INVITED REVIEW

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Flavonoid Natural Products: Chemistry and Biological Benefits on Human Health: A Review

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ABSTRACT: Flavonoids are one of the most common groups of plant's secondary metabolites. They are the low molecular weight polyphenolic compounds with a composition of various substituted three-ring structure and responsible for the vibrant colors of many plants. Flavonoids are recognized for their participation in plant's defensive mechanisms by preventing insect, microbe attacks and ultraviolet radiation. Several plants and spices containing flavonoid derivatives have found to be applicable as disease preventive and therapeutic agents in traditional medicine in Asia for thousands of years. More than 8000 varieties of flavonoids have been isolated yet. Due to their broad spectrum of biological benefits on human health, flavonoids are referred as "nutraceuticals". This review aims to categorical documentation about the chemistry and biological benefits on human health of flavonoids natural products in the light of available scientific literatures.

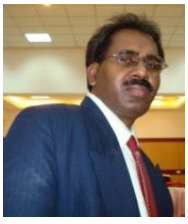
Keywords: Flavonoid; Biosynthesis; Chemistry; Anticancer; Polyphenol.

INTRODUCTION

Pigments that responsible for the color of the most flowers, fruits and seed in the plant kingdom are flavonoids. Initial discovery of these classes of molecules was in 1930 by Szent-Gyorgyi once they have isolated a new substance from oranges. But at that time it was believed as a new class of vitamin and classified as vitamin P. Later researches confirm that the new substance was a flavonoid namely rutine.[1] With the progress of science today we have more than 8000 of flavonoids isolated from natural sources.[2] These classes of secondary metabolites are not only important for participation in plant's defensive mechanisms they are important for the biological benefits on human health. Although lots of literatures[3] are available about the flavonoids chemistry and biology, this review is an effort to update the topic once again.

CHEMISTRY OF FLAVONOIDS

Flavonoids are largely planar molecules and their structural variation comes in part from the pattern of substitution such as hydroxylation, methoxylation, prenylation, or glycosylation. All flavonoids contain fifteen carbon atoms in their basic nucleus: two six-membered rings linked with a three carbon unit which may or may not be a part of a third ring.Flavones, flavonols, flavanones, isoflavones, anthocyanidins and



Prof. Dr. Parasuraman Jaisankar was born in Cheyyar, Tamilnadu, India in 1966. He received his B.Sc. degree from A. A. Govt. Arts College, Cheyyar, Madras University in 1987. In 1989 he received his M.Sc. degree from the Presidency College, Chennai, Madras University. He then joined the Indian Institute of Chemical Biology as scientist in 1990 and received his Ph.D. degree in 1995 from Jadavpur University. From 1996 to 1998 he received DAAD fellowship and worked with Prof. H. Seliger, Ulm University, Germany. From 2001 to 2002 he received RCMS fellowship and worked with Nobel laureate Prof. Ryoji Noyori, Nagoya University, Nagoya, Japan. From 2010 to 2011 he was awarded CSIR-Raman Fellowship to

Nagoya University, Japan. He is life member of Indian Association for the Cultivation of Science, Indian Science Congress Association, Institution of Chemists, India. He is the life member and founding joint secretary of Chemical Biology Society of India and member of International Chemical Biology Society. He has published more than 82 articles in international journals. He also has 6 national and international patents to his credit. Presently he is working as Senior Principal Scientist in the Laboratory of Catalysis and Chemical Biology, Department of Chemistry, CSIR-Indian Institute of Chemical Biology (CSIR-IICB), Kolkata-700032, India. His research focused on 1) Development of chiral ligands and their transition metal complexes for asymmetric organic transformations. 2) Synthesis of Lead Compounds and their analogues having various biological activities, with special focus on anticancer and anti-asthmatic, anti-microbial, and anti-inflammatory compounds. 3) Development of synthetic strategy for the generation of structurally unique bioactive molecules especially nitrogen heterocycles. 4) Identification and structure elucidation of antibiotics isolated from marine *actinobacterium*. 5) Search for safe drugs from herbal sources.

Rahul L. Gajbhiye was born in 1985 in Nagpur, Maharashtra, India. He received his B.Pharma degree from S.K.B College of pharmacy, Nagpur University in 2008 and obtained his M.S. (Pharm.) degree in Natural Products from National Institute of Pharmaceutical Education and Research (NIPER), Kolkata in 2010. Currently he carries out his doctoral research at Laboratory of Catalysis and Chemical Biology, Department of Chemistry, CSIR-Indian Institute of Chemical Biology, Kolkata under the supervision of Prof. Dr. Parasuraman Jaisankar.

Dr. Sanjit kumar Mahato was born in Baraurma, Purulia, West Bengal, India in 1979. He received his B.Sc. degree from the University of Burdwan in 2002. In 2004 he received his M.Sc. degree from the Barkatullah University (formerly Bhopal University), Bhopal, India. He qualified GATE-2005 with 97.71 percentile (all India rank 78). He worked at Chembiotek Research International Pvt. Ltd. Kolkata, India for short time (Sept' 2005- May 2006) as a Project Fellow. Then he joined (May' 2006) the research group of Prof. Dr. P. Jaisankar at the Laboratory of Catalysis and Chemical Biology, Department of Chemistry, CSIR-IICB, India and received his Ph.D. degree in August' 2012. His contribution in synthetic organic chemistry published in peer review journals and filled three patents (USA & India). He was awarded Young Scientist Award 2010 (J. M. Dasgupta Award) by Indian Chemical Society for his contribution in organic and biomolecular chemistry. During his doctoral studies he visited (May-July' 2009) the laboratory of Professor Emeritus Marek Zaidlewicz at Nicolaus Copernicus University, Poland and worked on asymmetric catalysis by borane-oxazaborolidine. He got his post doctoral training (Nov' 2012 to Oct 2013) in catalysis at the University of Johannesburg with Prof. K. Mallick and then with Prof. Emeritus Eli Breuer's group for short time (Nov' 2013 – Jan' 2014) at the Institute for Drug Research, School of Pharmacy, Hebrew University of Jerusalem, Israel worked on design and synthesis of carbamoylphosphonic based autotoxin inhibitors. He is life member of Indian Science Congress Association and Indian Chemical Society, India. Presently he is working as Assistant Professor in organic chemistry at Career Point University Hamirpur, India and supervising two master students on design and synthesis of thiadiazole derivatives for inhibiting cancer-promoting enzymes.

Dr. Debkumar Nandi was born in Kaliyan, West Midnapore, West Bengal, India in 1982. He received his B.Sc. degree from Behala College, University of Calcutta in 2002. In 2004 he received his M.Sc. degree from the Indian Institute of Engineering Science and Technology, Shibpur, India. He has qualified NET-CSIR in 2004. He then joined the research group of Prof. Dr. Parasuraman Jaisankar at the Laboratory of Catalysis and Chemical Biology, Department of Chemistry, CSIR-IICB, India and received his Ph.D. degree in 2009. From 2010 to 2013 he received NSC postdoctoral fellowship and worked with Prof. Hon Man Lee, National Changhua University of Education, Taiwan. From 2013 to 2014 he worked as research associate in a sponsored DBT project in CSIR-Indian Institute of Chemical Biology, India. He has more than 15 international publications to his credit. Presently he is working as GES post doctoral research fellow In the University of Johannesburg, South Africa. His research focused on search for safe drugs from herbal sources. Transition metal catalyzed C-H, C-C bond activation reactions and synthesis of biologically active natural products.

chalcones are the most common classes of flavonoids which account for approximately 80 % of total flavonoids identified yet. The various subclasses back bone of this group of natural products is depicted in figure 1. Flavones are different from flavonols due to lack of hydroxyl group at the 3-position. Common flavonols are quercetin (3,5,7,3´,4´-pentahyroxyflavone), kaempferol (3,5,7,4´- tetrahydoxyflavone), myrcetin (3,5,7,3´,4´,5´-hexahydroxyflavone), and isorhamnetin (3,5,7,4´-tetrahydroxy-3methoxy flavone). The most abundant flavones in plants are luteolin (5,7,3,4-tetrahydroxyflavone) and apigenin (5,7,4´-trihydroxyflavone). Flavonoid glycosides are molecules in which sugar parts are bound to the flavonoid moiety. Many plants store important chemicals in the form of its glycosides. As and where

required during plant metabolism, the glycosides are hydrolyzed by specific enzymes present in the plant making available of the aglycon metabolites. Flavonoids generally occur as glycosides in plant, although occasionally they are also found as aglycones.

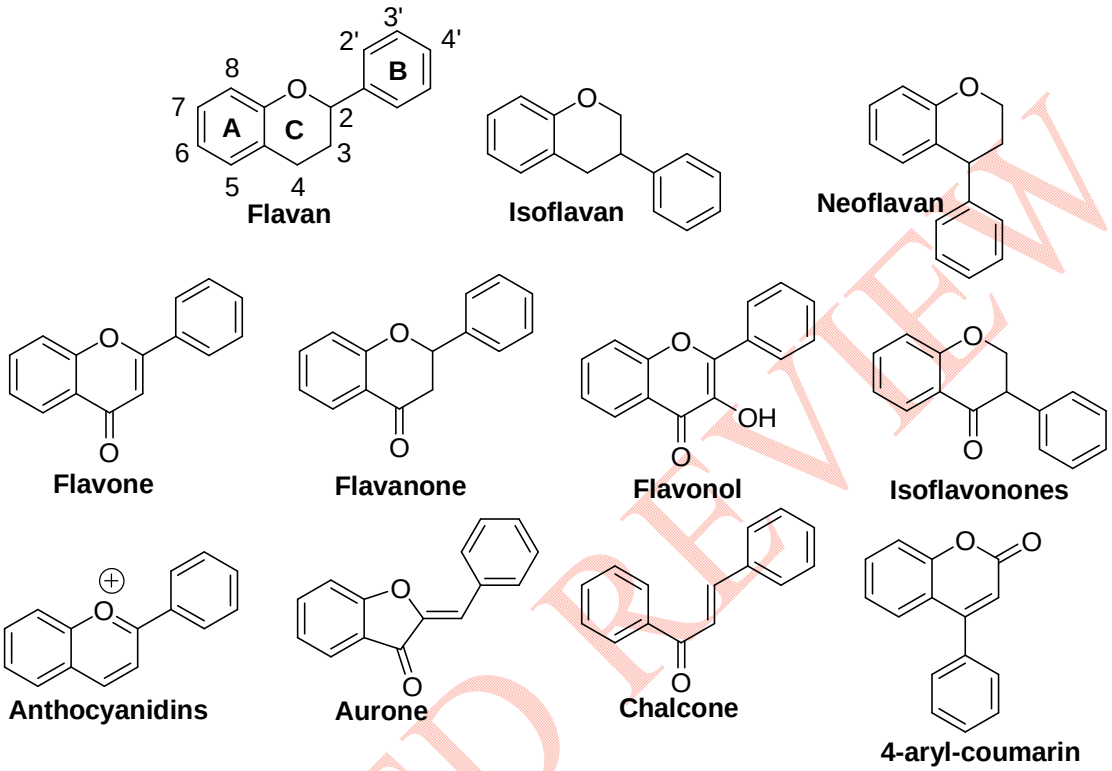


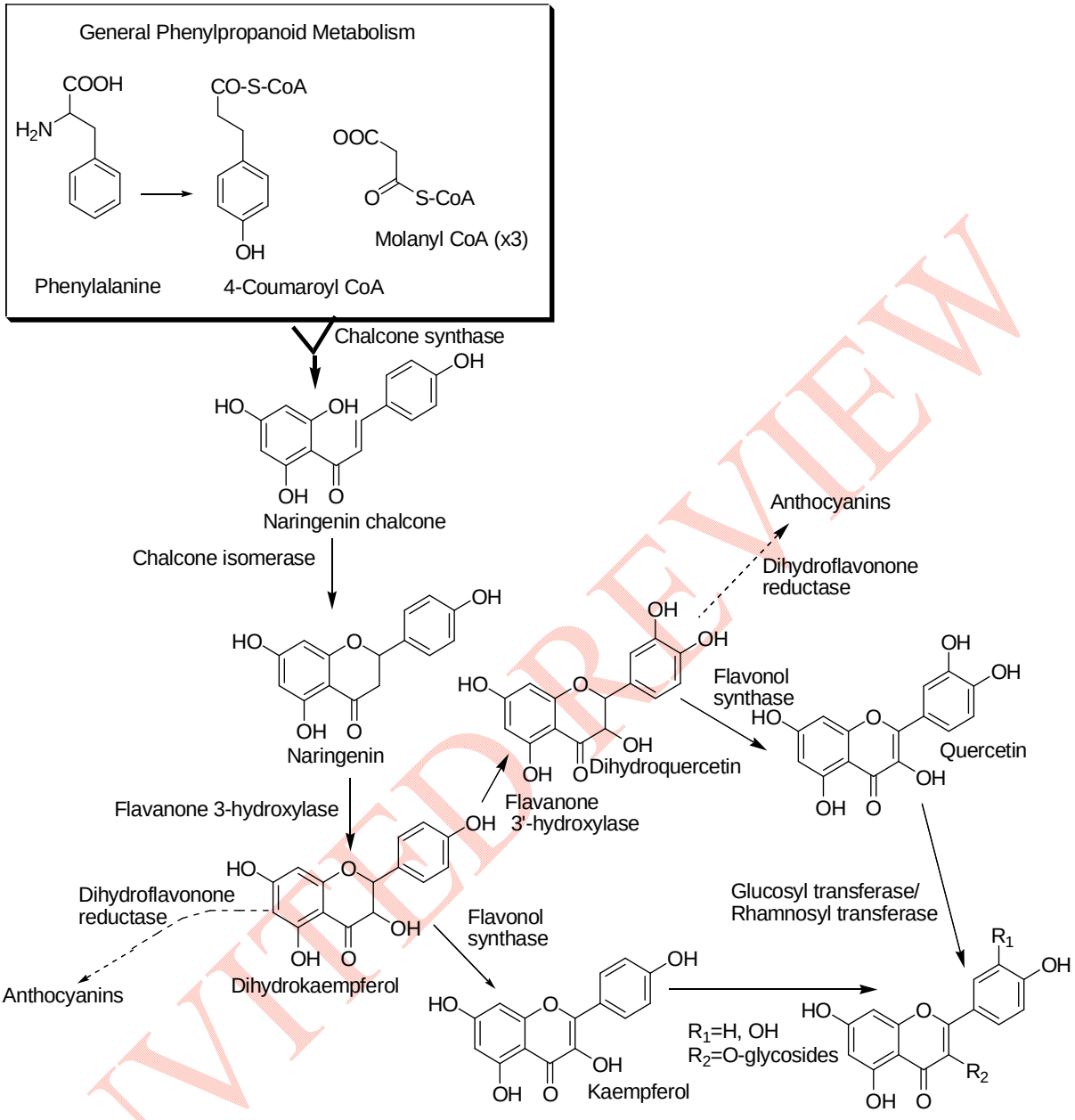
Figure 1: Structural backbones of different subclasses of flavonoids.

BIOSYNTHETIC PATHWAY

Bio synthetic route of any class of compound is important as it will helps to visualize the chemical synthesis of the same. The favonoid biosynthetic pathway (scheme 1) and its regulation have been well studied in plants[4] and many enzymes required for the production of different favonoid classes have been characterized.[5-7] The biosynthesis involves of a chalchone from L-phenylalanine which is metabolized to cinnamic acid derivatives. These then condences with malonyl-CoA to a chalcone. In plants cinnamic acid is hydroxylated by cinnamic-4-hydroxylases (C4H) to *p*-4-hydroxy-cinnamic acid, activated by 4-coumarate/ cinnamate coenzyme A, coupled with 3 malonyl-CoA units and converted by chalcone synthase (CHS) to a chalcone derivate as the first committed precursor for the flavonoid biosynthesis. Chalcones are converted to flavonoids by a ring closing step promoted chalconeisomerase resulting in the heterocyclic C ring. The chalcone, Naringenin is a key intermediate leading to the biosynthesis of some of the isoflavones, flavones, flavonols, flavannones, flavonols, and also then condensed tannin precursors. The corresponding secondary metabolites are formed involving hydroxylation, glycosylation, prenylation or alkylation.

NATURAL SOURCES

India has a rich heritage of traditional medicinal plants. The recognized Indian systems of medicine are Ayurveda, Siddha and Unani, which use the herbs and minerals in the formulations. Statistics shows that India contains 15 agro-climatic zones and 47000 plant species of which 15000 are reported to have medicinal properties of varying degrees.[3] The preferred bonding site of the sugar is the 3-position, less frequently the 7-position, and only in rare cases the 3´, 4´ and 5 positions.

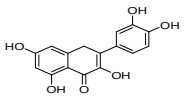
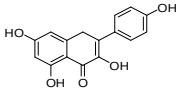
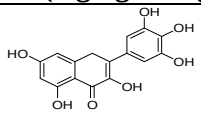
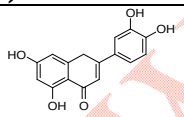
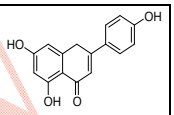


Scheme 1: A general bio-synthetic pathway of natural products of flavonoids and their glycosides

Flavones occur mainly as 7-*O*-glycososides, but *C*-glycosides (where the sugar is directly attached to an aromatic carbon atom) are also known. Comparatively less data are available for the flavone-*C*-glycosides, although they occur in teas[8 & 9] and cereals [10-12]. Apart from this, the most common D-Glucose, the other sugar residues found are D-galactose, L-rhamnose, L-arabinose, D-xylose and D-apiose, and also D-glucuronic acid. In general, the sugars of the D-configuration occur as β-glycosides, whereas those of the L-configuration occur as α-glycosides. In onions the major glycosides are quercetin-4´-glucoside and quercetin-3,4´-diglucoside.[13 & 14] Green beans contain mainly quercetin-3-*O*-glucuronide (4-14 mg/kg).[15] Commercial processing did not result in chemical break-down of the conjugates. The two main glycosides of broccoli are quercetin-3-*O*-sophoroside (65 mg kg^{-1}) and kaempferol-3-*O*-sophoroside (166 mg kg^{-1}).[16] The total flavonols and flavones contents of fresh and processed vegetables, fruits

beverages commonly consumed in the Netherlands have been determined[17-18] after hydrolysis of their glycosides. Peak identity and purity were confirmed by photodiode array detection[19] or by application of on line LC-MS analysis.[20] Recent investigation has shown that the dietary intakes of bioflavonoids ranged between 23 mg d^{-1} (estimated in the Netherlands) and 170 mg d^{-1} (estimated in the USA).[21-22] Distribution of flavonoid either in free from or as glycosides in some common food have been listed in the table 1.

Table1: Content of flavonols and flavones (mg/kg or mg/L) in foods*

Food	 Quercetin	 Kaempferol	 Myrcetin	 Luteolin	 Apigenin
Apple	20-36	-	-	-	-
Apple juice	2.5	-	-	-	-
Apricot	25-26	-	-	-	-
Bean, French	39	<12	-	-	-
Bean, French processed	17	<3.8	-	-	-
Bean, Green	16	-		-	-
Bean, Slicing	29	-	-	-	-
Bean, Broad	20	-	26	-	-
Bean, Broad processed	5.5	<7	-	-	-
Broccoli	30-37	60-72	-	-	-
Brussels spourts	0-6	7.4-9	-	-	-
Cabbage, green	-	-	-	-	-
Cabbage, red	4.6	-	-	-	-
Cabbage, red processed	2.8	-	-	-	-
Cabbage, white	-	-	-	-	-
Cauliflower	-	-	-	-	-
Celery, leaf	-	-	-	200	750
Celery, Stalk	-	-	-	2-20	16-61
Cherry	10-15	-	-	-	-
Cherry, processed	-	-	-	-	-
Cucumber	-	-	-	-	-
Currant, black	37	1	-	-	-
Currant, red	8-13	-	-	-	-
Endive	<1.3	46	-	-	-
Grape, black	15-37	-	4.5	-	-
Grape, white	2-12	-	4.5	-	-
Grape, juice	4.4	-	6.2	-	-
Grapefruit juice (fresh)	4.9	-	-	-	
Kale	110-120	211-470	-	-	-
Leek	-	30-31	-	-	-
Lettuce	14-79	-	-	-	-
Mushroom	-	-	-	-	-
Onion	340-347	-	-	-	-
Orange juice	5.7	-	-	-	-
Pea	-	-	-	-	-

Food	Quercetin	Kaempferol	Myrcetin	Leuteolin	Apigenin
Pea, processed	4	-	-	-	-
Peach	-	-	-	-	-
Pear	3.4	-	-	-	-
Plum	-	-	-	-	-
Spinach	-	-	-	-	-
Strawberry	6-8.6	5-12	-	-	-
Tea, black	14-17	14-16	3.0	-	-
Tomato	2-14	-	-	-	-
Cherry tomato	63	-	-	-	-
Tomato juice	13	-	-	-	-
Wine, red	8.3	-	7.9	-	-

*Only edible portions are considered. '–' denotes below the detection limit.

BIOLOGICAL BENEFITS ON HUMAN HEALTH

The diversity in the chemical structure of the flavonoids leads them to a wide range of biological activities. Their actions in humans have been the subject of extensive research.

Antioxidative effects: Most studied property of almost every group of flavonoids is their capacity for protecting the body against reactive oxygen species (ROS)thus act as antioxidants.[23] Cells and tissues of human body are continuously damaged due to free radicals which are produced during normal oxygen metabolism or inducing by exogenous damage.[24 & 25] Though cellular functions are not fully understood yet, one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. Cellular damages resulted to the shift in the net charge of the cell. As a result final outcome observed as change in the osmotic pressure, swelling and eventually cell death. Living organisms have developed several effective mechanisms to protect themselves from reactive oxygen species.[26] There are various antioxidant-defense mechanisms of the body which include enzymatic (superoxide dismutase, catalase andglutatione peroxidase) and also non-enzymatic counterparts (glutathione, ascorbic acid and α-tocopherol). In addition to these self protections dietary uptake of flavonoids natural products can prevent injury caused by free radicals. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation.[27]

$$\text{Flavonoid(OH)} + R^{\cdot} \rightarrow \text{Flavonoid } (O^{\cdot}) + RH$$

Where, R• is a free radical and O• is an oxygen free radical. Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite. Epicatechin and rutin are also powerful radical scavengers .[28] The scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase. Low-density lipoprotein (LDL) belongs to the lipoprotein particle family which transports cholesterol and triglycerides from the liver to peripheral tissues and appears to be harmless until oxidized by free-radicals.[29] By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro.[30] This action protects the LDL particles and, theoretically, flavonoids may have preventive action against atherosclerosis.

Nitric oxide: Several different types of cells including endothelial cells and macrophages produce nitric oxide. Early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels[31], but much higher concentrations of nitric oxide produced by inducible nitric-oxidesynthase in macrophages can result in oxidative damage. Activated macrophages greatly increase their simultaneous production of both nitric oxide and superoxide anions. Nitric oxide reacts with free radicals, there by producing the highly damaging peroxynitrite, which can directly oxidize LDLs, resulting in irreversible damage to the cell membrane. Several flavonoids, including quercetin, result in a reduction in ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity.[32] When flavonoids are used as antioxidants, free radicals are scavenged

and therefore can no longer react with nitric oxide, resulting in less damage.[33] It was reported that nitric oxide molecules are directly scavenged by flavonoids and it has also been speculated that nitric oxide scavenging plays a big role in the therapeutic effects of flavonoids.[34] Silibin is a flavonoid that has been reported to inhibit nitric oxide dose dependently.[35]

Xanthine oxidase: The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues, especially after ischemia-reperfusion.[36] Both xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid. Xanthine dehydrogenase is the form of the enzyme present under physiological conditions, but its configuration is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (ie, reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals. At least 2 flavonoids, quercetin and silibin, inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury.[32, 37 & 38] According to Cos et al.[39] luteolin (3,4,5,7-tetrahydroxyflavone) is the most potent inhibitor of xanthine oxidase.

Leukocyte immobilization: Under normal conditions, leukocytes move freely along the endothelial wall. However, during ischemia and inflammation, various endothelium-derived mediators and complement factors may cause adhesion ofthe leukocytes to the endothelial wall, thereby immobilizing them and stimulating degranulation of the neutrophil. As a result, oxidants and inflammatory mediators are released, resulting in injuryto tissues. Oral administration of a purified flavonoid fraction was reported to decrease the number of immobilized leukocytes during reperfusion.[40] The decrease in the number of immobilized leukocytes by flavonoids may be related to the decrease in total serum complement and is a protective mechanism against inflammation-like conditions associated with, for example, reperfusion injury.[40-41] Some flavonoids can inhibit degranulation of neutrophils without affecting superoxide production.[42] The inhibitory effect of some flavonoids on mast cell degranulation has beenshown to be due to modulation of the receptor-directed Ca^{2+} channels in the plasma membrane.[43]

Interaction with other enzyme systems: Compared to their antioxidant capacities, relatively little research have been doneon other beneficial effects of flavonoids. The major effects of flavonoids (eg, antiallergic effects) may be the result of radical scavenging. Alternatively they may interact with various enzyme systems and some effects may be due to combination of radical scavenging process and an interaction with enzyme functions. Iron with reactive oxygen species cause lipid peroxidation [44] and specific flavonoids are known to chelate iron,[45] thereby removing a causal factor for the development of free radicals. Quercetin in particular is known for its iron-chelating and iron-stabilizing properties. Direct inhibition of lipid peroxidation is another protective measure.[46] Another feature of flavonoids is a reduction in the release of peroxidase. This reduction inhibits the production of reactive oxygen species from neutrophils by interfering with α_1-antitrypsin activation.[47] An interesting effect of flavonoids on enzyme systems is the inhibition of the metabolism of arachidonic acid.[48] This feature gives flavonoids anti-inflammatory and anti-thrombogenic properties. The release of arachidonic acid is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines.

Anti-atherosclerotic effects: Clinical studies have pointed out that flavonoid intake protects against coronary heart disease.[49-50] Thus, flavonoids in regularly consumed foods might reduce the risk of death from coronary heart disease in the elderly. Furthermore, a Japanese study has reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations.[51] Oxidative stress and vascular damage are postulated to play a key role in dementia, and the intake of red wine is reported to prevent the development of dementia.[52] The intake of flavonoids was reported to be inversely related to the risk of incident dementia.[53]

Anti-inflammatory effects: Cyclooxygenase and lipooxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Neutrophils containing lipooxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines. Selected phenolic compounds were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways.[48, 54 & 55] This inhibition reduces the release of arachidonic acid.[56] The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus diminishing the formation of these inflammatory metabolites.[57-58] Another antiinflammatory feature is the ability of flavonoids to inhibit eicosanoid biosynthesis, cytosolic and membranal tyrosine kinase.[58-59] Eicosanoids, such as prostaglandins, are involved in various immunologic responses[60] and are the end products of the cyclooxygenase and lipooxygenase pathways. It has been observed that flavonoids are able to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells.[61-62]

Anti-tumor effect: Damage from reactive oxygen species is proposed to be involved in carcinogenesis.[63 & 64] Reactive oxygen species can damage DNA and division of cells with unrepaired or misrepaired damage leads to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression cancer is the outcome. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens. It has been stated that flavonoids, as antioxidants, can inhibit carcinogenesis.[65] Some flavonoids (such as fisetin, apigenin, and luteolin) are stated to be potent inhibitors of cell proliferation.[66] Integral membrane proteins, such as tyrosine 3-monooxygenase kinase, are involved in a variety of functions, like enzyme catalysis, transport across membranes, transduction of signals that function as receptors of hormones and growth factors, and energy transfer in ATP synthesis. Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation. A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequentincidence of lung cancer. This effect was mainly focused on quercetin, which provided >95% of the total flavonoid intake in that particular study.[67] Quercetin and apigenin inhibited melanoma growth and influenced the metastatic potential in mice.[68] It has been speculated that flavonoids can inhibit angiogenesis.[69] Angiogenesis is normally a strictly controlled process in the human body. The process of angiogenesis is regulated by a variety of endogenous angiogenic and angiostatic factors. It is switched on, for example, during wound healing. Pathologic, unregulated angiogenesisoccurs in cancer.[70] Angiogenesis inhibitors can interfere with various steps in angiogenesis, such as the proliferation and migration of endothelial cells and lumen formation. Among the known angiogenesis inhibitors, flavonoids seem to play an important role.[69 & 71] However, the mechanism behind the antiangiogenetic effect of flavonoids is still unclear. A possible mechanism could be inhibition of protein kinases.[72] These enzymes are implicated to play an important role in signal transduction and are known for their effects on angiogenesis.

Anti-thrombogenic effects: Development of atherosclerosis, acute platelet thrombus formation, and embolization of stenosed arteries arise due to Platelet aggregation. Activated platelets adheringto vascular endothelium generates lipid peroxides and oxygen free radicals, which inhibit the endothelial formation of prostacyclin and nitrous oxide. It has been observed that tea pigment can reduce blood coagulability, increase fibrinolysis, and prevent platelet adhesion and aggregation.[73] Selected flavonoids, such as quercetin, kaempferol, and myricetin have beenshown to be effective inhibitors of platelet aggregation in dogs and monkeys.[74] Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide.[75] Flavonoids are powerful antithrombotic agents both in vitro and in vivo because of their ability to inhibit of the activity of cyclooxygenase and lipooxygenase pathways.[76] It is well known that arachidonic acid, which is released in inflammatory conditions, is metabolized by platelets to form

prostaglandin, endoperoxides, and thromboxane A2, leading to platelet activation and aggregation.[77] The main antiaggregatory effect of flavonoids is thought to be by inhibition of thromboxane A2formation. Flavonoids affect arachidonic acid metabolism in different ways. Some flavonoids specifically block cyclooxygenase or lipooxygenase, whereas others block both enzymes.[78] In vitro studies have shown that flavonoids bind to platelet membranes and may therefore have an accumulative effect over time.[79]

Antiviral effects: The anti-viral activity of the flavonoids has beendetected out in a study by Wang *et al*[80]. Some of the viruses have beenreported to be affected by flavonoids (e.g. herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus). Quercetin has beenreported to exhibit both antiinfective and antireplicative abilities. The interaction of flavonoids at the different stages in the replication cycle of viruses has previously been described.[81] For example, some flavonoids work on the intracellular replication of viruses, whereas others inhibit the infectious properties of the viruses. By far, most studies of the effects on viruses have been performed in vitro and little is known about the antiviral effect of flavonoids in vivo. There has been some evidence that flavonoids in their glycone form seem to inhibit rotavirus than in their aglycone form.

Because of the worldwide spread of HIV since the 1980s, investigations of the antiviral activity of flavonoids have been mainly focused on HIV. Many natural products can inhibitvarious stages of the replication cycle of the virus.[82] The discovery and development of flavonoids as anti-HIV agents has expanded in the past 2 decades. Most of these studies focused on the inhibitory activity of reverse transcriptase, or RNA-directed DNA polymerase[83], but antiintegrase and antiprotease activities were also described.[84] Again, flavonoids have mainly been studied in in-vitro experiments, and hence no clear contribution of flavonoids to the treatment of HIV-infected patients has yet been shown.[85]

Anti-steoporotic effects: Hegarty *et al*. [86] have compared bone mineral density between older women who consumed tea and those who did not in England. Women in the study who drank tea had higher bone mineral density measurements than those who did not. The flavonoids in tea might be responsible for the prevention of osteoporosis.

Effect on central nervous system: Many flavonoids were found to be ligands for the γ-aminobutyric acid type A ($GABA_A$) receptors in the central nervous system (CNS); which led to the hypothesis that they act as benzodiazepine-like molecules. This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion.[87] Kang *et al*.[88] detected sedative action in mice of two flavonol glycosides, quercitrin and isoquercitrin, isolated from the flowers of Albizzia julibrissin Durazz. Du *et al*.[89] have found that goodyerin, a flavonol glycoside isolated from *Goodyera schlechtendaliana*, possesses sedative and anticonvulsant activities in mice and Datta et al.[90] havedescribed that the flavonol glycoside quercetin-3-O-(6´´-feruloyl)-β-D-galactopyranoside, isolated from the aerial parts of Polygonumviscosum, possessed CNS depressant activity. Flavanones glycosides 2S-hesperidin[91] and linarin[92] which were identified in the roots and rhizomes of two valerian species have also beenreported to possess sedative-hypnotic effects. Although, there is not enough information to establish a full structure-sedative activity correlation, partial conclusion apparent from the behavioral results point that the linkage to a sugar moiety is important to preserve the action.[93]

Anti-diabetic: Quercetin has been found to be an inhibitor of the enzyme aldose reductase, which plays a role in converting glucose (sugar) to sorbitol (a sugar alcohol) in the body. People with diabetes develop secondary problems, such as neuropathy, retinopathy, diabetic cataracts, and nephropathy because of sorbitol buildup in the body. Quercetin may therefore be beneficial in the nutritional management of diabetes, but clinical studies need to be conducted to verify these effects, which have been observed in non-human experiments.[94]

ABSORPTION

Naturally occurring flavones exist predominantly in a glycosylated form rather than in their aglycone form. The form of the flavonoid seems to influence the rate of absorption. Data on the absorption,

metabolism, and excretion of flavonoids in humans are contradictory and scarce. Some studies have showed that the most intensely studied dietary flavonoid, quercetin, is absorbed in significant amounts.[95] Hollman *et al*.[96] have suggested that the glycosylated forms of quercetin are absorbed more readily than are the aglycone forms. The role of flavonoid glycosylation in facilitating absorption has been questioned by the fact that catechin, which is not glycosylated in nature, is absorbed relatively efficiently.[97]

TOXICITY

There is much controversy regarding the toxic and mutagenic properties of quercetin. Dunnick *et al*.[98] have reported that high doses of quercetin over several years might result in the formation of tumors in mice. However, in other long-term studies, no carcinogenicity was found.[99] In contrast with the potential mutagenic effects of flavonoids in earlier studies, several more recent reports have indicated that flavonoids, including quercetin, seem to be antimutagenic *in vivo*.[59,100 & 101] A large clinical study by Knekt *et al*.[67] in which 9959 men and women have been followed for 24 years, showed an inverse relation between the intake of flavonoids (e.g., quercetin) and lung cancer. One possible explanation for these conflicting data is that flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less toxic to normal cells.

CONCLUSION

Flavonoids have received much attention in the literature over the past few decades due to their divers' health beneficial effects. However, most of the research involved *in vitro* studies. Thus it is difficult to draw definite conclusions about the usefulness of flavonoids in the diet. The study of flavonoids is complex because of the heterogeneity of their vast varieties in molecular structures. Thus it is essential to improve analytic techniques to allow collection of more accurate data. In conclusion, the *in vivo* studies that have been performed do give a hopeful picture for the future. More epidemiologic studies and cohort studies are mandatory to make recommendations on daily flavonoid intakes.

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