Review Article

ROLE OF FLAVONOIDS IN HUMAN NUTRITION AS HEALTH PROMOTING NATURAL CHEMICALS - A REVIEW

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ABSTRACT

Flavonoids are a class of secondary plant metabolites with significant antioxidant activities. There are many biological activities attributed to flavonoids. Their cardio protective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuate other processes involving reactive oxygen species.The majority of them could be beneficial and some of them may be detrimental, depending on specific circumstances. They are structurally similar to estrogens, exerting both estrogenic and antiestrogenic properties in various tissues. The results of epidemiologic studies exploring the role of flavonoids in human health have been inconclusive. Some studies support the notion of a protective effect of their consumption in immunomodulation, cognition, and risk reduction of certain cancers, cardiovascular and skin diseases, osteoporosis and obesity, as well as relief of menopausal symptoms. Other studies failed to demonstrate any effects.

Key words: Flavonoids, plant metabolites, antioxidant, immunomodulation, cardio protective.

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INTRODUCTION

Flavonoids are shown to exert beneficial effects in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders. Many of the biological actions of flavonoids are due to their antioxidant properties, through their reducing capacities either per se or through their possible influences on intracellular redox status. The precise mechanisms by which flavonoids exert their beneficial or toxic actions remain unclear. Flavonoids (the term is derived from the Latin word “flavus”, meaning yellow) are ubiquitous plant secondary products that are best known as the characteristic red, blue, and purple anthocyanin pigments of plant tissues [1]. Apart from their physiological roles in the plants, flavonoids are important components of the human diet.

The dietary anomaly called French paradox was first observed in French population and found later in other Mediterranean populations. Epidemiological studies revealed that flavonoid-rich in diet is correlate with the increased longevity and decreased incidence of cardiovascular diseases seen in these populations despite their high intake of fat [2–5]. In addition to their antioxidant properties, flavonoids have been reported to exhibit other multiple biological effects, e.g. antiviral, antibacterial, anti-inflammatory, vasodilatory, anticancer, and anti-ischemic. Moreover, they are able to inhibit lipid peroxidation and platelet aggregation and improve increased capillary permeability and fragility [6–8].

Flavonoids as antioxidants

The term antioxidant is commonly used in scientific literature but it can be defined in multiple ways according to the methods used to measure antioxidant activity. Therefore, Halliwell and Gutteridge [9] proposed a definition of an antioxidant as “any substance that delays, prevents or removes oxidative damage to a target molecule”. Physiological role of these compounds, as this definition suggests, is to

prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals.

The flavonoid constituents of red wine and red grapes are factors of particular interest due to the French paradox: the Southern French have a very low incidence of coronary heart disease despite their high fat diet, low exercise, and smoking habits. The exact molecular mechanism underlying this prevention remains poorly defined. One of the features that has been highlighted relates to the high consumption of red wine in the French population and the question as to whether the polyphenolic antioxidants from this dietary source contribute to the protection from coronary heart disease along with the antioxidants in the olive oil and the high intake of antioxidant nutrients from the fresh fruit and vegetable-rich Mediterranean diet [10].

Polyphenolic grape extract of red wine was described to stimulate inhibition receptor PECAM-1 (platelet endothelial cell adhesion molecule-1) thereby inhibiting platelet activation. The flavonol intake also predicted a reduced incidence of first myocardial infarction in elderly men. The antioxidant capacities of many flavonoids are much stronger than those of vitamins C and E. For example, the one-electron reduction potential of epigallocatechin gallate under standard conditions is 550 mV, a value lower than that of glutathione (920 mV) and comparable to that of α-tocopherol (480 mV).

Flavonoids can prevent injury caused by free radicals by the following mechanisms:

(1) Direct scavenging of reactive oxygen species (ROS),

(2) Activation of antioxidant enzymes

(3) Metal chelating activity

(4) Reduction of α-tocopheryl radicals

(5) Inhibition of oxidases

(6) Mitigation of oxidative stress caused by nitric oxide

(7) Increase in uric acid levels

(8) Increase in antioxidant properties of low molecular antioxidants.

Direct scavenging of ROS

The in vitro flavonoid antioxidant activity depends on the arrangement of functional groups on its core structure. Both the configuration and total number of hydroxyl groups substantially influence the mechanism of the antioxidant activity [11]. The B ring hydroxyl configuration is the most significant determinant of ROS scavenging [12], whereas substitution of the rings A and C has little impact on superoxide anion radical scavenging rate constants.

The in vitro antioxidant activity could be increased by polymerization of flavonoid monomers, e.g. proanthocyanidins (also known as condensed tannins), the polymers of catechins, are excellent in vitro antioxidants due to the high number of hydroxyl groups in their molecules.

The antioxidant capacity of proanthocyanidins depends on their oligomer chain length and the type of ROS with which they react [13]. The glycosylation of flavonoids reduces their in vitro antioxidant activity when compared to the corresponding aglycons. Comparison of TEAC values of quercetin (4.42 mM) and rutin (2.02 mM), quercetin-3-O-rutinoside, shows that glycosylation of the 3-OH group has strongly suppressive effect on the antioxidant activity. Similar results were observed for other pairs of flavonoid aglycon and glycoside (e.g. hesperetin–hesperidin, luteolin–luteolin 4'-gGlucoside; luteolin–luteolin 7-glucoside; baicalein–baicalin; and quercetin–quercitrin).

Quercetin glycosylation also significantly reduced its superoxide scavenging ability, hypochlorite scavenging activity and power to reduce Fe (III) to Fe (II) (determined by FRAP assay). The main

structural features of flavonoids required for efficient radical scavenging can be summarized as follows [14, 15]:

a) An ortho-dihydroxy (catechol) structure in the B ring, for electron delocalization.

b) 2, 3-double bond in conjugation with a 4-oxo function in the C ring provides electron delocalization from the B ring.

c) Hydroxyl groups at positions 3 and 5 provide hydrogen bonding to the oxo group.

According to the previously stated criteria, flavonols quercetin and myricetin should be the most effective radical scavengers in the aqueous phase, which has been confirmed experiment.

Ability to activate antioxidant enzymes

Other possible mechanism by which flavonoids act is through interaction with various antioxidant enzymes. Furthermore, some effects may be a reset of a combination of radical scavenging and the interaction with enzyme functions.

Flavonoids are able to induce phase II detoxifying enzymes (e.g. NAD (P) H-quinone oxidoreductase, glutathione Stransferase, and UDP-glucuronosyl transferase), which are the major defense enzymes against electrophilic toxicants and oxidative stress. Regulation of this protective gene expression can be mediated by an electrophile responsive element (EpRE), which is a regulatory sequence of a number of genes encoding these phase II enzymes [16, 17].

The ability of flavonoids to activate the EpRE-mediated response correlates with their redox properties. Lee-Hilz et al. [18] observed activation of firefly luciferase reporter gene in Hepa-1c1c7 mouse hepatoma cells upon induction with flavonoids of different structure. The most effective inducers were flavonoids containing a hydroxyl group at the 3-position in the ring C (quercetin and myricetin), whereas flavonoids without this hydroxyl group only (luteolin and galangin) were low luciferase inducers. Therefore, flavonoids with a higher intrinsic potential to generate oxidative stress and redox cycling are the most potent inducers of EpRE-mediated gene expression. It can be conclude that the prooxidant activity of flavonoids can contribute to their health-promoting activity by inducing important detoxifying enzymes, pointing to a beneficial effect of a supposed toxic chemical reaction.

Nagata et al [19] investigated cytoprotective effect of quercetin and catechin against hydrogen peroxide cytotoxicity in cultured rat hepatocytes BL-9, which are cells highly expressing cytosolic glutathione peroxidase (GPx). The authors described that the protective activity of tested flavonoids was related to the activation of GPx. Martín et al [20] described activation of survival signalling proteins (protein kinase B and extracellular regulated kinases) and increase in the activities of GPx and glutathione reductase (GR) in human hepatocytes caused by cocoa flavonoids.

Leung et al [21] provided evidence that luteolin-induced human lung carcinoma CH27 cell apoptosis were accompanied by activation of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), but not through the production of ROS and disruption of mitochondrial membrane potential.

Therefore, the effects of luteolin on CH27 cell apoptosis were suspected to result from the antioxidant rather than the prooxidant action of this compound. Administration of the flavonoid-rich fraction along with a high fat diet caused a significant increase of SOD, CAT and GPx activities in rat erythrocytes. Similar results were also observed after administration of naringin to hypercholesterolemic volunteer [22].

However, some contradictory observations in this field were obtained, e.g. glutathione S-transferase activity (GST) was significantly induced by apigenin, genistein and tangeretin in the rat heart but not in colon or liver. In red blood cells chrysin, quercetin and genistein significantly decreased the activity of GR, CAT and GPx, whereas SOD was only significantly decreased by genistein.

Metal chelating activity

It chelates iron and copper, thereby removing a causal factor for the development of free radicals, known specific flavonoids. Quercetin was able to prevent oxidative injury induced in the erythrocyte membrane by a number of oxidizing agents (e.g. acrolein and phenylhydrazine), which cause release of iron in its free, redox active form. The catechol moiety in the B ring have been shown to be important for Cu2+-chelate formation and thus being the major contributory site of the metal chelation [23]. Quercetin, in particular, is well known for its iron-chelating and iron stabilizing properties. Morin and quercetin were shown to form complexes with Cd (II) and exhibit strong antioxidant activity in the in vitro studies. Their sulfonic water-soluble derivatives exert low toxicity and therefore could be potential antidotes in cadmium intoxication.

Reducing α-tocopherol radicals

The α-tocopherol represents a major antioxidant in cell membranes and in human low-density lipoproteins (LDL) which protects lipoprotein particles against oxidative damage. Flavonoids could act as hydrogen donors to α-tocopheryl radical, which is a potential pro oxidant. Furthermore, by interaction with α-tocopheryl radical, they possess a great potential to delay the oxidation of LDL. Flavonoids (kaempferol, morin, myricetin and quercetin) showed a varying protective activity against depletion of α-tocopherol in LDL, with kaempferol and morin being less effective than myricetin and quercetin. Catechins may be evenmore effective than ascorbate in regeneratingα-tocopherol in micellar solution [24]. Similarly, the addition of green tea catechin extracts (epigallocatechin, epigallocatechin gallate, epicatechin, and epicatechin gallate) demonstrated a gradual regeneration of α-tocopherol in human LDL. These observations are supported by the low value of redox potentials of some flavonoids (e.g. quercetin E7=0.2 V or catechin E7=0.4 V).

Ability to inhibit oxidases

Flavonoids inhibit the enzymes responsible for superoxide production, such as xanthine oxidase and protein kinase C. Quercetin and silybin inhibited xanthine oxidase activity, thereby resulting in decreased oxidative injury. Flavonoids have been shown to inhibit cyclooxygenase, lipoxygenase, microsomal succinoxidase and NADH oxidase [25].

NADPH oxidase is membrane-associated system catalyzing production of $O_2^{\bullet-}$ in activated neutrophils. The mechanism of its activation includes interaction of an agonist with specific receptor on neutrophil membrane, activation of phosholipase C with subsequent formation of second messengers, which activate protein kinase C. This enzyme phosphorylates p47phox subunit of NADPH oxidase, a key component of this enzyme, and thus causes activation of respiratory burst [26, 27].

The inhibition of protein kinase C was suggested to be a mechanism of an inhibition of NADPH oxidase by quercetin. Potent flavonoid inhibitors of protein kinase C (e.g. quercetin, fisetin, and luteolin) possess a coplanar flavone structure with free hydroxyl substituents at the 3', 4' and 7-positions [28].

Mitigating oxidative stress caused by nitric oxide

Nitric oxide (NO) is important in maintaining the dilation of blood vessels [78] but its high concentrations may result in oxidative damage. NO is produced by the oxidation of L-arginine catalyzed by NO synthases (NOS). Nitric oxide toxicity is mainly mediated by peroxinitrite, which is formed in the reaction of NO with $O_2^{\bullet-}$ [29].

Flavonoids exerted NO production inhibitory activity in several lipopolysaccharide-activated cell lines and cultures (mouse peritoneal macrophages, RAW 264.7 cells, and J774.2).This effect was probably caused by flavonoid inhibitory effect on expression of inducible NOS but not by the inhibition of its activity. Several structural requirements of flavonoids for this activity were elucidated: presence of 2, 3-

double bond with 4-oxo group and 3,5,4'-trihydroxyl group were crucial. Their activity was even enhanced by methylation of 3-, 5-, or 4'-hydroxyl group and reduced by glycoside moiety and catechol or pyrrogallol arrangement of the B-ring.

Thus, apigenin, diosmetin, and luteolin belong among naturally occurring flavones with the most potent inhibitory activity [30–32]. These flavonoids may at the same time increase the activity of endothelial NOS. Several flavonoids, including quercetin, result in a reduction in ischemia–reperfusion injury by interfering with inducible NOS activity. Flavonoids also possess ability to directly scavenge molecules of NO.

How flavonoids inhibit induction of NOS and NO production is not clearly understood yet, but several explanations are argued. The first possibility might be derived from the antioxidant property of flavonoids by which these compounds scavenge ROS. The second possibility is that flavonoids may act as lipopolysaccharide-signalling molecule inhibitors. Flavonoids are known to scavenge peroxynitrite directly [33]. The most significant determinant of their activity against peroxynitrite is the 3',4'-catechol arrangement, followed by an unsubstituted 3-hydroxyl group. An apparent positive correlation between number of hydroxyl groups, particularly of the B-ring, and antiradical activity has been described.

Increasing uric acid levels

Interestingly, there are the great discrepancies between plasma or serum total antioxidant capacity and plasma concentrations of flavonoids. Cao et al [34] described the significant increase in plasma or serum urate after consumption of strawberries, spinach or red wine. Similar increase of plasma or serum urate was described after drinking of port wine, French Bordeaux, tea or coffee. Thus, several studies indicate that the consumption of flavonoid-rich foods may increase plasma urate, although the underlying mechanism remains unclear. On the other hand, since elevated urate may be a risk factor for some diseases, the alleged 'antioxidant benefit' may not be what it seems [35].

Modification of pro-oxidant properties of low molecular antioxidants

Several authors described pro-oxidative activity of ß-carotene under certain conditions (e.g. UVA irradiation) and suggested that its combination with an antioxidant may have preventive effect [36, 37]. When each flavonoid was combined with β-carotene during preincubation, UVA-induced cellular DNA damage was significantly suppressed and the effects were in the order of naringin ≥ rutin and quercetin. Results of this study suggested that a combination of β-Carotene with naringin, rutin or quercetin might increase the safety of β-carotene.

CONCLUSION

Flavonoids are a class of secondary plant metabolites with significant antioxidant activities. The majority of them could be beneficial and some of them may be detrimental, depending on specific circumstances.There are many biological activities attributed to flavonoids. Apart from their physiological roles in the plants, flavonoids are important components of the human diet.

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