

TOPICAL REVIEW

Effects of red wine polyphenolic compounds on the cardiovascular system

Zenebe W, Pechanova O

*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia. birhanuz@unpf.savba.sk***Abstract**

Phenolic phytochemicals are widely distributed in the plant kingdom. Regarding the protective effects on organisms, the polyphenol group is the most important. In different experiments, it has been shown that selected polyphenols, mainly flavonoids, possess protective effects on the cardiovascular system, as well as anticancer, antiviral and anti-allergic properties. In coronary heart disease, the protective effects include mainly antithrombotic, antioxidant, anti-ischaemic and vasorelaxant properties of flavonoids. It has been hypothesised that the phenomenon of a low incidence of coronary heart disease in French people may be partially related to the pharmacological properties of polyphenolic compounds included in red wine. Many epidemiological studies have shown that regular flavonoid intake is associated with reduced risk of cardiovascular diseases.

This review article discusses the chemical structure of polyphenols and their beneficial properties in the cardiovascular system. (Fig. 1, Ref. 74.)

Key words: polyphenols, flavonoids, antithrombotic effect, antioxidant activity, anti-ischaemic effect, vasorelaxant properties.

Consumption of diets high in saturated fat and cholesterol is associated with increased risk of cardiovascular disease. However, epidemiological evidence has shown that cardiovascular disease is less prevalent in the French than expected in the light of their saturated fat intake and serum cholesterol levels. This paradoxical finding has been attributed to regular consumption of fresh vegetables, fruit and red wine (1, 2).

Law and Wald (3), however, predicted that it would be only a matter of time before the “French paradox” resolved itself as the only recently comparable pattern of risk factors (animal fat consumption, serum cholesterol concentrations and blood pressure) between France and Britain would become translated into similar death rates from coronary disease. Although red wine consumption remains higher in France than in Britain, the authors rejected this as a possible explanation because they consider that wine intake greater than one unit a day confers no greater benefit (3). However, the protective effect of moderate consumption (2–3 units) of red wine on the risk of cardiovascular disease morbidity and mortality has been consistently seen in many epidemiological studies (2). Phenolic compounds and especially a group of flavonoids seem to be responsible for the majority of the protective effects of red wine. One of the major activities of

plant polyphenols is their antioxidant property that may explain many of their beneficial effects on cardiovascular function (4, 5, 6). Polyphenols also act on other targets involved in the metabolism of mammalian cells, including nitric oxide (NO), which by itself regulates haemostasis (7, 5), thrombus development (8) and vascular tone (9, 10). The beneficial properties of NO may therefore explain in part the anti-ischaemic activities of plant polyphenols.

The purpose of this review is to describe protective effects of red wine polyphenolic compounds on cardiovascular disease, particularly their antithrombotic, antioxidant, anti-ischaemic, vasorelaxant and antihypertensive properties and clinical application.

Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

Address for correspondence: Woineshet Zenebe, MVD, Institute of Normal and Pathological Physiology, SAV, Sienkiewiczova 1, SK-813 71 Bratislava 1, Slovakia.

Phone: +421.2.52926271, Fax: +421.2.52968516

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Chemical structure

From the chemical point of view, natural polyphenols are derivatives of chroman. There is a wide range of polyphenols which have desirable biological properties for man. Among them are flavonoids, phenolic acids, 3,4-trihydroxystilbens and leucocyanidols (11).

Due to the beneficial effects on the cardiovascular system, the group of flavonoids has been the most studied. The presence of over 4000 naturally available flavonoids is widespread among plants and plant products (5). Flavonoids are derivatives of phenylchroman. Depending on structural features, they can be divided into different subgroups: flavanes, flavanones, flavones, flavonoles and anthocyanidins. The basic structure is comprised of two benzene rings linked through a heterocyclic pyran or pyrone ring in the middle (Fig. 1). Flavonoids and tocopherols (vitamin E) share a common structure, i.e., the chromane ring (12). Flavonoids differ from one to another in the orientation of hydroxylation or methylation, in the position of benzoid substituent and in the degree of unsaturation. They usually occur in the form of glycosides. The characteristics of flavonoids appear also to be required especially for their antioxidant and antiproliferative activity (5, 12, 13).

As mentioned in different works, polyphenols act as reducing agents, hydrogen donating antioxidants and metal chelators. Since little is known about the relationship between their structure and antioxidant activity, Cao et al (14), Arora et al (15) and Wang and Joseph (16) examined structurally different polyphenols. They reported that hydroxyl substituents on the flavonoid ring increased the antioxidant activity while substitution by methoxy groups decreased this activity. Mabry et al (17) and Markham (18) reported the relationship between spectroscopic identification and structural characteristics of polyphenols. According to their measurements, most flavones and flavonols showed two absorption bands in ultraviolet visible region, representing B ring and A ring absorptions. They also observed that an increase in the numbers of hydroxyl groups results in a red shift, for example, from 367 nm in kaempferol with hydroxyl substitutions in positions 3,5,7,4' to 371 nm in quercetin with hydroxyl groups in positions 3,5,7,3',4'. The shorter wave length in flavones is the result of the absence of a 3-OH group in their structure. In flavanones with a saturated heterocyclic C ring, lack of conjugation between ring A and B is defined by the very strong maximum UV spectra and with lower antioxidant activity (14, 15).

A flavonol, quercetin, has the same number of hydroxyl groups in the same positions as catechin which is a flavane, hence it also contains the 2,3 double bond in the C ring and the 4-oxo junction. This conjugation of 3-OH group with the 2,3-double bond adjacent to the 4-carbonyl in the C ring gives to quercetin its antioxidant activity (19). Shahidi et al (20) and Rice Evans et al (19) reported that flavanones with only one hydroxyl group on the B ring possessed little antioxidant activity. As a result of the unsaturated heterocyclic ring quercetin has a two fold higher antioxidant activity than the saturated heterocyclic ring of catechin (19).

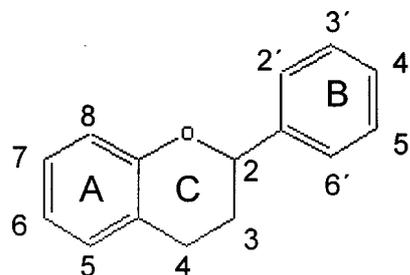


Fig. 1. The three ring structure of flavonoids.

Other authors also reported that the position and degree of hydroxylation is the key to the antioxidant activity of flavonoids (21, 22). Bors et al (23) and Sichel et al (24) generalised that for flavonoids to accomplish radical scavenging activity they need the o-dihydroxy structure in the B ring which gives a higher stability to the radical form and participates in electron delocalisation. The 2,3 double bond in conjugation with a 4-oxo junction in the C ring is responsible for electron delocalisation from the B ring, while the 3- and 5-OH groups with 4-oxo junction are responsible in A and C rings. It was also reported that glycosylation of flavonoids reduces their activity compared to the aglycones (20).

It can be concluded that the structure of different flavonoids is responsible for their different reducing capacity and antioxidant activity.

Antithrombotic effects

Polyphenols have been shown to be able to modulate the process of thrombosis in several systems. Fuster et al (25) reported that reduction of the rate of atherosclerosis and coronary heart disease caused by daily intake of flavonoids was based mainly on the ability of flavonoids to inhibit acute thrombus formation.

In both platelets and leukocytes, interference with the arachidonic acid metabolism has been demonstrated, resulting in inhibition of platelet aggregation and reduction of prothrombic and proinflammatory mediator synthesis in humans (26, 27) and also in experimental models (28, 29). In humans, Pace-Asciak et al (27) showed that polyphenolic compounds from red wine, especially quercetin, catechin and resveratrol, inhibited the synthesis of thromboxane in platelets and of leukotrienes in neutrophils. In their experiments, resveratrol and quercetin exhibited a dose-dependent inhibition of thromboxane-induced and ADP-induced platelet aggregation, while epicatechin, α -tocopherol and butylated hydroxytoluene were inactive. *Trans*-resveratrol also inhibited the synthesis of thromboxane B₂, hydroxyheptadecatrienoate, and slightly of 12-hydroxyeicosatetraenoate (12-HETE). Alcohol-free red wine inhibited the synthesis of thromboxane B₂

but not that of 12-HETE. Other investigators studying platelet aggregation and lipid levels in humans consuming red or white wine found an increase in high density lipoprotein (HDL) levels in both groups and a decrease in ADP-induced platelet aggregation in humans consuming red wine (30).

With the goal of studying the platelet inhibitory effects of different compounds including wine, the Folt's coronary thrombosis model of platelet aggregation and thrombus formation based on measurement of cyclic reductions in coronary flow (CFRs) in mechanically stenosed coronary artery was used (28). The authors showed that CFRs were eliminated by red wine and grape juice when given intravenously as well as intragastrically, however a 2.5-times greater amount of grape juice than red wine was needed for the elimination of CFRs. In the case of white wine, the elimination of CFRs was not significant. These results suggest that there are antiaggregative compounds present in red wine and grape juice that are absent in white wine. Quercetin and rutin were also found to eliminate CFRs in the same model. Measurement of quercetin, rutin and resveratrol content of red wine, white wine and grape juice indicated that the flavonoid content was severalfold higher in red wine and grape juice compared to white wine (28, 8). Red wine polyphenols were capable of reducing the level of thromboxane A_2 , similar to aspirin. Polyphenols had a shorter-term effect on coronary blood flow in contrast to aspirin, however they could interfere with glycoprotein receptors on endothelial cells. As a result of this interference, platelets were not able to adhere on the vessel wall and therefore, thrombus formation was inhibited (31).

Ruf et al (29) reported that except decreasing prostanoid synthesis from arachidonate, wine polyphenols were able to reduce platelet activity by nitric oxide mediation. In agreement with this study, Wollny et al (8) found that red wine supplementation, either after 10-day or acute administration in rats, markedly prolonged bleeding time and inhibited platelet adhesion to fibrillar collagen by NO-dependent mechanism. N^G -nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO formation, prevented these effects of red wine. The possibility to revert the effect of L-NAME by L-arginine, the substrate for NO synthase, but not by its stereoisomer D-arginine, strengthens the role of NO in red wine-induced effects. Regarding the mechanisms of NO increase, it is possible that red wine polyphenols decrease degradation of basal levels of NO, preventing its destruction by superoxides and/or stimulate NO synthase in endothelial cells. It is conceivable that both mechanisms are active in *in vivo* conditions (10, 8).

Adhesion of platelets to the subendothelial matrix, after vessel damage, is a triggering mechanism of thrombus formation, and thus platelet inhibition by red wine could partially explain the prevention of thrombus growth.

Antioxidant activity

Increase in reactive oxygen species in the organism leads to oxidative stress with subsequent damage of many biological molecules. Proteins, DNA and lipids of cell membranes are significant targets of cellular injury (32). Lipid peroxidation *in vivo* involves

a radical chain reaction leading to lipid hydroperoxides, which can stimulate vascular cell to produce monocyte-chemotactic and macrophage-stimulating factors, resulting in formation of so-called foam cells and atherosclerotic plaques (32, 33). Oxidised low-density lipoproteins (LDLs) also play a role in thrombus development, since they stimulate procoagulant activities in endothelial cells and monocytes and inhibit vasodilatation by decreasing expression of endothelial NO synthase. In the prevention of cardiovascular disease, many of the observed effects of polyphenols can therefore be attributed to their recognised antioxidant and radical scavenging properties, which may delay the onset of atherogenesis by reducing chemically and enzymatically mediated peroxidative reactions (34). According to the work of Rice Evans and Miller (35), which contributed to the understanding of structure-activity relationship of the antioxidant effects of flavonoids, quercetin, the common red wine flavonoid, appears to be an extremely efficient radical scavenger. Indeed, reduced progression of atherosclerosis in apolipoprotein E-deficient mice was shown after consumption of quercetin or red wine. Reduction in atherosclerosis progression was associated with decreased susceptibility of LDL to oxidation and aggregation (36). The studies of De Whally et al (37) and Miyagi et al (38) showed that red wine significantly inhibited Cu^{2+} -catalysed LDL oxidation, yet white wine and beer failed to do so. Similarly to red wine, grape juice with a large amount of flavonoids also significantly inhibited oxidation of LDL. However, in the experiment on 20 volunteers, the antioxidant activity was not significantly increased after ingestion of grape juice, suggesting that flavonoids in red wine can be absorbed from the intestine more easily than those in grape juice. It was concluded that the antioxidant properties of flavonoids concerning LDL oxidation were associated with absorption of flavonoids from the intestine into the circulation (6). Recently, Nidgikar et al (39) confirmed the finding of De Whally et al (37) and Miyagi et al (38), showing that 2-week consumption of red wine, but not white wine, enhanced antioxidant capacity, as measured by decreased plasma lipid peroxides, conjugated dienes and Cu^{2+} -catalysed peroxidation of LDL. Serafini et al (40) showed that the total radical-trapping antioxidant capacity of red wine was at least 20-times stronger than that of white wine. Frankel et al (4) reported that quercetin and *trans*-resveratrol were more effective than α -tocopherol in inhibiting the oxidation of human LDL. Moreover, flavonoids may protect α -tocopherol from oxidation by being themselves oxidised by free radicals or by regenerating active α -tocopherol (12). The antioxidant activities of flavonoids and their glycosides were even higher than those of vitamin C and E (19). Red wine consumption was also found to increase plasma HDL levels characterised by their antiatherogenic effects (41). All the mechanisms by which red wine polyphenols exert their antiatherogenic effect appear to be crucial in the prevention and treatment of cardiovascular disease.

Anti-ischaeamic activity

The protective role of flavonoids in cardiac ischaemia may be related mainly to their ability to scavenge oxygen free radi-

cals (42), to maintain NO concentration (43, 10) and to inhibit mast cell secretion (44).

The study of Sato et al (42) found that an ethanol-free red wine extract as well as *trans*-resveratrol protected the hearts from detrimental effects of ischaemia/reperfusion injury, as evidenced by improved postischaemic ventricular function and reduced myocardial infarction. Both the red wine extracts and *trans*-resveratrol reduced the oxidative stress in the heart, as indicated by decreasing malondialdehyde formation. These compounds were also found to be highly effective in direct scavenging of peroxyl radicals. Direct perfusion of ethanol into the hearts may however cause development of oxidative stress resulting in a completely different effects on the hearts (45). A reduction effect of several flavonoids on acute regional myocardial ischaemia in isolated rabbit hearts was also reported (45). Ning et al (46) showed that flavone administration improved functional recovery in the reperfused heart after a bout of global ischaemia. The effect of flavone on postischaemic recovery was proposed to be caused by its stimulation of the cytochrome P450 system. It has been suggested that flavone might act as an allosteric effector of cytochrome P450 reductase, which improves catalytic efficiency, thereby diminishing production of free radicals. Quercetin was reported to exert a protective effect by preventing the decrease in the xanthine dehydrogenase/oxidase ratio observed during ischaemia-reperfusion in rats (47). Inhibition of xanthine oxidase by flavonoids was also described (48). In addition, flavonoids were found to possess positive chronotropic and antiarrhythmic effects and to minimise mitochondrial ischaemia/reperfusion injury (49).

Since changes in NO concentration were found during ischaemia-reperfusion in rats (50), the effects of red wine polyphenols on NO stability and generation are crucial in prevention against ischaemia. Recent studies reported an increase in NO synthase activity due to the red wine polyphenolic compounds treatment in the heart (51) and also in aorta (51, 52). The protective effects of flavonoids in cardiac ischaemia are also associated with their ability to inhibit mast cell secretion, which may be involved in cardiovascular inflammation, at present considered one of the key factors in coronary artery disease (44). Quercetin and some other flavonoids were found to inhibit the release of rat mast cell histamine in a concentration-dependent manner (53, 54).

Vasorelaxant activity

The idea that phenolic compounds in grape juice, red wine and in some other beverages have a protective effect on cardiovascular disease led investigators to think about the effect of these compounds on vascular functions. Polyphenolic compounds have the ability to relax precontracted smooth muscle of aortic rings with intact endothelium, moreover, some of them are able to relax endothelium-denuded arteries (9, 10).

Fitzpatrick et al (9) reported that in aortic rings with endothelium, skin extracts from red grapes caused relaxation. On the contrary, in endothelium-denuded aortic rings, relaxation was

not observed. However Andriambeloson et al (10) reported relaxation induced by leucocyanidol and catechin in aortic rings both with and without endothelium. However a 1000-times greater amount of these substances was needed to induce relaxation in endothelium-denuded arteries. N^G-monomethyl-L-arginine and N^G-nitro-L-arginine, inhibitors of NO synthesis, reversed the relaxation induced by grape skin extracts. Using electron paramagnetic resonance, acute administration of red wine polyphenolic compounds was found to elevate NO synthesis in the endothelium (55). However, the mechanism of NO synthase activation by red wine polyphenolic compounds is not satisfactorily understood. Recently, Andriambeloson et al (51) observed that red wine polyphenolic compounds produced NO-induced Ca²⁺-dependent vasorelaxation of the rat aorta. Martin et al (56) suggested that the rise in intercellular Ca²⁺ involves both Ca²⁺ release and Ca²⁺ entry, the latter being an essential step for NO production in the endothelial cells. However, the mechanisms of Ca²⁺ handling among different polyphenolic compounds could differ with regard to the type of intracellular Ca²⁺ stores mobilized and the nature of G proteins implicated. It has been suggested that phospholipase C and tyrosine kinase pathways are involved in the Ca²⁺ signalling (56).

Since red wine polyphenols consist of hydroxycinnamic acid, proanthocyanidins, anthocyanins, flavanes, and flavonols, the question which substance(s) may be responsible for the elevation of NO synthesis had to be addressed. From anthocyanin-enriched wine extracts, aglycone-, monoglycoside- as well as diglycoside-enriched fractions were capable to induce endothelium-dependent vasorelaxation, similar to that elicited by original red wine polyphenolic extract. Of the anthocyanins, only delphinidin, but not cyanidin or malvidin, mimicked the effect of the original extract (57). The representative derivatives of phenolic acid (benzoic, vanillic and gallic acid), hydroxycinnamic acid (*p*-coumaric and caffeic acid), flavanols (catechine and epicatechine), as well as the higher polymer-enriched fraction of condensed tannins failed to induce endothelium-dependent vasorelaxation (57, 58).

Mechanisms implicated in the vasorelaxant effects of flavonoids may include also inhibition of cyclic nucleotide phosphodiesterases (59) and activation of Ca²⁺-activated K⁺ channels (60), both pathways are linked to the effects of flavonoids on smooth muscle cells rather than the endothelium. Ferrell et al (61) proposed that the flavonoid inhibitory activity on phosphodiesterases could be ascribed to the structural mimicry of the purine ring in cAMP and cGMP and the pyranone ring of active flavonoids. Both increase in NO synthase activity and decrease in phosphodiesterase activity may lead to increase in cGMP concentration resulting in vasorelaxation and inhibition of platelet aggregation.

Antihypertensive effect

The ability of polyphenolic compounds to activate the NO-cGMP system seems to be associated also with their antihypertensive effect. The effects of the red wine extract provinol was

tested on the experimental model of L-NAME-induced hypertension, which was developed by long-term inhibition of NO synthase activity. Provinol partially prevented increase in blood pressure when given simultaneously with NO synthase inhibitor L-NAME (51). In rats with developed hypertension, provinol-treatment resulted in a greater readiness to blood pressure decrease and higher elevation of NO synthase activity in the heart and aorta compared to rats with spontaneous recovery (62). Similarly, Hara (63) and Mitzuani et al (64) reported that in vivo administration of an extract of polyphenolic compounds from tea and wine, respectively, resulted in the former study in an attenuation of elevated blood pressure in spontaneously hypertensive rats, and in the later study in a reduced blood pressure and decreased risk of stroke in susceptible rats. The improvement of the biomechanical properties of aorta, lowering of cholesterol levels and inhibition of LDL oxidation were suggested as the mechanisms responsible for blood pressure reduction (63, 64). Recently Diebolt et al (65) found that short term oral administration of red wine polyphenolic compounds produced a decrease in blood pressure in normotensive rats. This hemodynamic effect of red wine polyphenolic compounds was associated with an augmentation of endothelium-dependent relaxation and a modest induction of gene expression of inducible NO synthase and cyclooxygenase 2 within the arterial wall which together maintained unchanged agonist-induced contractility (65).

The effects of decaffeinated green tea were studied on mice exposed to various intensities of social stress associated with hypertension. In decaffeinated green tea drinking mice, blood pressure fell from 150 to 133 mmHg. It is speculated that the sympathetic adrenal medullary system may be involved in the mechanisms of blood pressure reduction in this model since the heart rate was also affected (66). The mechanisms by which red wine polyphenols exert their antihypertensive effect appear to be important in the prevention and treatment of cardiovascular disease.

Clinical application

Several reports have documented the protective effects of natural polyphenols present in wine or different fruits and vegetables in human volunteers.

In 1993 Hertog et al (67) investigated the effect of high flavonoid intake (42 mg) in 800 human volunteers and reported a lower risk of dying from coronary heart disease than in those receiving only 12 mg of flavonoids (67). Later in 1997, the authors completed the work with results strengthening the previous one (68). Consumption of 400 ml red wine in 17 human volunteers resulted in reduction in the susceptibility of plasma and low density lipoprotein to lipid peroxidation in the presence of free radical generating substances (69).

The Copenhagen city heart study in 13285 humans, which conducted from 1976 until 1988 studied beer, spirits and wine with respect to their relation to mortality from cardiovascular disease. The study showed that unlike beer and spirits, moderate drinking of wine was associated with a decrease of death from

coronary heart disease (1). A cohort study based on data collected at the Finnish mobile clinic was carried out over 27 years. Similar to Grønbaek et al (1) these authors reported an inverse relation between flavonoid intake and mortality from coronary heart disease. The major sources of flavonoids in this study were apples and onions (70). The protective effect of tea polyphenols as a result of their antioxidative effect has been done in human volunteers by Serafini et al (71) and they expressed both green and black tea as the source of the antioxidant polyphenols.

Flavonol and flavone intake and the risk of stroke had been studied in a group of 550 men for 15 years. The result showed that men with the highest intake of flavonol and flavone showed a decreased risk of coronary heart disease (72). A non-significant inverse association between flavonoid intake and mortality from coronary heart disease was found in men with previous history of coronary heart disease (73). Schmidt et al (74) reported a decrease in blood pressure and heart rate in men with chronic heart disease who received flavonoids from hawthorn extract.

To summarise the above mentioned results, epidemiological evidence demonstrates that regularly consumption of flavonoids reduces the risk of cardiovascular disease.

As shown in the above review, it is evident that natural polyphenolic compounds possess antithrombic, antioxidant, anti-ischaemic, vasorelaxant and antihypertensive properties. All these effects improve cardiovascular functions and potentially result in decreased morbidity and mortality due to the coronary heart diseases. Red wine, as opposed to other sources of polyphenols is unique in a number of selected dietary polyphenols as well as in their ingestion and bioavailability.

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