Bioactive effects of citrus flavonoids and role in the prevention of atherosclerosis and cancer

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Abstract

Citrus fruits are the main fruits of the Mediterranean diet and have been long recognized for their beneficial effects on human health. Observational studies have shown a significant association between dietary flavonoid intake and reduced risk of cardiovascular and malignant diseases. The beneficial effects of citrus fruits on human health appear to be due to their high content in vitamins, minerals and fibers. In particular, the antioxidant and anti-inflammatory activities have been indicated as some of the mechanisms through which citrus fruits may thwart the development of chronic degenerative diseases such as atherosclerosis and cancer. This review would critically examine the results from numerous experimental and clinical studies carried out in order assess the contribute of citrus flavonoids to the prevention of chronic pathological conditions including atherosclerosis and cancer.

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Introduction

Growing experimental and observational studies provide evidence that the beneficial effects exerted by foods on human health appears to be due to their content in vitamins, minerals, fiber and antioxidant nutraceutical components that provide functional characteristics to foods and account for their preventive effects on chronic-degenerative diseases.[1] Several antioxidant phytochemicals that are present in fruits and vegetables, can modulate the metabolic functions and redox balance of human cells in order to maintain their integrity and tissue homeostasis to prevent the onset of chronic-degenerative diseases.[2] Plants biodiversity is related to the presence of a wide range of different chemical compounds, including phythochemicals, many of which are endowed with important pharmacological properties and whose beneficial effects on human health are currently under extensive investigations.[3] In this setting, numerous observational studies have highlighted the fact that an increased consumption of fruits and vegetables may protect humans against chronic degenerative diseases, such as cancer and atherosclerosis (Table 1).[4-5] For instance, flavonoids are an heterogeneous group of substances largely present in plants which show to possess several pharmacological functions.[6] These molecules protect plants from UV radiation and pathogens.[7] Dietary flavonoids are important components of the human diet.[6] These compounds are present in significant amount in a wide variety of foods including fruit, vegetables, nuts, cocoa, soy, coffee, tea, and wine.[8] Epidemiological studies have shown a significant correlation between dietary flavonoid intake and decreased incidence of cardiovascular diseases, cancer,[8,9] type 2 diabetes,[10] neurodegenerative disorders,[11] and osteoporosis.[12] Although some observational studies failed to show significant correlation between total flavonoids intake and reduced risk of stroke, they reported a significant association between increased intake of citrus flavonone subclass from orange and grapefruit and reduced risk of ischemic stroke in women.[13] This review would provide insight into the most recent findings and advances in understanding the cellular mechanisms underlying the preventive effects of citrus flavonoids on the onset of some chronic pathological conditions such as atherosclerosis and human tumors.

Classification and distribution of flavonoids

Flavonoids are a heterogeneous group of substances widely present in many foods. Flavonoids are a subclass of calorie-free polyphenols.[14] These molecules belong to a class of secondary plant metabolites showing a polyphenolic structure, which are widely present, in particular, in fruit, vegetables and which are endowed of various biological activities.[15] The common chemical structure of flavonoids consists of two aromatic rings linked by three carbon atoms that form an oxygenated heterocycle (Figure 1). The differences in the chemical structure of each group of flavonoids are given by the different number and arrangement of the hydroxyl groups and by their degree of alkylation and glycosylation. These molecules can be detected as free aglycone form. However, they are often linked to glycosides. In this form these compounds are soluble in water.[16] Flavonoids are endowed with antioxidant activity that account for their preventive effects on the onset of chronic diseases such as cancer, atherosclerosis and neurodegenerative diseases (Figure 2 and 3).[15] In this setting, accumulating evidence has indicated that the presence of these substances into the daily diet can contribute to the beneficial effects on human health and to prevent on the onset of degenerative diseases.[1] Flavonoids are the major water-soluble plants pigments which are involved in the production of the colors needed to attract pollinating insects. In particular, citrus peel and seeds are rich in phenolic compounds, being peels richer in flavonoids than seeds.[17] The juices of bergamot, grapefruit and bitter orange are rich in naringin, neohesperidin and neoeriocitrin. While bergamot, orange, mandarin, and lemon juices has high contet of hesperidin, narirutin and didimin.[18,19] Eriocitrin and hesperidin are contained in good quantities in lemon, while neoesperidin and naringin are found in bitter orange. Bergamot seeds are rich in glycosylated flavanones, naringin and neohesperidin.[17,20] Naringin has been found in lemon peel and mandarin seeds. However it is not present in the juices of these fruits.[21] According to their molecular structures, flavonoids are divided into six major subtypes, which include fla-

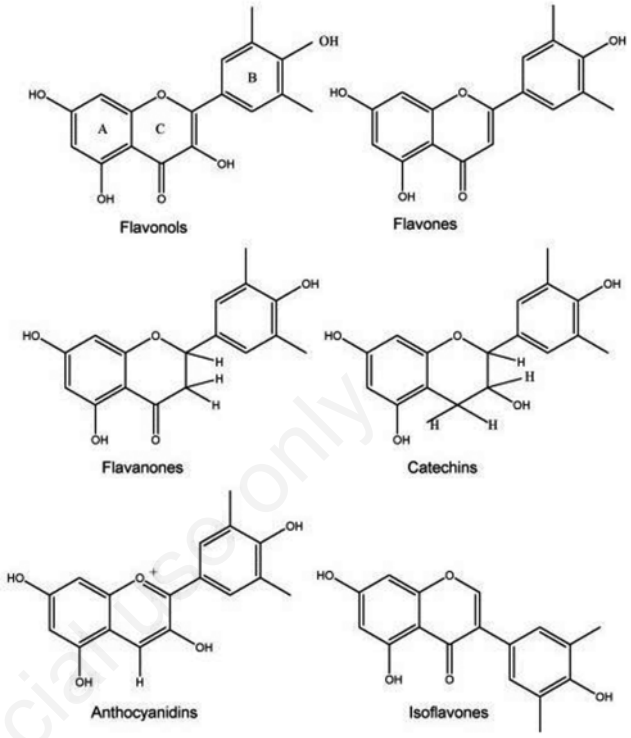

Figure 1. Structures of flavonoids.

Table 1. Citrus flavonoid and atherosclerosis.

Phytochemical, Flavonoid, food	Experimental system	Results	References
Genistein, apigenin, biochanin A, naringin, quercetin	*In vitro* studies	Inhibit *in vitro* LDL oxidation	[49]
Hesperetin, m-hydroxycinnamic acid (m-HC), 3,4-dihydroxyphenylpropionic acid (3,4-DHPP), and 3-methoxy-4-hydroxycinnamic acid (ferulic acid)	Animal model	Supplementation of hesperetin and its metabolites significantly lowered the plasma total cholesterol and triglyceride	[52]
Naringenin	Animal model	Decreased the plasma fatty acid, hepatic pro-inflammatory mediators, expression of genes including tumor necrosis factor-α, interlukin-6, interleukin-1β, inducible nitric oxide synthase and matrix metalloproteinases (MMP-2, 9)	[53]
Naringenin	Animal model	Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyltransferase	[56]
Naringin	Animal model	Reduced plaque progression only in wild-type mice fed the high-fat/high-cholesterol diet	[58]
Orange juice	Clinical study	Reduction in blood levels of total cholesterol, LDL cholesterol, apo B and LDL / HDL ratio	[65]
Glucosyl hesperidin (G-hesperidin)	Clinical study	Reduction in triglycerides in subjects with hyprtriglyceridemia	[68]
Glucosyl hesperidin (G-hesperidin)	Clinical study	Reduction in triglycerides in subjects with hyprtriglyceridemia and reduction of small dense low-density lipoprotein	[69]
Naringin	Clinical study	Reduction of total plasma cholesterol and low density lipoprotein cholesterol in hypercholesterolemic subjects	[70]
Orange juice	Clinical study	Reduction diastolic blood pressure	[73]

vanols, flavanones, flavonols, isoflavones, flavones and anthocyanins, depending on the differences in their structures.[22,23] More than 4000 varieties of flavonoids have been identified to date.[24] Among the aglycone forms naringenin and hesperetin are considered the most important flavanones, while the glycoside forms includes neohesperidosides and rutinosides.[25] The neohesperidosides, flavanones, naringin, neohesperidin and neoeriocitrin are constituted by a flavanone with neohesperidose. Rutinosides (flavanones, hesperidin, narirutin and didymin) have a disaccharidic residue such as rutinose.[25]

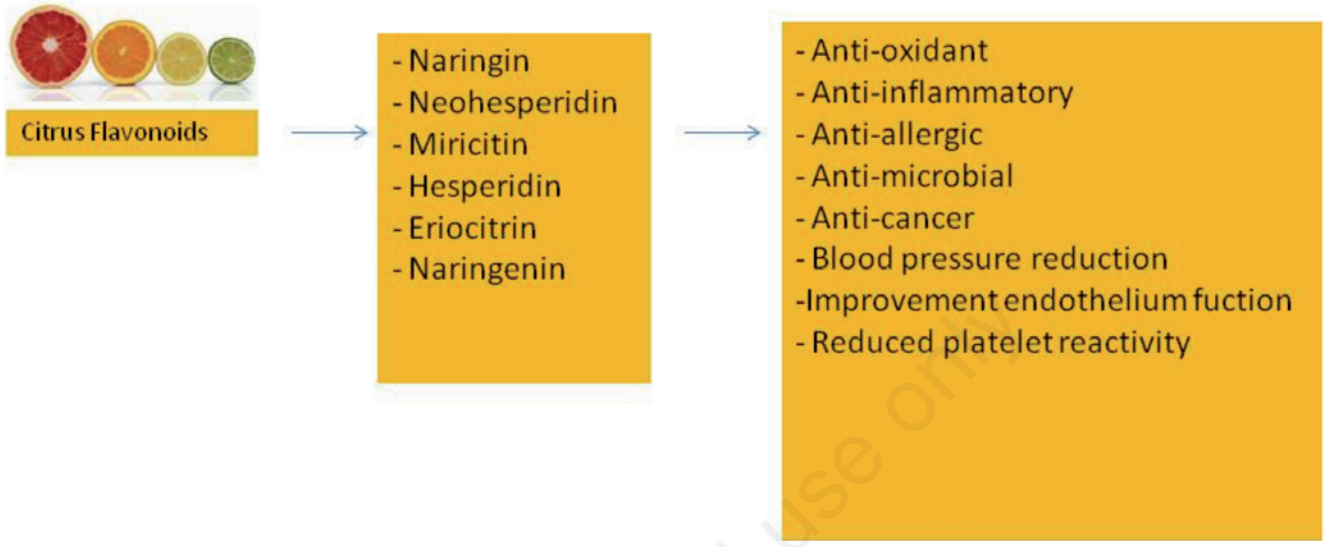

Figure 2. Effects of citrus flavonoids.

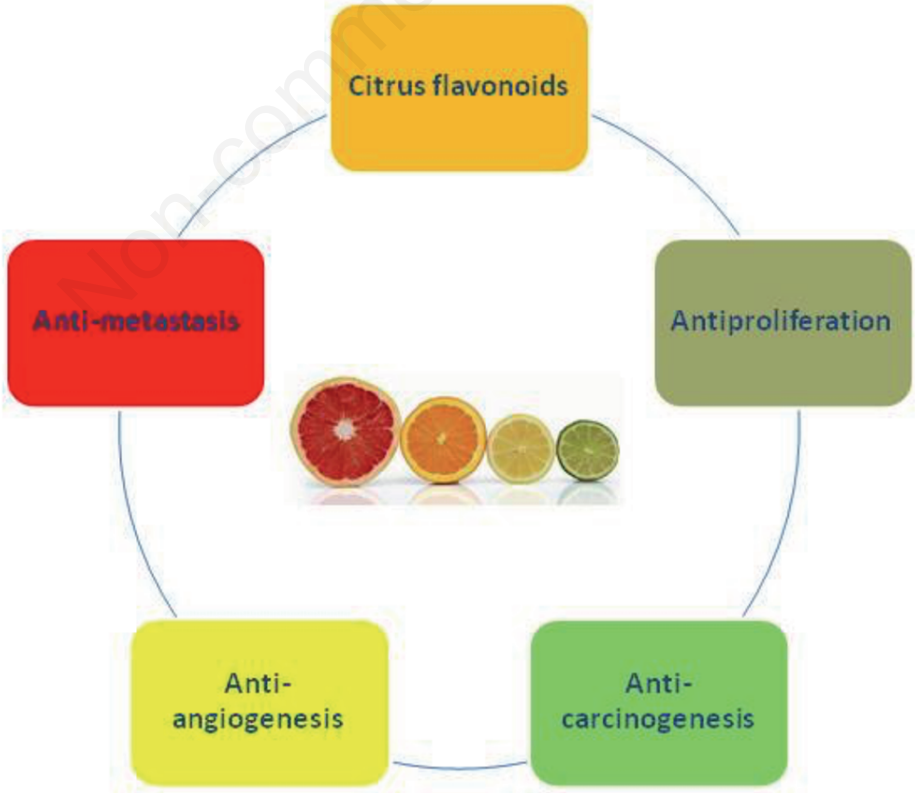

Figure 3. Anti-cancer effects of citrus flavonoids.

Bioavailability

Flavonoid glycosides are hydrolized into their aglycone form by the intestinal microflora and then, in this form, can be absorbed. Flavonoids undergo extensive hepatic metabolism by hydroxilation or demethylation and then conjugated with glicuroinic acid or sulphates. The metabolism of flavonoids depends on hydroxylation which modifies their sensitivity to hydrolysis and to the cleavage of the heterocyclic ring by the bacterial flora.[8] Therefore, due to the intestinal flora, the metabolism of the different flavonoids can vary according to the specific chemical structure.[26] The flavanone, hesperidin and naringin glycosides are both rutinosides linked to disaccarides ramnose and rutinose in position 7. Consequently, they are not hydrolyzed by intestinal bacterial β-glucosidases but however the flavonoids are metabolized in the distal part of the small intestine and colon by the microflora.[27] The 7-glucoside forms of naringenin and hesperin are rapidly absorbed as they are hydrolyzed in the small intestine.[27] Naringenin and esperperin are absorbed within minutes when administered orally, with a peak plasma concentration at approximately three hours.[28] Flavonoids can accumulate in the body, in one study, naringin was administered to rats resulting in increased concentrations mainly in the liver, followed by spleen, heart, brain and kidneys.[29]

Antioxidant activity

Flavonoids show anti-oxidant effects against free radicals.[30] The scavenger ability of these molecules has been suggested to be due to their hydrogen-donating capability so that a subsequent production of radicals can be delocalized on the flavonoid structure.[31,32] The antioxidant potential of flavonoids is determined by the presence of glycosides and free hydroxyl groups or by the number and position of esterified hydroxyl groups.[33] The concentration of flavonoids and several environmental factors influences the antioxidant activity. The common structural element is the configuration of the C-ring with the 3-hydroxyl group, which activates the double bond in position 2-3. Only when the concentration is lower than 100μM the presence of the hydroxyl groups in the ring B is important for the radical scavenger activity.[34] Furthermore, pH has been also shown to affect the antioxidant activity of some polyphenols.[35] The rate of flavonoids activity appears also to be related to their chemical structure. Flavonoids has been shown to be and are excellent scavengers of the hydroxyl radical.[36] In this context *Rapisarda et al.* determined the antioxidant capacity of polyphenols, flavonoids, anthocyanins, hydroxycinnamic acids and ascorbic acid which were present in of the juice pigmented oranges of Moro, Sanguinella, Tarocco and Washington varieties. The juice from all these varieties showed an antioxidant activity, which appears correlated with the total amount of phenol and that is influenced by the pigmented component of anthocyanins.[37,38] An *in vitro* study showed that quercetin and kaempferol are able to cross the erythrocyte membrane and increase antioxidant activity of erythrocyte by 15% and 13% respectively.[39] These findings indicate that flavonoids are able to form stable complexes with erythrocytes and may influence intracellular redox homeostasis. Therefore, these observations support the hypothesis that polyphenols are able to protect erythrocytes from reactive oxygen species (ROS) induced cell damage.[39] Other observational studies carried out to compare the activity of catalase in erythrocytes of smokers versus non-smokers, showed that the enzymatic activity of catalase was significantly lower in smokers than in non-smokers. Furthermore, these studies also reported that exposures of erythrocytes from smokers to quercetin at a concentration of 100 μM resulted in an return of catalase levels to normal values.[40]

Anti-inflammatory activity

Many experimental studies have shown that flavonoids can inhibit the expression of enzymes or transcription factors that regulate the biological functions of signalling molecules involved in inflammation.[40] The effects on the immune and inflammatory responses induced by flavonoids appear to be, in part, the result of the inhibition of the expression levels of enzymes such as protein-kinase C, phosphodiesterase, phospholipase, lipoxygenase and cyclooxygenase, which regulate the synthesis of biological effectors responsible for the activation of endothelial cells and cells of the immune system involved in inflammation.[40] Flavonoids has been also shown to inhibit enzymes such as aldose-reductase, xanthine-oxidase, phosphodiesterase, $Ca^{(+2)}$-ATPase, lipoxygenase, and cyclooxygenase, which play a role in promoting the transduction and activation of cellular signals and to modulate the activation of cells involved in the immune response.[41,42] In this setting, experimental *in vivo* studies have shown that hesperidin inhibited carrageenan-induced pleurisy and reduced yeast-induced hyperthermia in rats.[43] Hesperidin, in particular, has been shown to exert inhibitory effects on Lipopolysaccharide (LPS) induced by the expression of cyclooxygenase-2, inducible nitric oxide synthase (iNOS), hyperproduction of prostaglandin E2 and nitric oxide (NO).[44] On the other hand, nobiletin has been reported to selectively downregulate cyclooxygenase-2, and the gene expression of pro-inflammatory cytokines, by mechanisms resembling those of dexamethasone.[45]

Prevention of atherosclerosis and cardiovascular diseases

Citrus flavonoids have attracted particular attention due to their unique and effective therapeutic activity against various chronic diseases, in particular atherosclerosis (Table 1).[46] Inflammation of the vessel wall and increased adhesion of mononuclear cells to the altered endothelium are the the first step of the atherosclerosis. In response to inflammation, Low-Density Liproteins (LDL) can penetrate the intima of the arterial wall and, following LDL-laden foam cells, can form atherosclerotic plaques.[47,48] Citrus flavonoids, including naringin, have shown to exert inhibitory effects on the oxidation of LDL cholesterol.[49] Citrus consumption has been associated with an assessmented of cardiovascular events, suggesting that citrus flavonoid intake may be cardioprotective.[50]

Animal studies

Numerous preclinical *in vivo* studies have reported positive effects of the intake of citrus fruits, derived from peels and seeds or molecules administered individually or in combination. For instance Kurosawa *et al*. investigated the hypocholesterolemic effects of citrus juices in mildly hypercholesterolemic rats in which high levels of LDL were obtained following the intake of high-fat diet. These studies showed that orange juice and grapefruit juice affected cholesterol metabolism. In particular, the administration to rats of a diet in which water was replaced with orange juice and 32% with grapefruit juice, induced an evident decrease of serum LDL cholesterol in 43% of the animals.[51] Hesperetin was also noted to exert a hypolipidemic effect. In fact, in male rats fed a high cholesterol diet, the administration of this compound reduced the circulating level of triglycerides and cholesterol.[52] Furthermore, Chtourou *et al.* reported that in Wistar rats fed with a

high cholesterol diet, the administration of naringenin, resulted in a reduction of plasma lipids, liver lipids and liver fibrosis. These effects were associated with the decreased expression levels of matrix metalloproteinase and that of macrophage infiltration markers.[53] Furthermore, studies carried out on hamsters fed with diet-induced hypercholesterolemia, showed that formulations containing citrus polymethoxylated flavones, mainly tangeretin or citrus flavanone glucosides, hesperidin and naringin, significantly reduced the circulating levels of the Very Low Density Lipoproteins (VLDL) and serum cholesterol.[54] In addition, the administration to db/db mice of cross-linked Citrus peel extract caused a decrease in fat liver and in plasma lipids.[55] Other studies have shown that a dietary supplementation with naringenin reduced cholesterol plasma levels and those of triacylglycerol, in the liver of rats fed a high cholesterol diet. These effects resulted also associated with a decrease in 3-hydroxy-3-activity methylglutaryl-coenzyme A reductase (HMG-CoA) and Acyl-CoA: Cholesterol Acyltransferase (ACAT).[56,57] Experimental studies on wild type mice fed a integrated diet high fat/cholesterol/high-naringin, reported significant anti-atherogenic effects, in particular, in the case of diet-induced atherosclerosis.[58] On the other hand, rabbits fed high cholesterol diet showed that the integration of naringin and naringenin reduced the area of fat strips in the thoracic aorta. Interestingly, this effect was associated with reduced expression levels of adhesion of vascular cell adhesion molecule-1 (VCAM-1) and Monocyte Chemoattractant Protein-1 (MCP-1) as compared to the control group.[59,60] On the other hand, low-density lipoprotein receptor-null mice (Ldlr-/-mice) fed the western diet and supplemented with 3% naringenin diet showed a reduction in the infiltration of monocyte/macrophage antibody-2 (MOMA-2) positive lesions and collagen deposition. These findings are suggestive of an antiatherogenic activity.[61] Other experimental investigations were carried out in order to assess, the effect of the administration of grapefruit juice and shaddock on the activity of the Angiotensin-1 Converting Enzyme (ACE) *in vitro* and on the hypocholesterolemic properties of juices in rats fed a high cholesterol diet. The results from these studies showed that, grapefruit juice had a higher total flavonoid content than shaddock juice, and that both juices inhibited ACE activity in a dose-dependent manner. In addition, the administration of juices to rats fed a high cholesterol diet, resulted in a significant reduction of total cholesterol, triglycerides and LDL-cholesterol levels, and in increase of HDL (High-Density Lipoprotein-cholesterol) plasma levels.[62] Studies aimed at assessing the effects of quercetin and myricetin on isolated and perfused Wistar rat hearts showed that low concentration of quercetin induced inotropic and lusitropic effects while, myricetin low doses pure induced coronary dilation. On the other hand, the simultaneous administration of these two flavonoids produced only vasodilation. Cardiomodulation induced by the basic mechanical performance of quercetin and the selective vasodilatation induced by myricetin indicate these flavonoids as powerful cardioactive molecules which are able to protect the heart from cardiovascular diseases.[63]

Human studies

Observational studies carried out to evaluate the effects of daily intake of citrus fruits in humans have shown a beneficial impact of these compounds on human health. For example Mink *et al.* have highlighted the fact that a daily glass of orange reduces the risk of stroke in men by 25% while, a dietary intake of grapefruit was associated with a significant reduction in mortality due to coronary heart disease in women.[64] A study performed on employees of an orange juice factory with mild hypercholesterolemia showed that the daily intake of 480 ml of juice was associated with a significant reduction of serum concentration of total cholesterol, LDL cholesterol and apoB.[65] Furthermore, a study undertaken on 10,623 subjects who took citrus fruit 6 times a week showed an inverse association with cardiovascular events and in particular ischemic stroke.[66] Studies of Gorinstein *et al.* have demonstrated that the consumption of citrus fruits reduced the plasma levels of triglycerides in patients with cardiovascular diseases.[67] Other reports have shown a significant reduction of triglycerides in subjects with hyperlipidemia and hypertriglyceridemia following the daily intake of glucosyl-hesperidin.[68,69] Moreover, studies in patients with hypercholesterolemia revealed that a daily intake of naringin (400 mg/day for 8 weeks) resulted in a 17% reduction of LDL-C and apoB plasma levels.[70] Clinical observations by Roza *et al.* highlighted the fact that subjects with hypercholesterolaemia, receiving 270 mg of citrus flavonoids and 30 mg of palm tocotrienols per day for four-week, had a significant reductions of total cholesterol (20-30%), low density lipoprotein (19-27%), triglycerides (24-34%) and apolipoprotein B (21%) plasma levels. Furthermore, subjects underwent a longest period of diet (*i.e.* up to 12 weeks) had an increase in HDL levels (4%) and a significant increase in apolipoprotein A1 (5%).[71] Daily consumption of Sweetie Fruit, a flavanone-rich fruit, administered up to 4-5 weeks has been shown to reduce diastolic blood pressure.[72] In healthy men, daily consumption of orange juice or hesperidin for 4 weeks significantly improved endothelium-dependent vasodilation starting from six hours after ingestion.[73] Other clinical investigations undertaken in subjects treated with 500mg/day of pure hesperidin showed a significant increase in brachial artery-mediated flow dilation compared to control subjects.[74] Citrus flavonoids have been shown to possess also anti-platelet and anti-adhesive activity. For instance, methoxylated flavonoids nobiletine and tangeretin, which are much more active than hydroxylated flavonoids showed to have an antiaggregant activity similar to acetylsalicylic acid.[75] Some authors have pointed out that the inhibition of platelet aggregation depends on the aggregation state of the various chemical structures of several flavonoids.[76] In this context, *in vitro* studies have shown that flavonoids, by interacting with platelet membranes, can therefore induce cumulative effects over the time.[77] On the other hand, platelet aggregation induced by arachidonic acid is more inhibited by fisetin, kaempferol and quercetin than by myricetin. Furthermore, quercetin, fisetin and myricetin showed a more marked inhibitory effect on collagen-induced aggregation.[78] Alcaraz *et al.* have shown that flavonoids are antithrombotic factors that act by inhibiting the activity of cyclooxygenase and lipoxygenase, and a consequent reduction of thromboxane A2 and the production of 4-series leukotrienes. The anti-thrombotic effects exerted by flavonols are the consequence of their binding to platelet mural thrombus. In addition, their ability to eliminate free radicals result in an activation of the biosynthesis and activity of endothelial prostacycline.[79] Therefore, flavonols stimulate the release of thrombolytic and vasoprotective endothelial mediators.[79]

Citrus flavonoids and cancer

Cancer is the second leading cause of death worldwide.[80] A growing number of investigations undertaken in the aim to identify natural products endowed with chemo-preventive activity against malignant diseases has, ultimately, led to the development of the conceptual definition of "*functional foods*" in the prevention of carcinogenesis.[81] Growing evidence has identified dietary flavonoids as potential chemopreventive and/or anticancer

agents.[82] *In vitro* studies carried out to clarify the mechanisms by which these molecules induce growth inhibitory effects on tumor cells, have shown that their antioxidant properties can likely account for these effects.[83] For instance, naringin has been reported to exert its antioxidant effects by up-regulating the gene expressions of some antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx).[84] Moreover, flavonoids have been shown to prevent DNA damage and carcinogenesis by directly interacting with carcinogens and by inactivating them.[85] Furthermore, hesperetin and naringenin have been shown to inhibit the tumor promoting effects of 7,12-dimethylbenz [a] anthracene on breast cancer.[86] Interestingly, citrus flavonoids have been shown to enhance the effects of antitumor drugs through the modulation of some of the molecular mechanisms which foster the onset of tumor cell resistance.[87] In addition, quercetin has been reported to increase the cytotoxic effects of adriamycin on the multi-drug resistent human breast cancer MCF-7 cells.[88] Moreover, apigenin, kaempferol and quercetin have been shown to inhibit the proliferation of human breast, prostate and lung cancer cell lines.[89] While flavonoids inhibit the proliferation of human leukemia, gastric carcinoma and ovarian carcinoma cells.[90-92]

Colon cancer

Colon cancer is one of the most common cancers in western countries being its incidence increasing even in subjects <50 years of age.[93] Experimental investigations carried out in a mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate showed that mice fed with nobiletin diet had a reduced risk of developing colon carcinoma.[94] Furthermore, other *in vivo* studies highlighted the fact that the administration of flavonoids such chrysin, quercetin and nobiletine, azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db mice, reduced the incidence of aberrant cryptic foci which are known to be closely associated with the development colon adenocarcinoma.[95] Experimental *in vivo* studies undertaken to assess, the therapeutic effectiveness of a product formulated from the extraction of citrus peels, in a model of colon tumorigenesis induced by oxymethanes, showed that the oral feeding of the extract of mixed citrus peel reduced the number of large, aberrant cryptic foci in the colon tissues of these mice. Citrus peel extract was also observed to decrease iNOS, COX-2, ODC, VEGF and matrix metalloproteinase-9 (MMP-9) protein levels in the colon tissues of mice.[96] Furthermore, other *in vitro* studies have shown that apinella and quercetagetin inhibited cell proliferation of human SW480, colon cancer cells and modulate the expression of apoptosis-related genes/proteins.[97] On the other hand, other experimental studies showed that apigenin reduced the number of aberrant crypt foci and azoxymethane-induced tumor formation in CF-1 mice.[98] Moreover, diets containing hesperitin have been shown to decrease the number of aberrant crypt foci in Wistar rats treated with 1,2 dimethylhydrazine.[99] Leonardi *et al.* investigated the effects of four citrus flavonoids, namely naringenin, apigenin, hesperidine and nobiletine, and a mixture of limonoid (limonin glucoside/obacunone glucoside) on the azoxymethane-induced colon cancer promotion in rats. These studies showed that apigenin lowered the number of aberrant crypt foci compared to rats fed a controlled diet, while naringenin lowered the number of foci and the proliferation index. Both apigenin and naringenin increased apoptosis of the luminal surface colonocytes. On the other hand a glucose mixture of hesperidin, nobiletin and the glucoside/obacunone limonin did not show to exert these effects.[100] Some clinical investigations have reported that a mixture of apigenin and epigallocatechin gallate suppressed the recurrence of colon cancer in humans underwent surgery.[101] These findings have provided evidence regarding a possible clinical role of naringenin and apigenin as natural chemo-preventive agents against colon carcinogenesis.[102]

Lung cancer

Lung cancer is the leading cause of cancer death among men and the second leading cause of cancer death among women worldwide.[103] The correlation between flavonoid intake and rate of lung cancer risk has been investigated by a food-frequency questionnaire study carried out in a population-based control case study of 1061 cases and 1425 controls.[104] The results from this study showed a significant correlation between intake of food containing low levels of flavonoids and increased the risk of lung cancer.[104] In line with these findings, other case-control study have reported a significant inverse associations between lung cancer risk and the main food sources of flavonoids including quercetin, deriving from the intake of onions and apples and naringin deriving from white grapefruit.[105] One of the main citrus flavonoids, *i.e.* nobiletin, has shown to inhibit A549 human lung cancer cell growth *in vitro* by inducing apoptosis by inhibiting the expression of Bcl-2 protein, and increasing Bax (higher Bax/Bcl-2 protein ratio) and that of p53, while this compound showed to cause cell cycle arrest at the G2/M phase.[106]

Consistent with these results, Park *et al.* have recently shown that Korean Citrus aurantium L. has antimetastasis activity. *C. aurantium* L., known as bitter orange, is used as a flavoring and acidifying agent for foods,[107] the major flavonoids present in these fruits are nobiletin, naringin, and hesperidin, while the most commonly detected free flavones are apigenin, luteolin, and diosmetin.[108] This study highlighted the fact that flavonoids isolated from *C. aurantium* were able to prevent the homing and dissemination of A549 human lung adenocarcinoma cells in lung tissues of syngenic mice.[108] These effects have been attributed to the pro-apoptotic and anti-migratory effects induced by flavonoids.[108] These findings further suggest a potential clinical use of flavonoids isolated from *C. aurantium* in the prevention and treatment of human lung cancer. In this setting Bruno *et al*. carried out some *in vitro* experiments to investigate the antitumor effects of apigenin on human A549 lung adenocarcinoma cell line. These studies demonstrated that this molecule significantly reduced the rate of cell proliferation and increased the spontaneous release of ROS thus inducing tumor cell death. These data are suggestive for a future complementary therapeutic approach with apigenin in patients with lung adenocarcinoma.[109]

Breast cancer

Epidemiological studies show an inverse association between increased intake of food flavonoids and the reduced risk of breast cancer.[110] Flavonoids are endowed with antioxidant activity. Furthermore, their estrogen-like chemical structure allows them to interact with estrogen receptors, thus exerting a phytoestrogen-like activity.[111,112] These effects, which account also for their modulating activity on endogenous estrogens and their metabolism, has been suggested as possible mechanisms which mediate the anti-proliferative properties of these molecules.[111,112] This hypothesis has been corroborated by the results from *in vitro* studies showing that hesperetin, naringenin, baicalein, galangin, genistein and quercetin inhibited the proliferation of MDA-MB-435 human breast cancer cells.[113] Furthermore, hesperetin, naringenin, baicalein, galangine, genistein and quercetin and grapefruit concentrate and orange juice, have been shown to inhibit the proliferation of 7.12-dimethylbenz [a] anthracene (DMBA) induced breast

tumors in female rats.[113] In line with these observations, recent findings have reported that the exposure of various breast cancer cell lines to Oncamex, a second-generation flavonoid analogue, reduced cell viability and induced cytotoxicity and apoptosis, concomitant with increased caspase activation.[114] Furthermore, it has been reported that the polymethoxyflavones of sweet orange peel induced apoptosis in human breast cancer cells (MCF-7),[115] while hesperetin inhibited the proliferation of MCF-7 breast cancer by causing cell cycle arrest in G1 phase.[115]

Prostate cancer

Prostate cancer is one of the most common cancers and is the third most common cause of cancer death in men of all ages.[116,117] Flavonoid-rich diets have been associated with a reduced incidence and mortality of prostate cancer.[118] The lowest incidence of prostate cancer worldwide has been observed in populations consuming the largest amount of flavonoids.[119] As many experimental studies highlighted the protective effects of these molecule on prostate cancer, a greater intake of flavonoids through an increased consumption of fruit and vegetables, may be an useful approach in preventing prostate cancer.[119] Naringenin, has been shown to stimulate DNA repair following oxidative damage in human prostate cancer cells.[120] On the other hand, hesperidin, has been shown to inhibit the growth of prostate cancer cells by multiple mechanisms, in addition this compound may inhibit testosterone-induced proliferation of prostate cancer cells probably, by interacting with androgen receptors.[121] Furthermore, nobiletin extracted from a flavonoid mixture decreased the viability of PC-3 and DU-145 prostate cancer cell lines. Therefore, these studies suggest a possible clinical role of nobiletin, eventually given in association with other therapeurtic options, to improve the survival rates of prostate cancer patients.[122] Further, myricetin has been shown to exert inhibitory effects on PC-3 human prostate cancer cell lines. The combination of myricetin and myricitrin resulted in synergic antiproliferative effects on cancer cells, the rate of apoptosis increases in a dose-dependent manner after treatment with flavonoids.[123] An extract of multiple varieties of citrus peels containing high concentrations of flavonoids, has been shown to suppress the growth of cancer cells *in vivo* by using a human prostate tumor xenograft mouse model.[124]

Thyroid cancer

Thyroid cancer is the most common malignant tumor of the endocrine system. Its incidence has increased worldwide.[125] However, the causes of this phenomenon are still highly debated. In many cases, the normal mechanisms which regulate the Iodine uptake by thyrocites, remail unaltered also differentiated thyroid carcinoma. In this context, the aim of the radioiodometabolic therapy performed after total thyroidectomy, is directed to kill metastatic cancer cells by radioactive iodine picked up by any thyroid residues or malignant tissue. This therapeutic approach, results, in general, in an excellent prognosis for these tumors.[126] In some cases, due to the down-regulation of the iodine transport protein in the thyrocytes (sodium-iodide symporter-NIS), cancer cells partially lose the ability to concentrate iodine inside the cells, thus making radioiodine therapy less effective. In this setting, some experimental observations have shown that some flavonoids reduced the rate of cell proliferation and increased cell death, as well as increase NIS mRNA levels and iodine absorption.[127] In line with these findings Allegri *et al.* have carried out some studies to assess the antitumor activity of resveratrol, genistein, and epigallocatechin-3-gallate on two cell lines derived from anaplastic thyroid carcinoma namely, SW1736 and 8505C. These authors demonstrated a decrease in cell viability and an increase of apoptosis in cells of this very aggressive tumor which usually does not respond to radiodometabolic therapy.[128] Experimental studies on the effects of flavonoids extracted from mandarin juice on the proliferation and migration of CAL-62, C-643 and 8505C human anaplastic thyroid carcinoma cell lines showed that flavonoid components of the tangerine juice extract significantly reduced the proliferation of all these cancer cell lines by blocking them in the G2/M phase of the cell cycle. Furthermore, the extract also caused a reduction of cell migration, which was associated with a reduced expression of matrix-metalloproteinase-2 (MMP-2).[129] Moreover, another study on an anaplastic thyroid carcinoma cell line (HTh7) showed that hesperedine was effective in reducing cell proliferation and survival mainly by inducing apoptosis. On the other hand, hesperetin also induced cellular re-differentiation of anaplatic thyroid cancer.[130]

Discussion

The studies reported in the present review indicate that flavonoids appear to be useful functional food compounds, in the prevention of some chronic pathological conditions.[131,132] In particular, experimental *in vitro* and *in vivo* investigations indicate that citrus flavonoids may play an important role in the prevention and treatment of atherosclerosis and human tumors.[133,134,135] Besides vitamin C, folate, dietary fiber and carotenoids,[136] the beneficial effects of diet citrus fruits can be attributed, in particular, to the antioxidant activity of flavonoids present in this fruit. The studies on the therapeutic effects of flavonoids is complex due to the heterogeneity of various molecules. Cardiovascular diseases and cancers are pathological conditions associated with the highest incidence and mortality worldwide.[137] On the other hand, multiple environmental and/or genetic factors have been shown to contribute to these increasing incidence of these non communicable diseases. In these recent years, balanced diets have gained a significant attention with regard to the clinical treatment of atherosclerosis, cardiovascular diseases and tumors. The use of citrus fruits in the daily diet not only provides important nutrients such as vitamins, mineral salts, trace elements but also numerous functional molecules that are useful to maintain normal body's homeostasis. In particular flavonoids present in citrus fruits possess antioxidant, anti-inflammatory, hypolipidemic, antidiabetic and anticancer effects. These observations suggest that, citrus flavonoids may be of clinical relevance in the prevention and treatment of atherosclerosis and human tumors. Current dietary recommendations are based on the advice to consume fruits, vegetables and drinks, such as red wine, containing flavonoids on daily bases. However, current efforts are directed to find out most suitable molecules and/or novel, effective associations among multiple flavonoid molecules that can result in an improved therapeutic activity. On the other hand, further investigations are needed to better assess the pharmacokinetics pathways of these molecules following their long-term daily intake. The role of citrus flavonoids on human health currently is an a growing area of research interest. The advances in the technologies used for improving the separation and purificationof flavonoids will contribute to better define the biological activity of these compounds. Further investigations may better assess the pharmacokinetics pathways of these molecules following their long-term daily intake.

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