

Invited critical review

Modulation of endogenous antioxidant system by wine polyphenols in human disease

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ABSTRACT

Numerous studies indicate that moderate red wine consumption is associated with a protective effect against all-cause mortality. Since oxidative stress constitutes a unifying mechanism of injury of many types of disease processes, it should be expected that polyphenolic antioxidants account for this beneficial effect. Nevertheless, beyond the well-known antioxidant properties of these compounds, they may exert several other protective mechanisms. Indeed, the overall protective effect of polyphenols is due to their large array of biological actions, such as free radical-scavenging, metal chelation, enzyme modulation, cell signalling pathways modulation and gene expression effects, among others. Wine possesses a variety of polyphenols, being resveratrol its most outstanding representative, due to its pleiotropic biological properties. The presence of ethanol in wine aids to polyphenol absorption, thereby contributing to their bioavailability. Before absorption, polyphenols must be hydrolyzed by intestinal enzymes or by colonic microflora. Then, they undergo intestinal and liver metabolism. There have been no reported polyphenol adverse effects derived from intakes currently associated with the normal diet. However, supplements for health-protection should be cautiously used as no level definition has been given to make sure the dose is safe. The role of oxidative stress and the beneficial effects of wine polyphenols against cardiovascular, cancer, diabetes, microbial, inflammatory, neurodegenerative and kidney diseases and ageing are reviewed. Future large scale randomized clinical trials should be conducted to fully establish the therapeutic use of each individual wine polyphenol against human disease.

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1. Introduction

Dietary polyphenols are derived from plants, and are consumed in the forms of fruits, vegetables and wine. Dietary intakes of polyphenols widely fluctuate between cultures, ethnic groups, and even within a narrow geological location. Large percentages of dietary polyphenols are consumed in the form of flavonoids, although cultural and dietary habit will dictate which forms of polyphenols are taken up. Phenolic compounds have been receiving increasing interest from consumers and manufacturers because numerous epidemiological studies have suggested associations between consumption of polyphenol-rich foods or beverages and the prevention of certain chronic diseases such as cancers and cardiovascular diseases. Red wine has long been thought to have beneficial effects on cardiovascular health. This relation was clear in the French Paradox phenomenon as well as in the Mediterranean diet. The French Paradox is defined as a low incidence of coronary heart disease (CHD) while consuming a diet rich in saturated fat. The Mediterranean diet, rich in fruits and wine, was shown to protect against the occurrence of coronary events [1,2]. Over 50% of the population has some kind of chronic condition (high blood pressure, high cholesterol, arthritis, diabetes, asthma, osteoporosis ...), so the goal of the many researches in the last decades is improving the quality of life. Much of the interested was transferred to homeopathy, alternative, and folk medicine, evidencing polyphenols as one of the main nutraceuticals [3].

It is of interest to consider that red wine is one of the most abundant sources of polyphenols [4]. Among these compounds, flavonoids and phenolic acids have antibacterial, antifungal, antiviral, antineoplastic, hepatoprotective, immunomodulating, and anti-inflammatory properties. In addition, it has been reported their therapeutic use proven beneficial in allergies, asthma, diabetes, hypertension, micro bleeding, among others. These pharmacological effects are mostly associated with their antioxidant activity [5]. More than 500 polyphenols have been described in common foods and beverages [6]. During the last decade the interest for polyphenols has increased considerably, especially among food scientists, nutritionists, the agricultural/food industry and the consumers. This is mainly due to the discovery of their marked antioxidant effects and their role in the prevention of several chronic diseases, such as cardiovascular diseases, certain cancers or type 2 diabetes [7,8]. Dietary polyphenols differ widely in their physico-chemical properties, bioavailability, biological properties and health effects [9,10]. Typically a glass of red wine contains about 100 mg polyphenols [11].

2. Oxidative stress and antioxidant defense system

Oxidative stress constitutes a unifying mechanism of injury of many types of disease processes, it occurs when there is a serious imbalance between the generation of ROS and the antioxidant defence systems in the body so that the latter become overwhelmed [12]. ROS are a family of highly reactive species that are formed either enzymatically or non-enzymatically in mammalian cells and causing cell damage either directly or through behaving as intermediates in diverse signalling pathways. In the cellular metabolism, the oxygen molecule itself is reduced to water after forming, as successive

intermediates, superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radical. The generation of water from oxygen in mitochondria is an enzymatic process in which intermediates of oxygen reduction do not leave the system before the process is finished. Therefore, the question arises how superoxide is generated. The mitochondrion is a major site of cellular superoxide production, mainly derived from Complex I due to partial reduction of NADH-dehydrogenase bound FMN; and from Complex III also due to partial reduction of ubiquinone/ubisemiquinone/ubiquinol, by transfer of one electron to O_2 [13,14].

Superoxide is converted to H_2O_2 by superoxide dismutase (SOD). In the absence of transition metal ions, H_2O_2 is stable; it does, however allow neutrophils to oxidize chloride ions, via myeloperoxidase, into hypochlorous acid, providing additional cytotoxic activity, through the formation of oxygen singlet, another ROS. Catalase (CAT), glutathione peroxidase (GSH-Px), and other peroxidases convert hydrogen peroxide to water. Hydroxyl radicals can be formed by the reaction of superoxide with H_2O_2 in the presence of metal ions. Hydroxyl free radicals are much more reactive than superoxide anions [15]. In healthy aerobes, production of ROS is approximately balanced with antioxidant defense system. However, the balance is not perfect, so that some ROS-mediated damage occurs continuously. In pathological conditions, sources of ROS include the mitochondrial respiratory electron transport chain, xanthine oxidase activation because of ischemia–reperfusion, the respiratory burst associated with neutrophil activation, and arachidonic acid metabolism. Activated neutrophils produce superoxide as a cytotoxic agent as part of the respiratory burst via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the free radical nitric oxide (NO) that can react with superoxide to produce peroxynitrite, a powerful oxidant, which may decompose to form hydroxyl radical. In ischemia–reperfusion, enzymatic production of superoxide anion could result from the joined effect of NADPH oxidase, uncoupled eNOS and xanthine oxidase.

The precise tissue specificity on ischemia reperfusion has not been completely elucidated. Nevertheless, it has been reported that reperfusion results in a sustained generation of ROS derived from NADPH oxidase (isoforms NOX1–4), whose isoforms are being differentially expressed across the diverse tissues [16]. Superoxide release results in the recruitment and activation of neutrophils and their adherence to endothelial cells, thereby activating the formation of xanthine oxidase in the endothelium, with further superoxide production. Although the role of xanthine oxidase in oxidative damage by ischemia–reperfusion remains controversial, this enzyme appears to be an important source of ROS production in this pathological condition [17,18]. Accordingly, allopurinol, a xanthine oxidase inhibitor, has been demonstrated that blocks the superoxide production in ischemia–reperfusion settings involving organs such as liver [18], heart [19], kidney [20], and small intestine [21].

The product formed by ROS attack to biomolecules could be a useful tool for oxidative stress assessment. Oxidation of DNA and proteins may take place, along with membrane damage, because of lipid peroxidation, leading to alterations in membrane permeability, modification of protein structure and functional changes [22]. Oxidative damage to the mitochondrial membrane can also occur, resulting in membrane depolarization and the uncoupling of oxidative

phosphorylation, with altered cellular respiration [23]. This can ultimately lead to mitochondrial damage, with release of cytochrome c, activation of caspases and apoptosis [24]. Indeed, ROS have physiologically essential roles in mitochondrial respiration, prostaglandin production pathways and host defence [15].

Oxidative stress has been found to play a crucial role in the pathogenesis of several cardiovascular diseases. One of the most studies has been atrial fibrillation and particularly post operative atrial fibrillation [25–27]. Following cardiac surgery, and especially with extracorporeal circulation, ischemic phenomena and posterior reperfusion are mandatory. This leads to the synthesis of high concentration of ROS, which could impair the normal operation of several physiological processes in the organism [28].

3. Polyphenols

3.1. Naturally occurring polyphenols in wine

Grapes have a long and abundant history; indeed, during the ancient Greek and Roman civilizations, grapes were revered for their use in winemaking [29]. Grape is a phenol-rich plant, and these phenolics are mainly distributed in the skin, stem, leaf and seed of grape, rather than their juicy middle sections (Table 1). The total phenolic content of grape skins varied with cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections [39]. In red wine, anthocyanins and flavonoids are the major two groups of phenolic compounds, and (+)-catechin is an abundant flavonoid [32]. Wine, juice and even pomace from grapes had high antioxidant capacities [29]. Anthocyanins are the main polyphenolics in red grapes, while flavan-3-ols are more abundant in white varieties [40]. Resveratrol is the most famous polyphenolic compound occurring in grapes and wine. The levels of resveratrol found in wine vary greatly, but is generally more abundant in red grapes and red wine [41].

3.2. Chemistry

Wine contains many phenolic substances, most of which originate in the grape berry. Phenolic compounds in grapes and wine are grouped within the following major classes: stilbenes, flavan-3-ols, flavonols, anthocyanins, hydroxybenzoic acids, procyanidins, hydroxycinnamic acids (Table 2) [42]. Wine is an alcoholic beverage that contains a large amount of different polyphenols extracted from grapes during the processes of vinification. These molecules are responsible for color, acidity, flavor, and antioxidant properties of wine [43]. Red wine possesses the most antioxidant effect because of its high polyphenolic content being procyanidins its most abundant polyphenols. However the phenolic composition of wine depends not

Table 2

Wine polyphenolics chemical classification in decreasing content order.

Category	Compound
Flavonols	quercetin
	myricetin
	kaempferol
	isorhamnetin
Anthocyanins	cyanidin
	malvidin
	petunidin
	delphinidin
	peonidin
	pelargonidin
Hydroxycinnamic acids	caffeic
	ferulic
	p-coumaric
	chlorogenic
Hydroxybenzoic acids	gallic
	ellagic
	protocatechic
	vanillic
	syringic
	3,4-dihydroxybenzoic
	p-hydroxybenzoic
	(+)-catechin
Flavanols	(-)-epicatechin
Stilbenes	resveratrol
	piceid
Procyanidins	procyanidins A2, B1, B2, B3, B4

only on the grape variety from which it is made, but also on some external factors such as climate and winemaking technology. Grape seeds contain mainly phenols such as proanthocyanidins. Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C [44]. For a detailed chemical description of each polyphenol, refer to: <http://www.phenol-explorer.eu/>.

3.3. Bioavailability, metabolism and excretion

Several studies showed rapid absorption of the polyphenolics, such as procyanidins, quercetin and flavanols from grapes into plasma, with plasma concentrations peaking at 2 or 3 hours after ingestion [32,45–47]. Enteric polyphenol absorption occurs by passive transport through membranes that are mainly determined by the lipophilicity of the substance. Absorption is mainly greater than 60%, but this has to be taken with dose of doubt, as experimental data are contradictory [48]. Biological effects of polyphenols depend on their bioavailability that differs within each and every polyphenol. There is no relation between the quantity of polyphenols in food and their bioavailability in human body. Generally, aglycones can be absorbed from the small intestine. Nevertheless, most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in native form [49]. Before absorption, these compounds must be hydrolyzed by intestinal enzymes or by colonic microflora. During the course of the absorption, polyphenols undergo extensive modification; in fact they are conjugated in the intestinal cells and later in the liver by methylation, sulfation and/or glucuronidation [50]. After 2 weeks of daily low to moderate red wine consumption, plasma levels of total phenolic concentrations increased significantly, and trace levels of metabolites, mainly glucuronides and methyl glucuronides of (+)-catechin and (–)-epicatechin, were detected in plasma, which could not be found in a control group [51]. These results indicated that phenolic compounds could be absorbed by human digestion system, and entered the blood successfully. The mechanisms involved in the process of digestion and absorption of phenolic compounds in gastrointestinal lumen are complex and not very clear [29].

Table 1

Main polyphenols in different parts of grape, raisin and red wine.

Compound	Source					
	Seed	Skin	Leaf	Stem	Raisin	Red wine
Resveratrol		x		x		x
(+)-catechin	x					x
(–)-epicatechin	x					
Quercetin		x	x	x	x	x
Myricetin		x	x			
Malvidin						x
Kaempferol		x	x			
Gallic acid	x		x			
Procyanidin	x					x
Proanthocyanidins	x	x				
Rutin				x		
Others		x	x	x	x	x
References	[29–32]	[29,30]	[29]	[33]	[34]	[35–37]

Polyphenols and their conjugated metabolites circulate in the blood bound to albumin, the primary protein responsible for the binding. The affinity of polyphenols for albumin varies according to their chemical structure [52,53]. The effect of sulfation and glucuronidation is unknown, but it probably depends highly on the position of substitution.

The partitioning of polyphenols and their metabolites between aqueous and lipid phases is largely in favor of the aqueous phase because of their hydrophilicity and binding to albumin. At physiologic pH, most polyphenols interact with the polar head groups of phospholipids at the membrane surface via the formation of hydrogen bonds that involve the hydroxyl groups of the polyphenols [54]. This adsorption of polyphenols probably limits the access of aqueous oxidants to the membrane surface and their initial attack on that surface. Many studies have shown that various polyphenols have the ability to protect LDL from oxidation. Polyphenols are associated with lipoproteins only by ionic interactions with charged residues on the surface of the particles. Protection probably occurs at the interface between lipophilic and hydrophilic phases [55]. Further studies accounting for polyphenol diversity on these pharmacokinetic aspects are still lacking.

Most classes of polyphenols are sufficiently absorbed to exert biological effects. As a consequence, the forms reaching the blood and tissues are different from those present in food and it is very difficult to identify all the metabolites and to evaluate their biological activity [56]. The concentration of intact polyphenols (parent compounds and their tissue conjugated forms) in plasma rarely exceeds 1 $\mu\text{mol/L}$, and their urinary recovery ranges from 1% to 25% of the ingested dose [57].

Importantly, it is the chemical structure of polyphenols and not its concentration that determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma. Evidence, although indirect, of their absorption through the gut barrier is given by the increase in the antioxidant capacity of the plasma after the consumption of polyphenols-rich foods [58,59]. The higher excretion of aromatic acids by rats fed wine polyphenols is likely due to their poor absorption in the proximal part of the gut.

Due to the major biological effects of resveratrol (further detailed below), numerous studies have been performed *ex vivo* and in animal models, providing information on the absorption, metabolism, and consequent bioavailability of this polyphenol [60]. The oral bioavailability of resveratrol is low due to rapid excretion and extensive metabolism into various glucuronide and sulfate conjugates of unknown potential biological activities. The major metabolites identified in the urine in human after oral dosing of synthetic resveratrol are: resveratrol monosulphate, two isomeric forms of resveratrol monoglucuronide, dihydroresveratrol monosulphate, and dihydroresveratrol monoglucuronide. Total sulphate conjugates account for more than one-third of the metabolites in the urine and total glucuronide conjugates represent about 20% [61]. These pharmacokinetic studies cast doubt on the therapeutic potential of unmodified resveratrol. Nevertheless, *in vivo* data from several studies have clearly demonstrated in various organisms that resveratrol intake has protective properties against multiple illnesses, including cancer, cardiovascular disease, and ischemia, and was also found to confer resistance to stress and to extend life span [62].

Anthocyanins were found in human plasma after wine consumption [63]. Absorbed quercetin is metabolized to conjugated derivatives retaining antioxidant properties in plasma [64]. Indeed, after oral administration of red wine, resveratrol shows significant bioavailability and strong affinity for liver and kidney [65].

Even small changes in physical-chemical properties of hydroalcoholic solutions in which polyphenols are dissolved significantly influence their solubility, precipitation behavior, and their interactions with proteins, which in turn may influence their biochemical properties and bioavailability [66,67]. Indeed, proportionally more phenolics are absorbed from whisky than from wine, which is partly

ascribed to the greater ethanol content of whisky that aids phenolics absorption [58]. Nevertheless, it has also been reported that red wine is a poor source of bioavailable polyphenols in men [68], because wine flavonols are poorly absorbed relative to onions or tea flavonols [69]. The bioavailability of wine polyphenols remains to be fully established.

3.4. Polyphenols as antioxidants

Grape fruit contains various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and polyphenols. The overall protective effect of polyphenols is thought to be mainly due to their large array of biological actions, such as free radical-scavenging, metal chelation and enzyme modulation abilities, as well as their effects on cell signaling pathways and on gene expression [70,71]. Even though glutathione is the most important non-enzymatic cellular antioxidant, its modulation by wine polyphenols has yet to be determined [72].

3.4.1. Free Radical Scavenging properties

Being most the outstanding bioactivity of phenolic compounds from grapes, the antioxidative characteristics have been widely studied, including scavenging of free radicals, inhibition of lipid oxidation, reduction of hydroperoxide formation, quenching of electronically excited compounds and so on [73–77].

It has been showed that one hour after ingestion of 300 mL of red wine serum antioxidant capacity increased by 18%, which is comparable with 22% increase after ingestion of 1 g of vitamin C [78]. Another aspect of wine-related increase in plasma antioxidant activity is the effect of unabsorbed polyphenols that remain in gastrointestinal system following the wine consumption. In this location, they can scavenge free radicals, preventing lipid peroxidation and, at the same time, sparing other antioxidants from oxidation [79]. In such a way, although acting locally, the polyphenols could influence the whole organism and the plasma concentration of various antioxidants, which in turn could affect plasma antioxidant capacity [80,81]. Recently, a new beneficial function has been attributed to red wine polyphenols: prevention of cytotoxic lipid peroxidation products absorption [82].

Grape seed extracts protected the rat liver against oxidative damage induced by irradiation *in vivo*, and remained the activities of superoxide dismutase and catalase at normal level [83].

3.4.2. Metal chelating properties

Together with scavenging free radicals, polyphenols may inhibit their formation through the Haber–Weiss/Fenton reactions, due to their metal chelating properties. Accordingly, quercetin chelates intracellular iron [84], thereby avoiding its catalyzing effect on the formation of ROS. Flavonoids display higher antioxidant capacity against metal-ion-induced peroxidation than peroxyl-radical-induced peroxidation. The mechanism is mainly speculated to be related with phenoxyl radical generation. The number of OH group and its position on the ring of molecule determine the antioxidant capacity of flavonols [85].

3.5. Ethanol effect

Numerous epidemiological and controlled clinical studies have investigated the effects of varying levels and patterns of ethanol intake on cardiovascular health [86]. In contrast to high ethanol intake which is detrimental to cardiovascular health, chronic low ethanol has been shown to have a beneficial effect [86,87]. An extensive body of data shows concordant J-shaped associations between alcohol intake and a variety of adverse health outcomes, including coronary heart disease, diabetes, hypertension, congestive heart failure, ischemic stroke, dementia, Raynaud's phenomenon, and all-cause mortality [88]. Light to moderate alcohol consumption (up to 1 drink daily for

women and 1 or 2 drinks daily for men) is associated with cardio-protective benefits. Alcohol consumption confers cardiovascular protection predominantly through improvements in insulin sensitivity and high-density lipoprotein cholesterol [88].

Moderate alcohol consumption induces not only quantitative, but also qualitative changes of HDL fractions [89]. The increased lipidation of HDL found in alcohol consumers might augment the antiatherogenic effect of HDL-cholesterol increase. Until now, the exact mechanism of alcohol influence on HDL metabolism is not clear. The higher HDL-cholesterol associated with moderate alcohol consumption is not caused by an effect on plasma lecithin:cholesterol acyl transferase, cholesterol ester transfer protein and phospholipid transfer protein activity levels [90]. This ethanol effect could be controversial in the light of some studies reporting that while alcohol per se may increase the serum HDL level, alcoholism particularly alcoholic liver disease—probably negates the HDL related protection against coronary heart disease [91]. Therefore, more studies including randomized, blinded clinical trial data are still needed to further elucidate this point.

Ethanol is metabolized differently at high and low concentrations. With chronic high ethanol intake, the microsomal ethanol oxidizing system (MEOS) is induced. MEOS has a relatively high K_m for ethanol, and at high concentrations ethanol is metabolized to acetaldehyde without producing reduced nicotinamide adenine dinucleotide (NADH). Instead, this pathway utilizes reduced nicotinamide adenine dinucleotide phosphate (NADPH), another reducing equivalent, thus producing an oxidative environment [92]. This high ethanol intake thus associated with an enhanced rate of metabolism by the MEOS pathway results in a decrease in reducing equivalents, elevated acetaldehyde, and increased oxidative stress. These factors may account for the detrimental effects of high ethanol intake.

Induction of cytochrome P450 2E1 (CYP2E1) is a central pathway by which ethanol generates oxidative stress. Increases in nuclear factor erythroid 2-related factor 2 (Nrf2) protein and mRNA were observed in livers of hepatocytes of chronic alcohol-fed and of pyrazole-treated rats or mice, conditions known to elevate CYP2E1. The transcription factor Nrf2 regulates the expression of important cytoprotective enzymes [93]. However, at low ethanol blood levels, ethanol is metabolized very efficiently by low K_m alcohol dehydrogenase to acetaldehyde and then by aldehyde dehydrogenase to acetate, producing NADH in both reactions [90,92].

Glucose metabolism via the pentose cycle plays a crucial role in providing NADPH and, hence, maintaining the normal ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and a normal redox state in cells. Then, oxidized glutathione is converted back to GSH by the NADPH-dependent glutathione reductase. This process could contribute to the recycling of vitamin E following its oxidation by scavenging. α -tocopheroxyl radical reacts with vitamin C resulting in α -tocopherol recovery and oxidized vitamin C. The latter reacts with GSH leading to the production of GSSG and reduced vitamin C [94]. It is of interest to mention that vitamin C is the cornerstone in this chain of coupled redox reactions [95].

Although ethanol is primarily metabolized in the liver, it is also metabolized in other tissues, including vascular tissue. An increase in antioxidant capacity would offer protection against oxidative stress and secondary production of aldehydes through lipid peroxidation [90].

In humans, ethanol decreased urinary 8-hydroxy-2-deoxyguanosine, a measure of oxidative stress [96]. NADH may also increase overall antioxidant capacity, increasing tissue levels of cysteine by converting cystine to cysteine, via the NADH dependent enzyme, cystine reductase [97]. Cysteine is a precursor of glutathione, a major endogenous antioxidant.

Additionally, glutathione is a cofactor in methylglyoxal catabolism and cysteine has the ability to bind aldehydes to foster excretion and reduce advanced glycation endproducts (AGE) formation [98]. An

increase in the low molecular weight thiols, cysteine and glutathione, may also enhance the transfer of NO from protein-bound reserves improving endothelial dysfunction [99]. Decreasing levels of AGEs may also preserve eNOS and prevent breakdown of NO. Low ethanol increased the expression of eNOS [100] and stimulated calcium-activated potassium channels increasing production of NO in vascular endothelial cells in culture [101]. Humans consuming low amounts of ethanol showed significant dilation of brachial artery at rest and at reactive hyperaemic conditions [102]. Low ethanol decreases cytosolic free calcium, an initiator of smooth muscle cell contraction [91].

Low alcohol intake has increased insulin sensitivity in humans [103]. Improving insulin resistance, the source of excess aldehydes, would limit formation of AGEs and their subsequent hypertensive and atherosclerotic complications [91]. Moreover, it has a beneficial effect on lipoprotein profiles, as it has been shown to increase HDL [104]. This increase may be a result of mediating the AGE-induced inhibitory effect on reverse cholesterol transport. In hypertensive patients low ethanol decreased lipoprotein a (Lp[a]), an independent predictor for atherosclerosis [105].

3.6. Adverse effects and interactions

There have been no reported polyphenol adverse effects derived from intakes currently associated with the normal diet. However, diet supplements for health-protection should be cautiously used as no definition has been given for the levels or limits to make sure the dose is safe. Nevertheless, the potential toxicity of some polyphenols from grape, such as epicatechin to the fibroblast, and keratinocyte cell lines, has been investigated. The results show marked negative effects at concentration 3- to 7-fold higher than that of expressing positively antioxidant activity. Compounds having a gallate group exhibited more potential toxicity than those without this moiety [106]. In addition, marked DNA damage was reported in mice spleen cells following incubation with higher concentration (150 μ mol/L) of catechin [107]. The polyphenolic compounds of grape extract, caffeic acid, gallic acid, and rutin hydrate enhanced mitomycin C-induced clastogenicity at accordant concentrations. The results suggested that negative effects of phenolic compounds were related to the synergistic effect of some molecules, and the concentration was not always a crucial factor. Therefore, the dose and composition of grape extracts should be investigated further for secure and healthy application of grape products. In the future, the extraction methods of polyphenols from grape should be improved, and the by-products of wine industry should be utilized effectively. The crude extracts from grape could be used as diet supplements for health-protection after defining the levels or limits to make sure the dose is safe for health, but bioactive components at high purity should be used instead of crude extracts in medicinal preparations from grape [29]. On the other hand, although chronic hyperuricemia has been associated with gout [108] and ethanol intake is a well-established risk factor [109], recent epidemiological studies have shown that moderate consumption of wine is not associated with higher incidence of gout, in contrast to consumption of beer and spirits [110,111].

In relation to various nutritional supplements rich in polyphenols, some recommend the consumption of 50 mg/day isoflavones or 100–300 mg/day grape seed extracts rich in proanthocyanidins. These intake levels are close to those derived from the consumption of soy products in Japan or of grapes or wine in some European countries (55, 57). However, some supplement manufacturers recommend intakes far higher than those currently associated with the diet. This would result in intakes 100 times higher than the common intakes in a Western diet. Furthermore, some of these supplements may appear safe when isolated from food plants, but the method of extraction used to produce the supplements may influence the nature of the compounds ingested and thus the safety of the product. This occurred

with a hydroalcoholic extract of tea buds, sold as a slimming supplement, which was withdrawn from the market because of severe cases of liver toxicity [112]. Definition of a therapeutic polyphenol dose having safety practical certainty that no adverse effects will be observed is still lacking.

Although there are contrasting viewpoints on the effects of polyphenols on LDL oxidation variables, there is increasing evidence that these compounds possess additional cardioprotective functions including altering hepatic cholesterol absorption, triglyceride assembly and secretion, the processing of lipoproteins in plasma, and inflammation.

Polyphenols may be involved in interactions having positive effects, as occurs with ethanol that enhances the anti-inflammatory effect of quercetin and resveratrol [113]. In contrast, chemical interactions *in vitro* were detected between analgesic and non-steroidal anti-inflammatory agents such as acetylsalicylic acid and acacetin, galangin, chrysin, pinocembrin and cinnamic acid; ibuprofen and flavone, ketoprofen and pinocembrin, vitamin C and myricetin, morin and quercetin; and vitamin E with flavanone [4]. However, further *in vivo* studies have to demonstrate applicability of this model [114]. Polyphenols may affect drug bioavailability and pharmacokinetics. Some drugs, such as benzodiazepines and terfenadine, show up to 3-fold increases in bioavailability with grapefruit juice (rich in naringenin), because of inhibition of CYP3A4 [115,116]. These effects, which may be attributable in part to psoralens as well as naringenin, are clinically significant in the case of cyclosporine, because of a narrow therapeutic range (e.g., when used after organ transplants) [117].

The oxidation of phenols by polyphenol oxidase produces extremely reactive free radical intermediates, which following release from the enzyme, readily condense to yield polymeric products of variable stoichiometry. This may be associated with the pro-oxidant activity of polyphenols as a result of their tendency to autooxidation accompanied by the formation of ROS and H₂O₂ [118]. The production of H₂O₂ could lead to the oxidation of HNE resulting in the formation of its corresponding epoxide, forming adducts with vitamin E [119].

3.7. Supplementation, Therapeutic and Clinical studies

3.7.1. Cardioprotective effects

Moderate consumption of red wine has been putatively associated with lowering the risk of developing coronary heart disease. An increase body of evidence demonstrates that polyphenols play a role in the homeostasis of cardiovascular system, through the reduction of oxidative stress and inflammation, both key processes implicated in the pathogenesis of cardiovascular disease [120,121]. Polyphenols act on multiple levels on the cardiovascular system, such as endothelial function, atherosclerosis, platelet aggregation, and ischemia-reperfusion events.

3.7.2. Vascular function

Endothelial dysfunction constitutes a major event in the development of hypertension, atherosclerosis and stroke. ROS contribute to vascular dysfunction and remodeling through oxidative damage. In human hypertension, it has been found increased production of superoxide anion and hydrogen peroxide, decreased NO synthesis and bioavailability of antioxidants. As a consequence of oxidative stress, the reduced vascular bioavailability of NO, the most important endogenous vasodilator agent, leads to vasoconstriction, increasing the blood pressure. Supplementation with quercetin significantly reduced systolic blood pressure on individuals with a high-cardiometabolic risk phenotype on established cardiovascular disease risk biomarkers [122]. This effect was most pronounced in subjects aged 25–50 years. In addition, quercetin caused a significant reduction in plasma concentrations of atherogenic oxidised LDL. In contrast, there were no effects of quercetin on biomarkers of inflammation and metabo-

lism including body composition. They used a moderate supranutritional but non-pharmacological dose of quercetin, since these data should provide a rational basis for the development of functional foods.

The results of a recent placebo-controlled intervention study in healthy subjects suggested that pure quercetin can improve endothelial function by modulating the circulating concentrations of vasoactive NO products and endothelin-1 [123]. These effects may be explained by inhibition of NADPH oxidase and the activation of endothelial NO synthase.

There have been no reported changes in systolic or diastolic blood pressure when healthy volunteers were supplemented with a high (non-nutritional) dosage of quercetin [124]. In contrast, a 4-week supplementation with quercetin significantly reduced systolic and diastolic blood pressure in subjects with stage 1 hypertension but not in those with prehypertension [125]. However, a non-significant reduction in blood pressure was also observed in the placebo group and the authors did not report significant differences between the quercetin and placebo groups. It was concluded that a certain degree of hypertension might be required for quercetin to exert a blood pressure-lowering effect.

In this context, it has been demonstrated that oral administration of grape skin extract significantly reduced systolic, mean, and diastolic arterial blood pressure in a model of hypertensive rats [126]. Accordingly, the administration of purple grape juice in human hypertensive patients increases NO release and reduces superoxide production in the vessels [127], thereby improving the cardiac output in patients with coronary artery disease. Anthocyanins from wine inhibit phosphodiesterase-5 activity, thus reducing the risk of cardiovascular disease by vasorelaxation [71]. These effects are not only mediated by a direct modulation of polyphenols on eNOS activity, but also by increasing the expression of this enzyme through their action in the promoter gene region, thus explaining the long-term beneficial effects of red wine intake on the cardiovascular function [128].

Estrogen receptor α (ER α) has been identified as the key receptor transducing vascular effects exerted by red wine polyphenols, particularly delphinidin with respect to NO production [129]. Indeed, estradiol and 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole, as well as a polyphenolic extract from red wine (Provinols™) and delphinidin, are able to activate molecular pathways, involving Src, ERK1/2, eNOS and caveolin-1 phosphorylations, by a mechanism that required ER α activation, with subsequent increase of endothelial NO production and endothelium-dependent vascular relaxation. Moreover, using a binding assay and a docking, they showed that delphinidin fits on ER α 's activation site. The latter demonstrated for the first time the physiological relevance of this receptor in triggering the vascular protection induced by red wine polyphenols.

It is important to note that the ability of red wine to preserve vascular function depends on the type of strain, the area growing and the vinification processes [130]. Accordingly, the improvement of the endothelium-dependent relaxation level is not the same for all wines, and there is a positive correlation between the vasodilator effect and polyphenols bioavailability [131].

3.7.3. Atherosclerosis

Endothelial dysfunction and proliferation and migration of smooth muscle cells of the vessels are central events in the pathogenesis of atherosclerosis. Interactions between polyphenols and the development of atherosclerotic plaques have been widely studied [132,133]. Thus, in several animal models, the administration of red wine, grape juice and dealcoholized red wine attenuated the development of atherosclerotic lesion [134–136].

At the site of atherosclerotic lesion, the most important mitogenic factor for smooth muscle cells growth is the platelet derived growth factor (PDGF) released by platelets, endothelial cells and smooth

muscle cells. PDGF exerts its biological action by binding to tyrosine kinase receptor type B, thus activating the MAPK intracellular pathways [137,138]. In this context, the role of red wine polyphenols and the proliferation of vascular smooth muscle cells have been widely studied and several mechanisms have been proposed. It involves the downregulation of cyclin A gene expression, through the decreased expression of transcription factors AFT-1 and CREB. Another course of action is the decrease in phosphorylation of phosphatidylinositol 3-kinase (PI₃K) and p70 ribosomal protein S6 kinase (p70_{S6K}) pathways. These two proteins are involved in angiotensin II-induced smooth vascular cells proliferation [139].

Postprandial hyperlipemia and oxidative stress, a well-defined risk factor for atherosclerosis, could be reduced by grape seed extracts or phenolic-rich grape juice. These oxidative stress factors refer to plasma lipid hydroperoxides, serum lipid peroxidation products and malondialdehyde-modified-LDL (MDA-LDL). The lipid-bound polyphenols increasing in serum were found even 2 hours after intake of phenolics, and MDA-LDL was detected even 6 weeks later [45,140]. Grape seed extracts protected the rat liver against oxidative damage induced by irradiation *in vivo*, and remained the activities of superoxide dismutase and catalase at normal level [83].

Following grape juice intake plus hypercholesterolemic diet for 96 days, platelet aggregation in rabbits was significantly ameliorated and the development of atheroma was near 30% lower than that of the control group [141]. In comparison to the control group, aortic fatty streak areas of hamster also showed significant reduction of 84%, 80% and 76% in the groups receiving catechin, quercetin or resveratrol, respectively [38,142].

In addition, red wine consumption resulted in high concentrations of HDL cholesterol [51], which renders the risk of coronary heart disease to lower levels than controls. After feeding to hamsters at a moderate dose of grape extracts, the plasma cholesterol was reduced 11% on average [142]. Moreover, plasma apolipoprotein A1 concentration was increased 26%, 22%, and 19%, induced by catechin, quercetin, and resveratrol, respectively [38].

For hemodialysis patients, grape polyphenols are helpful to prevent inflammation. Red grape juice significantly reduced plasma monocyte chemoattractant protein 1, an inflammatory factor involved in cardiovascular disease risk [46]. After 2 weeks of daily red wine consumption (375 mL), the maximum concentrations of conjugated dienes and thiobarbituric acid reactive substances in Cu-oxidised LDL were reduced [51]. It was reported that red wine consumption reduced oxidative stress induced by Cu-oxidised LDL and increased HDL cholesterol concentrations [143].

3.7.4. Antiplatelet effects

Polyphenol rich grape seed extracts displayed reduction of platelet adhesion and aggregation, and generation of superoxide anion. In addition, they were more effective than resveratrol alone [144]. Platelets play a key role in all phases of atherosclerosis. Antiplatelet drugs, particularly aspirin, have proven beneficial effects on cardiovascular risk. Because polyphenols have shown platelet inhibitory effects, there is great interest in the possibility that grape consumption might provide similar protection.

In vitro studies have shown that grape-derived polyphenols inhibited platelet activity and accounted for a number of potential mechanisms. Flavonoids inhibited cyclooxygenase and reduced thromboxane A₂ production [145]. Red wine polyphenols also decreased platelet production of hydrogen peroxide and inhibited activation of phospholipase C and protein kinase C [146]. Dilute grape juice inhibited platelet aggregation and this effect was associated with decreased production of superoxide anion and increased platelet NO production [147].

Human studies have also demonstrated antiplatelet effects of grape-derived beverages. Grape juice consumption is able to decrease platelet aggregation and superoxide production and increased NO

production in healthy volunteers [147]. In that study, grape juice also inhibited protein kinase C and spared cellular antioxidants. Red wine has more potent antiplatelet effects than white wine [148].

3.7.5. Myocardial Ischemia

It has been reported that using a model of isolated rat heart made ischemic for 30 min followed by 2 hours of reperfusion, the ischemic reperfusion injury was significantly inhibited after consumption of flesh and skin of grapes [149]. The formation of new blood vessels is a potential mechanism to reduce ischemic damage to the heart [150]. Ischemia induces VEGF release to encourage the development of collateral coronary circulation. Reactive oxygen species may have an important role in activating this process of myocardial angiogenesis [151]. Resveratrol enhanced angiogenesis both *in vivo* and *in vitro* by induction of VEGF which was regulated by thioredoxin-1 and heme oxygenase-1 [152]. This could be an important antioxidant defense mechanism mediating cardioprotection in the chronic ischemic myocardium.

3.7.6. Anticancer effects

Anticancer activities of phenolic compounds from grapes have been widely studied. Phenolic compounds had dual effects on cells, modulating cell proliferation in a dose-dependent manner [153]. However, at high concentrations, they induced cells to death by a direct toxic effect [154]. The relationship between anticancer activity and structure of phenolic compounds was also investigated. The regulation target of grape skin extracts to cell apoptosis was the phosphatidylinositol 3-kinase-Akt and mitogen-activated protein kinase survival pathways. The extracts reduced Akt transcription, and enhanced proteasome degradation [155]. Resveratrol was determined mainly bearing *o*-diphenoxyl groups, which displayed inhibition on DNA damage induced by ROS, and enhancement of DNA damage induced by cupric ions, as well as inducing apoptosis [156].

There is growing evidence showing the anticarcinogenic properties of polyphenols, particularly resveratrol, in the prevention and treatment of a wide range of cancer settings [157,158]. As a chemoprevention agent, resveratrol has been shown to inhibit tumor initiation, promotion, and progression [159]. Resveratrol acts at multiple levels, controlling the cell cycle progression, regulating the signs of apoptosis and survival pathways, inhibiting tumor growth and angiogenesis and modulating the activity of transcription factors related with the pathogenesis of cancer, as NF- κ B [160–162]. Resveratrol has also been shown to reduce inflammation via inhibition of prostaglandin production and cyclooxygenase-2 activity [163]. It also potentiates the apoptotic effects of cytokines, chemotherapeutic agents and gamma-radiation [164,165].

Major evidence has shown that the extracts from grapes and its products had anticancer activity. It has been reported that the grape skin extract induced prostate tumor cell lines apoptosis with high rates [155]. The extract from pomace remaining after wine production inhibited activities of matrix metalloproteinases-2 and -9, and expressed a significant antiproliferative effect on human colon adenocarcinoma cells (Caco-2), which implied by-product of wine would help to fight against carcinogenesis [166,167]. Grape juice polyphenols also significantly inhibited carcinogen-induced DNA adducts formation in a rat model [168], and inhibited DNA synthesis in breast cancer cells [169].

Estrogens play an important role in breast cancer development [170]. Aromatase (CYP19), a cytochrome P450 isoenzyme, is the enzyme responsible for estrogen synthesis. Aromatase is expressed at a higher level in human breast cancer tissue than in normal breast tissue. Because of its structural resemblance to estrogen, resveratrol's agonistic and antagonistic properties on estrogen receptor have been examined and demonstrated [171]. Resveratrol inhibits aromatase activity and transcription in a human breast adenocarcinoma cell line [171]. Accordingly, the exposure to dealcoholized red wine decreased

cell proliferation in hormone sensitive and resistant human cancer cell lines [172]. Exposure to red wine polyphenols induced apoptosis in a dose dependent manner in neoplastic cells of colon cancer in vitro, increasing caspase-3 activity and Bax expression [173].

A similar chemopreventive effect have been observed in hepatocarcinoma cells of rats treated with resveratrol [174], and a diminution of the carcinogenic effects and progression to metaplasia in reflux esophagitis to Barrett's esophagus have been reported in rats supplemented with resveratrol [175]. Additionally, it has the ability to overcome chemotherapy-induced resistance in multiple myeloma, a major challenge in the treatment of this disease [160].

Moderate red wine consumption has shown a small inverse correlation with lung cancer risk [176]. In contrast, in a multiethnic cohort study, the moderate red wine consumption was not associated with reduced risk of colorectal [177] and prostate [178] cancer in a population of middle-aged men. Despite empirical evidence of the anticarcinogenic properties of polyphenols, clinical trials have not shown significant effects in terms of their potential chemopreventive effects.

3.7.7. Anti-diabetic effects

Long term effects of diabetes include progressive development of specific complements such as retinopathy, which affects eyes and lead to blindness; nephropathy in which the renal functions are altered or disturbed and neuropathy which is associated with the risks of amputations, foot ulcers and features of autonomic disturbance including sexual dysfunctions. Numerous studies report the antidiabetic effects of polyphenols, such as tea catechins [179,180]. Increased ROS levels are an important trigger for insulin resistance in numerous settings [181]. Grape seed extract given to type 2 diabetic patients for 4 weeks, had positive effect on several inflammatory markers and glycemia, but did not result in statistically significant changes in insulin resistance [182]. In addition, wine polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. The hypoglycemic effects of catechin are due to an inhibition of α -glucosidase, α -amylase and sucrase in rats [183,184]. In turn, red wine treatment has proven a substantial effect on lowering the 3-nitrotyrosine, a footprint of nitrosative stress, content in sciatic nerve, dorsal root ganglion neurons, spinal cord, kidney and retina of diabetic rats [185]. These effects may be due to red wine polyphenols-mediated oxidative stress diminution. In addition, protection of oxidative changes in diabetic patient erythrocytes has been reported for individual polyphenols such as resveratrol [11,186], myricetin [187] and quercetin [188] as well as a synergistic effect for resveratrol in combination with quercetin or pterostilbene [189].

Several mechanisms have been proposed to explain the anti-diabetic action of resveratrol, such as SIRT1 modulation which improves whole-body glucose homeostasis and insulin sensitivity in diabetic rats [190,191]. Sirtuins are enzymes with preferential deacetylase activity, coded by seven genes (SIRT1–7). The most investigated sirtuin is SIRT1, which is involved in several physiologic and pathologic processes including apoptosis, autophagy, diabetes, cancer, cardiovascular disorders, and neurodegeneration. Resveratrol inhibits diabetes-induced changes in the kidney (diabetic nephropathy) and significantly ameliorates renal dysfunction and oxidative stress in diabetic rats. Treatment with resveratrol also decreased insulin secretion and delayed the onset of insulin resistance. A possible mechanism was thought to be related to the inhibition of ATP- and voltage-dependent channels in beta cells [192].

Concordant studies have also revealed the beneficial effect of resveratrol in vivo on energy metabolism in diseases such as diet-induced obesity and insulin resistance [193–195]. It was reported that in cultured cell lines, high glucose induced cytotoxicity and oxidative stress was inhibited by grape seed polyphenols. Ferulic acid, another wine polyphenol, lowered blood glucose followed by a significant

increase in plasma insulin and a negative correlation between blood glucose and plasma insulin in rats [196]. Therapeutic use of this compound against diabetes as well as other oxidative stress mediated diseases has been proposed but clinical studies are still lacking [197]. Indeed, there are only a few controlled interventions studying the effects of specific polyphenols to ameliorate the symptoms of the metabolic syndrome [198].

3.7.8. Antimicrobial effects

Polyphenols have demonstrated potential antibacterial [45,199], antifungal [39,200] and antiviral [201] activities. Wine inhibited microbial, especially *Escherichia coli*, growth, and the inhibition increased as the polyphenol concentration increased, and clarified wines were inactive against all bacteria tested [203]. The extracts of alcohol-free red and white wine exhibited antimicrobial activity to some pathogens such as *Staphylococcus aureus*, *E. coli* and *Candida albicans* [203]. The results suggested that polyphenolic compounds contained in red wines were responsible for the antimicrobial effects. Some studies reported phenolic compounds inhibited other food-borne species such as *Salmonella typhimurium* [204] and *Listeria monocytogenes* [205]. High concentration of flavonoids and their derivatives in grape seeds and flavonoids, stilbenes, and phenolic acids in grape stem were responsible for the antimicrobial activity [206]. Resveratrol displayed potent antifungal activity against the human pathogenic fungi *C. albicans* [207].

Various bacterial species exhibit different sensitivities towards phenolic compounds, being *S. aureus* the most sensitive to wine extracts, followed by *E. coli* and the least effect of inhibition was detected in *C. albicans* [203,208]. Phenolic compounds from defatted grape seed extract inhibited the growth of *S. aureus* and *E. coli*, while they showed no effects on *Salmonella* sp. [209].

The phenolic compounds from different parts of grapes displayed different antimicrobial effects. The antimicrobial activity of fermented pomace was either as effective as or significantly better than whole fruit grape extracts [210]. Seed extracts were more effective antimicrobial than other parts of grapes. The extract from grape flesh did not exhibit any antimicrobial effect at all [211]. The grape skin possessed the strongest activity in anti-*Helicobacter pylori* [212]. The increase order of the antimicrobial activity was flesh, whole fruit grape extract, fermented pomace, skin, leaf and seed [29].

The application of phenolic compounds could be better in food preservation than in medical field [205,213], and the potent function of phenolics as perfect nature preservative and antimicrobial agents for food is very promising. In Turkish diet, unripe grape juice is used as flavoring and acidifying agent. It has acted as a practicable antimicrobial agent for salad vegetables unconsciously due to its immediate inhibition against *Salmonella typhimurium* [214].

3.7.9. Anti-inflammatory effects

Grape polyphenols have shown significant anti-inflammation effects on rats, mice and humans [37,40,215,216]. Extracts from grape skins and seeds inhibited mouse ear inflammation, edema, and polymorphonuclear leukocyte infiltration [217]. Procyanidins inhibit the release of proinflammation factors [29]. The anti-inflammation effect of grape phenolics has been mainly ascribed to immunomodulation and antioxidative action [37,40,218]. A remarkable decrease in NO concentration, prostaglandins E2 and ROS in human chondrocytes culture, compared to control groups, is achieved by treatment with a combination of wine extract and IL-1b. These effects were equal or greater to those of indomethacin [37]. Proanthocyanidins could prevent the increase of MDA in rat paws with arthritis induced by carrageenan [218]. NOS activity and N-acetyl- β -D-glucosaminidase were also successfully inhibited by proanthocyanidins.

Grape polyphenols modulates cytokine gene expression as basic pathway to anti-inflammation [40,216,218]. Human adipocytes and macrophage-like cell lines pre-treated with extracts of grape seed

procyanidins, produced less IL-6 and MCP-1 induced by inflammatory stimulus, and increased anti-inflammatory adipokine and adiponectin. Grape seed procyanidins inhibited the increase of C-reactive protein in rat plasma induced by high fat feed, and the same trend in IL-6 and TNF- α was detected in the mesenteric white adipose tissue [216].

3.7.10. Neuroprotective effects

Resveratrol has recently come to the attention due to its neuroprotective actions and activation of the sirtuins' family member SIRT1. The various functions of the sirtuins and its relationship with resveratrol effect will be further discussed below. Resveratrol has neuroprotective features both in vitro and in vivo in models of Alzheimer's disease (AD), but it has proved to be beneficial also in ischemic stroke, Parkinson's disease, Huntington's disease, and epilepsy [219].

No specific environmental risk factor has been definitively identified as being associated with Alzheimer's disease. However, the potentially important role for diet in the causation or prevention of AD is supported by several observations. For instance, there is evidence that homocysteine-related vitamins, fats, and red wine consumption have a role in the pathogenesis of AD [220]. Moderate to mild wine consumption was associated with a low risk of AD [221]. Wine intake by individuals aged 65 years and older was associated with a low risk of dementia, including AD [222,223]. Furthermore, a prospective analysis of risk factors for AD in the Canadian population determined that wine consumption was the most protective variable against AD by reducing its risk by 50% [224]. Interestingly, wine intake in this population was more protective than the use of nonsteroidal anti-inflammatory drugs [224]. Nevertheless, it should be noted that red wine intake lowers AD risk, although this view is still controversial and remains to be clearly demonstrated. Moderate red wine consumption lowers A β levels and the associated neuro-pathology in the AD mouse model, implying that red wine intake may have a beneficial effect against AD pathology by promoting anti-amyloidogenic mechanisms [225].

Resveratrol delays A β -induced toxicity in different neuronal culture models [226–228]. Independent biochemical approaches have demonstrated that the polyphenol has potent anti-amyloidogenic and anti-fibril effects in vitro [229–232], suggesting that resveratrol may act as an antioxidant by preventing the formation of toxic A β oligomers and protofibrillar intermediates [41].

3.7.11. Renoprotective effects

Kidney protection by polyphenols has also been reported. Both in rodents and humans red wine administration is associated with an increased antioxidant capacity of plasma. In addition, down regulation of rat kidney cytochrome P450 found in chronic exposure to alcohol free red wine could account for a diminished production of free radicals. This renal response to red wine exposure could contribute to an amelioration of the effects resulting from oxidative challenge, as shown by the decreased functional, biochemical and morphological renal damage induced by models such as ischemia-reperfusion and myoglobinuria known to cause acute renal failure [233]. It has been demonstrated that polyphenol rich red wine protects the kidney against rhabdomyolysis following glycerol injection. This renoprotective effect was significantly higher in chronic red wine intake, rather than alcohol free red wine or ethanol [234]. An enhancement of the antioxidant defenses could account for this wine effect [235]. Taking into account the pathogenic role of oxidative stress on kidney damage, it was suggested that the renoprotective effect of wine results from the actions of both ethanol and polyphenols [236].

3.7.12. Antiageing effects

It was found that polyphenolics present in foods might be beneficial in reversing the course of neuronal and behavioral ageing

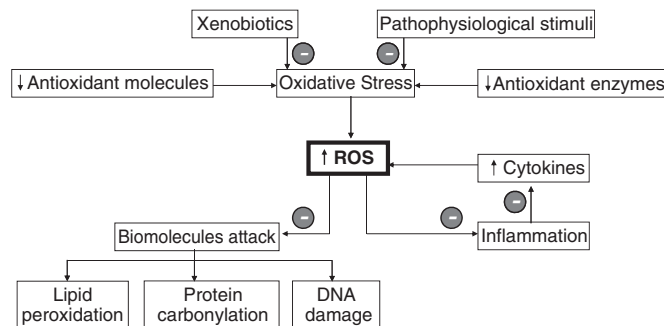


Fig. 1. Multiple wine polyphenol site of action against biomolecules oxidative damage. ROS, reactive oxygen species. $\ominus$: counteracting effect of polyphenols.

[29]. Due to their notable antioxidant activity, such as scavenging free radical, they could prevent organs and tissues from oxidative damage, and modify the body negative mechanism of redox status. Accordingly, supplementation with grape seed extracts inhibited the accumulation of age-related oxidative DNA damages in neural tissue [237] as well as a decreased incidence of free radical-induced lipid peroxidation in the central nervous system of aged rats [238].

Resveratrol is a potential candidate for preventing oxidative stress-induced aging in endothelial cells by preventing ROS-induced damage via increased endothelial silencing information regulator (SIRT1) expression. SIRT1 belongs to the Nicotinamide Adenine Dinucleotide (NAD $^{+}$) dependent histone deacetylase family, which regulates gene silencing. It has a fundamental role in the regulation of the cell cycle [239] and is an essential mediator of longevity in normal cells by calorie restriction [240]. Resveratrol might act as a dietary restriction mimetic by providing the health benefits of calorie restriction without requiring reduced food consumption [241].

Red wine possesses cardioprotective and nephroprotective effects not only mediated by eNOS upregulation but also through an enhancement of eNOS-independent NO production, involving the SIRT1 [240]. Resveratrol has been reported to activate SIRT1 through a mechanism dependent on AMP kinase, which has been related with increased longevity [242]. In addition, it has been reported to increase life span in several non-mammalian species, and to confer protection against a variety of aging-related maladies [243]. Furthermore, resveratrol via activation of the SIRT1 significantly decreases LPS-induced TNF, IL-6 and IL-8 gene expression and release, and COX-2 mRNA expression and resultant prostaglandin (PG) E2 and PGF2 α release in human tissues [244].

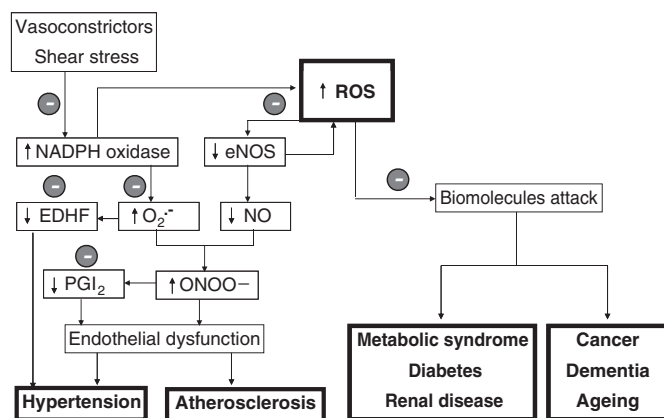


Fig. 2. Multiple wine polyphenol site of action against the mechanism of diseases and ageing mediated by oxidative stress. ROS, reactive oxygen species; EDHF, endothelium derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PGI $_2$, prostaglandin I $_2$; ONOO $^-$, peroxynitrite; O $_2^{\bullet -}$, superoxide anion; DNA, deoxyribonucleic acid. $\oplus$: counteracting effect of polyphenols.

A hypothesis accounting for the generation of oxidative stress, biomolecules attack by reactive oxygen species (Fig. 1), their involvement in the mechanism of development of human disease and the therapeutic targets for wine polyphenols (Fig. 2) is depicted.

A summary of current clinical trials of therapeutic use of polyphenols is presented in Table 3. The studies related with the mechanism of protection against human diseases, and their beneficial or adverse effects are listed according to their source of supplementation: red wine, grape juice, grape seed and other compounds [N1–NX]. Differences in the design of the studies and in the composition of the tested products (not always provided) could explain the dissimilar results of these studies. New dietary supplements, such as

SRT501, are currently being subjected to clinical trials (<http://clinicaltrials.gov/ct2/results?term=SRT501>).

4. Concluding remarks

Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of wine, fruits and vegetables. Red wine is protective against all-cause mortality. These protective effects could be due to one or many components of the complex mixture of bioavailable and bioactive compounds present in red wine including ethanol, resveratrol, flavonols, flavan-3-ols, anthocyanins, phenolic acids as well as their metabolites formed either in the tissues or in the colon by the microflora.

Table 3

Summary of current clinical trials of therapeutic use of polyphenols.

Compounds	Involved mechanism	Beneficial effects	Adverse effects	Reference
Red wine	Improvement of endothelial function by polyphenols	Systolic and diastolic blood pressure reduction in population at high cardiovascular risk	Not reported	[245]
	Increased plasma antioxidant capacity	Increased coronary flow reserve	Not reported	[246]
	Polyphenols-mediated attenuation of senescence and promotion of EPC adhesion, migration, and tube formation	Enhancement on circulating endothelial precursor cells number and improved function	Not reported	[247]
	Red wine polyphenols antioxidant ability	Reduced malondialdehyde, superoxide concentration in LDL particles and plasma oxidized LDL concentration	Reduced superoxide-dismutase activity	[248]
Armagnac	Polyphenols antioxidant effects	Increased HDL cholesterol, total antioxidant capacity and antioxidant compounds	Not reported	[51]
	Increased sympathetic output	Increased heart rate	Not reported	[249]
	Inhibition of ADP- and thrombin-induced aggregation by polyphenols	No difference in ovarian cancer incidence	Not reported	[250]
		Decreased platelet aggregation	Not reported	[251]
Red wine and grape extract Grape juice	Ethanol effect	Increased HDL cholesterol	Not reported	[252]
	Polyphenols vasodilating effects	Decreased systolic blood pressure 7.2 mm Hg and diastolic blood pressure in 6.2 mm Hg	Not reported	[253]
	Downregulation of neutrophil NADPH oxidase	Reduction on total cholesterol and LDL cholesterol	Not reported	[254]
	Improvement of endothelial function by polyphenols	Enhancement of flow-mediated vasodilation and reduction in LDL susceptibility to oxidation in coronary artery disease patients	Minor change in lipid profile	[255]
	Antioxidant and anti-inflammatory effects of polyphenols	Decreased oxidized LDL, apolipoprotein B100 and monocyte chemoattractant protein 1; increased HDL, apolipoprotein A1 and α -tocopherol in hemodialysis patients	Not reported	[46]
	Antioxidant-sparing and/or direct effects of polyphenols	Decreased platelet aggregation, increased platelet-derived NO release and decreased superoxide production	Not reported	[147]
	Platelet inhibitory effect of the polyphenols	Decreased platelet aggregation response to 1 mg/L of collagen by 77%	Not reported	[256]
	Protective polyphenols effect via increased thermogenesis and substrate oxidation	Weight gain prevention	Not reported	[257]
	Polyphenols antioxidant and anti-inflammatory properties and influence in neuronal signalling.	Improvement in a measure of verbal learning	Not reported	[258]
	Increased endothelial function and decreased oxidative stress	Enhancement of flow-mediated vasodilation	Not reported	[259]
Grape seed extract	Oxidative stress reduction	Cardioprotection by means of LDL cholesterol and TAG diminution	Not reported	[260]
	Platelet inhibitory effect of the polyphenols	Decreased platelet reactivity in smokers	Not reported	[261]
		No beneficial effect on flow mediated vasodilation, platelet function and blood lipids	Reported, considered not to be related with the supplementation	[262]
Grape extract	Enhancement of endothelial antioxidant status	Increased endothelial shear stress-induced vasorelaxation responses in healthy normal subjects	Not reported	[263]
Grape seed extract plus Vitamin C	Increased oxidative stress		Increased blood pressure (in combination)	[264]
Resveratrol	Cerebral vasodilation	Dose-dependent increases in prefrontal cerebral cortex blood flow during task performance and increased oxygen extraction	Not reported	[265]
Quercetin	Quercetin inhibition of src-family kinases, the tyrosine kinase Syk and PI3-K	Inhibition of platelet cell signaling and thrombus formation	Not reported	[266]
Topical Epigallocatechin-3-gallate	Decreased VEGF and HIF-1 α	Decreased facial erythema and telangiectasia	Not reported	[267]
Antioxidants including polyphenols	Reduction in NF- κ B and PPAR- γ activity	Reduced inflammation and oxidative stress	Diarrhea and change in bowel habits	[268]

Emerging findings suggest a large number of potential mechanisms of action of polyphenols in preventing disease, which may be beyond their conventional antioxidant activities. Red wine polyphenol metabolites affect the activity of different transcription factors playing a key role in the modulation of endothelial cell functions. The reported evidence of beneficial health effects of wine polyphenols includes inhibiting some degenerative diseases, such as cardiovascular diseases, neurodegenerative disorders, diabetes, and certain types of cancers, reducing plasma oxidative stress and slowing ageing. Definition of a safe therapeutic wine polyphenol dose having practical certainty that no adverse effects will be observed remains to be established. In addition, clinical studies confirming the health benefits of these compounds are still lacking. Therefore, future large scale randomized clinical trials should be conducted to fully establish the therapeutic use of each individual wine polyphenol against human disease.

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