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REVIEW

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Hydroxytyrosol in cancer research: recent and historical insights on discoveries and mechanisms of action

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Abstract

Background Cancer is a persistent global health challenge, demanding continuous exploration of innovative therapeutic strategies. Hydroxytyrosol (HT), derived from olive oil, has garnered attention for its potent antioxidant and anti-inflammatory properties, revitalizing interest due to recent breakthroughs in comprehending its intricate anticancer mechanisms.

Main Body This review conducts a detailed analysis of hydroxytyrosol's molecular mechanisms in cancer. Delve into the complex pathways and processes underlying its anticancer properties, including its impact on critical cellular events such as inhibiting cancer cell growth, proliferation, metastasis, and apoptosis. We meticulously evaluate HT efficacy and safety through scrutiny of preclinical and clinical studies. Additionally, we explore the potential synergistic effects of combining HT with conventional cancer therapies to improve treatment outcomes while reducing side effects, offering a comprehensive approach to cancer management.

Conclusion This review stands as a valuable resource for researchers, clinicians, and policymakers, providing profound insights into HT potent anticancer activity at the molecular level. It underscores the immense potential of natural compounds in the intricate realm of cancer management and highlights the urgent need for further research to translate these discoveries into effective clinical applications. Ultimately, it fosters the development of targeted and personalized therapeutic approaches, reigniting hope in the ongoing battle against cancer and enhancing the quality of life for those afflicted by this relentless disease.

Keywords Anticancer agent, Anti-inflammatory, Clinical studies, Hydroxytyrosol, Molecular mechanisms, Synergistic effects, Therapeutic approaches

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Background

Cancer refers to a set of functional abilities that human cells acquire when they transition from regular developmental stages to abnormal growth states, more especially abilities that are essential for the development of aggressive tumors [1]. Worldwide, the prevalence of cancer diseases is constantly rising, resulting in millions of fatalities every year. According to the most recent projections, the worldwide cancer burden will rise significantly over the coming decades, rising by 47% by 2040 compared to the year 2020 [2, 3]. Globally, about ten million cancer patients died in 2020 and 19 million cancer patients were newly diagnosed which is expected to increase in future [3, 4]. In the USA, approximately 2,001,140 new cancer patients and 611,720 cancer deaths are projected to occur in 2024 [5]. Deaths of cancer patients continued to be decreased by 2021, preventing over 4 million mortality since 1991 due to reductions in smoking, earlier diagnosis of cancers, and enhanced better quality treatment in both the adjuvant and metastatic settings. However, the rates of cancer cases increased in period of 2015–2019 by 0.6–1% every year for pancreas, breast and uterine corpus cancers and by 2–3% every year for kidney, prostate, liver (female) and human papillomavirus-associated oral cancers [5]. Therefore, finding innovative cancer treatment approaches is crucial to decreasing patient suffering and the cost of the current cost-prohibitive treatments.

More and more scientists worldwide are attempting to find innovative anticancer drugs and develop new effective techniques to treat this terrible disease as a result of numerous flaws in conventional therapeutic formulations. The advancement of science has ushered in the creation of numerous treatments as well as diagnostic techniques that have played a pivotal role in managing and, to some extent, even curing various types of cancer. Approximately 70–95 percent of people in underdeveloped nations still utilize traditional medicines today. The vast majority of currently used chemotherapy medications with clinical approval was derived from numerous natural sources, including microorganisms and terrestrial and aquatic plants [6]. Natural compounds and their derivatives, with their diverse structures and favorable pharmacological properties, demonstrate remarkable potential for developing chemotherapeutic agents [7]. As a result of this early accomplishment, numerous research organizations worldwide are committed to isolating novel structural leads from various plant species and evaluating them for possible anticancer actions [8].

Hydroxytyrosol (HT) is one of these promising substances, being a primary phenolic compound found in virgin olive oil. Oxygen and nitrogen free radicals are scavenged by the potent antioxidant HT. Additionally, in neural hybridoma cells, HT guards DNA from oxidative damage by activating Nrf2, and HT promotes the expression of antioxidant enzyme [9]. It also displays analgesic and anti-inflammatory properties and inhibits the growth of colon and breast cancer cells by regulating gene expression, resulting in pro-apoptotic effects [10–14].

While the precise mechanism of HT's impact on cancer cells remains unclear, it may involve reduced Pin1 levels causing cyclin D1 to translocate to the cytoplasm, leading to its degradation. Cyclin D1 plays a vital role in driving the G1/S cell cycle transition, promoting the proliferation of tumor cells [15]. Pin1 is a peptidyl-prolyl cis/trans isomerase enzyme that plays a crucial role in the regulation of cell cycle progression. Pin1 specifically recognizes and binds to phosphorylated serine or threonine residues preceding proline in its substrate proteins. It has been implicated in the regulation of cyclin D1 stability [16]. The interaction between Pin1 and cyclin D1 has been shown to influence the subcellular localization of cyclin D1. Reduced levels of peptidyl-prolyl cis–trans isomerase NIMA-interacting 1 (Pin1) can disrupt the normal regulation of cyclin D1, leading to its translocation to the cytoplasm. In the cytoplasm, cyclin D1 is subjected to ubiquitin-mediated degradation, preventing its accumulation and promoting cell cycle arrest [17].

Furthermore, HT has shown protective properties against breast cancer development, safeguarding DNA in normal breast cells in vitro. Earlier research has shown its antioxidant, hypoglycemic, anti-thrombotic, hypocholesterolemic, anti-inflammatory, and antibacterial properties. Furthermore, hydroxytyrosol has demonstrated its ability to shield human erythrocytes from oxidative damage, promote eye health, modulate the immune system, and reduce the risk of atherosclerosis and coronary heart disease. It is also regarded as a crucial anticancer substance [12, 18, 19]. Many research studies have demonstrated that hydroxytyrosol exhibits both anti-inflammatory and antioxidant characteristics. Furthermore, it hinders the proliferation of various tumor cell lines by stimulating molecular signaling pathways that induce apoptosis and cell cycle arrest [20, 21]. The growth of pancreatic cancer cells has also been inhibited by this substance in a dose-dependent manner [22]. With a focus on plant by-products, we sought to study the most significant and promising studies relating to novel chemical hydroxytyrosol derived from natural products with anticarcinogenic potential.

Methodology

A systematic literature search was carried out on PubMed, Web of Science, Scopus, Embase, and Google Scholar databases to identify original articles on the biological activities of primary olive oil phenols. The search utilized keywords such as hydroxytyrosol, tyrosol, oleuropein, oleocanthal,

oleacein, olive oil phenols, along with terms like antioxidant, anti-inflammatory, cardioprotective, neuroprotective, osteoprotective, anticancer, antidiabetic, antiobesity, antimicrobial, metabolism, and bioavailability. The extensive literature gathered was then manually curated to ensure its relevance to the subject. This curation included both emerging insights and well-established findings. Additionally, literature dated prior to 2010 was included when deemed relevant to the topic.

Main text

Chemistry of hydroxytyrosol

Hydroxytyrosol is a colorless solid organic compound, chemically represented as $(HO)_2C_6H_3CH_2CH_2OH$, and it belongs to the group of phenolic phytochemicals. It is commonly found in olive fruit and oils, often in the form of esters with the secoiridoid elenolic acid [23, 24]. The hydroxytyrosol derivatives are obtained as by-products from olive trees and their leaves during the olive oil manufacturing process [25]. The synthesis of hydroxytyrosol occurs in single-step reaction from tyrosols. When the conversion of tyrosine into 3,4-dihydroxyphenylalanine (DOPA) happens that could lead to the synthesis of hydroxytyrosol. Hydroxytyrosol is readily soluble in organic solvents, while it exhibits only slight solubility in water, typically around 10 mg/mL, at room temperature [26]. The partitioning coefficients of hydroxytyrosol in between oil and water phases were found to be 0.010 [27]. The diverse biological properties of hydroxytyrosol stem from its potent antioxidant and radical-scavenging attributes. Its effectiveness is also influenced by the presence of an ortho-dihydroxy conformation in the aromatic ring, which is akin to catechol [28]. Moreover, hydroxytyrosol has demonstrated the ability to enhance endothelial function, reduce oxidative stress, provide neuro- and cardioprotection, positively affect lipid and hemostatic profiles, and exhibit anti-inflammatory properties [29] (Fig. 1).

Ethnopharmacological applicability

Hydroxytyrosol is found in both olive leaves and fruits, which belong to the Oleaceae family. It serves as a significant component of olive leaf extract, olive mill wastewater, and virgin olive oil. Notably, it is regarded as having the highest in vitro antioxidant potential among all the polyphenols in olive oil [10]. It is stable in its free form and readily get penetrated into tissues [30]. In olive fruits or olive oil, tyrosol and hydroxytyrosol are considered to be an important dietary phenolic component. They are well known for their scavenging attribute and either can be found in ester form of secoiridoid elenolic acid or in free form [27, 31]. Both hydroxytyrosol and tyrosol are natural compounds known for their diverse pharmacological properties. They demonstrate a wide array of effects, including anti-inflammatory, antioxidant, antigenotoxic, anti-hyperglycemic, anti-depressant, anticancer, neuroprotective, and anti-atherogenic properties, among others (Fig. 2). They also prevent keratinocytes apoptosis induced by radiation, mitochondrial dysfunction induced by acrylamide and acrolein-induced deoxyribonucleic acid (DNA) damaged, etc. [19, 31–36]. Hydroxytyrosol can be obtained from fat's main source in Mediterranean diet. The Mediterranean diet, originating in olive-growing regions in the Mediterranean Basin in the 1960s, emphasizes plant-based foods with a primary focus on various fats, predominantly sourced from olive oil. This dietary pattern leads to a significant consumption of mono-unsaturated and polyunsaturated fats while minimizing the intake of saturated fats [37]. Hydroxytyrosol is the sole polyphenol in the market with an authorized health claim, approved by the European Food Safety Authority [37, 38]. The Mediterranean diet primarily comprises fruits, vegetables, and olive oil as its main components. In Mediterranean nations, despite a relatively high fat intake, the prevalence of cardiovascular disease is significantly lower compared to countries like the USA, where fat consumption is also relatively high. The European Commission has established a scientific panel on dietetic products, nutrition, and allergies as a division under the jurisdiction of the European Food Safety Authority. This panel assesses hydroxytyrosol as a novel product, deeming it safe for the public while excluding children under the age of three and pregnant or nursing women. This assessment aligns with the standards set for novel food constituents, as outlined in Article 3(1) of Regulation (EC) 258/97. EFSA has evaluated hydroxytyrosol as safe for human consumption as a new food, establishing a daily limit for potential adverse effects at 50 mg/kg of body weight [39]. In the USA, a dosage of 5 mg of hydroxytyrosol per serving is considered safe for its inclusion in processed foods [40]. An accepted daily safe dosage for adults is 800 mg, and this compound contributes significantly to the health benefits associated with extra virgin olive oil [41]. Bioavailability studies indicate that hydroxytyrosol from olive oil is effectively absorbed after ingestion and demonstrates significant biological effects that are dependent on the dose [42]. Olives provide natural antioxidants that protect against oxidative stress, a factor linked to diseases

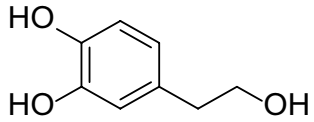

Fig. 1 Structure of hydroxytyrosol

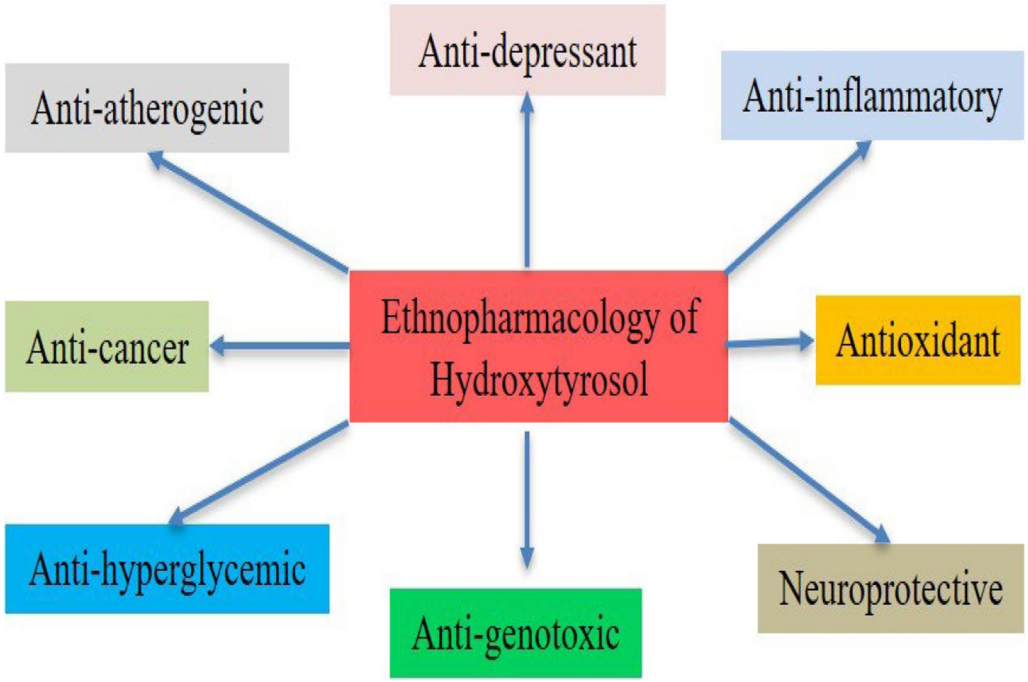

Fig. 2 Ethnopharmacological properties of hydroxytyrosol

such as coronary heart disease, cancer, and neurological disorders.

Absorption and metabolism studies

Preclinical and clinical studies have indicated that hydroxytyrosol is absorbed in the colon and small intestine, following a dose-dependent pattern [43, 44]. Transport through intestinal epithelium occurs via passive bidirectional diffusion. The absorption of hydroxytyrosol depends upon the nature of the vehicle it is carried in. A study found that rats absorbed 99% of hydroxytyrosol in olive oil, compared to 75% in an aqueous solution [45]. Also, the rate of absorption varies depending on the type of animal. For example, rats absorb at a rate that is different from that of humans since rats lack a gallbladder [46]. Tissue distribution studies in rats, conducted after intravenous administration of radioactive hydroxytyrosol, showed a short half-life in the blood (1–2 min), with the majority accumulating in the kidneys just 5-min post-injection [47]. Further, hydroxytyrosol is also widely distributed in different organs including the liver, lungs, skeletal muscle, and heart (Fig. 3). It easily crosses the blood–brain barrier and enters the brain. Additionally, it can also be synthesized endogenously from dihydroxyphenylacetic acid by dihydroxyphenylacetic acid reductase, an enzyme present in the brain [48]**.** It undergoes initial metabolic processes in enterocytes and subsequently in the liver**.** These stages are vital as hydroxytyrosol undergoes various transformations and modifications, which are believed to contribute to its therapeutic properties [49]. Three metabolic pathways have been proposed for hydroxytyrosol: (1) Oxidation, which is carried by enzymes aldehyde dehydrogenase and alcohol dehydrogenase, rendering dihydroxyphenylacetic acid (2) Methylation, which is carried by the enzyme catechol-O-methyltransferase, giving rise to dihydroxyphenylacetic acid, and (3) Methylation-Oxidation, which results to homovanillic acid [47]. Indeed, the primary metabolites of hydroxytyrosol encompass aldehydes, O-methylated forms, and acids, which are created through the oxidation of glucuronide, sulfates, aliphatic alcohol, as well as N-acetylcysteine and sulfated derivatives [44]. In rats, it takes around 5 h; in humans, it takes around 4 h for hydroxytyrosol and its metabolites to be discharged from the urine [43]. Similar to absorption, the elimination of hydroxytyrosol and its metabolites varies depending on the method of administration used for the compound. A study revealed that the elimination of hydroxytyrosol through urine is higher when it is administered as a natural component of olive oil compared to its external administration in low-fat yogurt or refined olive oil [46]. Absorption and urinary excretion of hydroxytyrosol and its metabolites differ between rats and humans, with both processes being lower in rats compared to humans. Therefore, these

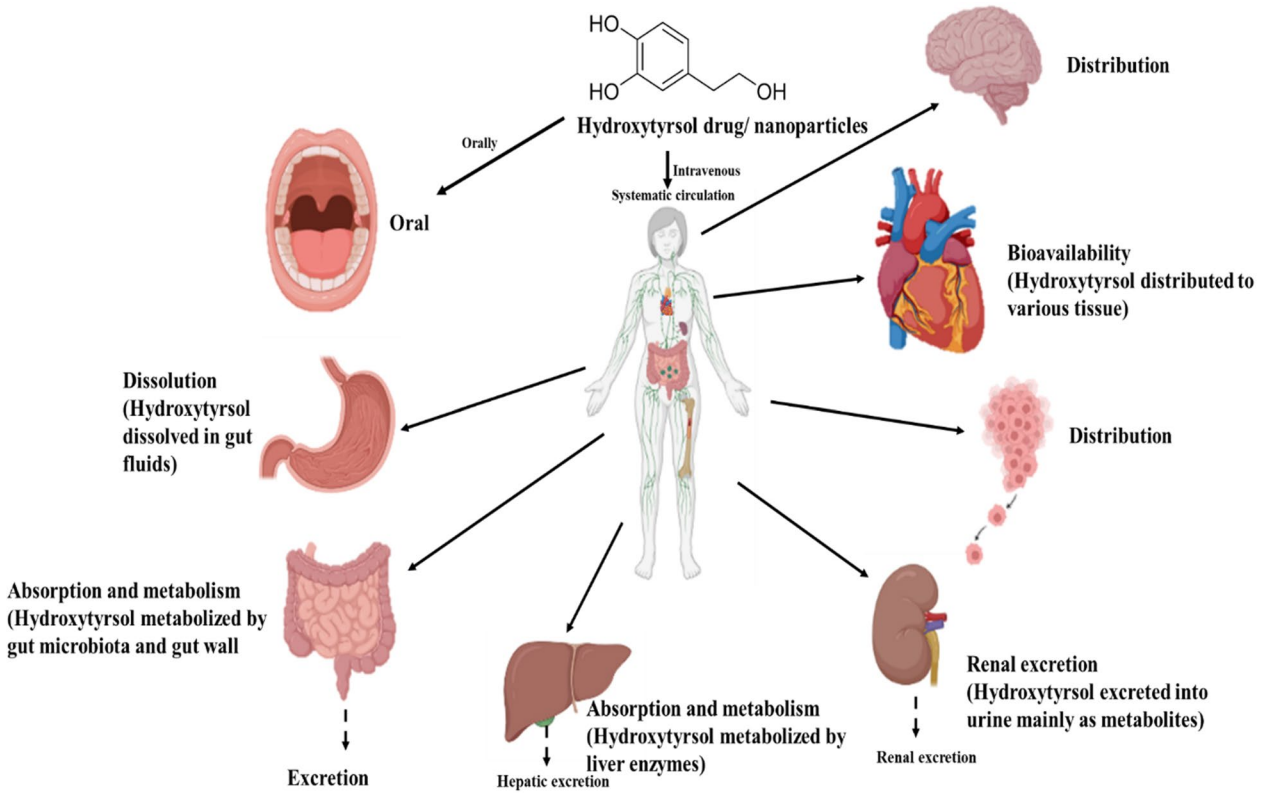

Fig. 3 Absorption and disposition of hydroxytyrosol in humans

findings indicate that rats may not be a suitable model for studying hydroxytyrosol metabolism.

Pharmacokinetics of hydroxytyrosol

Pharmacokinetics primarily involves the kinetic study of a compound's absorption, distribution, metabolism, and excretion in biofluids, tissues, and organs over a specific time period [50]. Understanding the pharmacokinetics of hydroxytyrosol is essential for optimizing its therapeutic potential and ensuring its safe and effective use. While there is limited research specifically focusing on the pharmacokinetics of hydroxytyrosol, some reports have shed light on its absorption and metabolism. When hydroxytyrosol is orally administered, whether with olive or an aqueous supplement, it is absorbed through the intestine and undergoes rapid metabolism involving both phase-I and phase-II metabolic reactions. Hydroxytyrosol metabolites were undetectable in fasting state plasma but quickly cleared during the postprandial phase and excreted in urine. The maximum concentration (Cmax) is reached within 30 min, and clearance occurs within 2–4 h [51]. In a preclinical study, hydroxytyrosol was found absent in the brains and cerebrospinal fluid of normal animals but crossed the blood–brain barrier in mice experiencing chronic unpredictable mild stress (CUMS). This suggests that hydroxytyrosol's beneficial effects primarily target the hippocampus, as it is distributed there due to BBB impairments in stressed mice after oral administration [52]. Metabolism of hydroxytyrosol primarily occurs in the liver. Studies using in vitro models have identified several metabolic pathways for hydroxytyrosol including glucuronidation and sulfation. These conjugation reactions facilitate the excretion of hydroxytyrosol from the body. The unchanged hydroxytyrosol (free form) is almost undetectable in urine and plasma samples by oral route than intraperitoneal. Food matrix significantly affects the absorption and metabolism of hydroxytyrosol, with extra virgin olive oil recognized as the most effective matrix for improving its bioavailability [51]. Hydroxytyrosol primarily metabolizes into HVA, DOPAC, and HT-3-S, and these metabolites can be detected in plasma samples from food supplements shortly after ingestion. Among these, DOPAC and HVA reach their peak plasma concentrations approximately 30 min after ingestion. Notably, DOPAC exhibits lower concentrations and faster elimination compared to HVA, largely due to its enzymatic conversion into HVA through the action of catechol methyltransferase enzyme [41, 53]. The pharmacokinetics of hydroxytyrosol are subject to influence by several factors, including the administered

dose, co-administration with food, and individual variations in metabolism. Finding from the literature regarding pharmacokinetics concluded that hydroxytyrosol showed rapid absorption, hepatic metabolism, and elimination through conjugation reactions. Further research is required to obtain a comprehensive understanding of the complete pharmacokinetic profile of hydroxytyrosol, with a particular focus on its distribution and excretion pathways. Nonetheless, the existing research provides valuable insights into the absorption and metabolism of hydroxytyrosol, which contribute to its potential therapeutic applications.

Anticancer cellular targets

Hydroxytyrosol demonstrates a multifaceted approach in targeting cellular components relevant to cancer development and progression. Research has demonstrated hydroxytyrosol's ability to interact with multiple critical molecular targets, making it a good candidate for cancer therapy [54–56].

Hydroxytyrosol notably focuses on regulating oxidative stress within cells. Hydroxytyrosol, as a strong antioxidant, counters reactive oxygen species (ROS) known to stimulate cancer growth and harm cellular components. By mitigating oxidative stress, hydroxytyrosol helps maintain cellular homeostasis and reduces the risk of cancer initiation (Fig. 4) [57].

Hydroxytyrosol also plays a crucial role in modulating inflammatory pathways. Chronic inflammation significantly contributes to the promotion of cancer development and metastasis [58]. Hydroxytyrosol's anti-inflammatory properties help suppress pro-inflammatory signaling molecules, thereby curbing cancer-promoting processes and reducing the tumor microenvironment's pro-tumorigenic effects [59–61]. Hydroxytyrosol has also shown promise in regulating cell cycle progression. It can induce cell cycle arrest, halting uncontrolled cell proliferation, a hallmark of cancer cells. Its impact on the cell cycle inhibits cancer cell growth and promotes their elimination through apoptosis [22, 59, 60, 62]. Furthermore, hydroxytyrosol has been found to target specific signaling pathways crucial for cancer survival and invasion. It can modulate various kinases, such as PI3K/AKT and MAPK (Fig. 4), which play key roles in cancer cell survival and metastasis [62, 63]. By interfering with these pathways, hydroxytyrosol hinders cancer cell growth and motility. In summary, the diverse capabilities of hydroxytyrosol in targeting various cellular components relevant to cancer progression highlight its potential as a promising anticancer agent. Its ability to regulate oxidative stress, inflammation, cell cycle progression, and signaling pathways makes it an intriguing candidate for further research and potential incorporation into cancer treatment strategies.

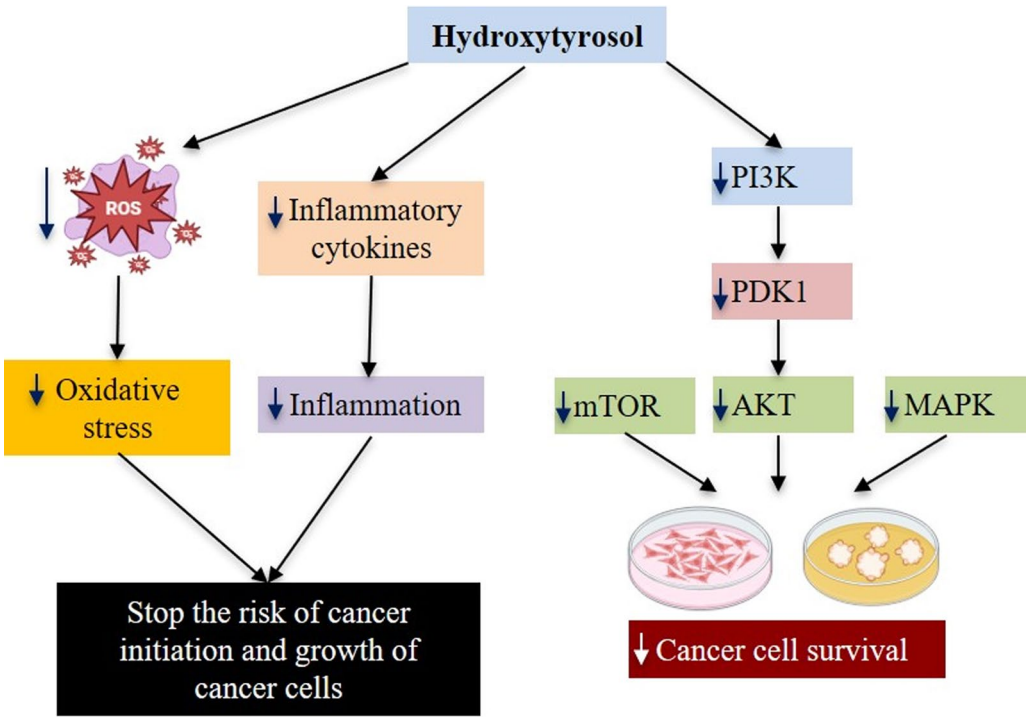

Fig. 4 Hydroxytyrosol targeting oxidative stress, inflammation, PI3K/Akt/mTOR and MAPK pathways in cancer cells

Zrelli et al. reported that hydroxytyrosol (HT) showed anticancer potential and induced apoptosis in vascular smooth muscle cells (VSMCs) via enhanced nitric oxide production, and reduced Akt phosphorylation levels [64]. Zubair et al., 2017 exhibited a study that evaluated the anticancer potential of hydroxytyrosol against prostate cancer (LNCaP and C4-2) cells, whereas non-toxic effects against normal cells. The treatment of hydroxytyrosol in prostate cancer significantly induced apoptosis, and that can also inhibit androgen receptor expression [22]. Calahora et al. (2020) found that hydroxytyrosol, a primary bioactive compound in olive oil, significantly reduces the growth of the breast cancer cell line MCF-7. This effect is likely attained by modulating HIF-1α protein expression, potentially by reducing oxidative stress and inhibiting the PI3K/Akt/mTOR pathway (Fig. 4) [65]. Costantini et al., 2020, reported that hydroxytyrosol primary component of olive oil showed anticancer potential toward human melanoma cell (A375, HT-144 and M74) lines through upregulation of ROS level and induction of apoptosis via increases p53 and γH2AX expression decreases AKT expression [66].

Jadid et al. (2021) explored the use of nano-encapsulated hydroxytyrosol and curcumin, both individually and in combination, and found that these formulations significantly reduced the proliferation of the pancreatic cancer cell line PANC-1. This effect was achieved by modulating the expression levels of key proteins including BCL-2, BAX, and Cas-9 [67]. Antonio et al., 2021 found that hydroxytyrosol showed cytotoxic potential against PC-3 and 22Rv1 treated cells but less cytotoxicity toward non-cancerous cells (RWPE-1 cells). Hydrotyrosol exhibited its anticancer potential toward these cell lines by modulating the phospho-AKT/AKT expression levels [68]. In 2022, Aghaei and colleagues discovered that hydroxytyrosol induced apoptosis in breast cancer cells (MDA-MB-231 and MCF-7) by upregulating pro-apoptotic genes (BAX and CASP3) and downregulating the anti-apoptotic BCL2 gene [69].

Apoptosis and cell cycle arrest

Apoptosis, essential for tissue balance, eliminates damaged cells. Dysregulation in cancer underscores its significance. Inducing apoptosis is a promising cancer therapy approach [70]. Hydroxytyrosol has been shown to activate caspases which are key enzymes involved in the apoptotic process. Caspase activation cleaves targets, instigating cancer cell apoptosis [71]. It modulates Bcl-2 proteins, central to apoptosis control. Hydroxytyrosol reduces the levels of the anti-apoptotic protein Bcl-2 while increasing the expression of the pro-apoptotic protein Bax, thereby promoting apoptosis in cancer cells [72]. Hydroxytyrosol disrupts cancer cell mitochondrial function, releasing pro-apoptotic factors like cytochrome C, activating c-Jun and c-Fos pathways, ultimately inducing apoptosis [73]. Hydroxytyrosol enhances apoptosis in breast cancer cells by increasing caspase-3 activity, inducing DNA fragmentation, and promoting mitochondrial membrane depolarization. Additionally, it upregulates the pro-apoptotic Bax and downregulates the anti-apoptotic Bcl-2, resulting in the release of cytochrome C and activation of the intrinsic apoptotic pathway [74]. In prostate cancer, hydroxytyrosol induces apoptosis through various mechanisms. It activates caspase-3 and caspase-9, triggers PARP cleavage, inhibits Akt/STAT3 phosphorylation, and retains NF-kB in the cytoplasm of prostate cancer cells [22]. Furthermore, hydroxytyrosol treatment activates critical signaling pathways, including MAPK, Akt, JAK/STAT, NF-κB, and TGF-β, which are instrumental in inducing apoptosis. [75, 76]. In colon cancer, hydroxytyrosol reduces cell viability and enhances caspase-3 activity, promoting apoptotic cell death [71, 77]. Additionally, hydroxytyrosol treatment upregulated pro-apoptotic proteins like p53 and Bax, while downregulating anti-apoptotic proteins like Bcl-2 [49, 63]. These findings define that hydroxytyrosol induces apoptosis in colon cancer cells by regulating apoptotic protein expression. Hydroxytyrosol's apoptotic effects have also been observed in other cancer types, such as liver cancer and leukemia. Hydroxytyrosol induces apoptosis in liver cancer cells via the mitochondrial pathway, with increased Bax expression and reduced Bcl-2 levels [78]. Hydroxytyrosol treatment induced apoptosis in leukemia cells by activating caspase-3 and caspase-8 while simultaneously inhibiting the NF-κB signaling pathway [63]. Hydroxytyrosol has been documented to provoke G1 phase arrest in cancer cells, a state often linked with the reduction in levels of cyclin D1, cyclin-dependent kinase 4 (CDK4), and CDK6, which play pivotal roles in regulating the G1-S transition [71]. Hydroxytyrosol has been shown to induce G2/M phase cell cycle arrest in cancer cells by downregulating cyclin B1 and CDK1, which are essential for the G2-M transition [61]. Numerous studies have delved into the cell cycle arrest mechanisms induced by hydroxytyrosol in various cancer types. A fundamental anticancer mechanism of hydroxytyrosol centers around cell cycle regulation, particularly in breast cancer. This is achieved by inducing G1 phase cell cycle arrest, involving the increase in cyclin-dependent kinase inhibitors, like p21 and p27, and the reduction in cyclins D1 and E. These actions collectively impede cell cycle progression, resulting in cell cycle arrest [79]. In colon cancer cells, hydroxytyrosol can induce G2/M phase cell cycle arrest by inhibiting cyclin-dependent kinases, specifically CDK1, and promoting the degradation of cyclin B1, both of which play crucial roles in regulating the G2/M

transition [71]. Hydroxytyrosol induces G1 phase cell cycle arrest in prostate cancer cells through the modulation of key cell cycle regulators, including cyclin D1 and p21 [22, 80].

Anti-angiogenic and anti-metastatic action

Angiogenesis, essential in numerous cancers, involves the formation of new blood vessels and plays a significant role in tumor growth and metastasis. Blocking angiogenesis can impede tumor progression by restricting the blood supply to tumors [81]. Angiogenesis, the formation of new blood vessels from existing ones, plays a vital role in embryogenesis, wound healing, and tumor growth. This process is controlled by a delicate balance between pro-angiogenic factors (vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor) and anti-angiogenic factors (Thrombospondin and Angiostatin). Angiogenesis, primarily regulated by VEGF and its receptor VEGFR-2, is pivotal in various diseases, including cancer, diabetic retinopathy, rheumatoid arthritis, and cardiovascular disorders. Especially in case of cancer, tumors require a blood supply to grow beyond a certain size, and they can induce angiogenesis to recruit new blood vessels and provide nutrients for their survival and expansion which is explained by a number of studies [42, 82]. Hydroxytyrosol has been shown to possess the capability to suppress the expression of vascular endothelial growth factor (VEGF), a crucial regulator of angiogenesis. By inhibiting VEGF expression, hydroxytyrosol can interfere with the development of new blood vessels, which are vital for tumor growth and metastasis [15]. Moreover, research has demonstrated that hydroxytyrosol can inhibit the proliferation of endothelial cells, a pivotal factor in the formation of new blood vessels. This anti-proliferative effect can further contribute to the suppression of angiogenesis [19]. According to studies by Li & Kroetz, (2018); Touyz et al., (2018) and Wu et al., (2008), Blocking VEGF receptor-2 binding with drugs like bevacizumab, sorafenib, and sunitinib inhibits angiogenesis. Yet, these drugs may cause side effects, such as hypertension [83–85]. Several natural products (Table 1) like epigallocatechin-3-gallate (EGCG), procyanidin oligomers, resveratrol, quercetin, caffeic acid phenethyl ester, urolithins, and ellagitannin show anti-angiogenic and anti-VEGF effect without causing hypertension like side effects. Besides these compounds, hydroxytyrosol (in fermented beverages & Olive oil) and Indole acetic acids (in wine) show VEGFR-2 inhibitory effect without causing adverse hypertensive effects, proving their advantage over synthetic drugs. Fortes et al. (2012) found that hydroxytyrosol inhibits endothelial cell apoptosis, alters cell cycle distribution, and impedes cell proliferation,

Table 1 List of HT sources and their physiological activities performed

S No	Source	Activity	Description	References
1	Olive oil	Anti-angiogenic effect	At low conc. (1–5 μM) of HT induces the expression of proto-oncogene tyrosine-protein kinase Src (Src), rho-associated protein kinase (ROCK) and MMP-2	[87]
2	Virgin olive oil	Apoptosis	Upregulation of Akt, mTOR, TGF-β1, and p53 were elevated	[108]
3	–	Anti-VEGF	Activated eNOS—all via Akt	[109]
4	Olive oil	Angiogenesis	inhibiting the PI3K/Akt/mTOR pathway	[65]
5	Olives	Cell cycle	Induced G1/S cell cycle arrest Inhibited the phosphorylation of Akt / STAT3, and induced cytoplasmic retention of NF-κB	[22]
6			Arrest at G_2/M phase downregulation of D3 and CDK6	[62]
7	Olive oil	Angiogenesis	Reduction in COX2 and TNF-α, and oxidative stress reduction	[61]
8	Aqueous olive extracts		Inhibition of NO and PGE_2 production (IC_{50} of 11.4 μM) Inhibition of cytokine secretion (IL-1 α, IL-1 β, IL-6, IL-12, TNF-α) Inhibition of chemokine secretion (CXCL10/IP-10, CCL2/MCP-1) Reduction in gene expression (iNOS, IL-1 α, CXCL10/IP-10, MIP-1 β, MMP-9, PGES)	[104]
9	Hydrolysis of Oleuropein in olive oil		Effective local treatment for inflammatory colitis	[110]
10	Olive leaf and oil		Increase expression of MMP-1 and MMP-3 Decreased in the expression of inflammatory interleukins (IL-1β, IL-6, IL-8)	[111]
11	Virgin olive oil extraction		Reduced the expression level of IL-1β, IL-6, TNF-α, NF-κB and MAPK suppressed NF-κB signaling and downregulated LPS-mediated expression of iNOS, cyclooxygenase-2, tumor necrosis factor alpha, and interleukin-1β at 12.5 μg/ml	[97]

migration, and differentiation into "capillary-like" tubes. Additionally, it inhibits MMP-9, cyclooxygenase 2, and VEGFR-2 phosphorylation, demonstrating its anti-angiogenic properties [86]. In a study by Lamy et al. (2014), it was noted that hydroxytyrosol inhibits angiogenesis by targeting specific phosphorylation sites (Tyr951, Tyr1059, Tyr1175, and Tyr1214) on vascular endothelial growth factors (VEGFR-2), leading to the inhibition of endothelial cell (EC) signaling and subsequent EC proliferation inhibition. All these studies suggested the hydroxytyrosol and its derivatives have potential anti-angiogenic properties and can be used in the prevention and therapy of cancer (Fig. 4) (Bernini et al., 2015). In breast cancer, hydroxytyrosol inhibits angiogenesis by reducing VEGF receptor expression and blocking the PI3K/AKT signaling pathway [65]. In an in vitro study on colorectal cancer, hydroxytyrosol inhibited angiogenesis by reducing VEGF expression, suppressing matrix metalloproteinase (MMP) activity, which is involved in angiogenesis and tumor invasion, and inhibiting the activation of the PI3K/AKT/mTOR signaling pathway [29, 87]. Metastasis is the process by which cancer cells disseminate from the primary tumor to distant organs through the bloodstream or lymphatic system. When cancer cells metastasize, they invade nearby tissues and enter the circulatory or lymphatic systems, allowing them to travel to distant organs or tissues. Metastasis, a fundamental characteristic of malignant tumors and a primary contributor to cancer-related mortality, entails the dissemination of cancer cells to various organs through the bloodstream or lymphatic system. Hydroxytyrosol has been demonstrated to inhibit MMPs, enzymes crucial for the degradation of the extracellular matrix, potentially impeding metastatic processes [88]. Hydroxytyrosol can hinder the invasion and metastasis of cancer cells by inhibiting matrix metalloproteinases (MMPs). [80, 89]. Hydroxytyrosol has been found to inhibit epithelial-mesenchymal transition (EMT), a fundamental stage in cancer metastasis. By preventing cancer cells from acquiring a more invasive and migratory phenotype through EMT, hydroxytyrosol can hinder metastatic spread [90]. Aghaei et al. in 2022, the anti-proliferative effects of hydroxytyrosol were demonstrated on both MDA-MB-231 and MCF-7 cancer cells, along with an increase in apoptotic activity. It also downregulates the anti-apoptotic (*BCL2*) gene and upregulates the pro-apoptotic (*BAX* and *CASP3*) genes. In a study by León-González et al. (2021) on prostate cancer cell lines (RWPE-1, LNCaP, 22Rv1, and PC-3), hydroxytyrosol and its derivatives demonstrated anti-proliferative effects. This included reduced cell migration in RWPE-1 and PC-3, as well as decreased prostatosphere size and colony formation in 22Rv1. In colorectal cancer cells, Hormozi et al. (2020) demonstrated that hydroxytyrosol induces apoptosis by upregulating the CASP3 gene expression and growing the BAX:BCL2 ratio. Furthermore, hydroxytyrosol enhances antioxidant enzyme activity and reduces LS180 cell proliferation by modifying the antioxidant-defense system in cancer cells. Méndez-Líter et al. (2019) documented the reduction in viability in the breast cancer cell line MCF-7 due to the impact of hydroxytyrosol. Hydroxytyrosol significantly reduced EGFR expression, leading to decreased cell proliferation in human colon cancer cells, and it also reduced tumor growth, along with EGFR expression levels, in HT-29 xenografts (Terzuoli et al., 2016). Hydroxytyrosol inhibits the migration and invasion of breast cancer cells by modulating crucial metastasis-related pathways, such as epithelial-mesenchymal transition (EMT) and matrix metalloproteinase (MMP) activation. The effects of hydroxytyrosol observed in both in vitro and in vivo studies underscore its potential as a promising anti-metastatic agent for breast cancer [89, 91, 92]. Li et al. 2014, the proliferation of human gallbladder cancer cell lines and human cholangiocarcinoma (CCA) was inhibited by hydroxytyrosol. Hydroxytyrosol disrupts (E2)-induced molecular mechanisms, leading to the inhibition of breast cancer cell proliferation (Fu et al., 2010). Some crucial proteins that are involved in the control of these processes were altered in expression by hydroxytyrosol. Additionally, hydroxytyrosol leads to reduced DNA synthesis, suppresses the cell cycle, lowers the levels of CDK-6, and increases cyclin D3 expression. All these factors induce apoptosis in HL 60 cells [93]. These studies collectively offer a comprehensive summary outlining the anti-angiogenic and anti-metastatic activities of hydroxytyrosol (Fig. 5).

Anti-inflammatory action

Fernández-Prior et al., 2021 found that the anti-inflammatory potential of hydroxytyrosol was investigated using the THP-1 cell line. Their analysis of pro-inflammatory cytokines, together with TNF-α, IL-6, and IL-1β, showed the reduction in the gene expression of these cytokines. This indicates the potential of hydroxytyrosol in treating diseases with inflammatory origins. Downregulation of cytokines (IL1, TNF, Cox2 and iNOs) expression was observed to be reduced as a result of hydroxytyrosol treatment and mice on olive oil diet show a significant reduction in cox2 and inducible nitric oxide synthase (iNOs) and its antioxidant activity exhibits the anti-inflammatory activity of hydroxytyrosol [59, 94, 95]. NF-κB, a crucial factor in inflammation, activates the transcription of various cytokine genes. Consequently, inhibiting NF-κB has been acknowledged as a strategy to control inflammatory cytokine. Hydroxytyrosol has been shown to inhibit the activation of both p53 and NF-κB in cells [94, 96]. Yonezawa et al. (2018, 2019) documented

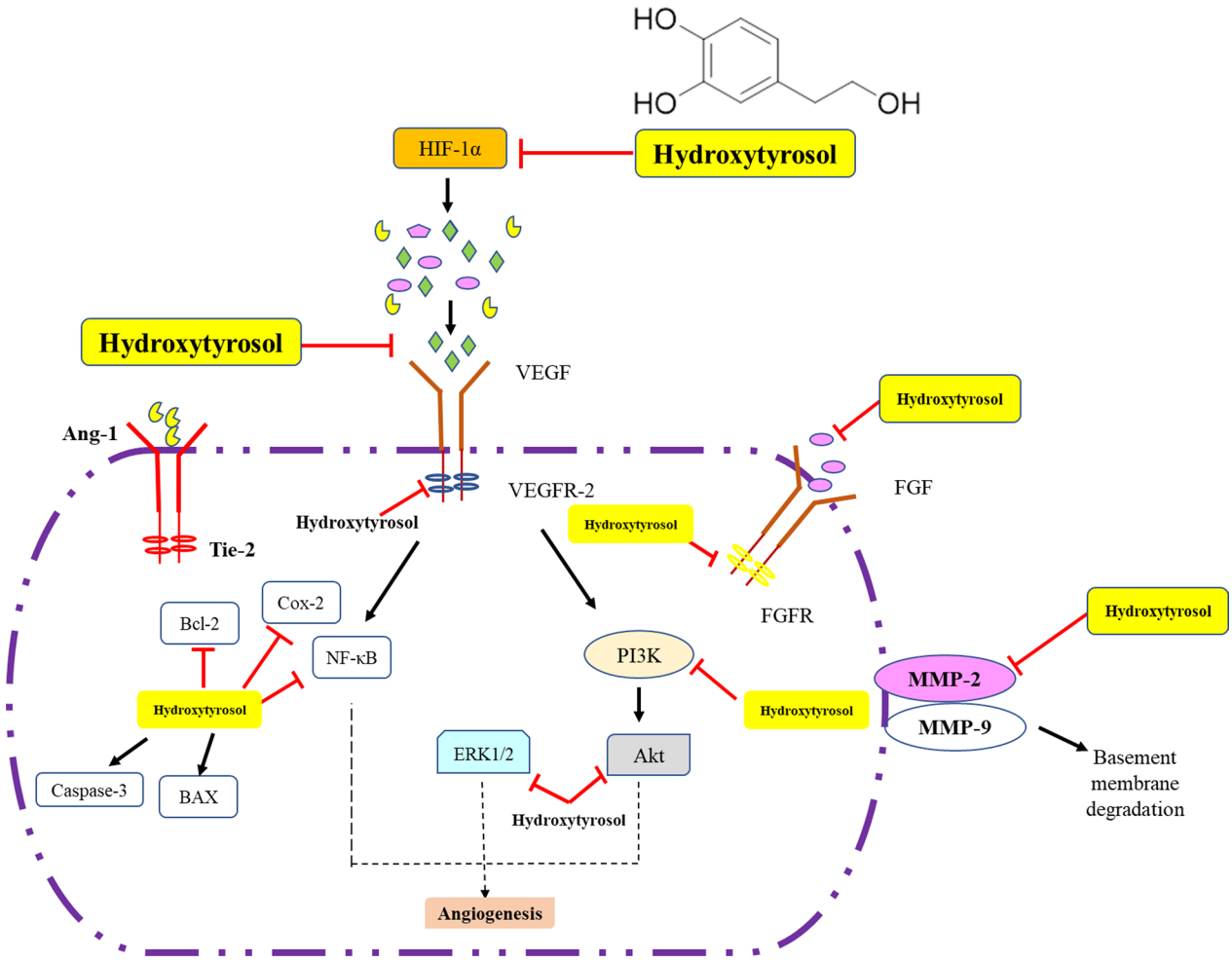

Fig. 5 Major signaling pathways targeted by hydroxytyrosol in angiogenesis and metastasis processes

that hydroxytyrosol inhibits the lipopolysaccharide-mediated stimulation of inducible nitric oxide synthase, cyclooxygenase-2 (COX-2), and interleukin-1 expression, leading to reduction in nitric oxide and prostaglandin E2 production [97, 98]. This underscores the anti-inflammatory activity of hydroxytyrosol. In the combination of hydroxytyrosol with pectin/alginate was found effective against TNBS-induced colitis, providing further evidence of the anti-inflammatory properties of hydroxytyrosol [99].

Hydroxytyrosol exhibited a dose-dependent reduction in SA-β-galactosidase activity in UVA-exposed human dermal fibroblasts (HDFs). Furthermore, it dose-dependently decreased the elevated expression of MMP-1 and MMP-3 induced by UVA exposure. Jeon & Choi (2018) noted that hydroxytyrosol reduced SA-β-galactosidase activity and inhibited the MMP-1 and MMP-3 expression. Moreover, hydroxytyrosol reduced the expression of genes associated with IL-1, IL-6, and IL-8 in UVA-exposed human dermal fibroblasts (HDFs) [100]. Fuccelli et al. (2018) illustrated that hydroxytyrosol treatment effectively reduced TNF-α production in plasma following intraperitoneal injection of lipopolysaccharide (LPS), indicating its anti-inflammatory potential [61]. FF-HT exhibited robust anti-inflammatory effects in vivo, resulting in a 16% reduction in plasma TNF levels and a 25% reduction in CRP levels when compared to the model group [101]. In TNF-activated human umbilical vein endothelial cells (hECs), Echeverría et al. (2017) found that hydroxytyrosol reduced the protein levels of phosphorylated inhibitor of κBα kinase (IKKαβ), inhibitor of κBα (IκBα), and p65, essential components of the NF-κB pathway. The suppression of NF-κB signaling highlights the involvement of NF-κB inactivation in the anti-inflammatory action of hydroxytyrosol [102]. Tutino et al. (2012) investigated the mechanisms through which hydroxytyrosol (HT) prevents oxidative stress and inflammation in human hepatoma cells while also

inhibiting cancer cell proliferation [103]. Richard et al. (2011) reported that hydroxytyrosol reduced the secretion of cytokines (IL-1α, IL-1β, IL-6, IL-12, TNF-α) and chemokines (CXCL10/IP-10, CCL2/MCP-1), highlighting its anti-inflammatory action [104]. In vitro, hydroxytyrosol (HTyr) inhibits pro-tumorigenic inflammatory reactions that are brought on by the activation of monocytes and macrophages (in vitro). For example, the anti-inflammatory effectiveness of hydroxytyrosol (HTyr) may be notably attributed to its ability to suppress the NF-κB signaling pathways [105]. Prolonged oxidative stress and inflammation can lead to the onset of autoimmune and chronic diseases. Several of the studies mentioned above have highlighted hydroxytyrosol's potential in inhibiting diseases like type II diabetes, rheumatoid arthritis, and inflammatory bowel disease through interactions with their respective receptors [106, 107].

Hydroxytyrosol antibacterial activity

Hydroxytyrosol ($C_8H_{10}O_3$) is reported as a phenolic phytochemical compound isolated from olive (*Olea europaea*) leaves and oils. Hydroxytyrosol (HT) from *Olea europaea* exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria among the isolated phenolic compounds [112]. In literature, many of the bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumonia*, *Campylobacter* spp. and *Acinetobacter baumannii* are responsible for causing different types of infections in human population [113]. Since ages, medicinal plants are used for the isolation of phytochemical compounds showing profound activities against pathogenic bacteria. Currently, HT is a desirable molecule among all the phenolic components of olives having profound applications in medical, nutraceutical, pharmaceuticals and food industries [114]. Numerous studies in the literature have explored the antimicrobial and antibacterial potential of hydroxytyrosol (HT) from olive leaves, as summarized in Table 2. These studies offer valuable insights into HT's potential as a natural antimicrobial agent. In addition to this, phenolic extracts of olive leaves showed potent activity against infections related to respiratory and gastro-intestinal tract [115].

HT exhibits potent antibacterial activity against a range of bacteria, including Staphylococcus aureus, Salmonella Typhimurium, Escherichia coli, Salmonella enterica, Shigella sonnei, Bacillus cereus, Listeria monocytogenes, Pseudomonas aeruginosa, Clostridium perfringens, and Yersinia sp [116, 117]. In 2019, Nazzaro et al. reported significant bacterial growth inhibition at very low concentrations in the polyphenolic extract obtained from three *Olea europaea* varieties: Ruvea, Ravece, and Ogliara [118]. Similarly, Rocha-Pimienta et al., (2020) reported significant antimicrobial activity of HT against Gram-negative *E. coli* and Gram-positive *Listeria innocua* [115]. Though, the action mechanism of HT and other phenolic compound is yet now clear but, Martillanes et al. [119] proposed that the antibacterial effects of phenolic compounds might be due to genetic material modifications, interactions with cell membranes, and the disruption of enzymatic systems. Silvan et al. [120] reported antibacterial activity in leaf extracts E1 and E2 of *Olea europaea* against *Helicobacter pylori* causing chronic gastritis in human population. Characterization of extracts E1 and E2 via HPLC-PAD-MS revealed that E1 is primarily composed of hydroxytyrosol (HT) and its glucosides, while E2 contains hydrophilic compounds like oleuropein (OLE).

In Silvan et al. [121] reported significant antibacterial activity in *Olea europaea* leaf extract E1, which comprises hydroxytyrosol and hydroxytyrosol glucosides. These compounds exhibited activity against antibiotic-resistant strains of *Campylobacter jejuni* and *Campylobacter coli*. Yuan et al. [122] reported the antibacterial

Table 2 Mechanisms of action of hydroxytyrosol (HT) in some of the bacteria

Compound name	Target organism	Mechanism of action	References
Hydroxytyrosol acetate	*Vibrio* spp.	Bacterial membrane permeabilization and interacting with DNA and inhibiting biological function	[128]
Hydroxytyrosol (HT)	*Staphylococcus aureus* and *Staphylococcus epidermidis*	NA	[129]
HT	*E. faecalis*, *S. aureus* and *C. albicans*, *P. gingivalis*, *F. nucleatum*, *S. mutans*	Damages cell envelope	[130]
HT	*Lactobacillus plantarum*	Reduction in expression of genes responsible for proliferation of cells	[127]
Olive oil polyphenol extract (OOPE) containing HT	*Listeria monocytogenes*	It impacts intracellular adenosine 5'-triphosphate (ATP) concentration, reduces bacterial protein/DNA levels, and alters cell morphology and membrane potential	[124, 131]
HT	*Mycoplasma gallisepticum*	Blocking activation of NF-kB/NLRP3/IL-1b signaling pathway	[124]

activity of HT, OL, 3,4-dihydroxybenzoic acid, and caffeic acid against *Klebsiella pneumoniae*, indicating its possible use as an important pharmaceutical compound. Pannucci et al. [123] reported antioxidant and antimicrobial activity of HT enriched extract from oil mill wastewater against two olive tree pathogens viz. *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* pv. *Savastanoi*. The chemical characterization of the extract identified hydroxytyrosol as the main component, demonstrating significant antimicrobial activity. Shan et al. [124] found that hydroxytyrosol exhibits anti-inflammatory and anti-apoptotic activity against pulmonary injury induced by *Mycoplasma gallisepticum* (MG) in chickens. This effect is achieved by reducing damage and inhibiting the activation of the NF-κB/NLRP3/IL-1β signaling pathway.

Hence, hydroxytyrosol can be seen as an important pharmacological compound having antibacterial activity against an array of different human pathogens.

In addition, it possesses antioxidative, anti-inflammatory, anti-atherogenic, anti-thrombotic, and anticancer properties [125]. The exact antibacterial mechanism of hydroxytyrosol is not fully understood, but some studies in the literature propose that HT may induce protein denaturation, alter cell membrane permeability, or downregulate genes responsible for cell proliferation [126, 127]. Table 2 provides information on the target organisms and mechanisms of action of hydroxytyrosol and its derivatives.

Synergistic action

The therapeutically active and anticancer activities of hydroxytyrosol have been linked to its concentrations. Hydroxytyrosol has been reported to exhibit diverse therapeutic activities in isolation, but its primary health-promoting properties often result from synergistic effects when combined with other compounds. It is a component found in virgin olive oil and has previously shown a beneficial role in breast epithelial cell cancer. However, it does not exhibit significant anticancer properties in highly invasive stages of breast cancer cells. [132, 133]. Treatment of highly metastatic human breast tumor cells with a combination of hydroxytyrosol and squalene inhibits cell proliferation, promotes apoptosis, and induces DNA damage in breast cancer cells [14]. Based on the studies mentioned above, there is a belief that a synergistic action may occur between hydroxytyrosol and other phenolic molecules found in olive oil or natural compounds like tyrosol, lycopene, oleuropein, and tea polyphenols. These interactions between various compounds could contribute to the observed therapeutic effects and health benefits associated with olive oil consumption [134]. Tyrosol is a common constituent of olive oil present as conjugated or free forms and showed several pharmacological functions. Furthermore, a synergistic interaction was observed when tyrosol and hydroxytyrosol were combined to inhibit tumor proliferation and reduce EGFR expression in HT-29 tumor cells. This suggests that the combined action of these compounds may have a more pronounced effect on cancer cell behavior than when used individually. This discovery highlights an intriguing mechanism of action for hydroxytyrosol and indicates that combining it with chemotherapy could be a promising approach for treating colon and rectal cancer [15]. Toteda and colleagues reported a synergistic effect of hydroxytyrosol in combination with doxorubicin for the treatment of leukemic K562 cells. The combination of hydroxytyrosol and doxorubicin results in a substantial reduction in cell viability, primarily through an increase in the apoptosis process and the induction of double-strand DNA breaks in K562 leukemia cells which suggests a potential synergistic effect of these compounds in combating leukemia [135]. Furthermore, a study has indicated that hydroxytyrosol demonstrates synergistic antioxidant activity when combined with other phenolic compounds like quercetin and resveratrol which suggests that the collective use of these antioxidants may enhance their effectiveness in combating oxidative stress and promoting overall health [136].

Role of nanotechnology

Nanotechnology holds significant promise in advancing cancer treatment, representing a cutting-edge frontier at the intersection of various disciplines. In vitro diagnosis and drug administration have recently attracted a lot of attention in the field of nanotechnology [137]. This technology is being created to win the battle against cancer. Drug resistance can now be reversed by active or passive mechanisms owing to studies focusing on nano-based medications [138]. Nanotechnology-based medications have effectively reduced side effects, enhanced treatment efficacy, and mitigated drug resistance. A wide range of nanoparticles (NPs) has been developed and extensively researched, including polymer-based nanoparticles, nanovesicles, and metal nanoparticles which exhibited potential in overcoming cancer's resistance to chemotherapy [139, 140]. Targeted therapy, photothermal therapy, nanomaterial-based chemotherapy, and sonodynamic therapy are now employed to treat cancer [141, 142]. Nanomedicine involves the use of nanoparticles and nanoscale materials for medical purposes, such as drug delivery, diagnostic imaging, and targeted therapy. When hydroxytyrosol is incorporated into nanomedicine formulations, it can enhance the therapeutic effects and improve the bioavailability of the compound.

Mechanism of nanoparticle in chemotherapy

It includes active as well as passive targeting. Nanoparticles achieve active targeting via ligand-receptor interactions, employing molecules such as siRNAs, proteins, vitamins, amino acids, monoclonal antibodies, and peptides on the surfaces of cancer cells. Nanoparticles' ligand-mediated targets in cancer cells contribute to their ability to discriminate between tumor cells and healthy ones [143]. As a result of this interaction, NPs can release the medicine at the target spot by receptor-mediated endocytosis. Passive targeting leverages the increased permeability and retention (EPR) effect, causing nanoparticles (NPs) to accumulate around cancer cells due to their restricted lymphatic circulation. This facilitates the delivery of medication to the intended site using nanocarriers. Since NPs are made of small particles, they are more permeable to cells than larger particles like conventional medications, which the immune system is likely to remove from the cell. A permeability advantage allows NPs to produce an EPR effect [144].

Many studies focus on the preparation and characterization of poly(lactide-co-glycolide) (PLGA) NPs loaded with hydroxytyrosol. Researchers investigated the stability and bioactivities of the hydroxytyrosol-loaded nanoparticles as follows.

Guan et al. [145] found that mPEG-PLGA co-loaded with syringopicroside and HT exhibited sustained release of drug, prominent liver distribution, and provided protection against hepatic injury in Sprague–Dawley rats. Jadid et al. [67] demonstrated that nano-encapsulated hydroxytyrosol (HT) and curcumin (Cur) within poly lactide-co-glycolide-co-polyacrylic acid (PLGA-co-PAA), individually and in combination (HT-Cur), exhibited substantial anti-proliferative, anti-migratory, and apoptosis-inducing effects on pancreatic cancer (PCNA-1) cells. These effects were achieved by modulating migration-related genes (MMP2 and MMP9) and apoptosis-related genes (BAX and Caspase-9).

Saini et al. [146] discovered that nano-capsulated hydroxytyrosol reduced colorectal cancer (HT-29) cell growth by modifying the expression of CDKN1A, CDKN1B, and CCND1 genes. Safi et al. [147] discovered that the employment of PLGA-co-PAA nano-encapsulated hydroxytyrosol in breast cancer (MCF-7) cells resulted in anti-proliferative effects. This was achieved by arresting the cell cycle through the upregulation of P21 and P27 expression, while concurrently downregulating Cyclin D1 expression. Zygouri et al. [148] utilized carbon nanotubes as biocompatible carriers for hydroxytyrosol. Their study revealed cytotoxic effects on NIH/3T3 and Tg/Tg cell lines, leading to cell cycle arrest and the generation of ROS.

Toxicological studies

To study the effect of hydroxytyrosol Fernández-Prior et al. [149] in THP-1 derived monocytes, treatment of different concentrations (10–100 ppm) were given. A very high cellular viability (100%) was recorded elucidating HT had no negative effects on the integrity of the cells in this cellular model. HT also showed high viability in living human cells without any inhibition effect. Hence, hydroxytyrosol (HT) has potential applications in the treatment of various diseases. Haloui et al. [12] reported no toxicity associated symptoms like convulsion, diarrhea, locomotor ataxia and mortality after doses (different concentrations) of hydroxytyrosol given to mice concluded that it does not cause any toxicological effect. D'Angelo et al. [47] found that orally administered hydroxytyrosol in rats did not result in significant adverse effects, after assessing its molecular pharmacokinetics and toxicity. While studying the anti-inflammatory effect of dietary hydroxytyrosol supplement, Voltes et al. [110] did not notice any symptoms of toxic effect of the hydroxytyrosol. Non-toxic and chemopreventive effects of hydroxytyrosol (HT) have been documented in various healthy and normal cell cultures [150]. There were not any signs of toxicity such as grip strength, locomotory activity, food consumption, or loss of body weight, observed in treated rats [151].

Limitations and challenges of hydroxytyrosol as an anticancer agent

Much of the evidence supporting the anticancer properties of hydroxytyrosol comes from in vitro and animal studies. While these findings are encouraging, the translation to clinical applications in humans which is not straightforward. More clinical trials are needed to establish the safety and efficacy of hydroxytyrosol in cancer prevention or treatment. Moreover, the variability in the composition of natural sources of hydroxytyrosol, such as olive oil, can make it challenging to achieve standardized doses. Standardization is crucial for ensuring consistent and reproducible results in both research and potential clinical applications. The potential synergistic or antagonistic effects of these compounds with hydroxytyrosol need further investigation. The complexity of natural products makes it challenging to isolate the specific contribution of hydroxytyrosol alone. The precise molecular mechanisms by which hydroxytyrosol exerts its anticancer effects are not fully understood. More research is needed to elucidate the signaling pathways and targets involved. Future studies and clinical trials will help address these issues and provide a clearer understanding of its potential therapeutic role.

Strengths and limitations of the study

Hydroxytyrosol as an anticancer agent exhibits several strengths in elucidating its potential therapeutic benefits. It effectively synthesizes existing research, highlighting hydroxytyrosol's promising anticancer properties, such as its antioxidant and anti-inflammatory effects, along with its ability to induce apoptosis and inhibit tumor cell proliferation. Moreover, the review likely discusses the mechanisms underlying hydroxytyrosol's action, shedding light on its molecular targets and signaling pathways involved in cancer inhibition. However, this review study might encounter limitations stemming from the heterogeneity of research methodologies, dosages, and cancer types studied, which could affect the generalizability of findings. Additionally, the scarcity of human clinical trials and long-term studies could impede the translation of hydroxytyrosol's potential into clinical practice, highlighting the need for further investigation to validate its efficacy and safety profile. Nonetheless, despite these challenges, the review provides valuable insights into hydroxytyrosol's anticancer properties, serving as a foundation for future research and clinical trials in this promising field.

Conclusions

The review underscores hydroxytyrosol as an exciting and innovative frontier in cancer research, as recent findings unveil its robust anticancer effects and elucidate the associated molecular mechanisms. Hydroxytyrosol has demonstrated its ability to impede cancer cell proliferation, trigger apoptosis, and suppress metastasis, rendering it a compelling candidate for cancer therapy. Furthermore, its antioxidant and anti-inflammatory properties have proven crucial in mitigating oxidative stress and fostering a less pro-tumorigenic tumor microenvironment. The review emphasizes the importance of recent discoveries in unveiling hydroxytyrosol's unique mechanisms of action, which are critical for its potential integration into personalized cancer treatment approaches. By targeting various cellular components crucial to cancer development, hydroxytyrosol shows potential in augmenting the effectiveness of current cancer treatments and overcoming drug resistance. Future research should focus on further elucidating the precise molecular interactions and signaling pathways involved in hydroxytyrosol's anticancer effects, opening up avenues for developing targeted therapies and innovative treatment combinations. Exploring its potential in different cancer types and addressing potential drug interactions are crucial considerations for its successful translation to clinical applications.

Abbreviations

%	Percentage
ATP	Adenosine 5'-triphosphate
BAX	Bcl-2-associated X protein
CCND1	Cyclin D1
CDKN1A	Cyclin-Dependent Kinase Inhibitor 1A
Cmax	Maximum concentration
Cox2	Cyclooxygenase 2
DNA	Deoxyribonucleic Acid
EC	Endothelial cell
EGCG	Epigallocatechin-3-gallate
HDF	Human dermal fibroblasts
HPLC-PAD-MS	High-Performance Liquid Chromatography with Photodiode Array Detection and Mass Spectrometry
HT	Hydroxytyrosol
Hecs	Human umbilical vein endothelial cells
IL	Interleukins
iNOs	Inducible nitric oxide synthase
HDF	Lipopolysaccharide
MG	*Mycoplasma gallisepticum*
MMP	Matrix metalloproteinase
NF-κB	Nuclear Factor-Kappa B
NPs	Nanoparticles
Pin1	Peptidyl-prolyl cis–trans isomerase NIMA-interacting 1
PLGA	Poly(lactide-co-glycolide)
PLGA-co-PAA	Polylactide-co-glycolide-co-polyacrylic acid
ppm	Part per million
ROS	Reactive oxygen species
THP-1	Tamm-Horsfall Protein 1

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