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Review

Flavonoids in health and disease

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ABSTRACT

The aim of this review is to summarize the biological actions and the potential health benefits of flavonoids. Flavonoids occur naturally in fruit, vegetables, tea and wine. Flavonoids are classified as flavonols, flavones, isoflavones, flavanones, flavanols and anthocyanidins. These flavonoids have different levels of bioavailability depending on their chemical status. Data presented support the concept that certain flavonoids in the diet can be associated with significant health benefits, including anti-inflammation, anti-cancer, cardiovascular protective activities and neurological protective functions. This review covers laboratory and clinical mechanisms of action on effects of flavonoids on these chronic conditions.

KEYWORDS: flavonoids, dietary intakes, inflammation, cardiovascular disease, cancer, degenerative disease

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1. INTRODUCTION

Flavonoids are part of the polyphenols family widely distributed in plants and plant products, including a variety of fruits, vegetables, cereals,

tea and wine. Flavonoids are low molecular weight secondary metabolites. These compounds play important roles in plants themselves, especially as pigments and defense compounds [1, 2]. Over 9000 phenolic compounds have been isolated from different natural products [3]. A significant number of flavonoids are identified to have health benefits to humans. They include anti-inflammation, anti-cancer, cardiovascular protective activities and neurological protective functions [4-6].

2. Flavonoid chemistry structure and classification

Flavonoids are a large family of compounds which belong to polyphenols subclass which are characterized by 2 aromatic rings (A and B rings), linked by a 3-carbon chain which forms an oxygenated heterocyclic ring (C ring). Each ring links at least one aromatic hydroxyl and connected with a heterocyclic pyran. A large number of compounds are generated from the different combinations of multiple hydroxyl, methoxyl, and *O*-glycoside group substituents on this basic benzo-*γ*-pyrone (C6-C3-C6) [7] (Figure 1).

Depending on the positions of the linkage of the aromatic rings (rings A and C) and the oxidation state and functional groups of the C ring, flavonoids are categorized into six subtypes (Figure 2), (1) flavonols (e.g., kaempferol, quercetin), which are found in onions, apple, leeks, and broccoli; (2) flavones (e.g., apigenin, luteolin), which are found in green leaf spices such as parsley and celery, (3) isoflavones (e.g., daidzein, genistein), which are mainly found in soy, legume and soy products; (4) flavanones (e.g., hesperetin, naringenin), which are mainly found in citrus fruit and tomatoes; (5) flavanols (e.g., catechin, epicatechin, epigallocatechin, epigallocatechin gallate (EGCG), which are abundant in green tea, red wine, red grape and chocolate; and (6) anthocyanidins (e.g., pelargonidin, cyanidin, malvidin), whose sources include red wine, plum and berry fruits [8].

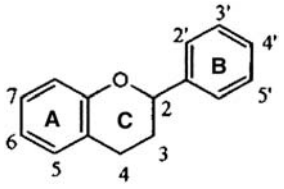

Figure 1. Basic structure of a flavonoid.

3. Types and distribution of flavonoids in foods

Although flavonoids are widely distributed in plant and plant products, plants have their unique flavonoid profiles. In nature, they are present principally as glycosylated, esterified, and polymerized derivatives. Flavonoids in plants play important roles as color definitions, antioxidants to protect plants against UV-radiation, defense against the bacteria and fungal attack, signal molecules to facilitate nitrogen fixation, and bitter or astringent taste attributes [9-12].

The most widespread flavonoids in the fruits and vegetables were the flavonol quercetin and kaempferol, followed by the anthocyanins derived from cyanidin. Flavones, chalcones, and catechins were found only in selected vegetables as summarized in Table 1.

Flavonoids in lettuce

The total flavonoids content in individual types of lettuce exhibited very wide differences. Paola and his colleagues found that red lettuce contains highest level of flavonoids, ~67 mg/100 g of fresh weight (FW). Cyanidin contributed 28-31% of the total flavonoids content of red lettuce, quercetin contributed 56-67%. The mean luteolin content of lettuce was of 2 mg/100 g of FW [13].

Flavonoids in sweet pepper

It was found that sweet pepper contained both quercetin and luteolin conjugates. Quercetin conjugates, in a range of 0.3-4.1 mg/100 g of FW, and luteolin conjugates, in a range of 0.5-2.1 mg/100 g of FW. Justesen and his colleagues detected 0.1, 0.2, and 0.5 mg of luteolin per 100 g of FW in red, yellow and green sweet peppers [13, 14].

Flavonoids in onion

Significant amounts of quercetin conjugates were detected in onion types. In white onions, quercetin conjugates were detected in range of 48 and 56 mg of aglycon/100 g of FW. Crozier *et al.* reported in the ranges of 19-63 mg/100 g of FW [15], and 28-49 mg/100 g of FW reported by Hertog *et al.* [16]. In red onions, 38-94 mg/100 g

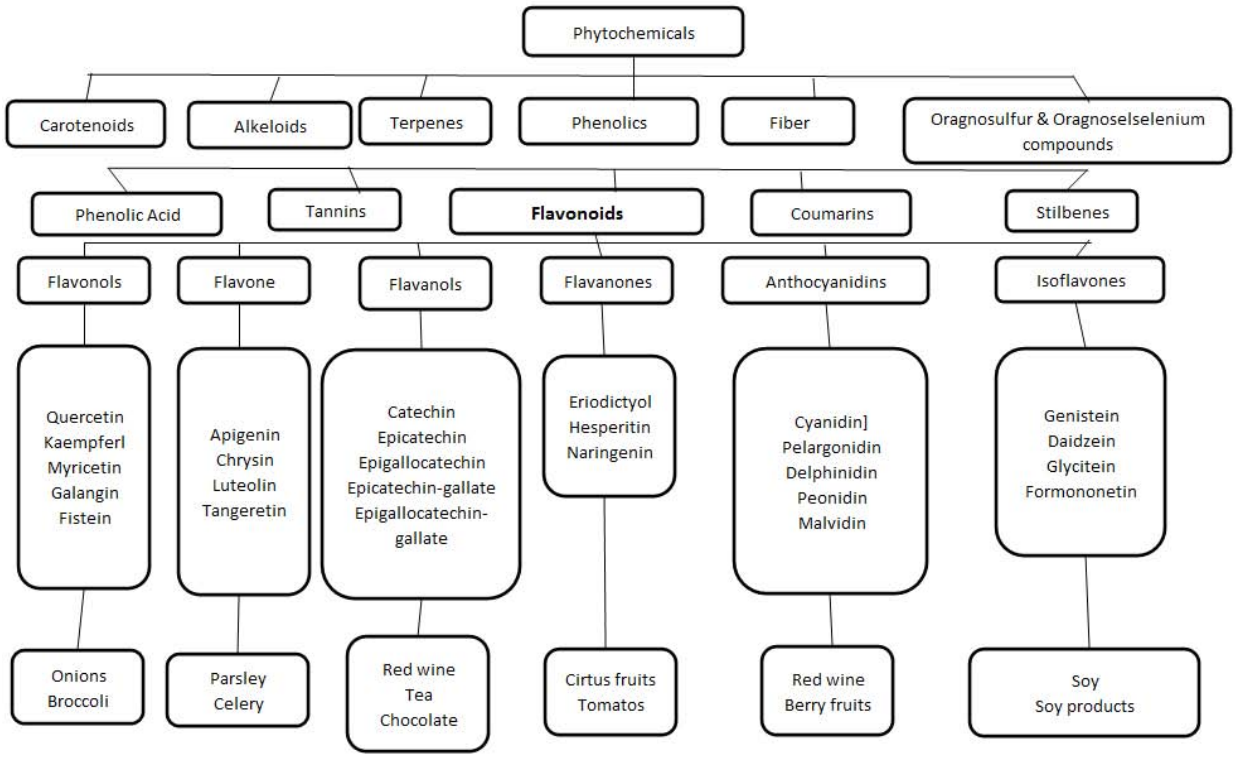

Figure 2. Classification of dietary phytochemicals (Adapted from Reference 9 & 10 with permission from American Society for Nutrition and Springer).

of FW of quercetin were detected. Rhodes and Price [17] reported quercetin values were 92 mg of /100 g of FW in red onions and 71 and 80 mg/100 g of FW in pink and brown onions, respectively. Cyanidin conjugates in the red onion range from 5%-9.2%, in red onions the content of anthocyanin was up to 25 mg/100 g [18].

Flavonoids in dark green leaves

Significant amounts of the flavonol kaempferol were detected in the samples of bitter leaves. Kaempferol made up the largest concentrations, 41 and 104 mg/100 g of FW, and quercetin derivatives were in a much lower concentration (14 mg/100 g of FW), depending on time of harvesting. Chicory contains similar amounts of quercetin (4-25 mg/100 g of FW) and kaempferol (4-11 mg/100 g of FW) glycosides but also has apigenin and luteolin derivatives [13].

Flavonoids in tomato

Tomatoes have significant amount of quercetin. Quercetin levels are 0.5 and 4.2 mg/100 g of FW in ripen salad and cherry tomatoes, respectively. The main classes identified in the cuticles of tomato cultivars were the quercetin derivatives and the chalconaringenin. Chalconaringenin is present in tomato peel. Stewart *et al.* [19] found that the flavonoid content of tomatoes varies due to different cultivars, size, and agronomic and climatic conditions. Fruit flesh accumulated most amounts of flavonoids [13].

Flavonoids in orange

In citrus fruits flavanones are the predominant flavonoids. The flavanones naringenin and hesperetin were present in the pulp and peel of oranges. The amounts of flavanone presented in the peel were 105-147 mg/ 100 g of FW and 34-44 mg/100 g of FW in the pulp, respectively. In the peel of orange, Larrauri *et al.* [20] found that hesperetin was the major component. Justesen *et al.* [21] reported naringenin and hesperetin contents of orange pulp were 11 and 31 mg/100 g of FW. Quercetin derivatives were in amounts of around 1 and 4 mg/100 g of pulp and peel, respectively.

Table 1. Types and distribution of flavonoids in selected foods.

Vegetables or fruits	Flavonoids subclass	Major compounds and concentration	Reference
Lettuce	Anthocyanidins Flavonols Flavonones	Cyanidin 18.8~20.8 mg/100 g of FW Quercetin 37.5~44.9 mg/100 g of FW Luteolin 2 mg/100 g FW	[13]
Sweet Pepper	Flavonols Flavonones	Quercetin conjugates 0.3~4.1 mg/100 g of FW Luteolin conjugates 0.5-2.1 mg/100 g of FW	[13, 14]
Red Onions	Flavonols Anthocyanidins	Quercetin 38-94 mg/100 g of FW Anthocyanin 25 mg/100 g of FW	[15-18]
Dark Green Leaves	Flavonols	Kaempferol 41 and 104 mg/100 g of FW Quercetin 14 mg/100 g of FW	[13]
Tomato	Flavonols	Quercetin 0.5~4.2 mg/100 g of FW	[13, 19]
Orange	Flavanones Flavonols	Naringenin 11 mg/100 g of FW Hesperetin 31 mg/100 g of FW Quercetin 1~4 mg/100 g of FW	[20, 21]
Apple	Flavonols Flavanols	Quercetin 0.4-10.1 mg/100 g of FW Catechin 1.3~5.1 mg/100 g of FW Epicatechin 5.4~10.4 mg/100 g of FW	[22, 23]
Tea	Flavanols	Catechins 12-18% dry weight (epicatechin 1-3% dry weight, epicatechin gallate 3-6%, epigallocatechin 3-6%, and epigallocatechin gallate 9-13%)	[24]
Soybean	Isoflavonoids	Genistein and daidzein ~3 mg/g	[25, 26]
Grape (seeds)	Flavanols	Catechin 42~64 mg/100 g Epicatechin 79~99 mg/100 g	[27, 28]
Berries	Anthocyanidins Flavonols	Anthocyanins 62.6~560mg/100 g of FW Quercetin 10.4~25mg/100 g of FW Myricetin 1.1-24.9mg/100 g of FW Kaempferol 28.4-124mg/100 g of FW	[29-31]

Flavonoids in apple

Whole apple with skin contains significant amount of quercetin (0.4-10.1 mg/100 g of FW). Flavanols (catechin and epicatechin) were present in high concentrations in apples, 1.3-5.1 mg/100 g of FW for catechin and 5.4-10.4 mg/100 g of FW for Epicatechin. The apple variety and growing conditions have an impact on the flavonoids levels. Arts *et al.* [22] observed that removing the apple skin resulted in a 23% decrease of total catechin content. Flavonols and dihydrochalcones (phloridzin) are present only in minor quantities but distinguish apple from other fruit. The level of phloridzin increased from the skin to the seeds, and it was the main flavonoid in the seeds, where it contributed 98% of the total flavonoid content [23].

Flavonoids in tea

All tea is produced from the leaves of Camellia sinensis, but differences in processing result in several types of tea, of which, green and black tea are the most consumed worldwide. Green tea is produced by steaming fresh leaves for 1 min to inactivate polyphenol oxidase, followed by drying. In contrast, black tea undergoes a fermentation procedure in which the leaves are kept at room temperature for 16-24 h and then cut and dried. One cup of tea (2g of tea leaves infused in hot water for 1 to 3 min) will provide 150 to 200 mg of flavonoids. Green tea is a rich source of flavonoids, the predominant is catechins (12-18% dry weight), particularly Epicatechin (1-3% dry weight), epicatechin gallate 3-6%, epigallocatechin

3-6%, and epigallocatechin gallate 9-13%). These catechins comprise 30-50% of the solids in green tea and 90% of total flavonoids. The fermentation process used to produce black tea results in the conversion of catechins to theaflavins (4% dry weight), theaflavin-3-gallate and thearubigin polymers. Thearubigin polymers, which is the major fraction of black tea polyphenols account for 20% of the solids and 47% of the total flavonoids [24].

Flavonoids in soybean

It has been known since 1931 that soybeans contain relatively high concentrations of isoflavones. Isoflavones are the predominant flavonoids in soybean seeds. Other flavonoids are present in different soybean plant. Genistein and daidzein and their glucoside conjugates are present in high concentrations (up to 3 mg/g) in soybeans [25]. Asian soy foods (soy milk, tofu, soy flour, soy powder, and soy nuts) had total concentrations of isoflavone (expressed as milligrams per gram of dry weight) in the range 1.3-3.8 mg/g. Mixing soy with other components such as barley, rice, or wheat, contained lower isoflavone concentrations in the range 0.6-1.4 mg/g of dry weight. The commercial soy products used in American foods all contained isoflavones which had isoflavone concentrations ranging from 0.58 to 1.2 mg/g of dry weight [26].

Flavonoids in grape seeds

Grape seeds and skins are good sources of polyphenolic tannins that provide the astringent taste to wine. The monomers catechin and epicatechin and phenolic acid (gallic acid) are the main phenolic compounds in grape seeds. Levels of the flavonoids present in grape skin and seeds vary by location, prevailing climatic conditions, and postharvest handling. It is reported that fresh Merlot and Chardonnay seeds in Ontario, Canada, contained catechin and epicatechin at 64 and 79 mg/100g in Merlot and 42 and 99 mg/100g in Chardonnay seeds, respectively, on a wet basis [27]. While air-dried byproduct of the winery industry has about 127 mg of catechin and 115 mg of epicatechin/100 g of dry matter (dm) in Merlot seeds, Chardonnay seeds had about 358 mg of catechin and 421 mg of epicatechin/100g of dm [28].

Flavonoids in berries

The contents of flavonoids and phenolic acids in berries vary widely mainly due to varieties, maturity stage, growing and processing conditions. Anthocyanins are major phenolic compounds in berries. Anthocyanins are high in blueberry, crowberry and strawberry. They contain 626-4840 mg/kg fresh weight, 3200-5600 mg/kg and 786-3851 mg/kg, respectively. Cranberry contains the highest quercetin (104-250 mg/kg) and Myricetin (11-249 mg/kg). Kaempferol is high in Raspberry (284-1240 mg/kg) and Cranberry (120 mg/kg). Generally, the content of flavonols is higher than that of flavan-3-ols in berries [29-31].

4. Dietary intake of flavonoids

It is difficult to estimate dietary consumption of flavonoids because of their structural diversity, wide distribution in foods, and variations in their content in a given food. Two ways are used to measure flavonoids intake: absolute and relative intakes. Absolute intake is obtained by gathering data of all the flavonoids content of foods and calculating the amount ingested by humans that need accurate and comprehensive food composition tables to calculate. Relative intake uses biomarkers of flavonoid intakes in blood/urine to evaluate the level of flavonoids consumed [5]. Data of absolute intake of flavonoids is limited due to low accuracy of both the food composition and the dietary intake data. The main flavonoids dietary sources are fruit and beverages (fruit juice, wine, tea, coffee, chocolate and beer) and, to a lesser extent vegetables, dry legumes and cereals. The most abundant flavonoids in the diet are flavanols (catechins and proanthocyanidins), anthocyanins and their oxidation products. In Western populations, crude estimate of average intake is 65-250 mg/day [32]. The average intake of flavonols and flavones in the Netherlands was 23 mg/day, of which the flavonol quercetin contributed 16 mg/day, kaempferol 3.9 mg/day and myricetin 1.4 mg/day. Thus, flavones contributed only a minor fraction, about 7%. The main sources of favonols were tea in Japan and the Netherlands, red wine in Italy and onions in the USA and Greece. In Finland, berries are an important source of favonols. Flavonol intake was highest in Japan (64 mg/day) and lowest in Finland (6 mg/day). Average intakes of favonols published so far are 20 mg/day in middle-aged to older American males [33-37]. Our research group found that average intakes of the flavonoids were 150 mg/day based on the data from What We Eat in America 2001-2002 survey.

The intake of flavan-3-ols was the highest (65 mg), followed by flavonol (43.21 mg), flavanones (16.87 mg) and flavones (13.63 mg) [237]. A latest research which combined the USDA flavonoids database and 24h DR in the NHANES 1999-2002 found daily flavonoids intake in Americans 19 or older was 189.7 mg mainly from falvan-3-ols (83.5%), followed by falvanones, flavonols, anthocyanidins, flavones and isoflavones [38].

5. Digestion, metabolism, absorption and excretion

Many of the flavonoid compounds in the plant are in a pro-form, i.e., they are chemically modified to limit their biological activity or to cause them to be sequestered within the plant in concentrated forms. This allows them to be made available quickly to the plant in times of stress. Flavonoids are usually found as *O*-glycosides [39].

Processing and cooking often change the chemical status of flavonoids. These changes may have strong influence on how well the flavonoids are absorbed in the intestines and carried via the bloodstream to the organs of the body.

Digestion and absorption of flavonoids begin in the stomach. Most flavonoid glycosides reach the small intestine intact. However, EGCG can be hydrolyzed to EGC in human saliva by the esterase [40]. Another exception is anthocyanins, which are absorbed intact in the stomach [41]. Flavonoids are extensively metabolized after absorption in the small intestine, and subsequently go through liver metabolism, which includes conjugates of O-methylation, sulfation and glucuronidation. The nonabsorbed fraction in small intestine, particularly flavonoid glycosides, reaches the colon and is degraded by colonic microflora. The metabolites are absorbed by the colon and further metabolized in the liver. Based on the size of conjugates, the metabolites are eliminated through biliary or urinary routes. Large conjugates are more likely to be excreted through the biliary way, whereas small conjugates preferentially follow the urinary route. Biliary excretion is important for all polyphenols. Urinary excretion is an important pathway for flavanones, isoflavones, and flavan-3-ols. About 10% or more of these three classes of flavonoids are excreted via urine.

Much remain unknown about absorption and metabolism of flavonoids. In the future, much more efforts should be made to investigate the biological activity of conjugated and microbial metabolites of flavonoids. This may give us more accurate insights into the contribution of flavonoids to health promotion [5, 41, 42].

6. Bioavailability and bioefficacy

Bioavailability is assessed by how much of the phytochemicals that was orally consumed appears in urine. The greater the proportion of the dose found in the urine, the more bioavailable it must be. It failed to take into account the portion that is retained in tissues and excreted more slowly in the urine and how well a compound is reabsorbed in the proximal tubules of the kidney after initial filtration by the renal glomeruli or is excreted by the organic anion transporter [43]. The bioavailability of the flavonoids varies among the subtypes.

Bioavailability is an overall effect of absorption, metabolism, distribution, and excretion. It has been indicated that the biological activities of flavonoids *in vivo* depend on bioavailability and their metabolites generated during metabolism. Although parent flavonoids have been described as powerful beneficial agents, these compounds undergo extensive metabolism after oral administration leading to biotransformation such as methylation, glucuronidation, sulfation as well as degradation into phenolic acids or other compounds. Biotransformation can dramatically alter the biological properties [42].

Anthocyanins were monitored after a single dose of 150 mg to 2 g total given to the volunteers. The mean time to reach C-max was 1.5 h for plasma and 2.5 h for urine. Concentrations of anthocyanins in plasma were very low, on the order of 10-50 nmol/ L. Most studies reported low relative urinary excretions, ranging from 0.004% to 0.1% of the intake [44], at most up to 5% [45]. It was concluded that anthocyanins are very rapidly absorbed and eliminated.

For quercetin, Hollman *et al.* [46] found that the absorption of orally administered quercetin aglycone was approximately 24%. However, the absorption of quercetin glycosides from onions was 52%, suggesting that the glycoside moiety actually enhanced absorption. It has been reported

that quercetin was readily absorbed, but the absorption of quercetin from tea, apple or pure quercetin-3-rutinoside was lower than that of onions. The bioavailability of quercetin from both apples and pure quercetin rutinoside was only 30% of that in onions and the bioavailability of quercetin in tea was only half of that in onions [46, 47]. The elimination of quercetin metabolites is quite slow with reported half-lives ranging from 11 to 28 hr. Bioavailability differs among food sources, depending on the type of glycosides they contain. Glucosides of quercetin were more efficiently absorbed than aglycones. The bioavailability of rutin was about 20% of that of quercetin glucosides [48, 49].

It was reported that the total urinary excretion of conjugated flavanones accounted for 8.6% of the intake for hesperidin and 8.8% for naringin. Plasma concentrations of hesperetin metabolites reached 1.3-2.2 μmol/L when 130-220 mg hesperetin was consumed in the form of orange juice. About 6 μmol/L of naringenin metabolites was detected when 200 mg naringenin is consumed in the form of grapefruit juice [50, 51].

Bioavailability differs markedly among catechins. Giving pure catechins individually, Van Amelsvoort *et al.* [52] found that only epigallocatechin was methylated and that 4'-*O*-methyl-epigallocatechin accounted for 30-40% of the total metabolites of epigallocatechin. In another study, the 4'-*O*-methyl-epigallocatechin concentration was 5 times higher than that of epigallocatechin in plasma and 3 times higher than that in urine [53]. Epigallocatechin gallate (EGCG) is the only known polyphenol present in plasma in large proportion (77-90%) in a free form. The other catechins are highly conjugated with glucuronic acid and/or sulfate groups [54, 55].

Isoflavones, found in soybean-derived products, can be present as aglycones or glycosides, depending on the soy preparation. No significant differences were found in the absorption efficiency for aglycones and glycosides. Interestedly equol production was significantly higher after ingestion of daidzein than after ingestion of daidzein [56]. Equol is a bacterial metabolite that has been shown to be more estrogenic than its precursor daidzein in many studies. Only 30-40% of the Western population and 50% of Asians are “equol producers”. Equol producers may gain more benefits from soy consumption than do nonproducers [57-59].

7. Flavonoids and inflammation

As Nathan [60] pointed out “Inflammation is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injury”. Usually inflammation is a part of immune response. Over activation immune response can cause chronic infection or chronic inflammation. Chronic inflammation is involved in the development of several chronic diseases, such as cancers, cardiovascular diseases, obesity, diabetes, and neurodegenerative diseases. Among them, cardiovascular diseases and cancers are main causes of mortality in many developed countries [61-65].

7.1. Inflammation and cancer

Several clinical and epidemiological studies have demonstrated a strong association among chronic infection, inflammation, and cancer. Inflammation plays an important role in malignant processes. Chronic inflammation is involved in tumor initiation, promotion and progression [66-69]. Multiple mechanisms have been identified explaining the ways by which inflammatory status can promote cancer development (Figure 3). Accumulating evidence has convincingly demonstrated the cancer preventive effects of nonsteroidal anti-inflammatory drugs (NSAIDs), especially in colorectal cancer. The cells and mediators of chronic inflammation act as tumor promoters at distinct phases of malignant progression. Deletion of inflammatory mediators inhibits development of experimental cancers, and long-term use of nonsteroidal anti-inflammatory agents reduces the risk of some cancers [70].

Plants extracts rich in flavonoids have been traditionally used for treating acute and chronic inflammation in Asia. Many studies have proven that different flavonoids exhibit anti-inflammatory properties, especially citrus flavonoids. Flavanones (hespertin, naringin and neohesperidin), flavonols (quercetin, ruin and morin) and flavones (diosmin, apigenin and luteolin) were investigated in acute and chronic inflammation animal models [70]. Benavente-Garcia and his colleagues [71] demonstrated Apigenin and their glucosides showed a good anti-inflammatory activity without side effects as shown in other anti-inflammation agents.

Anti-inflammatory properties are the underlying mechanism for other potential health benefits,

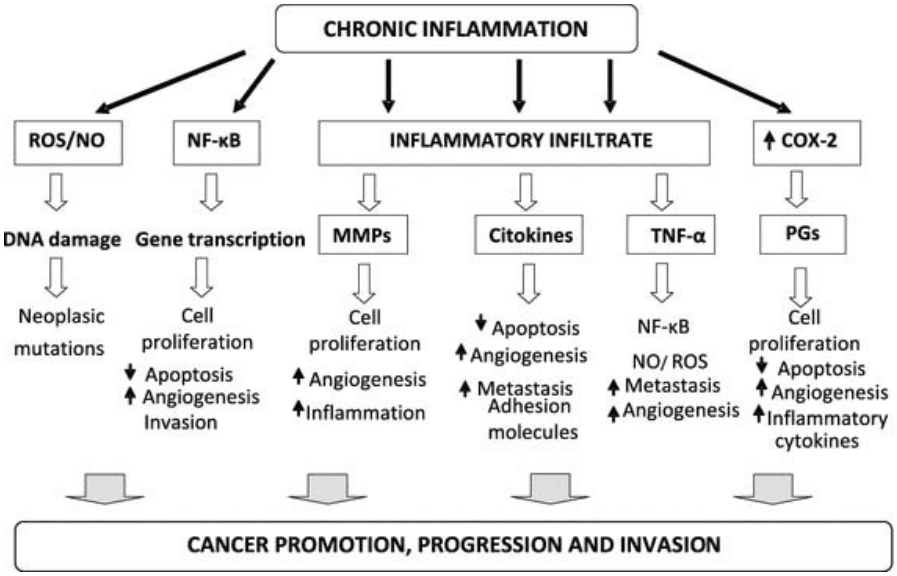

Figure 3. Mechanisms of cancer promotion and progression by chronic inflammation (Reprinted from Garcı´a-Lafuente, A. and Guillamo´, E. 2009, Inflamm. Res., 58, 537 with permission from Birkhaeuser Verlag AG).

including anticancer, antimicrobial, antiviral, immunomodulatory and antithrombotic activities [73, 74]. Therefore, the study about anti-inflammation properties are imperative and provides foundation for prevention and treatment of many chronic conditions [75, 76].

7.2. Anti-inflammatory mechanisms of flavonoids

Garcı´a-Lafuente and colleagues [72] summarized several mechanisms for anti-inflammatory activity of flavonoids (Figure 4). They include (a) anti-oxidative and radical scavenging activities, (b) regulation of cellular activities of inflammation-related cells, (c) modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase, lipoxygenase) and nitric oxide synthase, (d) modulation of the production of other pro-inflammatory molecules, and (e) modulation of pro-inflammatory gene expression (Figure 4).

7.2.1. Anti-oxidative and radical scavenging activities

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants [77]. In a multitude of disease states ranging from inflammatory injury to myocardial infarction and cancers, excess free radicals can accumulate resulting in oxidative stress. Free radicals also can attract various inflammatory mediators contributing to a generalized inflammatory response and tissue damage [78]. For example, high concentrations of nitric oxide, produced by inducible nitric oxide synthase in macrophages during inflammation which accumulated, would cause oxidative damage. In such condition, activated macrophages greatly increase the simultaneous production of both nitric oxide and superoxide anions. Excessive nitric oxide reacts with free radicals which result in producing the highly damaging peroxynitrite. Peroxynitrite has various toxic effects including direct oxidization of LDL, resulting in atherosclerosis [79]. Antioxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress. Flavonoids are powerful antioxidants that prevent formation of free radicals and also scavenge those already formed. Haenen and his colleagues found flavonoids are potent scavengers of peroxynitrite [80]. The antioxidant property of flavonoids is directed mostly toward $HO^{\cdot}$ and O_2^{-} as well as peroxyl and alkoxyl radicals [81]. Quercetin is reported to exhibit the highest antiradical property toward hydroxyl and peroxyl radicals and superoxide anions, and this property has been well attributed to its structural characteristics [82].

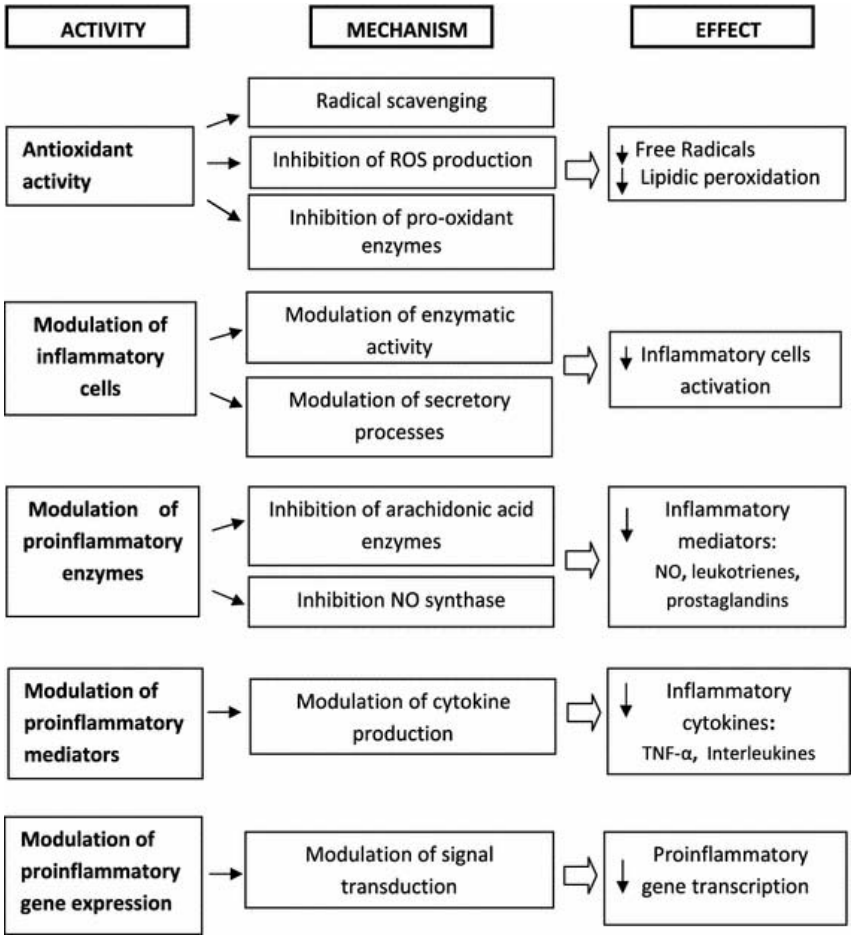

Figure 4. Anti-inflammatory mechanisms of flavonoids (Reprinted from Garcı´a-Lafuente, A. and Guillamo´, E. 2009, Inflamm. Res., 58, 537 with permission from Birkhaeuser Verlag AG).

Antonella, *et al.* demonstrated that rutin had an antioxidant effect comparable to that of quercetin [83].

Halliwell and Gutteridge [84] concluded that the mechanisms of antioxidant action include (1) suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging reactive oxygen species; and (3) upregulating or protecting antioxidant defenses. Flavonoids can function in most of the above mechanisms. First, flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase [85] and protein kinase C [86]. Flavonoids have also been shown to inhibit enzymes involved in reactive oxygen species generation: cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione *S*-transferase, mitochondrial succinoxidase, and NADH oxidase [87]. Flavonoids could efficiently chelate trace metals, which play an important role in oxygen metabolism. The proposed binding sites for trace metals to flavonoids are the catechol moiety in ring B, the 3-hydroxyl, 4-oxo groups in the heterocyclic ring, and the 4-oxo, 5-hydroxyl groups between the heterocyclic and the A rings. Due to their lower redox potentials ($0.23 < E7 < 0.75$ V), flavonoids are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 2.13-1.0 V, such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation. The overall capacity of flavonoids to act as antioxidants depends not only on the redox potential of the

couple aroxyl radical but also on possible side reactions of the aroxyl radical [88]. The radical scavenging activity depends on the structure and the substituents of the heterocyclic and B rings: (i) the presence of a catechol group in ring B, and (ii) a 2, 3- double bond conjugated with the 4-oxo group. The presence of a 3-hydroxyl group in the heterocyclic ring also increases the radical-scavenging activity. Glycosylation of this group, as in rutin, reduces greatly the radical-scavenging capacity. In conclusion, the antioxidant activity of flavonoids depends not only on their structural features but also by their location in the membrane [89].

7.2.2. Regulation of cellular activities of inflammation-related cells

The immune system is a highly regulated group of cells which may interact in a cell-cell manner and also may respond to intercellular messages including hormones, cytokines, and autacoids [90]. Flavonoids have effects on the function of all kinds of immune cells, including T cells, B cells, macrophages, NK cells, basophils, mast cells, neutrophils, eosinophils, and platelets. Hunter [91] reviewed several flavonoids that specifically impact the function of enzymic systems critically involved in inflammatory processes. Tyrosine and serine-threonine protein kinases are two great examples. These enzymes are directly involved in signal transduction and cell activation of the immune system. The members of a family of protein tyrosine kinases (PTKs) generated the proliferative signal by catalyzing the phosphorylation of cellular substrates, which in turn leads to T cell proliferation. Certain flavonoids could affect the activity of PTKs. Flavonoids primarily inhibit, sometimes stimulate T and B lymphocytes. The direction of effects depends on flavonoids concentration and experiment conditions [92]. Quercetin, kaempferol, and myricetin were potent inhibitors of histamine release from mast cells and basophils cells. The level of activity depends on their structure [93]. Tordera and coworkers reported quercetagetin-7-O-glucoside, apigenin, fisetin, kaempferol, luteolin and quercetin were the most potent inhibitors of beta-glucuronidase and lysozyme released in neutrophils by inhibiting arachidonic acid release from membranes [94].

7.2.3. Modulation of the activities of arachidonic acid metabolism enzymes and nitric oxide synthase

Arachidonic acid (AA), which is a starting point for a general inflammatory response, are involved in cyclooxygenase (COX) and lipoxygenase (LOX) release. COX and LOX play an important role as inflammatory mediators. Different flavonoid molecules have been shown modulating the activity of AA metabolizing enzymes such as phospholipase A2 (PLA2), COX and LOX and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS) [95, 96]. The inhibition of these enzymes leads to reduced production of many inflammatory factors, such as AA, prostaglandins, leucotrienes, and NO. Thus, the inhibition of these enzymes by flavonoids may be one of the most important mechanisms of their anti-inflammatory activity. The exact mechanism by which flavonoids inhibit these enzymes is not clear.

Quercetin was the first described flavonoid inhibitor of PLA2, which also inhibits COX and LOX and diminishes the formation of these inflammatory metabolites [97, 98]. Other flavonoids such as luteolin, galangin or morin were also demonstrated as inhibitors of COX [99].

Nitric oxide is produced by several different types of enzymes, including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) in endothelial cells and macrophages. iNOS is an inducible enzyme that is highly activated in inflammatory reaction [100]. The early release of nitric oxide by activating nitric-oxide synthase is important in maintaining the dilation of blood vessels, but the much higher concentrations of nitric oxide produced in macrophages may result in oxidative damage. iNOS is responsible for the overproduction of NO during inflammation [101]. Raso, G. M. *et al.* demonstrated quercetin, galangin, apigenin, and naringenin markedly decreased PGE2 release and COX-2, iNOS expression in a concentration-dependent manner which suggested that inhibition of iNOS and COX-2 expression by flavonoids may be one of the mechanisms responsible for their anti-inflammatory effects [102]. Fuu Sheu and coworkers studied effects and mechanisms of the soy isoflavones on the inducible nitric oxide synthase (iNOS) system in

lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. They found that soy isoflavones might attenuate excessive NO generation at inflammatory sites. The suppression of NO production by genistein, daidzein, and glycitein might be due to the inhibition of both the activity and expression of iNOS in LPS-activated macrophages [103].

7.2.4. Modulation of the production of other pro-inflammatory molecules

In addition to COX-2 and iNOS, several cytokines are strongly associated with inflammatory diseases. In particular, tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β, IL-6 are important contributors to chronic inflammatory disorders. It was shown that hesperetin and naringenin inhibited TNF-α-stimulated free fatty acid (FFA) secretion from mouse adipocytes. Obese adipose tissue is characterized by an excessive production of inflammatory adipokines including TNF-α. TNF-α stimulates FFA secretion through adipocyte lipolysis, and increased plasma levels of FFA promote insulin resistance. Hesperetin and naringenin could block the TNF-α-induced activation of the NF-kappaB and ERK pathways [104]. Rao, *et al.* demonstrated Epicatechin and quercetin significantly and dose-dependently inhibited the production of the inflammatory mediator nitric oxide (NO), and the cytokines (TNF-α and IL-12), in lipopolysaccharide (LPS) and interferon IFN-γ activated murine peritoneal macrophages, without displaying cytotoxicity [105]. Hougee and colleagues reported apigenin and luteolin dose-dependently inhibited both pro-inflammatory cytokine (TNF-α, IFN-γ) production and metabolic activity of LPS-stimulated peripheral blood mononuclear cell (PBMC) [106].

7.2.5. Modulation of proinflammatory gene expression

Xagorari, *et al.* compared the ability of several flavonoids to modulate the production of proinflammatory molecules from lipopolysaccharide (LPS)-stimulated murine macrophages and investigated their mechanism(s) of action. They found that quercetin, and genistein inhibited both the LPS-stimulated TNF-α and IL-6 release, whereas hesperetin only inhibited TNF-α release. Among them luteolin and quercetin were the most potent inhibitors of TNF-α production with an IC(50) of less than 1 and 5 microM, respectively. The underlying mechanism of this effect is inhibition of protein tyrosine phosphorylation and nuclear factor-kappaB-mediated gene expression [107]. Quercetin inhibited TNF-α induced IFN-γ-inducible protein 10 (IP-10) and macrophage inflammatory protein 2 (MIP-2) gene expression in Mode-K cells with effective inhibitory concentration of 40 and 44 microM, respectively. The anti-inflammatory effect of quercetin in epithelial cells is due to the inhibition of cofactor recruitment at the chromatin of proinflammatory genes [108]. Ruiz and Haller characterized the molecular mechanisms of flavonoids including apigenin, luteolin, genistein, 3'-hydroxy-flavone, and flavone in inhibiting TNF-α-induced interferon-induced protein (IP)-10 gene expression in the murine intestinal epithelial cell (IEC) line Mode-K. They demonstrated that it was 3'-hydroxy-flavone, not the chemical core structure flavone, blocked TNF-α-induced NF-kappaB transcriptional activity and IP-10 expression by inhibiting IkappaB kinase activity. In addition to the compound-specific inhibition of TNF-induced NF-kappaB DNA binding and NF-kappaB transcriptional activity, apigenin and luteolin selectively blocked a serine/threonine protein kinase (Akt) phosphorylation activity. Genistein blocked IP-10 but not IL-6 expression through NF-kappaB, IRF, and Akt independent mechanisms [109]. Another study reported that flavone, the isoflavones daidzein and genistein, the flavonols isorhamnetin, kaempferol and quercetin, the flavanone naringenin, and the anthocyanin pelargonidin inhibited iNOS protein and mRNA expression and also NO production in a dose-dependent manner and inhibited the activation of NF-kappaB, which is a significant transcription factor for iNOS. Genistein, kaempferol, quercetin, and daidzein also inhibited the activation of the signal transducer and activator of transcription 1 (STAT-1), another important transcription factor for iNOS [110].

These studies demonstrated the functional diversity of flavonoids in inhibiting proinflammatory processes. Table 2 shows the possible mechanism of some flavonoids in modulating expression of inflammatory genes.

Table 2. Possible mechanism of some flavonoids in modulating expression of inflammatory genes (Modified with permission from Pan, M. H., Lai, C. S., Dushenkov, S., and Ho, C. T. 2009, J. Agric. Food Chem., 57, 4467 Copyright (2009) American Chemical Society).

Class	Compound	Dietary source	Anti-inflammatory mechanisms	Refs
Flavones	Apigenin	Parsley and	Inhibits HIF-1R and VEGF expression by blocking PI3K/Akt signaling	[112-114]
		celery	Blocks LPS-induced pro-inflammatory cytokines expression by inactivating NF-κB through the suppression of p65 phosphorylation	
			Inhibits UVB-induced CBP binding to the ATF/CRE of the COX-2 promoter	
	Tangeretin	Citrus peels	Inhibits LPS-induced NO production and suppresses IL-1β-induced COX-2 expression through inhibition of p38 MAPK, JNK, and AKT activation	[115, 116]
	Luteolin	Parsley and celery	Suppressed the expression of TNF-alpha, IL-8, IL-6, GM-CSF, and COX-2 through a decrease in the intracellular Ca2+ levels, and suppressed the ERK 1/2, JNK 1/2, and NF- kappaB activation	[130]
Flavonols	Kaempferol	Broccoli and	Inhibits iNOS expression and NO production by suppressing STAT-1 and NF-κB activations in activated macrophages	[117, 118]
		tea	Inhibits cytokine-induced expression of iNOS, COX-2, and adhesion molecules by blocking signaling of NF-κB and AP-1 in human endothelial cells	
	Quercetin	Onion, broccoli, apples, and	Inhibits phorbol-12-myristate-13-acetate (PMA)- induced histamine release and expression of pro-inflammatory cytokines through inhibiting nuclear translocation,	[118, 109]
		berries	DNA binding and transcriptional activity of NF-κB in mast cells	
			Suppresses TNF-induced NF-κB and CBP/p300 recruitment to pro-inflammatory gene promoters and down-regulation of interferon-inducible protein-10 (IP-10) and macrophage inflammatory protein-2 (MIP-2) gene expression	
Flavanones	Naringenin	Orange peel	Inhibits LPS-induced IL-1β, TNF-R production by suppressing phosphorylation on serines 63 and 73 of Jun proto-oncogene-encoded AP-1 transcription factor	[117, 119]
			Inhibits iNOS protein and mRNA expression and also NO production through blocking activation of NF-κB	

Table 2 continued..

Flavanols	Epicatechin gallate (ECG)	Tea	Inhibits TPA-induced O2 generation and nuclear translocation of p65 and subsequent DNA binding of NF-κB by blocking the degradation of IκBR	[120]
		Tea	Suppresses Akt phosphorylation as well as TNF activation of tumor necrosis factor receptor (TNFR)-1, which subsequently resulted in reduced MCP-1 production	
	Epigallocatechin-3-gallate (EGCG)		Inhibits IL-1β-dependent pro-inflammatory signal transduction through mediated receptor-associated kinase (IRAK) degradation, IKK activation, and NF-κB signaling	[121-123]
			Inhibits TPA-induced DNA binding of NF-κB and CBP by blocking activation of p38 MAPK	
Isoflavonoids	Genistein	Soybean	Reduces LPS-induced IL-6 cytokine production and affects NF-κB subcellular localization and DNA binding transcription	[124, 125]
	Daidizein	Soybean	Down-regulates phytohemagglutinin (PHA)-induced activation of p42/44 and JNK	[110]
			Inhibited iNOS protein and mRNA expression by inhibiting the activation of the signal transducer and activator of transcription 1 (STAT-1)	
Anthocyanidins	Cyanidin	Cherry and strawberry	Inhibits PDGF-induced VEGF expression by preventing activation of p38 MAPK and JNK	[126-129]
			Inhibits LPS/IFN-γ-induced NO production and LPS-induced iNOS and COX-2 expression by suppressing the functional activation of NF-κB but not nuclear translocation	
	Procyanidins	Grape seed	Inhibits UVB- and TPA-induced transactivation of NF-κB and AP-1 and expression of COX-2 and TNF-R through the inhibition of MAPK activity	[131]
			Reduces the expression of IL-6 and MCP-1 and enhances the production of the anti-inflammatory adipokine adiponectin	

8. Flavonoids and cancer

8.1. Introduction

Epidemiological studies indicate an inverse association between the consumption of flavonoid-rich fruits and vegetables and the incidence of different types of cancers [132-136]. Consumption of vegetables and fruits prevent carcinogenesis through a variety of mechanisms. Phytochemicals may reduce cancer susceptibility. Among them flavonoids are especially promising candidates for cancer prevention [137, 138]. Flavonoids have been studied for about 50 years. The cellular mechanisms involved in their biological action are still not completely understood. In the cancer development process,

phytochemicals can serve as suppressing, blocking and transforming agents. Suppressing agents prevent the formation of new cancers from pre-cancer cells, blocking agents prevent carcinogenic compounds from reaching critical initiation sites, while transformation agents act to facilitate the metabolism of carcinogenic components into less toxic materials or to prevent their biological actions [139]. Flavonoids can act as all three types of agents [140-142].

8.2. Anti-proliferation

Cancer is a hyper proliferative disorder. So cancer prevention is generally associated with inhibition, reversion, or retardation of cellular hyper proliferation. Although most flavonoids appear to be non-toxic to humans and animals, they have demonstrated the ability to inhibit proliferation in many kinds of cancerous cell lines.

It has been reported that citrus flavonoids (flavanones, flavones, flavonols, and anthocyanins) exhibited anti-proliferative activities of the human lung DMS-114, colon HT-29, breast ER+ MCF-7 and ER- MDA-MB-435, prostate DU-145, and melanoma SK-MEL5 cancer cell lines. The highly methoxylated flavones exhibited the highest activities against this six cancer cell lines [143]. Anti-proliferative activity has also been shown with these compounds against squamous cell carcinoma HBT43 [144], human lung carcinoma A549, gastric cancer, metastatic lymph node (TGBC11TKB), A549 and B16 melanoma [145], ovarian cancer cells OVCAR-3 [146], leukemia CEM/ADR5000 and colon cancer cell lines [147]. These findings indicate that citrus poly-methoxylated flavones exhibit anti-proliferative activity against a wide spectrum of human cancer cell lines.

Many studies have shown that the anti-proliferative activities of flavonoids are dependent on particular structural motifs. The position, number, and substitution of the hydroxyl in the flavonoid A and B rings may be important factors affecting anti-proliferative and/or cytotoxic activities of flavonoids [148-150]. Firstly, the presence of a double bond at C2-C3 in polyhydroxylated flavonoids increased the anti-proliferative activity. So flavones have greater activity compared with flavanones which are observed in many important cancer cell lines including those of lung, colon, breast, prostate, and skin [151]. Secondly, the number and position of the substituents in the flavonoid base structure may influence anti-proliferative activity. It was found that three adjacent hydroxyl groups in the B ring provided a strong anti-proliferative effect. Agullo and his colleagues confirmed this [152]. Thirdly, the presence of the glycosidic substituents is linked to the absence of anti-proliferative activities. Recent studies showed the anti-proliferative activities of the hydroxylated flavone and flavanone aglycons against a number of human cancer cell lines [153-155]. Beside these natural substituents, the presence of a methoxyl group in position C-4 may be related to increased cytostatic activity. Furthermore, the flavone tangeretin (tetrahydroxylated in the A ring) had a greater anti-proliferative compared to other flavonoids. The less polar and planar structure of tangeretin may play a critical role in its biological activity [156].

Flavonoids have been shown to inhibit several kinases involved in signal transduction, such as protein kinases C, tyrosine kinases, PI 3-kinases, or S6 kinase [157] and human cytochrome P450 (CYP) family enzymes [158]. In eukaryotic cells, cell cycle consists of four distinct phases, G1, S, G2, and M. Cell cycle progression is timely regulated by cyclin-dependent kinases (CDKs) and their cyclin subunits at the two checkpoints, G1/S and G2/M [159]. Flavonoids also have been found to inhibit the proliferation of many cancer cells by arresting cell-cycle progression at either G1/S or G2/M checkpoint [160]. It can be concluded that the anti-proliferation effects of flavonoids depend on the inhibition of several kinases and their involvement in cell-cycle arrest. These inhibitions are structure related. A double bond at C2-C3, three adjacent hydroxyl groups in the B ring and being aglycones are essential.

8.3. Anti-invasive effects

In addition to rapid and continuous division and proliferation, another important and unique feature of cancer cells is metastasis. Metastasis is the ability of cancer cells to invade surrounding tissues and to migrate from their primary site to secondary sites. The invasion of surrounding

tissues by cancer cells involves several steps, including matrix metalloproteinase (MMP) secretion, migration, invasion, and adhesion. *Citrus* flavonoids have shown to impact all of the steps [4].

It is reported that the flavonoid nobiletin inhibited enzymatic activity of MMP-9 in a concentration-dependent manner *in vivo* [161]. Vijayababu, *et al.* demonstrated quercetin also downregulated the expression of MMP-2 and MMP-9 in prostate cancer PC-3 cells. The inhibition of metastasis-specific MMPs in cancer cells may be one of the mechanisms for anticancer function of quercetin [162]. Tangeretin has inhibitory effects on a number of intracellular processes, which leads to an inhibition of cell mobility and hence of invasion in *a vivo* study [163]. Luteolin was shown to suppress production and secretion of cytokines such as TNF-α and IL-6, which in turn can stimulate cancer cell migration and metastasis. Focal adhesion kinase (FAK) activity in human carcinoma cells is associated with increased invasive potential. Luteolin inhibited FAK phosphorylation which may contribute to suppressing FAK's cell invasion ability. *In vitro* studies have shown that luteolin potently inhibits migration and invasion of cancer cells through blocking the MAPK (mitogen-activated protein kinase)/ERKs (extracellular signal-regulated kinases) and PI3K-Akt (phosphoinositide 3-kinase-serine-threonine protein kinase) pathways [164-166].

8.4. Anti-angiogenic effects

Angiogenesis is the generation of new blood vessels which is critical for solid tumor growth and metastasis. Tumor cells are able to produce several angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast-like growth factor (bFGF), interleukin 8 (IL-8) and matrix metalloproteases (MMP) to trigger angiogenesis [167]. Luteolin was found to be a potent angiogenesis inhibitor. Eleni *et al.* found that certain flavonoids not only inhibit *in vitro* angiogenesis, but luteolin also inhibited tumor growth and angiogenesis in a murine xenograft model [168].

Suppression of VEGF secretion and signaling induced by VEGF may be the main mechanism of flavonoids-induced anti-angiogenesis. Transcription of the VEGF gene is enhanced by hypoxia-inducible factor-1α (HIF-1α). Yuki and colleagues found that luteolin, quercetin, and methyl ophiopogonanone B (MOB, homoisoflavonoids) suppressed VEGF expression by inhibiting HIF-1α through p53-mediated proteasomal degradation of this transcription factor [169]. It was demonstrated that luteolin inhibited VEGF-induced PI3K activity in HUVECs (human umbilical vein endothelial cells). P13K is responsible for conveying both survival and mitotic downstream signals [170].

MMP inhibitors can block angiogenesis because MMP is needed for this process [171]. Luteolin, the strongest among the eight flavonoids tested, was demonstrated as a potent MMP inhibitor. It suppresses MMP expression through suppressing NF-κB or directly inhibiting MMP activity. So flavonoids additional anti-angiogenesis mechanism may be via suppression of MMPs [172].

8.5. Estrogen motif (isoflavones and breast, prostate cancer)

The risk of prostate and breast cancers is lower in regions with high soy intake, especially in Asia. The risk increases for Asian people who migrate to Western countries or adopt a Western lifestyle [173-176]. Meta-analysis studies demonstrated that high soy food intake can be related to a 30% reduction in prostate cancer risk and a smaller risk reduction in breast cancer [177,178].

Soy and its processed products (tofu, soymilk, tempeh, miso, natto, soy-based yoghurts, desserts and soy protein and soy flour added to a variety of foods) are the main sources of isoflavones in the human diet. For Western people, major sources were tofu, doughnuts, soymilk, white bread, and canned tuna. Doughnuts accounted for about 20% of the average of daily intake of genistein and 15% of daidzein intake [179].

Isoflavones are known as phytoestrogens, mainly include daidzein, genistein and glycitein, which are present in glycosylated or aglycone forms. Isoflavones are similar in structure to the estrogenic steroids (17β-estradiol) which provides them the capacity to bind estrogen receptors (ERs) and to induce hormone-like effects. Estrogens regulate many physiological activities, including cell growth and differentiation, apoptosis and

tissue-specific gene regulation and also hormone-dependent diseases such as breast and prostate cancers. Estrogens have been implicated in the initiation and promotion stages of breast and prostate cancer. The biological functions of estrogens are mediated by the binding to one of the two specific nuclear receptors, ERα and ERβ and induce transcription of estrogen-responsive target genes. Although numerous studies have been conducted to understand the effects of soy isoflavones on breast and prostate cancer, the mechanisms of action are still not entirely elucidated [180].

Estrogens binding ERα exert strong proliferation stimulatory effects while those interacting with ERβ tend to reduce this stimulation. Therefore, cell response would depend on the balance between ERα and ERβ expression levels. ERα signaling pathway is promising protective target in cancer prevention [181]. *In vitro* and *in vivo* studies reported genistein at 1-10 mM levels downregulated ERα and upregulated of ERβ (mRNA and protein levels) in breast cancer cells [182, 183]. In the dorsolateral prostate of rats, dietary genistein down-regulated expression of the androgen receptor (AR) and ERα in the rat prostate at concentrations comparable to those found in humans on a soy diet [184, 185]. Soy isoflavones have higher affinity to ERβ than to ERα at normal soy intake level [186]. The activation of ERs by isoflavones subsequently lead to a modulation of the expression of their target genes, and then to a modulation of cellular processes such as proliferation and apoptosis. Well-known target genes for the ERs are TFF-1/pS2 (a gene expressed primarily in gastric mucosa and breast epithelia), cathepsin D (a lysosomal proteinase), PR, pS2, bcl-2 c-Myc and cyclin D1 [187-189].

Androgen receptor (AR) pathway plays a pivotal role in prostate cell growth, differentiation and function. Agents that could eliminate or antagonist AR activation are considered useful to prevent and treat prostate cancer [180]. Genistein has been shown to exert anti-androgenic effects in prostate cells, and downregulates prostate specific antigen (PSA, the typical androgen-responsive gene) mRNA and the protein expression and secretion [190]. The mechanisms underlying isoflavone anti-androgenic effects are still not completely elucidated. Isoflavones may also decrease androgen levels without affecting gonadotropin or testicular steroidogenic acute regulatory peptide levels [191].

9. Flavonoids and cardiovascular diseases

9.1. Introduction

Research has shown that reactive oxygen species (ROS) play a role in the pathophysiology of cardiovascular diseases [192]. In animal models of hyperlipidemia [193], hypertension [194], and diabetes [195], elevated levels of vascular superoxide radicals production have been observed. The following mechanisms may explain why excessive production of ROS leads to vascular pathology. Firstly, ROS initiates the oxidation of low-density lipoprotein (LDL)[196]. Secondly, superoxide anion rapidly inactivates endothelium derived nitric oxide (NO), a molecule with intrinsic anti-atherogenic properties, and leads to endothelial dysfunction, a hallmark of early atherosclerosis [197]. Thirdly, ROS participate in vascular smooth muscle cell growth and migration, modulation of endothelial function, and modification of the extracellular matrix. Flavonoids are normally anti-oxidants and deactivate DOS [198].

Epidemiological and experimental studies have demonstrated an inverse relationship between consumption of foods rich in flavonoids and risk of CVD [199-200]. Dietary flavonoids are known for their antiplatelet activity resulting in cardiovascular protection, although the specific mechanisms by which this inhibition occurs has not been fully established. The protective effects of flavonoids mainly include antithrombotic, anti-ischemic, anti-oxidant, and vaso-relaxant. Thus, it could be concluded that flavonoids decrease the risk of coronary heart disease through three major actions: (A) improving coronary vasodilatation, (B) decreasing the ability of platelets in the blood to clot, and (C) preventing LDLs from oxidizing [71].

9.2. Vasorelaxant and Vasoprotective Effects

Endothelium is critical to normal coronary vascular function. Endothelial cells synthesize and release a number of factors, including prostacyclin, NO, endothelium derived hyperpolarizing factor (EDHF), and endothelin. They are important in the regulation of vascular tone, platelet and

leukocyte adhesion, aggregation, and migration. Endothelial dysfunction, indicated by an impairment of endothelium-dependent vasodilatation, is an important component of coronary artery disease (CAD). NO is the critical factor in maintaining normal coronary vascular functions, and it is a well-established correlation between CAD and low NO level [71].

Chan, *et al.* examined flavonols (fisetin, quercetin, and 3,3',4'-trihydroxyflavone) and flavones (apigenin, chrysin, and luteolin) vasorelaxant activity using rat isolated thoracic aorta. They found that the hydroxyl substitution in the carbon 3 position which is characteristic of the flavonols is important in stimulating endothelium-dependent vasorelaxation, and the absence of hydroxyl substitution on the A phenolic ring enhances the relaxant action [201]. It was showed that quercetin exhibited vasodilator effects in isolated rat aorta and the relaxation induced by quercetin and other related flavonoids are endothelium-independent. These effects are modulated by endothelial factors [202].

Francisco and coworkers concluded that the primary mechanism of endothelium-independent flavonoid-induced vasodilation results from inhibition of protein kinases such as myosin light chain kinase and protein kinase C. Flavonoids relax resistance vessels and have antihypertensive effects [203]. Other recent studies show that vasorelaxant effects of *citrus*-fruit flavonoids naringenin and hesperetin are basically related to the inhibition of different cyclic nucleotide phosphodiesterase (PDE) iso-enzymes [204].

9.3. Antithrombotic properties

Platelet aggregation is the critical event during the initiation of coronary thrombosis. Flavonoids have been reported to modulate platelet function, and reduce the risk of clot formation [205]. The effect of distinct structural types of flavonoids on agonist induced platelet responses has been investigated. Structural features such as the presence of the double bond in C2-C3 and a keto group in C4 are related to tight binding of flavonoids to the Thromboxane A2 (TxA2) receptor. The level of inhibition on platelet aggregation by specificflavonoids *in vitro* is related to their ability to bind with the TxA2 receptor [206]. TxA2 is a strong platelet agonist involved in the pathogenesis of thrombotic diseases that elicits platelet aggregation and vasoconstriction through the activation of its specific membrane receptors. Another research showed that C7 and C8 carbons in the A ring, gamma-pyrone structure conjugated with a double bond between C2 and C3 carbons in the C ring, and C2', C3', and C4' carbons in the B ring are the structural determinants that create the active flavonoid skeleton in TP (specific membrane receptor) blockade [207].

9.4. Effects on serum lipids

Dauchet and his colleagues conducted an epidemiological study to assess the relationship between frequency of citrus fruit and vegetable intake and CHD risk in France and Northern Ireland, two European populations with contrasting cardiovascular incidence rates. They found frequency of citrus fruits, but not other fruits, intake is associated with lower rates of acute coronary events in both France and Northern Ireland and there was no evidence for any association between vegetable intake and total CHD events [208]. Increased LDL, especially oxidized LDL is recognized as trigger factors in CHD. It has been shown that consumption of fruit and vegetables is conversely related to the LDL cholesterol levels [209]. Flavonoids appeared to protect LDL from oxidation caused by the macrophages as it reduced the generation of lipid hydroperoxides and protects R-tocopherol. Flavonoids could protect α-tocopherol in LDL from oxidation, maintain their levels for longer periods of time, and delay the onset of lipid peroxidation. Flavonoids inhibit LDL oxidation through several possible mechanisms. Firstly, flavonoids may reduce the free radicals generation or release. Secondly, flavonoids could regenerate active α-tocopherol by donating a hydrogen atom to the α-tocopheryl radical. The latter is formed when it transfers its own OH hydrogen atom to a lipid peroxyl radical to terminate the chain reaction of lipid peroxidation. Thirdly, flavonoids may chelate metal ions, such as iron and copper, thereby diminishing the free radicals production in the medium [210]. *Citrus* flavonoids also can act as regulators of apolipoprotein B (apoB). Both naringenin and hesperetin were shown to decrease the availability of lipids for assembly of apoB

containing lipoproteins, an effect mediated by (1) reduced activities of acyl CoA: cholesterol acyltransferase (ACAT1) and ACAT2, (2) a selective decrease in ACAT2 expression, and (3) reduced microsomal triglyceride transfer protein (MTP) activity. Other dietary experiment studies on rats with diet- and nutrition-induced hyperlipidemia demonstrated that hesperidin significantly increased HDL and lowered cholesterol, LDL, total lipid and triglyceride plasma levels [211].

Flavonoids are protective against CAD through several processes, such as (1) decrease in LDL oxidation, (2) increase in HDL levels, (3) reduction of cardiac mast-cell-mediator release, and (4) decrease in cardiovascular inflammation [210].

10. Flavonoids and neurological degenerative diseases

10.1. Introduction

In neuropathology, epidemiological studies have indicated the positive effects of flavonoids, in particular towards deficits commonly observed with aging. They include reduced performance of cognitive, memory and learning tasks, Alzheimer disease, Parkinson disease, Huntington's disease, and Amyotrophic lateral sclerosis (ALS). Flavonoids could improve memory, learning and cognitive performance [212-215]. These neurological functions can be influenced by supplementation of single dietary flavonoids, or as part of a flavonoid-rich preparation. Nutritional studies have demonstrated that consumption of green tea could have a beneficial role in reducing the risk of pathology of Parkinson disease. Administration of the polyphenol EGCG significantly delayed the onset of symptoms and prevented the loss of substantia nigra dopaminergic neurons in experimental mice models of Parkinson's disease [216]. Treatment with other sources of flavonoids, such as those from the Ginkgo biloba extract EGb 761, can also improve the cognitive performance of Alzheimer's patients [217]. Catechins from green tea have been demonstrated to improve special cognition and learning ability in rodents and to reduce amyloidosis in transgenic mice, an animal model for Alzheimer disease [218]. Both animal and human studies have shown that nutritional interventions and increasing dietary intake of fruits and vegetables can retard and even reverse age-related declines in brain function and in cognitive and motor performance [219, 220]. Although experimental data are consistent in demonstrating the neuroprotective effects of polyphenols, especially flavonoids *in vitro* and in animal models, the clinical evidence that these agents may prevent or slow the course of these diseases is still relatively unsatisfactory, and is insufficient to strongly modify clinical practice [221].

10.2. Mechanisms

In order to modulate brain function, flavonoids must enter into the brain; they must first cross the blood-brain barrier (BBB). Studies have indicated that the flavanones hesperetin, naringenin and their relevant *in vivo* metabolites, some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, are able to across the BBB [222, 223]. Animal feeding studies also indicate that flavonoids may access the brain, the tea flavanol, EGCG, being reported to access the brain after oral administration to mice [224]. Oral ingestion of pure epicatechin resulted in the detection of epicatechin metabolites (glucuronide and 30-O-methyl-epicatechin glucuronide) in rat brain tissue [225]. After the animals were fed with blueberry, anthocyanidins have also been detected in the brain, with several anthocyanidins being identified in different regions of rat brain [226]. Furthermore, it was shown that the potential of flavonoid penetration is dependent upon the compound lipophilicity and be influenced by their interactions with specific efflux transporters expressed in the BBB. One of such transporters is P-glycoprotein, which plays an important role in drug absorption and brain uptake [227].

How flavonoids exert their neuroprotective actions is unclear. They may be linked to antioxidant actions, the modulation of neurotransmitter release, and the stimulation of hippocampal neurogenesis via the modulation of signaling or an ability to improve cerebrovascular blood flow [228, 229]. Increased neuronal activity requires an increase in blood flow to support the metabolic requirements. Human age-related degenerative diseases are associated with a decline in brain blood flow. Flavonoid-rich foods, in particular those containing flavanols, have been observed to

improve peripheral blood flow. Other studies demonstrated that total brain grey matter blood flow (measured by arterial spin-labeling and MRI) of healthy young women was increased by a single large dose of cocoa flavanols [230] and consumption of a drink with a high cocoa flavanol content for 14 days was associated with an increase in brain blood flow in older individuals [231]. It has been shown that consumption of cocoa flavanols for 5 days enhanced the functional magnetic resonance imaging (fMRI) response to a complex cognitive task in healthy young women. The enhanced fMRI response indicated an increased cortical blood flow response to a task. Increased cerebrovascular function, especially in the hippocampus, a brain region important for memory and new neuronal growth may facilitate adult neurogenesis and are considered vital for learning and memory [232]. The enhancement of both short-term and long-term memory is controlled at the molecular level. Four signaling pathways control this process: (i) cAMP-dependent protein kinase (protein kinase A), (ii) calcium-calmodulin kinases, (iii) protein kinase C, and (iv) mitogen-activated protein kinase (MAPK). All four pathways converge to signal to the cAMP-response element-binding protein (CREB). CREB binds to the promoter regions of genes which related to memory and synaptic plasticity [233]. Flavonoids are potential neuroprotective, neuromodulatory or anti-neuroinflammatory agents. Those beneficial properties are mediated by their abilities to interact with both protein and lipid kinase signaling cascades, rather than *via* their potential to act as classical antioxidants. One potential mechanism of flavonoids in modulating neuronal function, synaptic plasticity and synaptogenesis may be related to CREB. Interactions within the MAPK pathway are thought to be central for flavonoids found in berries, tea and cocoa, to mediate the cellular function. For example, the flavanol, (2)-epicatechin, induces both ERK1/2 and CREB activation in cortical neurons and subsequently increases CREB regulated gene expression [234]. Another flavonoid, fisetin, was shown to improve long-term potentiation (LTP) and memory through a CREB/ ERK mechanism [235].

The challenge now is to determine the precise site(s) of action of flavonoids within the signaling pathways and the sequence of events that allow them to regulate neuronal function in the central nervous system. A clear understanding of their mechanisms of action as modulators of cell signaling will be critical for evaluating their roles as inhibitors of neurodegeneration or as modulators of brain function [236].

11. CONCLUSION

Although an extensive amount of information has been presented on flavonoids and a variety of potential beneficial effects have been elucidated, the need for further research in this area is clearly evident. The study of flavonoids is complex because of the heterogeneity of the different molecular structures and the insufficient data on bioavailability. Moreover, most of the studies were *in vitro* and in *in vivo* animal models. It is difficult to draw definite conclusions about the usefulness of dietary flavonoids. More bioavailability and intervention studies are needed in order to establish their effectiveness in the treatment of chronic diseases such as chronic inflammation, cancers, cardiovascular diseases and degenerative diseases.

DECLARATION OF INTERESTS

The author has no relevant interests to declare.

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