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Hydroxytyrosol: A comprehensive approach to Mitigate Maladies

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The olive oil has been found essential part of human daily diet with a healthy way of eating and living nowadays. Oilve tree is promising source of phytochemicals such as hydroxytyrosol, oleocanthal, tyrosol, oleuropein, and oleacein along with numerous health endorsing perspectives in an invitro and in vivo studies such as prevention from cancer insurgence, diabetes, obesity, oxidative stress, microbial contamination and cardiac complications, respectively. In this regard, hydroxytyrosol inhibited the cancer proliferation stages such as cell growth, invasion and proliferation. It also lowered the expressions of nuclear factor kappa B (NF-κB), Akt and signal transducer and transcript 3 activator (STAT3). It also reduces the oxidative, & nitrosative stress, and brain inflammatory mediators with decreasing the TBARS level, neutralizing the free radicals, decreasing the higher glucose level, and increasing the insulin sensitivity. The current review article highlights the absorption and metabolism of hydroxytyrosol, in addition pharmacological potential, further studies are still required.

Keywords: Olive oil, hydroxytyrosol, polyphenol, cancer, anti-diabetic

INTRODUCTION

Olive oil is the most important part of Mediterranean diet associated with multiple health benefits beneficial for cardiovascular disease, diabetes mellitus, cancer and metabolic syndromes (Di Daniele et al. 2017; Schwingshackl et al. 2017). Aging is mainly linked with epigenetic alterations, cellular senescence, stem cell exhaustion, genomic instability and mitochondrial dysfunction. Oleic acid major component of olive oil and its bioactivity modulate the gene expression, improves cellular aging via directly and indirectly mechanisms (López-Miranda et al. 2010). Scoiridoids have been showed in both in vitro and in vivo animal models as well as in human studies to entail the multiple pathways involved in aging (Fernández del Río and Villalba, 2016).

Absorption

Multiple studies in vivo and in vitro along with human trial have described the absorption, metabolism and distribution after ingestion of HT. It is absorbed in a dose-dependent manner via passive transport in the small intestine and colon with efficacy range (from 75%-100%) (Suárez et al. 2011; Visioli et al. 2003). Moreover, it has highest hepatic/intestinal metabolism in human and low bio-availability in the plasma. HT goes o phase-I metabolism after ingestion via primarily hydrolyzed in the enterocytes then with the help of phase-II it is subsequently metabolized in to glucuronide, methylated and sulphate (bio-

products) (Rubió et al. 2012; Suárez et al. 2011).After being metabolized 98% HT found in glucuronide form in the plasma and urine while merely 2% in free form (Miro-Casas et al. 2003). The probable half-life of HT is around 8-min in humans and 2 min in rats, moreover it was reported to be maximum around 7 minutes after injestion in rats (González-Santiago and Lopez-Huertas, 2010; Granados-Principal et al. 2014). Domínguez-Perles et al. in 2017 reported plasma concentration of HT between 30min-2 hafter oral ingestion being practically undetectable after 4 h in rats (Domínguez-Perles, Auñón, Ferreres, and Gil-Izquierdo, 2017). However, in human the maximum concentration is noticed after 13 min, decreasing till undetectable levels 1 h after administration showed that HT once absorbed, becomes instant part of plasma-lipoproteins (Fernández-Ávila et al. 2015). HT is also have the ability to instantly cross the blood brain barrier in spite of less plasma half-life in wister rats and distributed in skeletal muscles, heart, liver and testis, while its metabolism ends in the liver and in kidneys (Serra et al. 2012). In the kidney, HT is stored till it excrete in the conjugated catabolites form perform there nephron-protective role due to its antioxidant properties (Chashmi, Emadi, and Khastar, 2017). It takes maximum 6-hours in human and 4-hours in rats for the elimination from the body (Rodriguez-Morato et al. 2016; Tuck and Stupans, 2001).

HEALTH PERSPECTIVES

Anticancer

Terzuoli et al. 2016 found anti-oxidative effects of Htyr in a hypoxic environment with low O2 pressure and high ROS levels, common in many strong tumors shown to reduce epidermal growth factor receptor (EGFR) levels. on HT-29 xenografts and CRC cells (Terzuoli, Giachetti, Ziche, and Donnini, 2016). EGFR includes growth control, apoptosis, angiogenesis and invasion. EGFR activation is followed by rapid infiltration and degradation by both lysosomal and proteasomal pathways. HTyr improved the process of EGFR reduction within cancer cells without affecting its level in colon cells and reduced the activity of nuclear factor kappa B (NF-κB), Akt and signal transducer and transcript 3 activator (STAT3) to show prostate cancer (PC) cell lines used in the proliferation, invasion and acquisition of cancer cells, and advances in aggressive PC anti-removal. Zhao and colleagues also looked at Htyr's anti-carcinogenic role in the Subdual of pathways (Akt and NF-κ) of human hepatocellular carcinoma (HCC), in vitro and in vivo (Zhao et al. 2014).

HTyr is very important in improving the carcinogenic effect of Transcript factor NF-κB involved in proliferation, apoptosis, invasion, angiogenesis and metastasis. The anti-cancer action of HTyr was studied by xenografts mice in a group of cancers called cholangiocarcinomas (CCA) in the form of bile ductsas causing apoptosis(H. Li, Lei, Wang, Skog, and He, 2005). This effect is due to the inhibition of extracellular signal-regulated mkinase (ERK), another signature mechanism that is critical for cancer growth and development. The anticancer effects of HTyr were also investigated in BC(Sirianni et al. 2010), human papillary, pancreatic cancer, glioma and follicular thyroid cancer (Chimento et al. 2014). It also was able to bind and activate G-protein-coupled receptor (GPER), and cause stabilization ERK 1/2 activation leading to an apoptotic effect in ER-negative BC cells (Chimento et al. 2014).

Proapoptotic Bax protein-up-regulation and decreased anti-apoptotic Bcl-expression, as well as the activation of cytosolic cytochrome c secreted by GPER, revealed a mitochondrial apoptotic process, performed by hydroxytyrosol. In addition, caspase-9, caspase-3 cleavage and peroxisome proliferator-activated receptor 1 (PARP-1) inactivation support the proposed mitochondrial apoptotic pathway. Similar studies have shown higher regulation of p21 and p53 proteins, poor cell cycle regulation, and decreased expression of cyclin D1 were reported (Elamin et al. 2013; Liu et al. 2019). In prostate cancer cells lines, hydroxytyrosol is significantly suppressed the proliferation, invasion and metastasis stages, signal transducer, Akt activation, inhibited activator of transcription 3 (STAT3) signaling pathways along with suppression of nuclear factor kappa B (NF-κB) (Zubair et al. 2017). In mouse xenografts, it also prevents from tumor development, and exhibits inhibition of extracellular signal-regated (ERK) kinase (Sirianni et al. 2010;Chimento et al. 2014) against different cancer cells lines such as human papillary, pancreatic cancer (Goldsmith et al. 2018), and follicular thyroid cancer and glioma (Ramírez-Expósito and Martínez-Martos, 2018; Toteda et al. 2017).

Table 1: Anticancer perspectives of hydroxytyrosol against different cancer cells lines

References	Cancer types	Cell lines types	Mechanisms
(Han, Talorete, Yamada, and Isoda, 2009)	Breast	SKBR3 and T-47D	Delay cell cycle in G2/M phase
(Bouallagui, Han, Isoda, and Sayadi, 2011)		MCF-7	G0/G1 cell cycle arrest
(Granados-Principal et al., 2014)			Inhibition of estradiol induced ERK1/2 phosphorylation
(El-Azem et al., 2019)		MB231	Inhibits CCL5 accumulation and consequently the increase in the ERK1/2-cyclin D1 pro-proliferative pathway
(Roberto Fabiani et al., 2008; Imran et al., 2018)	Leukemia	HL60	Cell cycle arrest (G0/G1 or G2/M)
(Aydar et al., 2017; Bernini et al., 2011; Della Ragione et al., 2000; Han et al., 2009)			Inhibition on DNA synthesis Antiproliferative and pro-apoptotic Induction of differentiation
(Lewandowska, Gorlach, Owczarek, Hrabec, and Szewczyk, 2014; S. Li et al., 2014; Vilaplana-Pérez, Auñón, García-Flores, and Gil-Izquierdo, 2014)	Cholangio-carcinoma	TFK-1 KMBC GBS-SD	G2/M phase cell cycle arrest Inhibition of phospho-ERK
(Corona et al., 2009; RDBA Fabiani et al., 2002; Sun et al., 2014; Terzuoli et al., 2016)	Colon	SW620 (90)	S phase cell cycle arrest. Inhibition of FAS expression
(RDBA Fabiani et al., 2002)		HT29 Growth arrest. Stress of the endoplasmic reticulum. Inhibits NF-kB Antiproliferativeand pro-apoptotic (86) G2/M phasecyclearrest	G2/M phase cell cycle arrest
(Fusco et al., 2020)		Caco-2 Inhibitionofp38/CREB phosphorylationandreduction COX-2expression Antiproliferative (87) Inhibition ofERK1/2 phophorilationandcyclin D1levelsAntiproliferative (88) Up-regulating p21andCCNG2and down-regulating CCNB1 proteinexpression	
(Fusco et al., 2020)		HT115Inhibitsinvasionof cancercoloncells Anti-invasive (91)	

Anti-diabetic

A study conducted by Reyes and their co-workers, they investigated that different concentrations of hydroxytyrosol (1, 5 or 10 mg/kg/day) treated with diabetic subjects exhibited neuro protective effect on brain damage via lowering the oxidative, and nitrosative stress, and brain inflammatory mediators. In another study reported by Ristagno and their colleagues, they explored the anti-diabetic role of hydroxytyrosol through lowering the TBARS level, neutralizing the free radicals, decreasing the higher glucose level, increasing the insulin sensitivity, enhancing the thermal nociception impairment and nerve conduction velocity, and abolishing the reduction sciatic nerve Na(+), K(+)-ATPase activity (Rosignoli and Morozzi, 2013). In the Caco-2 cells, inhibition of α-amylase and α-glucosidase, and reduction of viability were reported after administrated hydroxytyrosol (600 μM). Hydrogen peroxide has been found to lower the glucose utilization, phosphorylation of mitogen-activated protein kinases, insulin sensitivity via insulin-dependent and insulin independent in C2C12 cells whereas hydroxytyrosol reverted these changes. Different authors and researchers also explored the preventive role of hydroxytyrosol against diabetes volunteers through multiple processes such as increasing the insulin secretion, providing protection against destruction of pancreatic β-cells, inhibiting the ATP-sensitive potassium K(ATP) channels, enhancing the voltage-dependent calcium channel, and peripheral uptake of glucose, respectively (Hassan et al. 2012; Mao et al. 2012; Shamshoum and Tsiani, 2017). Being strong potent antioxidant agent, hydroxytyrosol has protective role against damage of pancreatic β-cells via reducing the glucose level, increasing the concentrations of hexokinase and pyruvate kinase via phosphorylation of glucose and insulin secretion. Moreover, enzymes concentrations are lowered which are used to catalyze the dephosphorylation of glucose-6-phosphate to glucose-6-phosphatase and fructose-1, 6-bisphosphatase (Kimura and Sumiyoshi, 2009). A study conducted by Cao and their colleagues, they found that hydroxytyrosol has protective role against high-fat-diet (HFD)-induced obesity C57BL/6J mice via multiple mechanisms such as enhancing the antioxidant enzymes (glutathione, superoxide dismutase), ameliorating HFD-induced oxidative stress by normalize expression of mitochondrial fission marker Drp1and mitochondrial complex subunits, and inhibiting the SREBP-1c/FAS pathway. Moreover, it also caused reduction in carbonyl protein, fasting glucose, serum lipids, and mitochondrial complexes (Cao et al. 2014). Similarly, administrated hydroxytyrosol at the rate of 100 and 150 μM has been found to suppress the lipid deposition and glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity dose-dependently (Casaburi et al. 2013).

Cardioprotective

Production of free radicals in type 2 diabetic patients caused dysfunction in Ca^{2+} homeostasis of platelet aggregation whereas hydroxytyrosol exhibited reduction in size of atherosclerotic lesions and improved their anti-oxidant status (Noce et al. 2021). Different groups of researchers and investigators, they found that hydroxytyrosol has been found to lower the lipopolysaccharide (LPS)-stimulated expression of monocyte cell adhesion to endothelial cells, vascular adhesion molecule-1 (VCAM-1) along with provided protection against vascular damages, and platelet aggregation (Bertelli et al. 2020). Abe and their colleagues, they found that hydroxytyrosol suppressed the cyclooxygenase (COX)-2, enhanced the vascular nitric oxide production, inhibited the vascular smooth muscle cell proliferation and decreased the thromboxane A2 blood levels (Noce et al. 2021). Administration of hydroxytyrosol (20mg/kg/d for 8 weeks) exhibited improvement in impaired glucose and insulin tolerance, showed reduction in systolic blood pressure, adiposity, resultant diastolic stiffness, left ventricular fibrosis, and liver damage markers in a diet-induced rat model of metabolic syndrome (Poudyal et al. 2017). Moreover, reduction in biomarkers of oxidative stress are linked with reduction in infiltration of monocytes/macrophages into the heart after hydroxytyrosol treatment (Vlavcheski and Tsiani, 2019).

Oxidative Stress

Hydroxytyrosol being phytochemical prevents from oxidative stress markers including cholesterol-conjugated dienes, hydroxy-fatty acids, and DNA oxidative damage in healthy male subjects (Granados-Principal et al. 2014; Visioli et al. 2000; Zhang, and Zhong, 2009). In erythrocytes cells of human, it also suppresses the free radicals, mediates cytotoxicity and also suppresses the passive smoking-induced oxidative damage (El-Azem et al. 2019) (Soni, Prakash, Dabur, and Kumar, 2018). oxidized glutathione, lipid peroxides, glutathione

peroxidase, F2-isoprostanes, and reduced glutathione F2-isoprostanes are used to determine oxidative stress and Hydroxy-tyrosol decrease the excretion via urine of F2-isoprostanes (Crupi et al. 2020) (Manna, Galletti, Cucciolla, Montedoro, and Zappia, 1999). Multiple researchers found that hydroxytyrosol improved level of oxidative stress caused by NO-mediated relaxation and was shown to protect the aorta. It also exerts suppressive action on vascular endothelial cells adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) (Wang et al. 2019). Baryam and their fellows described the antioxidative role of hydroxytyrosol in the heart of SAMP8 mice through modulating Nrf2-dependent gene expression, decreasing the TBARS and protein carbonyls levels, increasing the paraoxonase-2 PON activity and inducing the Nrf2-dependent gene expression (Chang, Alasalvar, Bolling, and Shahidi, 2016).

Anti-microbial

Multiple human and animal maladies are produced by protozoan parasites whereas hydroxytyrol bioactive compound from olive fruit has potent antimicrobial activity against gram positive and gram negative bacteria's (Bisignano et al. 1999; Ghalandari et al. 2018; Medina-Martínez, Truchado, Castro-Ibáñez, and Allende, 2016). In another study conducted by Bedoya and their workers, they investigated that hydroxytyrosol significantly suppressed the HIV-1 infections of recombinant-type viruses with 50% inhibitory (Zoric et al. 2013). It also has been found to inhibit the herpes simplex virus type 1 (HSV-1), Coxsackievirus type B3 (Cox B3) in a model of HSV-1 infection (Botta et al. 2015). In another recent study investigated by the Martínez and their colleagues, they found that hydroxytyrosol exhibited the potent antimicrobial activity against *Escherichia Coli, Lysteria monocytogenes,* and *Staphilococcus Aureus* strains via delaying the lipid oxidation (Aydar, and Üçok, 2017). Multiple studies conducted by different researchers and scientists (Crisante et al. 2015; Robles-Almazan et al. 2018), hydroxytyrosol exhibited antifungal activity against Verticillium dahliae, Fusarium sambucinum, and Alternaria solani (Pereira-Caro et al. 2009).

Anti-inflammatory role

Hydroxytyrosol (12.5 to 50μM), significantly reduced the expression of chemokines (CCL5 / RANTES, and CCL4 / MIP1β), Cyclooxygenase-2, NOS, prostaglandin-endoperoxide synthase 2 (PTGS2), Inter 1α (IL-1) and the genes of Matrix metallopeptidase-9 (MMP-9) in the cell line of RAW264.7 macrophage, previously stimulated by the proxyfigueatory molecule LPS (lipopolysaccharides). The high concentration of Hydroxytyrosol 50 μM was effective as an anti-inflammatory compound with Nuclear Factor-B (NF-κB) inhibition(Richard et al. 2011). In human THP-1 monocytic cell lines, the mechanism of action of Hydroxytyrosol molecules has been shown to be associated with a significant decrease in protein kinase C (PKC) - β1 and PKCcy membrane translocation, which inhibits the production of proinfigueatory cytokine and activation (Kiani et al. 2020). Furthermore, in the human PMA-activated monocyte U937, 1-10μmol / L of Hydroxytyrosol was able to reduce the expression and activity of MMP-9 and COX-2 by inhibiting the nuclear transmission of NF-κβ and PKCcy and PKCβ1 activation (Fuccelli, Fabiani, and Rosignoli, 2018). Hydroxytyrosol can also suppress NO mechanical production on the NF-pathway. Rosignoli et al. performed in vitro tests on individual monocytes (Scoditti et al. 2019). Their data showed that hydroxytyrosol (100μM) significantly inhibited the production of superoxide anions (O-2), and reduced COX-2 exposure and PGE2 release (Rosignoli et al. 2013). In addition, in J774 murine macrophages, hydroxytyrosol also regulates proinfigueatory molecules, iNOS and COX-2, antagonizing NF-κB, STAT-1α and IRF- 1, mediated by ROS generation produced by LPS (Bertelli et al. 2020; Richard et al. 2011; Fuccelli et al. 2018).

Anti-thrombotic Role

Platelets aggregation is the basic reason of arterial and venous thrombosis and hydro-oxytyrosol has been showed by many researches to improve the CVD. A dose-dependent methody of Three compounds (48.25 mg/kg per day for Hydroxytyrosol, 16.05 mg/kg per day for Hydroxytyrosol -acetate, compared to 2.42 mg/kg per day of acetylsalicylic acid) inhibited collagen-induced platelet aggregation in whole blood and in a dose-dependent way, in Wistar rats (D'Angelo, Franceschelli, Quiles, and Speranza, 2020).

Ruano and coworkers stated that oxidative stress and endothelial-dependent vasodilatation favors thrombogenic state. Olive oil was given during breakfast meal in different proportion in the 21 hypercholesterolemic middle-aged volunteers at with natural phenolic compounds, 40 ppm or 80 ppm. Olive oil significantly improved

microvascular vasodilatation and NO levels 2 h after the breakfast intake (Granados-Principal, Quiles, Ramirez-Tortosa, Sanchez-Rovira, and Ramirez-Tortosa, 2010). Meal high in phenolic content has been showed to decrease levels of plasmatic plasminogen activator inhibitor-1 (PAI-1) and activated factor VII (FVIIa) made it less less thrombogenic (Schaffer et al. 2007).

Anti-Aging

Hydroxytyrolsol (10 μM, 180 minutes) treated with rat pheochromocytoma PC12 cells lowered the autotoxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde, and 5-S-cysteinyl-dopamine (Cys-DA) levels. It also showed suppression on enzymatic and spontaneous oxidation of endogenous dopamine (Jeon and Choi, 2018). Likewise, multiple mechanisms are involved in neurodegenerative diseases by administrating the hydroxytyrosol such as accumulated α-synuclein decreased in muscle cells, enhancement in locomotion, and prevention from neurodegeneration, respectively (de Pablos, and Arguelles, 2019). It also has been found to inhibit MAPKs, NF-κB, inflammasome, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Fernández-Mar, Mateos, Garcia-Parrilla, Puertas, and Cantos-Villar, 2012). In recent study investigated by Calahorra and their co-workers (2019), hydroxytyrosol has been shown to improve short recognition memory, functional connectivity, enhance strength in the forepaws, cerebral blood flow, expression of brain derived neurotrophic factor, as well as also lower the ionized calcium-binding adapter molecule 1 (IBA-1) level and transcription of the postsynaptic marker postsynaptic density protein 95 in experimental animals (Mukherjee, and Das, 2009).

Rheumatoid arthritis (RA) is an autoimmune disease leads to the production of inflammatory cytokines generates neutrophils, macro-phages along with increase in production of free radical and synovial fluid enzymes (Stone, and Darlington, 2000; Kohyama, and Sekiya, 1997; Kremastinos, 2008). Olive oil also has the ability to prevent ROS mediated cell injury via producing antioxidant enzymes along with inhibition of AP-1 and LPS-triggered NF-kB in in human umbilical vein endothelial cells (Serreli and Deiana, 2020). Olive oil polyphenols improved deranged level of cholesterol, phosphatidylcholine and cerebroside levels in the blood and brain (Rabiei, and Mirzajani, 2012). They inhibit free radical formation along with the alkyl and peroxyl radicals' formation and decrease activation of TNF-α to stop the brain damaging caused by LPO (Benavente-Garcıa, Castillo, Lorente, Ortuño, and Del Rio, 2000). Oleuropein may protect the dopaminergic neuron loss in midbrain of old people(Rehman and Masson, 2001). On the other side, hydroxytyrosol prevented from the cell death through enhancing glutathione contents and suppressing the NF-κB subunits(St-Laurent-Thibault, , and Ramassamy, 2011).

CONCLUSION

The studies and evidences in this review describe the significance of major olive oil phenolic acid such as hydroxytyrosol. In last twenty years,potential benefits of hydroxytyrosol has been studied by different researchers and investigators due to its nutraceutical role. Furthermore, encapsulated hydroxyrosol also opened the new horizons in the field of medicine to treat human disoders.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: IA, MI. SN, UF, MR, SJ, MA, MT, MNN and SS analyzed the data: IA and MI contributed reagents/ materials/ analysis tools: IA and MI wrote the paper:

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