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Flavonoids as Potent Phytochemicals in Cardiovascular Diseases Prevention

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ABSTRACT

Flavanoids are the major plant phenolic secondary metabolites and represent the most studied phytochemicals. This family includes five major classes different by their specific chemical structure,we note: flavones, flavonols, flavanones, flavanols and anthocyanidins. Many epidemiological and experimental studies have shown that flavonoid intake is inversely correlated with cardiovascular disease events. In this field, a number of flavonoids from medicinal plants, fruits, vegetables, spices and beverages have been shown to beneficially affect parameters associated with cardiovascular diseases, including hyperlipemia and especially hypercholesterolemia, low density lipoprotein oxidation, blood platelet aggregation, and vascular reactivity. Therapeutic effects of flavonoids on lipid metabolism disorder have been attributed to effective diminution of total cholesterol, Triglycerides, LDL-Cholesterol and Apo B levels. On the other hand, their beneficial effect results also in an increase of HDL-cholesterolol, bile acids secretion and lipid catabolism. Furthermore, flavonoids play a pivotal role in preventing CVD related to oxidative stress via four distinct mechanisms including direct free radicals scavenging, pro-oxidant metal ions chelating, lipid peroxidation attenuation by recycling other antioxidants and preservation of the HDL-associated paraoxonase activity which has been shown to prevent oxidation of LDL particles. Moreover, flavonoids are able to inhibit cellular enzymes, implicated in cell-mediated oxidation of LDL particles including phospholipase A_2, cyclooxygenase and lipoxygenase, GSH reductase, xanthine oxidase and NADPH-oxidase. Flavonoids also protect platelet aggregation and related vascular complications through an increase of cAMP levels leading to intracellular Ca^{++} lowering, an inhibition of thromboxane formation and thromboxane receptor antagonism. Plus, these phytochemicals are able to play an important role on the endothelial function in regard to preventing hypertension via eNOS stimulation. The promising findings on biological activities of plant flavonoids can guide studies of the biochemical mechanisms underlying the protective effect of these compounds at arterial and cardiac levels to uncover new strategies for the treatment of vascular diseases and cardiac related complications.

KEY WORDS - Flavonoids, cardiovascular diseases, hypercholesterolemia, LDL oxidation, vasorelaxation, platelet aggregation.

INTRODUCTION

Flavonoids constitute one of the most characteristic classes of compounds in plants. Many flavonoids are easily recognized as flower pigments. However, their occurrence is not restricted to flowers but include all parts of the plant such as roots, leaves and stems. Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms and they can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring. This class consists of 5 major subgroups: flavones, flavonols, flavanones, flavanols and anthocyanidins **(Fig. 1)**. The average consumption of polyphenol flavonoids with the diet is 1 g per day and the main sources are fruits, beverages such as tea, coffee, wine and fruit juices, chocolate and, to a lesser extent, vegetables, cereals and legume seeds (1, 2). Today, flavonoids are to receive considerable interest for their presumed role in the prevention of various degenerative diseases such as cancers and cardiovascular diseases (3, 4).

Flavonoids have been identified as the potent hypolipidemic agents in a number of experimental studies. These molecules seemed to be related to a better plasma lipid and lipoprotein profile which is negatively correlated with incidence of cardiovascular diseases (5, 6, 7). Furthermore, as reported by many investigators, flavonoids from medicinal and aromatic plant possess a high anti-oxidant potential due to their hydroxyl groups and protect more efficiently against free radical-related diseases such as atherosclerosis (8, 9, 10). The beneficial effect of these compounds at the vascular endothelium level is now well documented. The consumption of flavonoids may prevent the endothelial dysfunction by enhancing the vasorelaxant process leading to the reduction of arterial pressure (11, 12, 13). Flavonoids act also at the blood platelet level and prevent platelet activity-related thrombosis. In this field, a significant negative correlation between flavonoids intake and incidence of many types of CVD involving platelet function has been reported in a number of epidemiological, clinical and experimental researches (14, 15, 16).

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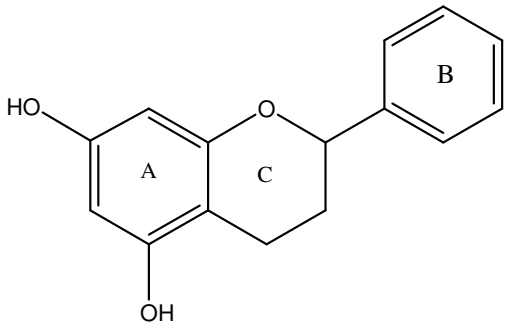
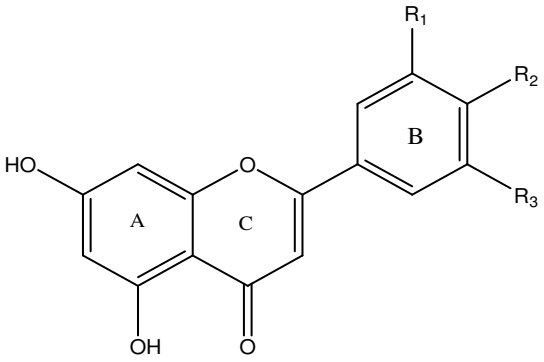
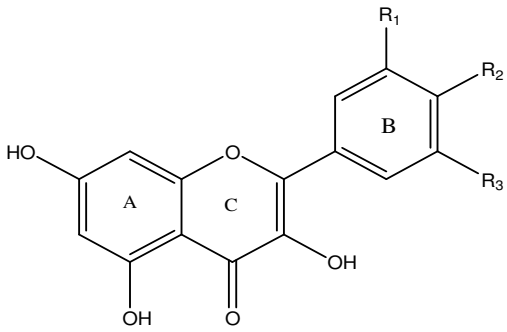


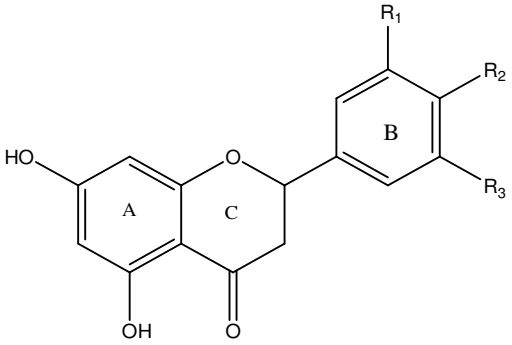
Base structure



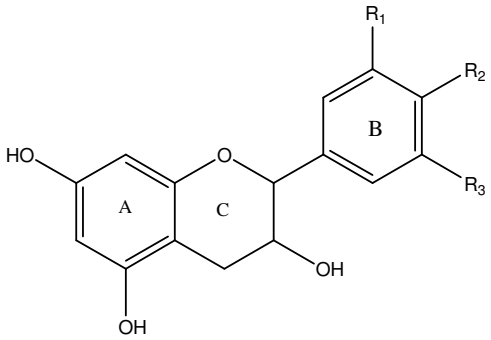
Flavones
Luteolin R_1: H, R_2: OH, R_3: OH
Apigenin R_1: H, R_2: OH, R_3: H



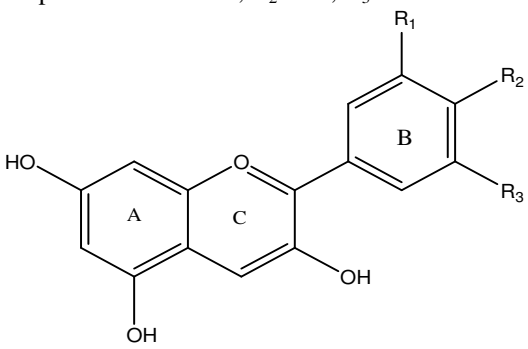
Flavonols
Quercetin R_1: H, R_2: OH, R_3: OH
Myricetin R_1: OH, R_2: OH, R_3: OH
Kaempferol R_1: H, R_2: OH, R_3: H



Flavanones
Cyanidin R_1: H, R_2: OH, R_3: OH
Malvidin R_1:OCH_3, R_2: OH, R_3: OCH_3
Delphinidin R1: OH, R_2: OH, R_3: OH



Flavanols
Catechin R_1: H, R_2: OH, R_3: OH
Epigallocatechin R1: OH, R_2: OH, R_3: O H



Anthocyanidins

Fig. 2 Chemical structure of some known flavonoids

In this review, we gave interest in the first time, on cardiovascular effects of the better known flavonoids including lipid metabolism, low density lipoprotein (LDL) oxidation, vasorelaxation-hypertension and platelet aggregation. Secondly we have elucidated the possible mechanisms by which these compounds can improve cardiovascular system dysfunction.

FLAVANOIDS AND LIPID METABOLISM

Flavonoids have been identified as the hypolipidemic components in a number of traditional ethnic remedies and this activity has been confirmed by many experimental studies. In fact, Narender et al. have isolated three known furanoflavonoids and a rare flavonoid glycoside for the first time from the aerial parts of *Indigofera tinctoria* and discovered the potent antidyslipidemic activity of isolated compounds in high fat diet (HFD) fed dyslipidemic hamsters (7). The results disclose that the treatment with mixture of flavonoids significantly decreased the plasma triglycerides (TG) total cholesterol (TC), glycerol (Gly), and free fatty acid (FFA) accompanied with increase in high density lipoproteins-cholesterol (HDL-C) which is negatively correlated with incidence of CVD.

The effects of favonoids on lipid parameters may be appear both at plasma and/or hepatic levels. Indeed, effect of naringenin on hepatic neutral and polar lipids were examined in rats that were fed either a high cornstarch or a high coconut oil diet, with or without supplementation with the assessed flavonoid for 40 days. When naringenin was added to the coconut diet, liver total lipids, and total neutral and polar lipids were significantly decreased.Neutral lipids, cholesterol ester, triglycerides, free fatty acids, mono- and diglycerides, and cholesterol levels were markedly reduced. Cholesterol ester and TG were more significantly decreased than cholesterol, while free fatty acids decreased most notably. Furthermore, naringin significantly suppressed the elevated levels of polar lipids induced by the high coconut oil diet. Authors concluded that the dietary naringenin supplementation significantly reduced both hepatic neutral and polar lipids in rats fed a high coconut oil diet, but not in the cornstarch-fed rats (17). Also, isoflavone seemed to be related to a better plasma lipid and lipoprotein profiles. Really, isoflavones from soy caused a significant decrease in the levels of plasma total lipids, total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein. Furthermore, plasmatic high density lipoprotein concentration was showed to be elevated (6).

In an experimental study designed by Lee et al. in order to understand the possible mechanism underlying the hypolipidemic effect of citrus biflavonoid naringenin, authors demonstrate that this phenolic lowers the plasma and hepatic cholesterol concentrations by suppressing the activity of the too key enzymes involved in cholesterol metabolism, hydroxymethyl glutaryl-CoA (HMG-CoA) reductase and acylCoA cholesterol acyltransferase (ACAT), and by the excretion of fecal neutral sterols in rats fed a high-cholesterol diet (5). The same mechanism on lipid metabolism was noticed with flavonoids from *Garcinia cambogia*, these phytochemicals significantly lowered lipid levels in rats fed normal and cholesterol-containing diets. HMG-CoA reductase showed significant reduction in normocholesterolemic rats. Highly stimulated activities of the enzymes lipoprotein lipase (LPL) and plasma ACAT were noted in flavonoid-administered animals. Hepatic and fecal bile acids and fecal neutral sterols were elevated substantially, indicating a higher rate of degradation of cholesterol. Thus, hypolipidemic activity of these flavonoids involves diminution of lipogenesis pathway and augmentation of lipid degradation rate (18). Furthermore, the effect of flavonoids on ACAT activity was recently confirmed by Lee et al. (19). Moreover, Jung et al. studied the mechanisms whereby these compounds exert their hypolipidemic action in type-2 diabetes. This study investigated the effect of the flavonoids hesperidin and naringin on lipid regulation in C57BL/KsJ*db*/*db* mice. The two flavonoids also led to a decrease in the plasma and hepatic cholesterol levels that have been partly due to the decreased hepatic HMG-CoA reductase and ACAT activities and increased fecal cholesterol secretion (20). This pathway was also investigated in human hepatocytes. The main active chemical of *Polygonum cuspidatum*, resveratrol, decreased ACAT activity in a dose-dependent manner. The results strongly suggest that the flavonoid reduces the cholesteryl ester by inhibiting ACAT (21). Again, the hesperetin played potent role in plasma lipid-lowering activities *in vivo* via a concomitant reduction in cholesterol biosynthesis and esterification as indicated by a decrease in HMG-CoA reductase and ACAT activities (22).

The effect of dietary hesperetin on the hepatic lipid content and the enzyme activities involved in triacylglycerol (TG) synthesis in rats fed diets with or without orotic acid was also studied. Cha et al. demonstrated that the compound reduces the hepatic TG accumulation induced by orotic acid and this was associated with reduced activity of TG synthetic enzyme, phosphatidate phosphohydrolase (PAP) (23).

Alterations in hepatic fatty acid oxidation constitute another crucial factor in the modification of serum lipid levels (24, 25). There is a possibility that flavonoids affect hepatic fatty acid oxidation and, hence, lower serum lipid levels. Some plant phenolic compounds have been reported to increase the activity and gene expression of enzymes involved in hepatic fatty acid oxidation in experimental animals (25, 26). Effectively, naringenin has the physiologic effect of increasing hepatic fatty acid oxidation through upregulation of gene expression of enzymes involved in peroxisomal-oxidation in mice. The change may account for the propensity of this compound to lower serum lipid levels (27).

Another mechanism was involved in the lipid lowering activity of phenolic flavonoids. In this case, the role of flavonoid taxifolin was examined on several aspects involving apolipoprotein B (apo-B) secretion and triglyceride (TG) availability in HepG2 cells. Taxifolin reduced apoB secretion by limiting TG availability via diacylglycerol acyltransferase (DGAT) and microsomal triglyceride transfer protein (MTP) activity (28). This mechanism was previously examined by Borradaile et al. (29). Authors conducted an experimental

study to determine if genistein and daidzein regulate hepatocyte cholesterol metabolism and apo-B secretion in cultured human hepatoma (HepG2) cells. The data found indicate that the studied flavonoids inhibit hepatocyte apoB secretion through several mechanisms, including inhibition of cholesterol synthesis and esterification, inhibition of expression of MTP and its activity and increased expression of the LDL-receptor. Also, these findings apparently validate the *in vitro* study undertaken by Borradaile et al. (30) demonstrating that hesperetin and naringenin dose-dependently reduced net apoB secretion by HepG2 cells through the inhibition of cholesteryl ester synthesis.

FLAVANOIDS PROTECT LOW DENSITY LIPOPROTEIN AGAINST OXIDATION

Low-density lipoprotein (LDL) is the major cholesterol-esters carrying lipoprotein in plasma (60% of total cholesterol). Linoleate is the predominant unsaturated fatty acyl group esterifying cholesterol in LDL particles. Because of this, The LDL is very susceptible to oxidative attack by free radicals (31).

The contribution of oxidized LDL on progression of atherosclerosis process and related cardiovascular events is now well documented (32, 33, 34, 35, 36). In fact, according to the oxidative-modification theory, when the LDL is oxidized in the subendothelial space of arterial wall (where it is protected from plasma antioxidants); it becomes "ox-LDL" which is very atherogenic and cytotoxic to vascular cells because of its high lipoperoxidation products content (37, 38, 39). To elucidate the mechanism by which the LDL is modified and implicated in the physiopathology of atherosclerotic plaques, a number of studies were undertaken and clearly indicate that the LDL peroxidation is initiated by free radical attack on a double bond of polyunsaturated fatty acids and removal of a hydrogen atom from a methylene (CH2) group, the rate of which indicates the rate of initiation phase of the oxidation process. The stabilization of the resulted unstable carbon radical by molecular rearrangement produces a more stable configuration called conjugated diene. This molecule reacts quickly with molecular oxygen to produce peroxyl radicals which are crucial intermediates in the general scheme of lipid peroxidation (40). A peroxyl radical in LDL may gain another hydrogen atom from an adjacent unsaturated fatty acid to form a hydroperoxide and another lipid radical, this reaction results in chain propagation. In addition, the gain of hydrogen atoms by the peroxyl radicals from other lipids such as cholesterol leads to oxysterol production. The resulting lipid hydroperoxides, after fragmentation, produce shorter-chain aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal which are potential reactive substances can bind to e-amino groups of the LDL apoprotein B-100 giving it an increased total negative charge. This alteration decreases the specificity of classical receptor (B/E) to LDL. In fact, LDL receptor recognizes a specific domain of positive charge from amino acid residues on apo-B including arginine, lysine and histidine. While, an increase in negative surface charge on apo-B 100 in response to the oxidative modification lead to the recognition of ox-LDL by the scavenger receptor and their preferential accumulation by macrophages which are transformed on foam cells, a key step on atherosclerotic plaque formation (40).

Antioxidants can scavenge and react with free radicals, and then terminate the free radical reaction. In this field, the suppression of the oxidative modification of LDL by anti-oxidants constituted one of the major targets of many anti-atherogenic agents and the preferable strategy to prevent the crises of coronary artery disorder (41, 42). Flavonoids may help to provide protection against cardiovascular diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. In addition, many experimental investigations have demonstrated that flavonoids from medicinal and aromatic plant possess a high anti-oxidant potential due to their hydroxyl groups and protect more efficiently against some free radical-related diseases (8). Indeed, flavonoids having more hydroxyl groups, or *ortho* hydroxyl groups, are more effective antioxidants. The B ring of these phenolic secondary metabolites is more electron rich than the A and C rings. That is why the B ring is primarily attacked by radicals. Hydroxyl groups act as electron donating substituent, and *ortho* hydroxylation helps to stabilize the phenoxyl radical (43, 44).

On the other hand, the efficacy of this phenolic antioxidant depends on the resonance stabilization of the phenoxy radical. This is influenced by the substituent linked to the aromatic ring (43, 44). Substitution at *ortho* and *para* positions (relative to the hydroxyl group) increases the reactivity and the resulted radicals are more stable.

Many investigators have proposed some mechanisms to explain the antioxidant activity of flavonoids. Firstly, these phenolic compounds may directly scavenge free radicals and consequently break chain reaction of lipid peroxidation (9, 10). Secondly, flavonoids may also chelate pro-oxidant metal ions such as iron and copper stimulating free radical formation (9, 10, 45). Thirdly, they may attenuate lipid peroxidation by recycling other antioxidants such as α-tocopherol (46). Finally, this phytochemicals may preserve the HDL-associated paraoxonase activity which has been shown to prevent oxidation of LDL particles (47). Moreover, Aviram and Fuhrman (48) demonstrate that flavonoids are able to inhibit cellular enzymes, implicated in cell-mediated oxidation of LDL particles including phospholipase A2, cyclooxygenase and lipoxygenase, GSH reductase, xanthine oxidase and NADPH-oxidase.

Recently, Park et al. (49) demonstrated that flavonoids from *Cudrania tricuspidata* exhibited significant antioxidant activity against LDL oxidation in thiobarbituric acid reactive substances (TBARS) assay. The result of an other study shows that the flavonoids of *Eriobotrya japonica* present strong ability to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and to attenuate human LDL peroxidation in vitro (50).

Rutin, one of ubiquitous flavonoids, in combination with other antioxidants may be helpful to reduce atherosclerosis risk, particularly since its vascular protective and santi-inflammatory properties are known for a long time. In fact, as reported by Milde et al. (51) rutin has been shown to can inhibit copper mediated LDL oxidation. Combination of rutin

with other – hydrophilic or lipophilic – antioxidants leads to a synergistic protection of LDL against oxidation of the lipid part as well as of the protein part.

In order to elucidate inhibition of LDL oxidation by flavonoids in relation to their structure, Vaya et al. (8) clearly demonstrate when studying twenty flavonoid compounds of five different subclasses that the most effective inhibitors, by either copper ion or 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) induction, were flavonols and/or flavonoids with two adjacent hydroxyl groups at ring B. In the presence of the later catechol group, the contribution of the double bond and the carbonyl group at ring C was negligible. Isoflavonoids were more effective inhibitors than other flavonoid subclasses with similar structure. Substituting ring B with hydroxyl group(s) at 20 position resulted in a significantly higher anti-oxidant effect than by substituting ring A or ring B at other positions.

Furthermore, several flavonoids from spices and medicinal plant have also been extensively studied with respect to their antioxidant activity. Chipault et al. have found high antioxidant activity in oregano, thyme, nutmeg, mace, turmeric and bell peppers (52, 53). A few years later, Sethi and Aggrawal reported the antioxidant effects of cinnamon, clove, turmeric, nutmeg and some other spices (54).

FLAVONOIDS AS VASORELAXANT AGENTS

Endothelial dysfunction represents critical event in the development of cardiovascular diseases and the major complication of atherosclerosis and arterial thrombus formation (55, 56). Normal physiologic function of arterial endothelium is of central importance in the maintenance of vascular tone, regulation of blood pressure and blood flow to organs and tissues (57). However, in the occurrence of endothelial impairment, this ability of the endothelium to maintain vascular tone is altered. A positive correlation between hypertension and endothelial dysfunction was reported by earlier experimental and clinical studies (58). Altered endothelial-mediated relaxation has been shown in patients with hypertension (59) and in hypertensive models [60]. Furthermore, the initial lesion of atherosclerosis induced by hyperlipidemic state involves an impairment of endothelial function (55). In fact, the lipidemic endothelial dysfunction theory was reported and confirmed by a number of previous works demonstrating that hypercholesterolemia constitute a prominent factor for premature atherosclerosis leading to the endothelial dysfunction (61, 55).

The consumption of flavonoids can be able to prevent a number of cardiovascular diseases including hypertension and atherosclerosis (62, 63, 64). Really, many experimental studies have shown that these phenolic compounds may reduce the arterial pressure in rats (12, 13) and enhance the vasorelaxant process (11). The endothelium-dependent relaxation induced by flavonoids has been well documented. Indeed, this activity was noticed with white and red wine, with polyphenol-rich extract and with purified flavonoids (65, 66, 67). In this field, investigators have demonstrated that anthocyanin delphinidin exerts a significant endothelium-dependent vasorelaxation (67). However, anthocyanins malvidine and cyanidin provoke no significant action on artery relaxation (65). In contrast, myricetin one of known biflavonoids exhibits endothelium-dependent contraction (68).

In order to understand possible mechanisms by which flavonoids improve the vasorelaxant property of arterial wall, many works have been designed using rat aorta rings as experimental model (65, 69). The results from these studies have clearly shown that phenolics play a direct effect on NO production by the endothelial cells (65). The intake of flavonoids significantly increases plasmatic NO levels in human, this is associated with a reduction in blood pressure (70). The production of NO by the endothelial cells in the response to flavonoids appears as result of an increase in $[Ca^{++}]i$ (71). In fact, quercetin and resveratrol induce a marked augmentation in $[Ca^{++}]i$ via the K^+ channel activation and the endoplasmic reticulum ATP-ases inhibition (72, 73). The stimulation of endothelial cells by flavonoids result both in an elevation of cytosolic $[Ca^{++}]i$ levels and phosphorylation on tyrosine residues of intracellular protein, mechanism involved in the regulation of eNOS leading to the production of NO (74). On the other hand, Diebolt et al., demonstrate in their study undertaken with normotensive rats that chronic intake of phenolics results in an improvement of the endothelial cells regarding to the production of NO and the suppression of acetylcholine-induced arterial contraction (13).

It is also established that certain flavonoids are able to enhance the production of NO through an inhibition of endothelial phosphodiesterases activity. In fact, inhibition of phosphodiesterases PDE_2 and PDE_4 by delphinidin can enhance endothelial cells to release NO (75, 76, 77).

Recently Morello et al. (78) suggest that galangin reduces the contractility of rat aortic rings through an endothelium-dependent mechanism, involving NO, and also through an endothelium-independent mechanism, inhibiting calcium movements through cell membranes. Chrysin, a flavonoid widely found in honey and propolis induced a relaxant response in precontracted mesenteric bed that was inhibited by the removal of endothelium, indicating the essential role of endothelium-derived factors for its vasodilator effect (79). Furthermore, when floranol isolated from the roots of *Dioclea grandiflora* was tested for its vasorelaxant activity in endothelium-containing aortic rings, it induced a concentration-dependent vasodilator effect in vessels precontracted with phenylephrine (80). Again, hesperetin (flavonoid mainly isolated from citrus fruits) concentration-dependently relaxed the isometric contractions induced by noradrenaline or by a high extracellular KCl concentration in intact rat isolated thoracic aorta rings. However, hesperetin did not affect the contractile response induced by okadaic acid. These results suggest that the vasorelaxant effects of hesperetin are basically due to the inhibition of PDE1 and PDE4 activities (81).

On the other hand, as demonstrated by other investigators, the *in vitro* vasodilator effects of some flavonoids are mainly endothelium-dependent and mediated by the prevention of superoxide-induced inactivation of endothelial derived NO and also by the enhancement of cGMP-induced vasodilatation

(82). However, the *in vivo* effects of polyphenols on the arterial wall are not very documented and a little data are available. Recently, the chronic oral administration of quercetin, one of known dietary flavonoids, has been shown to exert a marked antihypertensive activity in rats. These effects were associated with decreased plasma reactive oxygen species (ROS) level due to the antioxidant properties of the drug (83). This phenolic compound also prevented endothelium-dependent vasoconstriction induced by acetylcholine secondary to thromboxane A_2 release in aorta (84).

Flavonoids and blood platelet function

Platelet aggregation plays a pivotal role in the physiopathology of thrombotic diseases. Moreover, platelet activity may play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, antiplatelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina, and acute myocardial infarction (85, 86). Indeed, aspirin and abciximab have been proven to be of greet benefit to patients with cardiovascular events and to reduce the incidence of myocardial infarction and death from CVD in secondary prevention trials (87, 88, 89). Because of this, platelet inhibitors are to receive attention, and there has been a directed search for more effective and safe anti-platelet agents. In this field, there is growing interest in using naturally occurring compounds as potential anti-platelet aggregation agents in human populations, since a significant negative correlation between dietary intake of flavonoids and incidence of many types of CVD involving platelet aggregation has been established in epidemiological data (14). Besides, many dietary substances have been also documented to have anti-platelet properties in animal experiments (15, 16). These include green tea catechins, soy isoflavones, resveratrol, quercetin, rutin…etc.

Results from Neiva et al. experimental study on human blood platelets submitted to stimulation with arachidonic acid, adenosine diphospate (ADP) and epinephrine clearly demonstrated that treatment with catechin or epicatechin was sufficient to exhibit a potent dose dependent inhibitory effect of the three agonists (90). Some effects on platelet function were noticed with resveratrol and quercetin in *in vitro* and *in vivo* studies (15, 16). Genistein was also defined as promoter of platelet disaggregation and can serve as starting material for drug development programmes; this one acts by inhibiting collagen-induced platelet aggregation (91).

In a comparative study, the anti-platelet activity of xanthomicrol, kumatokenin, jaceidin and 3, 3'-di-O-methylquercetin on the aggregation of human plasma-rich platelet (PRP) induced by collagen and ADP was assessed by Afifi et al. (92). When platelet aggregation was induced by collagen, 3,3'-di-O-methylquercetin and xanthomicrol were found to be the most potent and kumatakenin less potent. However, jaceidine showed very weak activity. Furthermore, when platelet aggregation was induced by ADP, xanthomicrol was found to be the most active followed by the compounds kumatakenin and 3, 3'di-O-methylquercetin. Jaceidine revealed no anti-platelet activity.

In order to gain a more comprehensive understanding of the molecular mechanisms by which flavonoids might inhibit platelet aggregation, a number of experimental studies was undertaken using platelets from human and animal plasma. The effective inhibitory effect of flavonoid-rich extract of *Ginkgo biloba* on human platelet aggregation results in increased cAMP levels in platelets, which is known to inhibit platelet activation by lowering intracellular Ca^{++} levels. Thus, as reported by the authors, flavonoids from this medicinal plant affect metabolism of cAMP, TxA_2 and Ca^{++} in platelets (93). Kang et al. (94) demonstrate that the anti-platelet activity of flavonoids was mediated by the suppression of cytoplasmic Ca^{++} increase; this effect may be due to the inhibition of cytosolic calcium mobilization. It seems also that the inhibitory effect on calcium mobilization may implicate the elevation of cAMP level rather than inositol triphosphate (IP_3) inhibition.

On the other hand, inhibitory effects of five flavonoids from medicinal herbs on the aggregation and secretion of platelets were studied in rabbit plasma. These phytochemicals inhibited markedly platelet aggregation and ATP release induced by arachidonic acid or collagen, and slightly those by platelet-activating factor. ADP-induced platelet aggregation was suppressed by myricetin, fisetin and quercetin. The thromboxane B_2 formations were also inhibited by the flavonoids in platelets challenged with arachidonic acid. Fisetin, kaempferol, morin and quercetin antagonized the aggregation of washed platelets induced by U46619, a thromboxane A_2/prostaglandin endoperoxides mimetic receptor agonist. Quercetin prevented the secondary aggregation and blocked ATP release from platelets induced by epinephrine or ADP. These results demonstrate that the major antiplatelet effect of the tested flavonoids may be due to both the inhibition of thromboxane formation and thromboxane receptor antagonism (95).

To elucidate the possible mechanism of flavonoid quercetin isolated from *Flaveria bidentis* on the inhibition of platelet aggregation. Guglielmone et al. (96) assume that this phenolic might be a break of the TxA_2 formation or blocks TxA_2 receptors, so the investigators used the mimetic agonist of TxA_2, U-46619, as inducer in PRP. A marked inhibition of aggregation of the PRP incubated in presence of quercetin was observed. The results suggest that quercetin produce a blockade in the receptors of the TxA_2. Secondly, to test a possible interference in the formation of TxA_2, authors assayed the TxB_2 in the supernatant of washed platelet incubated with quercetin and stimulated with collagen and arachidonic acid. So, in this assay, the thrombin generation, an important stimulus for platelet activation and TxA_2 production, is totally excluded. Production of TxB_2 was inhibited by quercetin. These results are indicating that the thromboxane pathway is affected. However, the results show that this compound do not affect the interaction of the Von Willebrand factor and glycoprotein Ib–IX, a central key of the platelet adhesion. We conclude here that this inhibitory effect would involve at least the following two mechanisms: quercetin initially induce a deleterious effect on the production of TxA_2, as judged by TxB_2 formation in stimulated

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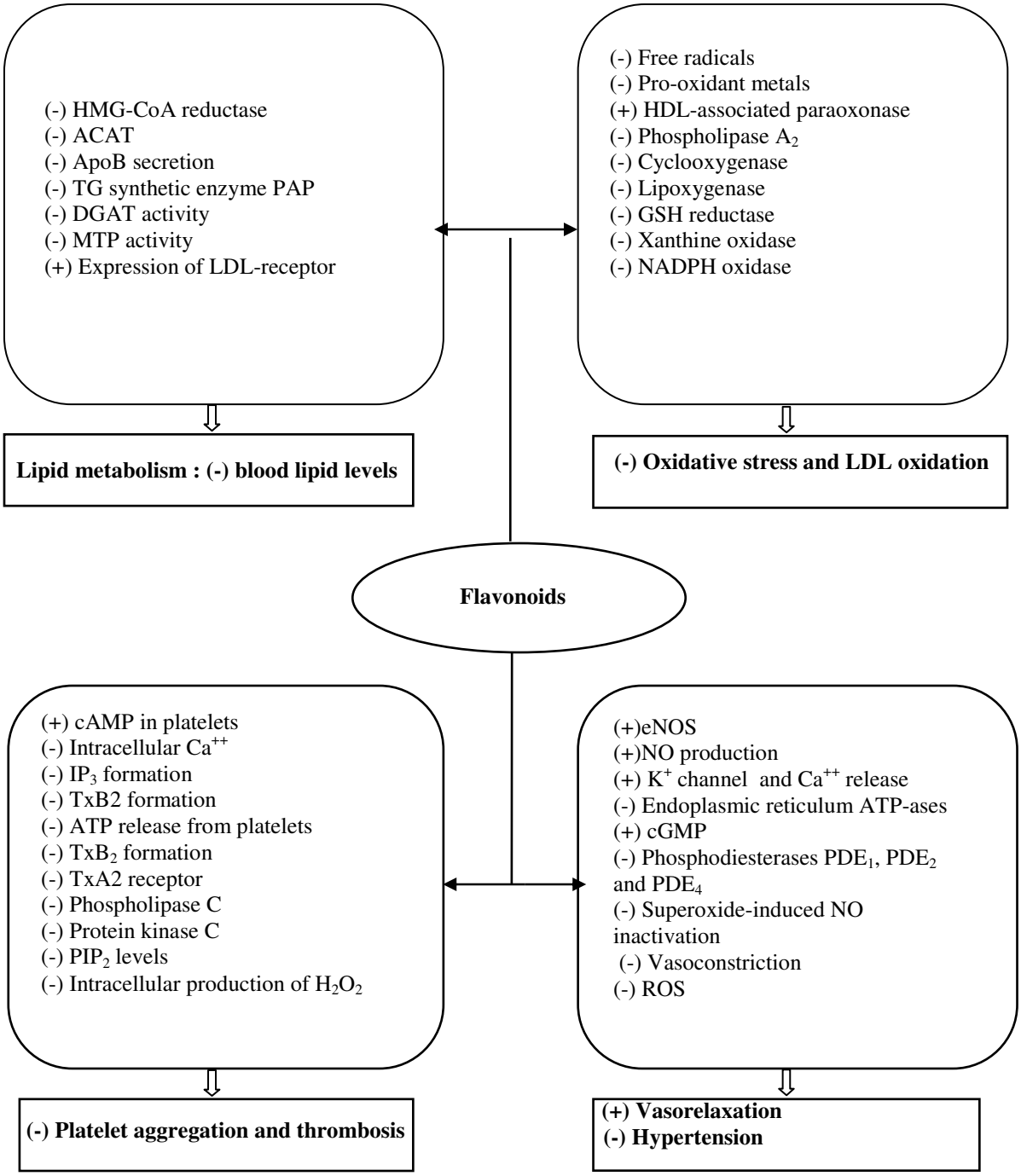

Fig. 2. Effects of flavonoids on cardiovascular system

(+) activation or increase; (-) inhibition or decrease; ACAT: acyl-CoA cholesterol acyl transferase; DGAT: diacylglycerol acyltransferase; eNOS: endothelial nitric oxide synthase; GSH reductase: glutathione reductase; HMG-CoA reductase: hydroxymethyl glutaryl-CoA reductase ; IP_3: inositol triphosphate; LCAT: lecithin cholesterol acyl transferase; MTP: microsomal triglyceride transfer protein; PAP: phosphatidate phosphohydrolase; PDE: phosphodiesterase; PIP: phosphatidylinositol biphosphate; ROS: reactive oxygen species; TxA_2: thromboxane A_2, TxB_2: thromboxane B_2.

washed platelets incubated with the studied flavonoid, and markedly inhibits the TxA_2 receptors according to the profile of inhibition of the agonist-induced platelet aggregation when using ADP, arachidonic acid, collagen and then confirmed with U-46619. In addition, quercetin can act synergistically together with catechin to inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide (97). Both quercetin and catechin significantly inhibited the release of platelet hydrogen peroxide elicited by collagen, with almost complete suppression when the 2 flavonoids were used in combination. An apparent discrepancy in this finding is that the inhibition of collagen-induced hydrogen peroxide release by quercetin or catechin, both alone and in combination, was more marked than the inhibition of platelet aggregation. This is likely dependent on the fact that hydrogen peroxide represents only one pathway through which collagen induces platelet aggregation.

According to a previous study showing that hydrogen peroxide contributes to activating the phosphoinositol pathway, quercetin and catechin alone or in combination significantly inhibited calcium mobilization and IP_3 formation because of their ability to quench hydrogen peroxide (97).

Flavones apigenin and luteolin and isoflavone genistein may also inhibit platelet function through binding to the thromboxane A_2 receptor. These flavonoids are shown to markedly abrogated arachidonic acid and collagen-induced platelet responses, such as aggregation and secretion, with a less substantial effect on TxA_2 synthesis. These compounds were identified as specific ligands of the TxA_2 receptor. Tight binding of flavonoids to the human TxA_2 receptor relies on structural features such as the presence of the double bond in C2–C3, and a keto group in C4. Antagonism of this TxA_2 receptor represents an additional mechanism for the inhibitory effect of these compounds in platelet function (98).

Another mechanism involved in anti-platelet function was elucidated by Sheu and colleagues (99) when examining the possible pathway of rutin in this field. The data obtained shows that rutin concentration-dependently inhibited the activation of phospholipase C, followed by inhibition of protein kinase C and thromboxane A_2 formation, thereby leading to inhibition of the phosphorylation of P_{47} and intracellular Ca^{++} mobilization, finally resulting in inhibition of platelet aggregation. One year after, Hsiao et al. (100) demonstrated that the same mechanism was involved by α-Naphthoflavone.

Finally, phosphatidylinositol 4, 5-biphosphate (PIP_2) was previously reported to play a direct role in phosphatidylserine exposure. Thrombin formation and platelet procoagulant activity are dependent on phosphatidylserine exposure. As flavonoids can inhibit phosphoinositide kinases, Buck et al. (101) examined whether changes in phosphoinositides metabolism in flavonoid-treated platelets could be involved in their anti-platelet property. Treatment with quercetin or catechin reduced phosphatidylserine exposure, thrombin formation, PIP_2 levels and resynthesis after platelet activation with collagen, thrombin or calcium inophore. Flavonoids also prevented $[Ca^{++}]i$ increase induced by collagen, but not by the inophore. Authors suggest that the ability of flavonoids to decrease phosphatidylserine exposure induced by inophore could result from the decrease of PIP_2 levels, whereas phosphatidylserine exposure induced by collagen could be diminished by flavonoids effects on calcium signaling on PIP_2 hydrolysis. These data favor the role of PIP_2 pathway in the anti-platelet effects of flavonoids.

CONCLUSION AND PERSPECTIVES

In the present review we have evoked the principal cardiovascular system benefits of flavonoids. Literature has documented the remarkable bioactivities ascribed to these molecules. Flavonoids represent an active principle of a grand number of medicinal plants and have been associated with potential beneficial health properties. These include the prevention of cardiovascular and cerebrovascular diseases and leading to an increasing interest in these compounds as potential constituents of functional food and future drugs. However, their medicinal potency, their impact on human nutrition and their targets on body physiology has still to be further investigated and there is a need to know more about their structure-activity relationship, metabolism and bioavailability. Many flavonoids have, as described above, beneficial effect on cardiovascular system. These natural compounds have several great advantages over other therapeutic agents since: i) Our diet is rich in these phenolics and they are daily consumed. ii) They rarely have any side effects. iii) They have relatively long half-life. iiii) They can easily absorbed in intestine after ingestion. The important cardiovascular feature of flavonoids is that they have a broad spectrum of targets at different tissues and metabolic levels **(Fig. 2)**. In contrast, a number of other characterized flavonoids have not or slight effects on cardiovascular system. Thus, pharmacologists, nutritionists and patients should not consider all flavonoids as potent cardiovascular drugs, especially within short-term intake. Furthermore, flavonoids constitute a best base chemical structure for new hemi-synthetic drugs. Finally, we would think that natural, hemisynthetic and synthetic flavonoids will constitute an important therapeutic tool in cardiovascular events treatment. These molecules, alone or in combination with other preventive and/or therapeutic strategies will become an effective future drugs against most common human degenerative diseases such as cancer, cardiovascular and cerebrovascular complications.

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