxytyrosol with a concentration of (100–400 μM) suppressed cell growth, a conclusion not detected in non-tumoral hepatic cells (HL-7702). This anti-proliferative activity is attributed to hydroxytyrosol pro-apoptotic mechanisms, including enhanced cleavage of PARP by procaspase-3, induction of cell cycle arrest at G2/M, and modulation of the PI3K/AKT and NF- κB signaling pathways (Table 1) [27].

Hydroxytyrosol-induced elevation of $[\text{Ca}^{2+}]_i$ in HepG2 cell lines, cell viability is presently being investigated. It was discovered that hydroxytyrosol inhibited the viability of HepG2 hepatoma cells at doses ranging from 40 to 100 μM . Furthermore, it has been noted that hydroxytyrosol raises intracellular calcium levels ($[\text{Ca}^{2+}]_i$), suggesting that calcium signaling plays a part in the compound's cytotoxic actions. The effects of hydroxytyrosol are mediated by store-operated, calcium entry that is sensitive to protein kinase C (PKC), suggesting that PKC regulates how cells respond to the material. The findings indicate that hydroxytyrosol activates release of calcium from thapsigargin-sensitive ER stores via a PLC-independent pathway, revealing additional ways that it influences cell death processes. Consequently, it proposed a different way whereby hydroxytyrosol might stop the development of hepatocellular carcinoma cells [28].

3. Olive-Derived Antioxidants: Anti-Inflammatory Properties in In Vitro Studies

3.1. Olive Polyphenols as Antioxidant and Anti-Inflammatory Agents in Animal Studies

Olive oil contains antioxidant compounds such as polyphenols are known to restore neuronal function by improving the redox status, and it is commonly acknowledged that inflammation and oxidative and nitrosative stress are the main abnormalities underlying neurodegeneration. Consuming EVOO polyphenols has been linked to several positive benefits of the MD, including anti-inflammatory, anti-aging, cardioprotective, antiviral, anticancer, antioxidant, hypoglycemic, and antibacterial properties [29]. EVOO polyphenols have been demonstrated to be neuroprotective functional decline related to aging and neurodegenerative

disorders while preserving endogenous antioxidant stability because by scavenging free radicals, they can defend the DNA against oxidative stress, which stop impaired mitochondrial function, and reduce lipid from oxidation [30]. Additionally, they can prevent the aggregation and toxicity of amyloid β ($\text{A}\beta$) and τ proteins, which are the primary culprits of the neurodegenerative pathway in Alzheimer disease [31, 32]. EVOO polyphenols contribute to the cell's redox stability by acting modest pro-oxidants and antioxidants, which causes the cell's antioxidant defenses to be upregulated. They may therefore be viewed as hormetic factors. For example, HT may go through a redox cycle that produces superoxide when peroxidases are present [30]. Moreover, tyrosol lengthens the life of *C. elegans* by triggering the heat shock response [32]. By promoting Nrf2-dependent gene expression, In transgenic mice with AD, HT has been demonstrated to reduce neuroinflammation and brain mitochondrial oxidative stress [33]. By triggering the Nrf2 pathway, oleuropein with a concentration of (60 mg/kg/day) enhanced the activity of mitochondria and decreased the oxidative stress in SHR rats after eight weeks of treatment [34]. Additionally, it was discovered that tyrosol (240 mg/kg) protected exerts protective effects against LPS-induced acute lung injury over the restriction of NF- κB and activating the Nrf-2 and AP-1 pathways. Additionally, EVOO polyphenols improve hepatic Nrf-2 activation and the subsequent release of antioxidant enzymes [35]. When Nrf2, the primary regulator of redox homeostasis, is activated, pro-inflammatory mediators such cytokines, COX-2, and iNOS are inhibited [36]. By downregulating NF- κB and AP-1 expression and activity, EVOO polyphenols help suppress inflammation that reduce inflammation by inhibiting free radical activity, dissolving radical chains, and reducing the HT reduces the formation of reactive oxygen and nitrogen species (ROS and RNS). Moreover, HT stops the progression of the inflammatory cascade following LPS and carrageenan injection by reducing DNA damage and downregulating the level of pro-inflammatory cytokines (TNF- α and IL-1 β), COX2, iNOS, NO, PGE2, and NF- κB [37]. Co-injecting OLE (450 μM) and $\text{A}\beta$ 2 (50 μM) into adult rats nucleus basalis magnocellularis was found to inhibit $\text{A}\beta$ aggregation, reduce astrocyte and microglia activation, and counteract $\text{A}\beta$ -mediated damage to choline acetyltransferase-positive neurons within the NBM [38]. According to a different study, OLE prevents motor impairments and $\text{A}\beta$ plaque load in transgenic *C. elegans* lines that continuously express $\text{A}\beta$ 3–42 [39]. Notably, the consumption of food with added oleuropein, hydroxytyrosol, or a combination of polyphenols from olive processing is of interest effluent was found to exhibit considerable with anti-aggregation and neuroprotective actions in the TgCRND8 mouse model of $\text{A}\beta$ accumulation. When given the OLE-supplemented food for eight weeks, mice that were three and six months old (during the initial and intermediate level of $\text{A}\beta$ accumulation, respectively) demonstrated a significant improvement in cognitive function as well as a substantial decline in the quantity, compactness of $\text{A}\beta$ plaques and size [40]. Despite treatment starting at 10 months, by which time $\text{A}\beta$ and pE3- $\text{A}\beta$ had become elevated in the mice deposition in the cortex and hippocampus, there was clear development

in the synaptic transmission and decrease in both the level and aggregation density of A β 42 and pE3-A β species. These findings suggest that oral OLE supplementation leads to a decrease in pE3-A β production, the disintegration of preformed plaques, and the avoidance of amyloid deposition [40]. By supplementing the diet with other polyphenols obtained from olive mill wastewater or HT administered at the same dosage as pure OLE, the dose-dependent impact of OLE on A β peptide aggregation can be reproduced [41, 42]. It is interesting to note that OLE therapy (delivered through the diet at 50 mg/kg for a period of 8 weeks) promoted significant neuronal autophagy, even in TgCRND8 mice at late level of disease. This was accompanied by increased histone H3 acetylation at lysine 9 (H3K9) and histone H4 acetylation at lysine 5 (H4K5) in the brain and hippocampus, along with a decrease in HDAC2 expression. Collectively, these findings suggest that HT and related olive polyphenols act as hormetic agents, mitigating oxidative stress, inflammation, and epigenetic dysregulation connected with aging and neurodegenerative disorders. [40].

3.2. Molecular mechanism of antioxidant activity

Defense mechanisms against increased reactive oxygen species (ROS) are essential for cell survival, because ROS overproduction is associated to lipid, protein, or DNA damage that precedes degenerative diseases [43]. To adapt to oxidative stress, organisms maintain complex antioxidant systems, primarily centered on glutathione (GSH), because antioxidants inhibit oxidation. Sadly, only a small number of medications and biological substances like vitamins have been shown to have antioxidant properties, but they may also have negative side effects [44]. These days, scientists are concentrating on the antioxidant qualities of organic substances that don't have any negative side effects. Specifically, the significance of the hydrophilic and lipophilic phenols' antioxidant activity in EVOO has come to light [45]. Plants biologically create this proportion to respond to wounds caused by different diseases or insects [46]. The primary phenolic compounds of EVOO, such as OLE, exhibit potent antioxidant activity and HT is fundamental to applying their metabolic and pharmacokinetic properties, and it is connected with their comparative bioavailability with a considerable degree of consumption [47]. Because of their catecholic structures, OLE and HT exhibit distinct antioxidant behaviors at the molecular level: (i) Through neutralization of peroxy radicals and interruption of peroxidative chain reactions, stable resonance structures are generated [48] and (ii) via chelating metal ions and protecting LDL from copper sulfate-induced oxidation [49]. OLE and HT may chelate metals through their hydroxyl groups, which donate electrons and form intramolecular H-bonds with free radicals [50]. In addition, the ROS-scavenging effects of OLE and HT were examined using non-metal oxidation models. Polyphenols effectively protect catalase (CAT) from inactivation by hypochlorous acid (HOCl), which inhibits atherosclerosis when HOCl oxidizes LDL by apoB-100 chlorination, according to in vitro data [11]. Furthermore, HT has been shown to raise GSH levels, which improves the cell's redox state [51]. It has recently been discovered that decreased level

of the transcriptional Nuclear factor erythroid 2 (NF-E2)-related aspect 2 (Nrf2) are the key source of oxidative damage in age-related disorders [52], and because of its role as a facilitator of the cell's general adaptive responses, such as inflammation and proteostasis, It has been identified as a promising target for treating metabolic diseases, including obesity [53, 54]. Nevertheless, Nrf2 plays a critical function in changing oxidation resistance [55]. Resulting the Nrf2 initiation and subsequent the nucleus translocation, the Nrf2 binding to antioxidant response elements (ARE) influences the transcriptional regulation of a number of antioxidant enzymes, with superoxide dismutase (SOD), c-glutamylcysteine synthetase (c-GCS), glutathione S-transferase (GST), and NADPH quinone oxidoreductase-1 (NQO1) [56].

3.3. Molecular mechanism of anti-inflammatory of olive plant in vitro

The immune system uses inflammation as a vital defense mechanism to identify and destroy pathogens and contaminated cells while also encouraging tissue healing and reestablishing equilibrium in the body. This process, which involves temporarily increased amounts of cytokines with the capacity to trigger both innate and adaptive immune responses systems, is incorporated into numerous coordinated processes. There are several negative effects on organismal homeostasis when an inflammatory response is not controlled. Chronic inflammation, which contains leukocytes collected by macrophages and lymphocytes, is a long-term, unresolved, and uncontrollable immune response that can result from an ongoing inflammatory response. This can cause injury to the mass or organs locally or systemically, as well as a breakdown in normal physiological function.

Chronic inflammation increases with age and is causally linked to the beginning or progression of disease. In actuality, cytokines, chemokines, and the regulation of proteins associated with irritation are all increased in older adults and patients with autoimmune diseases. These individuals also show higher rates of metabolic syndrome, cardiovascular disease, frailty, multimorbidity, and reduced physical and cognitive function. Therefore, treatments the interventions that modulate inflammation and correct dysregulated inflammatory responses can effectively halt disease progression. Regular consumption of foods high in polyphenol has been shown to help prevent chronic disorders like heart disease such as diabetes and obesity. The main reason of this advantageous effect is the poly phenolic molecule's anti-inflammatory qualities, which are demonstrated by a quantity of mechanisms, with antioxidant activity and the modification of signaling cascades and transcriptional processes.

4. In Vivo and In Vitro Studies of Antidiabetic Activities of Oleuropein and Hydroxytyrosol:

4.1. In vitro studies of oleuropein's impact on skeletal muscle cells

In C₂Cl₂ muscle cells, OLE treatment led to higher level of stimulated AMPK, ACC, and ERK proteins and enhanced

glucose utilization. Furthermore, OLE showed antioxidant qualities by dramatically reducing H₂O₂-induced (ROS) as compared to untreated cells [57]. When C₂C₁₂ myotubes were exposed to OLE, Fujiwara et al. observed a considerable rise in glucose uptake that was similar to what insulin had. In contrast to insulin, OLE raised the levels of protein of phosphorylated AMPK but not the content of protein of phosphorylated Akt, per the previously described Hadrich et al. study. Furthermore, the OLE pretreatment also enhanced the level of GLUT4 mRNA and rescued insulin-mediated uptake of glucose under palmitate exposure by reducing palmitate-induced decreases in phosphorylated/activated AMPK [58]. OLE treatment in cultured avian muscle cells promoted mitochondrial biogenesis, upregulated avUCP, PGC1- α , TFAM, NRF1, ATP5a1, and SIRT1 expression, increased cytochrome c oxidase activity, and reduced the superoxide levels of mitochondria, reflecting lower ROS

formation (Table 2) [59].

4.2. Oleuropein's effects on hepatocytes (In vitro)

OLE attenuated FFA-induced lipid accumulation, suggesting hepatoprotective effects against steatosis, in both human hepatoma-derived (HepG2) cell lines and mouse hepatocyte (FL83B) cell lines. Additionally, the OLE (10 μ M) dramatically reduced the size of lipid droplets in FL83B and HepG2 cells. FFA-induced elevations in phosphorylated/activated ERK protein levels were inhibited by the OLE treatment. Adipose differentiation-related protein (ADRP), which associates with lipid droplets, and tail interacting protein of 47 kDa (TIP47) mRNA levels were unaffected by OLE therapy. Additionally, OLE had no effect on JNK or phosphorylated/activated Akt protein levels (Table 3) [60].

Table 2. Oleuropein's effects on In vitro skeletal muscle cells.

Type of Cell	Treatment of Cells	Conclusions	References
C2C12 myotubes n = 3	With OLE the cells were exposed at a concentration of 200 μ M or 400 μ M for 30 minutes, followed by exposure to 400 μ M H ₂ O ₂ for 24 h.	Increased glucose consumption and insulin sensitivity, reduced H ₂ O ₂ -induced ROS, and elevated levels of phosphorylated AMPK, ACC, and ERK proteins	[58]
C2C12 myotubes n = 3	With OLE the cells were exposed at a concentration of 1 μ M, 10 μ M, or 100 μ M for 30 minutes to 24 h, followed by exposure to 250 μ M palmitic acid for a period of 24h.	Increased glucose uptake, elevated GLUT4 mRNA expression, and higher levels of phosphorylated AMPK (p-AMPK) protein.	[58]
Male chicks (Ross strain, Gallus Domesticus) n = 4-6	OLE was administered orally at 5 mg/kg body weight per day for 15 days	Increased mRNA levels of avUCP, PGC1- α , TFAM, NRF1, ATP5a1, and SIRT1, elevated cytochrome c oxidase activity, and reduced mitochondrial superoxide activity, indicating decreased ROS production	[59]

Table 3. In Vitro Studies of Oleuropein's Impact on Hepatocyte Function

Cell Types	Treatment of cells	Conclusions	References
HepG2 and FL83B hepatocytes n = 3	With OLE the cells were exposed at a concentration of 10 and 50 μ M for 24 h, followed by exposure to 0.5 mmol/L of a 2:1 oleic acid-palmitic acid mixture.	Lipid growth and size of droplet were decreased, TIP47 and ADRP mRNA levels were reduced, FFA-induced p-ERK protein levels were lowered, and p-JNK and p-Akt protein levels were unchanged/modulated.	[60]
FaO cells n = 3	With OLE the cells were exposed with at a concentration of 50 μ g/mL for 24 h, followed by exposure to 0.75 mM of a 2:1 oleate-palmitate mixture.	Triglyceride accumulation was decreased, and lipid peroxidation/oxidative stress was reduced.	[61]
HepG2 hepatocytes n = 6	Cells were treated with OLE at 10 μ M for 2 and 24 h.	OLE acts as a PPAR α ligand, increasing PPAR α mRNA and protein levels, as well as upregulating ACOX1, CYP4A14, Lipin 1, and ACOT4 mRNA levels.	[62]
HepG2 hepatocytes n = 5	Cells were treated with OLE at 10, 25, 50, 100, and 200 μ M for 24 h in a steatosis model using 0.5 mM palmitic acid/oleic acid (PA/OA).	Lipid accumulation was decreased at concentrations of 50, 100, and 200 μ M.	[63]

The cellular triglyceride content significantly decreased when Vergani et al. treated FaO rat hepatoma cells to OLE, suggesting that FFA-induced steatosis was attenuated. Furthermore, OLE decreased the rise in malondialdehyde (MDA) levels, a sign of lipid peroxidation, brought on by FFA (Table 3) [61]. According to Malliou et al.'s molecular docking simulations, OLE fits structurally within the PPAR α binding cavity in its integral form.

Similarly, a luciferase reporter gene experiment showed that the PPRE-luc plasmid activity had increased, suggesting that it might activate PPAR α and cause heterodimerization with RXR. Additionally, mRNA and protein levels of PPAR α , along with numerous downstream target proteins including acyl-CoA oxidase 1 (ACOX1), cytochrome P450 family 4 subfamily 3 polypeptide 14 (CYP4A14), lipin 1, and acyl-CoA thioesterase 4 (ACOT4), were elevated in HepG2 cells treated with OLE (Table 3) [62]. Using HepG2 cells, Santini et al. discovered that OLE administration significantly decreased palmitate-induced hepatic lipid buildup [63].

4.3. Effect of oleuropein on animal models of diet-induced diabetes (in vivo)

Numerous studies have investigated OLE's capacity to protect against the harmful consequences of a high-fat or "Western style" diet (HFD). Oi-Kano et al. discovered that OLE had anti-obesogenic qualities by protecting male Sprague-Dawley rats against weight gain brought on by an HFD. Administration of OLE resulted in a considerable decrease in the rises in plasma TG, FFA, TC, and leptin levels that were

produced by HFD. Rats given OLE supplementation presented advanced levels of UCP-1, a protein implicated in adipocyte thermogenesis, in their interscapular brown adipose tissue (iBAT) (Table 4) [64]. In a different study, Jemai et al. observed that how oleuropein affected a male Wistar rats assumed a high-cholesterol diet (HCD) and revealed that it prevented an increase in the liver-to-body weight ratio from growing as a result of HCD. According to serum lipid study, the OLE significantly reduced the growth in TC, TG, and LDL-C plasma levels carried on by HCD. The qualities of the phenolic compound's hypolipidemic were further verified by its capability to increase HDL-C levels in HCD rats, that had earlier dropped. OLE therapy restored the action of hepatic antioxidant enzymes SOD and CAT impaired by a high-cholesterol diet. Secoiridoid supplementation in HCD-fed rats enhanced ABTS radical scavenging and reduced the lipid peroxidation across the heart, aorta, liver and kidneys demonstrated that the OLE supplementation enhanced antioxidant activity. OLE treatment stopped the development of aortic wall lesioning and cardiac muscle hypertrophy brought on by HCD. Likewise, OLE stopped the development of fatty cysts and the movement showing peripheral localization of hepatocyte nuclei ((Table 4) [65]. Microarray research showed that OLE with a concentration of (0.03% w/w in diet) more than doubled the hepatic expression of over 90 genes in a mice fed with a high-fat diet. In specific, OLE treatment reduced the quantity of hepatic genes associated with inflammation and oxidative stress and also decrease the level of mRNA genes involved in the absorption pathway of hepatic fatty acid [66].

Table 4. Oleuropein's Effects on Animal Models of Diabetes produced by a high-fat diet (in vivo).

Cell Types	Treatment	Findings	References
Male Sprague-Dawley rats n = 6	OLE was administered at 1, 2, or 4 mg/kg, or provided as a 0.1%, 0.2%, or 0.4% dietary supplement for 28 days in a high-fat diet containing 30% shortening. Additionally, cells were treated with OLE at 10–50 mmol/L for 10 minutes	Body weight and weight gain, as well as epididymal and perirenal fat pad weights, were reduced. Plasma TG, TC, FFA, and leptin levels were lowered, while IBAT UCP-1 protein levels and urine and plasma norepinephrine and epinephrine levels were increased.	[64]
Male Wistar rats n = 10	OLE was administered at 3 mg/kg body weight for a period of 16 weeks in a high-cholesterol diet containing 1% cholesterol and 0.25% bile salts.	Liver-to-body weight ratio was decreased, along with reduced plasma levels of total cholesterol (TC), triglycerides (TG), and LDL-C. Plasma HDL-C levels were increased, as were SOD and CAT activities and overall antioxidant capacity measured by the TEAC assay. Lipid peroxidation was reduced, and OLE prohibited cardiac muscle hypertrophy, aortic wall lesions, and hepatic steatosis.	[65]
Male C57BL/6N mice n = 8	Oleuropein was provided as a 0.03% (w/w) dietary supplement for a period of 10 weeks.	Oxidative stress- and pro-inflammatory-related hepatic genes were downregulated, hepatic genes involved in lipid peroxidation product detoxification were reduced, and hepatic mRNA levels of fatty acid absorbed and transporter of genes were decreased.	[66]
Male C57BL/6N mice n = 8	OLE was administered ad libitum at 0.03% (w/w) for 10 weeks.	Body weight gain and liver weight were reduced, along with decreased plasma levels of AST and ALT. Plasma and liver levels of free fatty acids (FFA), total cholesterol (TC), and triglycerides (TG) were lowered. Hepatic mRNA levels of LXR, PPAR γ 2, LPL, aP2, Cyc-D, E2F1, CTSS, SFRP5, and DKK2 were decreased, and liver p-ERK protein levels were reduced, while β -catenin protein levels were increased.	[67]

Table 5. Hydroxytyrosol (HT) effect on hepatocytes (In vitro).

Cell Type	Hydroxytyrosol Concentration/Duration	Effect	References
Mouse hepatocytes	100 μ M for 4 h. (hypoxia); followed by reoxygenation	Cell apoptosis decreased, while hepatocyte viability and the activities of SOD1, SOD2, and CAT increased.	[68]
Rat hepatocytes	25 μ M for 2 h	Lipid synthesis, including fatty acids, cholesterol, and triglycerides was reduced, accompanied by decreased expression of ACC, diacylglycerol acyltransferase, and HMG-CoA reductase, while AMPK and ACC phosphorylation were increased.	[69]
Vit. E-deficient rat liver microsomes	0.05–2 mM for 30 min	Lipid peroxidation and TBARS levels were decreased.	[70]
Microsomal fractions from vitamin E-deficient rats treated with selenium- and sulfur-modified hydroxytyrosol derivatives	0.05–0.25 mM for 20 min	Selenium- and sulfur-modified hydroxytyrosol derivatives showed stronger inhibition of TBARS formation and lipid peroxidation compared with pure hydroxytyrosol.	[71]

Table 6. Hydroxytyrosol anti-diabetic effects: in vivo experiments on animals with diabetes produced by streptozotocin, alloxan, and genetics.

Model of Study	Hydroxytyrosol Concentration/Duration	Effects	References
Alloxan-induced diabetic male Wistar rats	20mg/kg for 2 months; intraperitoneal injection	decreased blood glucose levels, reduced liver TBARS, bilirubin, and fatty cysts; increased hepatic glycogen and HDL, enhanced antioxidant enzyme activities such as SOD, CAT, and GPX in the liver and kidney; and diminished β -cell damage	[72]
Alloxan-induced diabetic male Wistar rats	8 or 16 mg/kg orally for 4 weeks.	Reduced blood glucose levels, decrease TC and hepatic oxidative damage (TBARS), increased hepatic glycogen and antioxidant enzymes (SOD, CAT)	[73]

When mice were treated with OLE with a concentration of (0.03% w/w) ad libitum for a period of 10 weeks, a reduction in body and liver weight gain induced by a high-fat diet was observed. Elevated liver and plasma FFA, TC, and TG, as well as plasma AST and ALT, were attenuated after OLE therapy. Additionally, OLE markedly reduced expression levels of mRNAs encoding cyclin D (Cyc-D), E2F1, cathepsin S (CTSS), secreted frizzled-related protein 5 (SFRP5), dickkopf homolog 2 (DKK2), and lipid metabolism-related genes such as liver X receptor (LXR), fatty acid-binding protein 2 (aP2), lipoprotein lipase (LPL), and PPAR γ 2. Furthermore, HFD-induced increases in phosphorylated ERK decreases in β -catenin amount were mitigated by OLE treatment. Additionally, OLE exhibited anti-inflammatory effects by modulating TLR2/4, myeloid differentiation factor 88 (MyD88), IL-1 β , IL-6, TNF α , interferon beta (IFN β), TNF receptor superfamily member 6 (FAS), and TNF-related apoptosis-inducing ligand when OLE reduced the rise in mRNA levels brought on by HFD [67].

4.4. Hydroxytyrosol (HT) effects on hepatocytes (In vitro)

Hydroxytyrosol with a concentration of (100 μ M) treatment of primary mouse hepatocytes, under ischemia/reperfusion (I/R)-like circumstances led to a dose-dependent reduction in apoptosis and an increase in cell feasibility (Table 5) [68].

Furthermore, HT considerably reduced the decline in antioxidant enzymes like SOD1, SOD2, and catalase (CAT) brought on by I/R. Overall, HT therapy shielded mouse hepatocytes against oxidative damage and I/R-induced injury. Rat hepatocytes treated with HT (25 μ M) showed decreased de novo lipid production of cholesterol, triglycerides, and fatty acids without compromising cell viability. Furthermore, HT treatment markedly reduced the enzymatic activity that involved in cholesterologenesis (3-hydroxy-3-methyl-glutaryl-CoA reductase), fatty acid formation (ACC), and triglyceride formation (diacylglycerol acyltransferase) [69]. Additionally, HT enhanced AMPK and its downstream target ACC phosphorylation, indicating that AMPK mediates the process of HT's decreased lipid production [68]. These outcomes recommend that HT prevents the synthesis of lipids in hepatic tissue. According to another study, HT (0.05–2 mM) reduced the production of thiobarbituric acid reactive substances (TBARS), a biomarker of lipid peroxidation, in vitamin E-deficient rat liver microsomes, which in turn reduced lipid peroxidation [70]. Malonaldehyde, a byproduct of lipid oxidation, combines with thiobarbituric acid to generate TBARS, which damages cells. A current research found that when microsomal fractions from vitamin E-low rats were exposed to improved HT compounds (0.05–2 mM), the generation of TBARS and detrimental lipid peroxidation were

more strongly and potentially inhibited than when pure HT was used (Table 5) [71].

Overall, the aforementioned research has demonstrated that HT has hepatoprotective qualities by boosting antioxidant activity, hepatocyte survival, and reducing apoptosis [68, 70, 71]. More significantly, HT therapy dramatically decreased lipid production [69]. Lipid buildup in hepatocytes is frequently linked to hepatic steatosis, which in turn causes decreased glucose utilization, liver damage, and fibrosis, all of which exacerbate the onset of insulin resistance and type 2 diabetes. According to these investigations, HT may offer protection against the development of hepatic insulin resistance as well as hepatic steatosis.

4.5. Effect of hydroxytyrosol (HT) on in vivo diabetes in rodents caused by alloxan

Intraperitoneal injections of alloxan monohydrate (150 mg/kg) were administered to male Wistar rats to induce diabetes; animals with hyperglycemia (blood glucose levels of 2 g/L after two weeks) were kept for further study (Table 6) [72]. For two months, the treatment groups received daily intraperitoneal injections of pure hydroxytyrosol (F3), monomeric phenols (F1), or polymeric phenols (F2) from olive mill waste at a dose of 20 mg/kg. Blood glucose levels were markedly lowered by all three treatments, but pure HT (F3) was particularly effective. Animals treated with HT showed decreased levels of fatty cysts, bilirubin, and TBARS, markers of liver damage. HT enhanced circulating high-density lipoprotein (HDL), hepatic glycogen, and antioxidant enzymes (SOD, CAT, and GPX) in the kidney and liver. Furthermore, HT therapy reduced the harmful effects of alloxan on pancreatic β cells [72]. In 2009, Jemai et al. investigated the potential antioxidant and antidiabetic effects of administering HT and oleuropein to male Wistar rats. After four weeks of treatment with HT dissolved in drinking water to concentrations of 8 or 16 mg/kg, diabetes was caused by an intraperitoneal injection of alloxan (180 mg/kg) [73]. Hepatic glycogen and antioxidant enzymes (SOD, CAT) increased concurrently with reduction of hyperglycemia, hypercholesterolemia, and hepatic oxidative damage (TBARS) in all treatment groups. Dose-dependence was demonstrated by the higher effects of the 16 mg/kg dose compared to the 8 mg/kg dose [73]. In a diabetic rat model, this study shown that HT could maintain better lipid profiles, glucose levels, and antioxidant activity.

5. Conclusion

Oleuropein and hydroxytyrosol, the primary bioactive compounds of *Olea europaea*, exhibit significant antioxidant, anti-inflammatory, anticancer, and antidiabetic activities. These compounds play an important role in combating oxidative stress, regulating immune responses, and improving metabolic health, making them valuable in disease prevention and treatment. The antioxidant activity of these compounds protects against cellular damage and enhances mitochondrial function, which is essential for neuroprotection and cardiovascular health. Their anticancer effects, demonstrated in various In vitro and In vivo studies, highlight their potential

to inhibit tumor progression, induce apoptosis, and enhance the efficacy of chemotherapy. The anti-inflammatory properties of olive-derived bioactives help modulate cytokine levels, reduce chronic inflammation, and protect against neurodegenerative and autoimmune diseases. Additionally, their role in diabetes management is evident through improved glucose metabolism, enhanced insulin sensitivity, and reduced lipid accumulation in hepatic and adipose tissues. These findings suggest that oleuropein and hydroxytyrosol could serve as natural alternatives to conventional therapeutic agents for managing metabolic and inflammatory disorders. Despite extensive research, challenges remain regarding the clinical translation of these compounds. Bioavailability, stability, and optimal dosage require further exploration to maximize their therapeutic efficacy. Future research should focus on enhancing their absorption through innovative drug delivery systems and conducting well-structured clinical trials to establish their safety and effectiveness in humans. The integration of these compounds into nutraceuticals, functional foods, and pharmaceuticals presents a promising strategy for natural and sustainable healthcare solutions. Given the increasing global interest in plant-based therapeutic agents, *Olea europaea* bioactives offer immense potential for future biomedical research and clinical applications. Collaborative efforts between researchers, healthcare professionals, and the pharmaceutical industry will be essential in unlocking their full therapeutic benefits and ensuring their successful application in modern medicine.

6. Future Perspectives

Future research on oleuropein and hydroxytyrosol should focus on optimizing their pharmacokinetic properties to enhance bioavailability and stability through novel drug delivery systems such as nano formulations, liposomes, and polymeric nanoparticles. Further investigations into their precise molecular mechanisms and interactions with key cellular pathways in cancer, inflammation, and metabolic disorders are necessary to establish their therapeutic potential. To validate their efficacy, safety, and long-term outcomes in humans, large randomized clinical trials are indispensable. Additionally, exploring their synergistic interactions with conventional chemotherapeutic and metabolic drugs could enhance their clinical relevance. The incorporation of these bioactive compounds into nutraceuticals and functional foods could offer a natural and sustainable strategy for disease prevention and health promotion. A personalized medicine approach, considering genetic and metabolic variations, could further optimize their therapeutic benefits. Multidisciplinary collaborations between researchers, clinicians, and the pharmaceutical industry will be critical in translating these natural compounds into effective clinical applications, paving the way for their use in modern medicine.

Author contributions

Conceptualization and study design, Faryal Shams., Muhammad Ibrahim., Investigation, Faryal Shams. Immad Khan. and Faiq Firdous.; Visualization and original figure design, Faryal Shams. Muhammad Ibrahim. Sumaira Saeed.,

Shana Perrella., Carmen Lenti.; Writing original draft preparation, Faryal Shams. Muhammad Ibrahim. Sumaira Saeed., Shana Perrella.; Writing review and editing, Fabiana Monfrecola., Shana Perrella., Faiq Firdous., Immad Khan., and Carmen Lenti.; Supervision, Muhammad Ibrahim., and Sumaira Saeed., All authors have read and agreed to the published final version of the manuscript.

Ethical approval

Not applicable.

Conflicts of Interest

The authors report no conflicts of interest.

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