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Effects of High Potency Hydroxytyrosol from Natural Sources in Cardiovascular Disease and its Risk Factors: A Mini Review

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The fruit and leaves of the olive tree (Olea europaea L.), which produces olive oil, contain hydroxytyrosol, one of the major phenolic compounds. It can also be extracted from other natural sources like fermented grape. In this mini review, relevant studies were retrieved from PubMed, Elsevier, Wiley, Hindawi and Taylor & Francis for articles published since journal inception up to June 2013. Search terms related to hydroxytyrosol and how its effects to cardiovascular disease and its risk factor (e.g. diabetes, hypertension, hypercholesterolemia) were used. Preclinical investigations conducted over the past many decades have proved its health advantages and its ability to protect against a number of diseases. In this study, we highlight various research findings that demonstrate significant correlations between cardiovascular diseases, their risk factors, and the bioavailability of the substance in both animals and humans. The mechanisms of action include potent anti-oxidant and anti-inflammatory effects. European Food Safety Authority has emphasized the importance of hydroxytyrosol in reducing low-density lipoproteins, thus reducing the risk of cardiovascular diseases. They suggest a daily consumption of 5 mg of hydroxytyrosol and its derivatives to achieve this effect at a physiological level. Additionally, potential applications of this compound in supplements, nutraceutical foods, and topical formulations for disease risk reduction were explored. In conclusion, the potency amount is crucial to highlight and improve in next research explored in order to get the benefits especially for cardiovascular disease and its risk factor

Keywords: Review; hydroxytyrosol; cardiovascular; risk factor; potency; clinical

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Hydroxytyrosol (HT) is commonly found in both fruits and vegetables and is also produced within our bodies through the breakdown of dopamine [1]. Nonetheless, the primary origins of HT are traced back to the consumable components of the olive tree (Olea europaea), including its fruits, leaves, and extra virgin oil [2]. This compound is the primary phenolic compound found in virgin olive oil (VOO), existing in both free and complex forms commonly referred to as secoiridoids (SEC) or oleuropein aglycone derivatives. Hydroxytyrosol is also known by its IUPAC name, 4-(2-Hydroxyethyl)-1,2-benzenediol. This molecule exhibits antioxidant properties, as highlighted by Stefanon and Colitti in 2016 [3]. It remains stable in its free form and has the ability to easily permeate tissues. Its chemical formula is C8H10O3 and it closely resembles tyrosol, differing only in the addition of an extra hydroxyl group in the meta-position within the aromatic ring.

In spite of its classification as a phenolic compound, HT is found in a limited range of food sources. HT has been identified primarily in foods that are emblematic of the Mediterranean diet, notably including table olives, olive oil, and wine. Moreover, HT is produced by yeast through the process of alcoholic fermentation, subsequently becoming a constituent of wine. The spectrum of phenolic compounds found in olive fruits is expansive, encompassing as many as 36 varieties, with secoiridoids being the most widespread and plentiful among them. Notable among these are oleuropein and ligstroside, which stand as the foremost native compounds [4]. Through the breakdown of these compounds, various phenolic constituents emerge within the olive fruit, including HT, oleocanthal, elenolic acid, oleuropein aglycone, and tyrosol [4]. As the olive progresses through stages of ripening, storage, and processing, the hydrolysis of secoiridoid compounds gives rise to HT [5]. Generally, the reduction of oleuropein coincides with an escalation in HT levels during maturation, resulting in HT becoming the predominant compound in mature olives. HT has demonstrated a broad spectrum of biological functions, including antioxidant, anticancer, and neuroprotective activities, as well as positive effects on the cardiovascular system. Studies have indicated that this compound can beneficially influence lipid composition, platelet and cellular function, and reduce

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inflammation [6]. These effects have been associated with the lower incidence of cardiovascular disease (CVD) mortality observed in certain populations residing in Mediterranean countries.

The main objective of this mini review is to study the impacts of HT on cardiovascular disease and its risk factors. All the content compiled of this document is founded upon information sourced from primary documents and comprehensive evaluations. Our focus has been on hand-picking the most pertinent investigations pertaining to the pharmacological aspects of HT. A systematic electronic exploration was carried out in the PubMed, Elsevier, Hindawi, Wiley, and Taylor & Francis database using the search terms: hydroxytyrosol [text word] AND ("beneficial effects" [text word] OR "beneficial effect" [text word] OR "cardiovascular disease risk factor" [text word]). Only articles published in English up until 2013 that comprehensively delineated the attributes of HT and its impacts on both *in vitro* and *in vivo* models were covered.

Characteristic of Hydroxytyrosol and Its Safety

Hydroxytyrosol has get interest within the pharmaceutical sector due to its anti-inflammatory and antimicrobial properties. Its pivotal roles in combating cardiovascular diseases and metabolic syndrome, as well as its contributions to neuroprotection, anti-tumor effects, and modulation of chemotherapy, render it of paramount interest. This compound exhibits rapid absorption, reaching its peak plasma concentration approximately 7 minutes after ingestion [4]. Following absorption, hydroxytyrosol promptly incorporates itself into high-density lipoproteins within the bloodstream, operating both as an antioxidant and a guardian of cardiovascular health [7]. The remarkable bioactivity of hydroxytyrosol stems from its potent antioxidant capacity, which safeguards cellular integrity and arises from its structural compatibility with certain compounds [8]. Notably, hydroxytyrosol is amphipathic, exhibiting both water and fat solubility due to its lipophilic and hydrophilic ends. This unique characteristic endows it with exceptional transport capabilities throughout the human body, enabling it to traverse cellular membranes with greater ease.

Furthermore, hydroxytyrosol's amphipathic nature grants it a multifaceted utility within the physiological environment. Its water-soluble end enables interactions with hydrophilic cellular components [10]. This solubility is essential for its engagement with aqueous compartments of cells and tissues, contributing to its protective effects against oxidative stress and cellular damage. Conversely, its lipid-soluble end facilitates incorporation into lipid-rich structures such as cell membranes and lipoproteins. This property enables hydroxytyrosol to effectively traverse lipid barriers, enhancing its accessibility to intracellular compartments and lipid-based cellular structures. Its presence within cell membranes could potentially modulate membrane fluidity and integrity, influencing cellular signalling and function.

Hydroxytyrosol's unique structure and dual solubility make it an effective carrier of bioactive substances throughout the body. This attribute not only enhances its antioxidative and cardioprotective functions but also suggests its potential as a vehicle for delivering therapeutic compounds to specific cellular targets. Such versatility in traversing physiological barriers underscores the compound's promise for pharmaceutical applications, potentially aiding in the development of novel therapeutic strategies for various ailments.

At typical dietary levels, hydroxytyrosol is not associated with any significant toxicity or adverse effects. It's been consumed for centuries as part of the Mediterranean diet, which is considered to be one of the healthiest dietary patterns. Many of the potential health benefits associated with this diet are attributed to the presence of compounds like hydroxytyrosol. The main source of HT, extra virgin olive oil is generally recognized as safe (GRAS) by the US Food and Drug Administration and is marketed both as a food and natural medicine. To date, no toxicity studies have been carried out to stablish if HT could exert any toxicological effect at this level, Christian et al [11] employed a higher dose in rats (raising 5 g/kg) and they established a LD50 of around 3.5 g/kg of HT, no toxicity, no abnormalities behaviour after high dosage of application, the cell become healthy especially immune cells.

Potency of Hydroxytyrosol Towards Cardiovascular Disease Risk Factor

Cardiovascular disease (CVD) is a group of conditions that affect the heart and blood vessels, and it is a leading cause of morbidity and mortality worldwide. Several risk factors contribute to the development and progression of cardiovascular disease, and three significant risk factors are diabetes, hypercholesterolemia, and hypertension (high blood pressure) [10]. There are several important areas where a decrease in medication consumption has been proposed to avoid adverse effects, poor response and emerging resistance among patients. This indicates a need to encourage novel non-pharmacological treatments as a part of a healthy lifestyle.

In addition, the response of food industry was strongly engaged by developing functional foods, specifically phenolic compounds that could have an impact over the cardiovascular system. A high-nutrient diet can play a crucial role in managing cardiovascular disease (CVD) risk factors. By making thoughtful food choices, you can help control factors such as blood pressure, cholesterol levels, and blood sugar, all of which contribute to your heart health. Including foods rich in phenolic compounds in your diet can

contribute to managing cardiovascular disease (CVD) risk factors. HT acts as protector of the cardiovascular system, avoiding oxidation of LDL cholesterol by free radicals, maintaining normal blood HDL cholesterol concentrations and preventing atherosclerosis [12]. HT consumption influenced the major biochemical processes leading to diabetic vasculopathy and reduced cell proliferation in the vascular wall. This could influence the prevention of diseases such as cancer, diabetes, inflammation or cardiovascular and neuro-degenerative diseases, which aetiology and progression has been linked to the production of ROS on damaged tissues [12].

Cardiovascular Disease and its Risk Factor

Utilizing proteomics in cardiovascular tissues, such as the aorta or heart, holds great promise in unraveling the mechanisms of action of phenolic compounds in healthy animals. A study conducted by Catalán et al. 2016 [13] revealed that hydroxytyrosol (HT) in its complex form (SEC) exhibited higher fold change values, mainly attributed to the higher concentration of HT detected in heart tissue. For the study, twelve female Wistar rats were divided into three groups: one group received a standard diet, while the other two groups were supplemented with phenolic compounds (HT and SEC) at a dosage of 5mg/kg/day for a period of 21 days.

Proteomic analyses of the aorta and heart tissues were carried out using nano-LC and MS. The results showed that HT and SEC caused significant alterations in the proteome of both the aorta and heart compared to the standard diet. Notably, the most prominent interaction networks identified were related to the Cardiovascular System. Both HT and SEC led to the downregulation of proteins associated with endothelial cell proliferation and migration, as well as blood vessel occlusion in the aorta. Additionally, proteins related to heart failure in heart tissue were also downregulated by HT and SEC supplementation. Besides, there are the other efficient way to practice as a precaution before cardiovascular disease existed where the management of three main risk factor named as diabetes, hypercholesterolemia and hyper-tension using high potency active ingredient, hydro-xytyrosol [13].

Diabetes

Hydroxytyrosol acts as a potent antioxidant, effectively counteracting harmful free radicals within the body. This is especially relevant to diabetes, as oxidative stress can accelerate cell damage and contribute to complications. By mitigating oxidative stress, hydro-xytyrosol may play a protective role. Furthermore, emerging evidence suggests that hydroxytyrosol could enhance insulin sensitivity. Enhanced insulin sensitivity translates to improved cellular response to insulin, the hormone that regulates blood sugar levels. This potential effect could aid in managing fluctuations in blood sugar levels. Beyond this, hydroxytyrosol exhibits anti-inflammatory properties. Because chronic inflammation worsens insulin resistance and diabetes progression, the anti-inflammatory properties of hydroxytyrosol may help improve insulin sensitivity and overall health [14].

We also found elevated interleukin-6 levels (a pro-inflammatory cytokine) with olive leave extract, OLE supplementation. Interleukin-6 functions differently depending on its concentration and the tissue it acts upon is shown in Table 1. Rapid spikes boost insulin-regulated glucose metabolism in muscle [15], while chronically mildly elevated levels are associated with a pro-inflammatory insulin resistant state in the liver. Thus, OLE supplementation may improve insulin sensitivity and glucose uptake via interleukin-6, and possible mechanisms for this effect have been proposed.

Table 1. Summary of hydroxytyrosol action on diabetes (in-vivo and human).

	Dosage of HT	Result	Mechanism	Reference
In-vivo	HT was given once per day for 7 days before diabetes' induction. Then HT was given daily during 2 months to diabetes rats	MPOx, VCAM-1 and IL-6 reduced.	HT treatment reduced platelet activity, thromboxane B2 and ox-LDL levels.	[16]
In-vivo	Diabetes rat treated with 0.5 mg/kg/day HT+ DHPG	Reduction of oxidative and nitrosative stress, platelet aggregation, production of	The expression of the proteins that regulate the platelet fibrinogen receptor	[17]

		prostacyclin, myeloperoxidase, and VCAM-1		
In-vivo	HT administration (50 mg/kg/day) for 17 weeks	HT had beneficial effects on glucose in db/db mice, and it seems that HT was more effective than metformi	Reducing oxidative damage and improving mitochondrial activity might be primary contributors to regulating glucose and lipid metabolism.	[18]
In-vivo	HT group, 77 mg/kg HT for 4 weeks	Lowered blood glucose in DM mice	Regulate eNOS phosphorylation and NO production	[19]
In-vivo	HFD+HT (10 mg/kg/day by gavage) for 6 weeks	Improved glucose tolerance and insulin sensitivity	Strong effect of HT on phosphorylation of AMPK and ACC was consistent with the decrease in FFA and the increase in CTP1 and PPAR as indexes of increased fatty acid oxidation	[20]
In-vivo	20 mg/kg BW for 2 months	Decreases the glucose level in plasma by 55% compared to untreated diabetic rats, helpful in the prevention of diabetic complications associated with oxidative stress.	Protection of pancreatic cells from progressive damage enhanced by alloxan and/or the enhancement of the regeneration of these cells similar to other substances such as oxovanadium	[21]
Human	9.7 mg hydroxytyrosol for 12 weeks	15% improvement in insulin sensitivity compared to placebo	Elevated interleukin-6 levels (a pro-inflammatory cytokine) with OLE supplementation which improve the insulin-regulated glucose metabolism in the muscle	[15]

db:diabetes

Hypertension

Hydroxytyrosol has strong antioxidants that help combat oxidative stress. This stress contributes to the deterioration of blood vessels and worsen inflammation, both of which are intricately linked to the development of hypertension. By diminishing oxidative stress, hydroxytyrosol may contribute to the preservation of cardio-vascular health. Additionally, emerging evidence points to hydroxytyrosol's potential to induce vasodilation, a physiological process that widens blood vessels. Enhanced vasodilation holds the promise of improving blood circulation and subsequently reducing blood pressure, a particularly pertinent benefit for individuals grappling with hypertension. Another noteworthy aspect is hydroxytyrosol's suspected role in promoting the production of nitric oxide,NO a molecule that aids in blood vessel relaxation. The augmentation of nitric oxide production potentially improved blood vessel function, subsequently contributing to blood pressure reduction.

Furthermore, the regulation of blood pressure could be influenced by hydroxytyrosol's ability to bolster blood vessel health and function. This includes preventing excessive constriction of blood vessels, a facet pivotal to maintaining optimal blood pressure levels. Endothelial health, another key component, might be positively influenced by hydroxytyrosol. Endothelium, the inner lining of blood vessels, plays a crucial role in preserving vessel flexibility and, by extension, blood pressure equilibrium. There is study shown the magnitude of BP changes observed here (SBP by 3.33 and 3.95 mmHg and DBP by 2.42 and 3.00 mmHg (24 h and daytime values, respectively)

Table 2. Summary of hydroxytyrosol action on hypertension (in-vivo and human).

	Dosage of HT	Result	Mechanism	Reference
Human	9.9 mg of HT for 20 weeks	The systolic prehypertension and hypertension subgroups presented significant differences in systolic blood pressure compared to the placebo (-15.75 ± 9.9 vs. -2.67 ± 12.0 mmHg, $p < 0.05$).		[23]
Human	OLE (136 mg oleuropein; 6 mg hydroxytyrosol) for 6 weeks	The magnitude of BP changes observed here (SBP by 3.33 and 3.95 mmHg and DBP by 2.42 and 3.00 mmHg (24 h and daytime values, respectively))		[22]
In vivo	OLE consisting of an optimized mixture of compounds derived from olive leaf, hydroxytyrosol content (1% w/w) for 5 weeks	Progressive reduction in both systolic blood pressure (SBP) (-21.6 ± 5.5 mmHg)	Key mechanism of endothelial dysfunction in hypertension involves the vascular production of ROS, particularly superoxide (O2 .-), which reacts rapidly with NO and inactivates it	[24]

can be considered physiologically significant, as shown in Table 2 [22]. Data from observational studies suggest that 2 mm Hg reductions in SBP and DBP are associated with 6% and 7 % reductions in CHD risk and 10% and 15 % reductions in stroke and heart attack respectively. Extrapolating from this would suggest that regular OLE intake may be associated with a 9–14 % reduction in CHD risk and a 20–22.5 % reduction in risk of stroke and heart attack.

Hypercholesterolemia

Oxidative stress is closely linked to the development of high cholesterol, fostering inflammation and harming blood vessels. By counteracting oxidative stress, hydroxytyrosol might contribute to the preservation of cardiovascular health. Moreover, preliminary studies hint at hydroxytyrosol's potential to influence cholesterol metabolism. It may hold the capacity to lower LDL (low-density lipoprotein) cholesterol—commonly referred to as "bad" cholesterol—while elevating HDL (high-density lipoprotein) cholesterol, often dubbed "good" cholesterol. Chronic inflammation is a hallmark of high cholesterol and plays a pivotal role in advancing cardiovascular diseases. Hydroxytyrosol's anti-inflammatory traits could potentially mitigate inflammation, thus positively affecting cholesterol metabolism [25].

The health of the endothelium, the inner lining of blood vessels, is another aspect to consider. A well-functioning endothelium is crucial for effective cholesterol transport and the maintenance of healthy blood vessels. Given that high cholesterol often coincides with elevated blood pressure, the potential of hydroxytyrosol to bolster blood vessel health and function could aid in blood pressure regulation, addressing a multifaceted aspect of cardiovascular health. By potentially promoting a healthier lipid profile, hydroxytyrosol might contribute to reducing the risk of heart disease linked to high cholesterol. Additionally, its influence on blood vessel health might thwart the onset of atherosclerosis—a condition characterized by artery plaque buildup—commonly associated with high cholesterol [25].

Several studies (Table 3) showed significant reduction in total and LDL cholesterol was observed, especially in participants with hypercholesterolemia. A cholesterol-lowering effect of HT has been previously reported in the literature, particularly in animal models. In humans, the evidence is limited and controversial, with some studies showing positive effects on lipid status and others showing no effect. Possible mechanisms underlying the lipid-lowering effect of HT are the inhibitory effects on the synthesis of fatty acids, cholesterol, and triglyceride in the liver.

Table 3. Summary of hydroxytyrosol action on hypercholestrolemia (in-vivo and human).

Sources	Dosage of HT	Result	Mechanism	Reference
In-vivo	During 8 weeks: 0.04% HT to rats	LDL-c levels were reduced in cholesterol group.	Serum antioxidant activity were improved in HT and HT-Ac group in relation with cholesterol group	[25]
In-vivo	10 mg/kg/day orally). After 16 weeks to rats	Reduction of LDL-C and increase of HDL-C in the circulation	Mediating lipid metabolism-related pathways through regulating the activities of inflammatory signalling molecules.	[26]
In-vivo	HT administration (50 mg/kg/day) for 17 weeks	HT had beneficial effects on lipid metabolism in db/db mice, and it seems that HT was more effective than metformi	HT administration could successfully inhibit both SREBP1c and FAS expression in liver and muscle tissue, suggesting that HT exhibited an inhibitory effect on the SREBP-1c/FAS	[17]

			pathway, which contributed to the decreased lipid deposition in the liver and muscle	
In vivo	Hypercholesterolemic diet was supplemented with 0.04% of HT for 8 weeks	Significantly decreased the total cholesterol and LDL-cholesterol levels	Decrease the pro-inflammatory cytokines IL-1β and TNFα but not to increase the anti-inflammatory cytokine IL-10 in carrageenan induced acute inflammation and hyperalgesia in rats	[25]
Human	Hydroxytyrosol (5 mg) for 3 months	Reduced total cholesterol by 11.4% and 14.1%, LDL-c by 19.8% and 19.7%, and apolipoprotein B by 12.4% and 13.5%, respectively	Reduces plasma CRP and IL-6 concentrations after intervention	[27]
Human	9.9 mg of HT for 20 weeks	Reduced oxLDL by 28.74 ng/mL ($p < 0.05$) in subjects with higher levels of oxLDL, which was an improvement compared with the placebo	Modulating the expression of genes involved in atherogenesis	[23]
Human	30 mg/day of hydroxytyrosol) or placebo for 2 months	Significant reduction in plasma levels of total cholesterol (–10.8 mg/dL), LDL cholesterol (–10.8 mg/dL)	The inhibitory effects on the synthesis of fatty acids, cholesterol, and triglyceride in the liver	[28]
Human	7.5 mg HT +210 mg Almond seed for 8 weeks	Oxidized LDL (oxLDL) levels and the oxLDL/LDL ratio were lower in the EG than in the CG after 8 weeks of treatment (18.76 – 3.91 vs. 10.34 – 4.22, $P < .001$ and 0.151 – 0.025 vs. 0.08 – 0.023, $P < .001$, respectively).	Avoided the increase of IL-6 compared to the CG and tended to decrease IL-1b values, whereas it activated IL-10 at the midterm of the intervention.	[29]
Human	3.3 mg of HT for 20 weeks	Significantly decreased the plasma levels of	The regulation of cholesterol metabolism via decreasing the	[23]

	low-density lipoprotein cholesterol (LDL-C), significantly reduced the plasma levels of triglycerides (TG)	phosphorylation, followed by the activation of AMP-activated protein kinase (AMPK) and inactivation of nuclear factor-kappa B

The consumption of olive oil as a main natural source containing a substantial quantity (more than 5mg in 20g of olive oil) of hydroxytyrosol has been demonstrated to significantly enhance certain cardiovascular and metabolic risk factors, as illustrated in Figure 1. With its abundance of bioactive phenolic olive compounds, particularly free and bound HT, this oil could potentially serve as a valuable nutraceutical component for food production, akin to its incorporation into products like pasta, bread, and granola bars. Over 15 years after the initial study, the European Food Safety Authority (EFSA) issued a scientific opinion concerning polyphenols in olives.

The EFSA Panel on Dietetic Products, Nutrition, and Allergies highlighted the connection between olive oil polyphenol consumption and the protection of LDL particles against oxidative damage [30]. The panel established that a daily intake of 5mg of hydroxytyrosol and its derivatives in olive oil is required to elicit this effect at a physiological level. This quantity of hydroxytyrosol should ideally be present in a maximum of 20g of oil to align with a well-balanced diet. Given the crucial role of LDL oxidation in atherosclerosis development, this communication presents substantial evidence regarding the involvement of phenolic compounds in coronary heart disease. This scientific standpoint is reinforced by consistent studies that have underscored the link between phenolic compounds in olive oil and the oxidation of LDL.

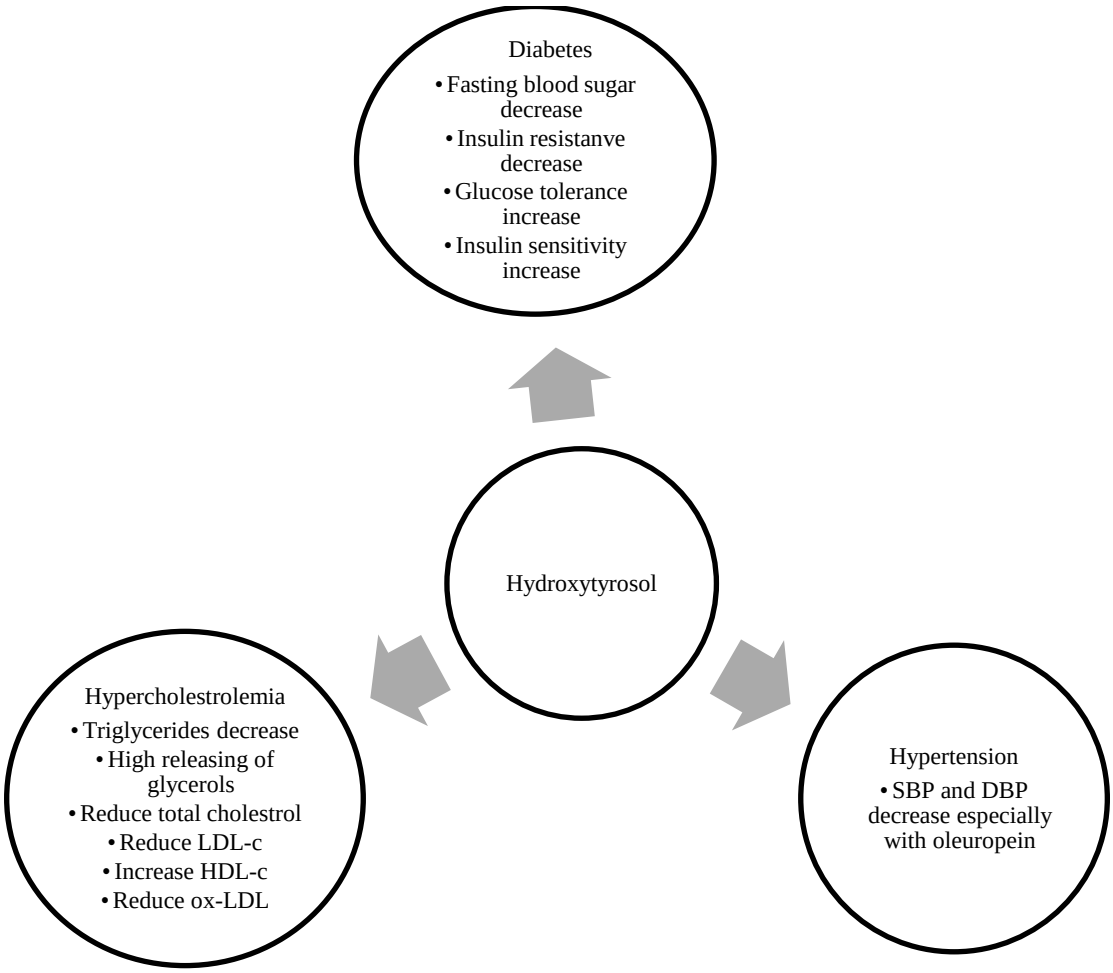

Figure 1. Overview of the mechanisms by which hydroxytyrosol affects cardiovascular risk factor.

CONCLUSION

In conclusion, the research presented in this article underscores the significant potential of high-potency hydroxytyrosol derived from natural sources as a promising avenue for addressing cardiovascular disease and its associated risk factors. The compelling evidence highlights the beneficial impact of hydroxytyrosol on various aspects of cardiovascular health, including its ability to improve lipid profiles, reduce oxidative stress, and enhance endothelial function. These findings offer a new perspective on the potential role of natural compounds in preventing and managing cardiovascular diseases, which remain a global health concern. While further studies and clinical trials are needed to elucidate the full scope of hydroxytyrosol's benefits and its mechanisms of action, the evidence presented here suggests that it holds substantial promise in the pursuit of improved cardiovascular health. Integrating high-potency hydroxytyrosol into dietary and therapeutic strategies may provide a novel approach to mitigating the burden of cardiovascular disease and its risk factors, ultimately contributing to better overall health and well-being.

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and Allergies (NDA) Scientific opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), anti-inflammatory properties (ID 1882), contributes to the upper respiratory tract health (ID 3468), can help to maintain a normal function of gastrointestinal tract (3779), and contributes to body defences against external agents (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. EFSA J (2011), **9(4)**, 2033.