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MINIREVIEW

FLAVONOIDS: OLD AND NEW ASPECTS OF A CLASS OF NATURAL THERAPEUTIC DRUGS

Giulia Di Carlo, Nicola Mascolo*, Angelo A. Izzo, and Francesco Capasso[1]

Department of Experimental Pharmacology, University of Naples "Federico II", Via D. Montesano 49, 80131 Naples, Italy and
*Department of Pharmaceutical Sciences, University of Salerno, Piazza Vittorio Emanuele 9, 84084 Penta di Fisciano, Salerno, Italy

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Summary

Flavonoids are natural products widely distributed in the vegetable kingdom and currently consumed in large amounts in the daily diet. Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems, suggesting that the compounds may possess significant antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic and even antitumor activities. This review summarizes available data on these beneficial effects of flavonoids.

Key Words: flavonoids, enzyme activity, antihepatotoxic activity, antiallergic activity, anti-inflammatory activity, antiosteoporotic activity, antitumor activity

Flavonoids (or bioflavonoids) are a group of about 4000 naturally occurring compounds that are ubiquitous in all vascular plants (1). They are pigments responsible for the autumnal burst of hues and the many shades of yellow, orange and red in flowers (2). They are also important for the normal growth, development and defense of plants (3). Flavonoids, important constituents of the human diet, are found in fruits (in citrus fruit they may represent up to 1% of fresh material) and vegetables. Beverages like red wine, tea, coffee and beer also contain large amounts of flavonoids: on the average, the daily diet contains approximately 1 g of flavonoids per day (4). Flavonoids are also found in several medicinal plants, and herbal remedies containing flavonoids have been used in folk medicine around the world.Therefore it seems that these compounds are important not only for plants, but also for animals, including humans. Flavonoids have probably existed in the plant kingdom for over one billion years. This long interaction between plant flavonoids and humans has stimulated much interest in the biochemical and physiological activities of these chemicals.

[1]Corresponding author: Prof. F. Capasso, Department of Experimental Pharmacology, University of Naples "Federico II", Via D. Montesano 49, 80131 Naples, Italy

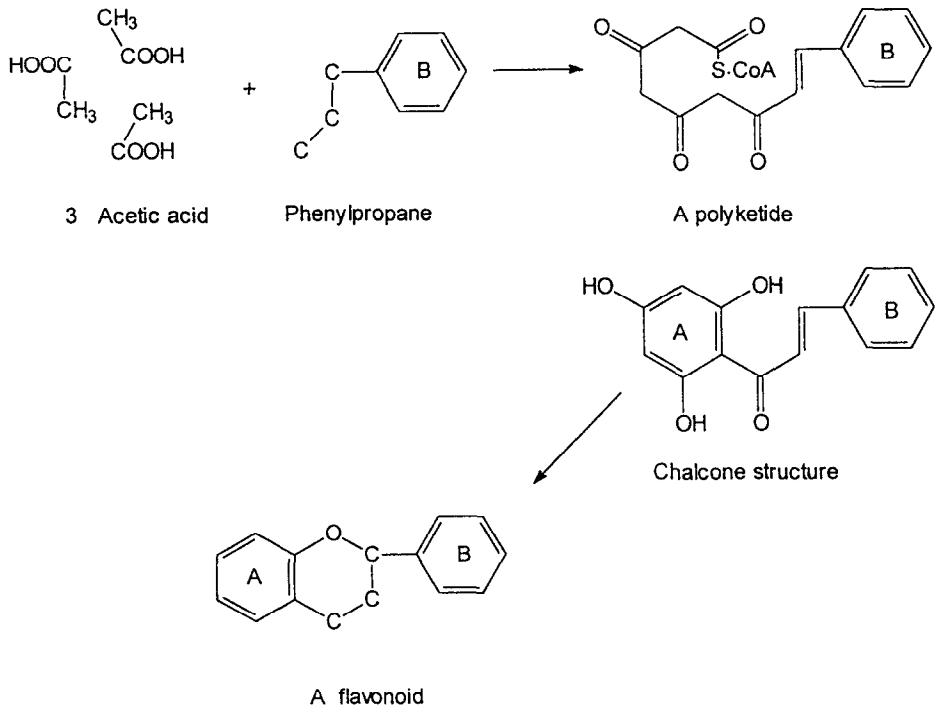


FIG. 1

Biosynthesis of flavonoids.

Many papers have been published in the last two decades, and this present article will be a review of a part of these data with a particular emphasis on the effects of flavonoids on the gastrointestinal tract.

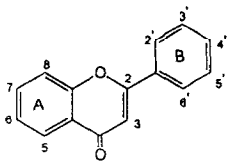
Biosynthesis of Flavonoids

Flavonoids are biosynthesized via a combination of the shikimic acid and acylpolymalonate pathways. A cinnamic acid derivative (phenylpropane), synthesized from shikimic acid, acts as the starting compound in a polyketide synthesis, in which an additional three acetate residues are incorporated into the structure (Fig.1). This is followed by ring closure. Through subsequent hydroxylations and reductions, plants are then able to form different classes of flavonoids (5).

Structure of Flavonoids

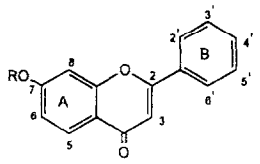
Flavonoids are benzo-γ-pirone derivatives that can be grouped according to the presence of different substituents on the rings and to the degree of benzo-γ-pirone ring saturation (Fig.2). Flavonoids *per se* are compounds in which the benzenoid substitution is at the 2 position; compounds with substitution at the 3 position are properly termed *isoflavonoids*. Common isoflavonoids include genistein, daidzein and biochanin A.

In addition, the six member ring could be either γ-pirone or its dihydroderivative: the first case compounds are termed *flavones* and *flavonols*, the latter case *flavanones* and *flavanols* Flavonoids may also be aglycones (consisting of a benzene ring condensed with a six member ring which possesses a phenyl ring at the 2 position), glycosides (that carry one or more sugar residues on the ring) or



Flavone

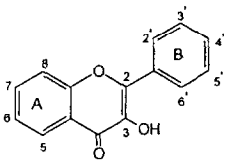
	5	6	7	4'
Apigenn	OH	-	OH	OH
Baicalein	OH	OH	OH	-
Chrysin	OH	-	OH	-



Flavone glycosides

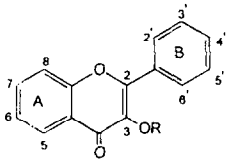
	5	6	7
Baicalin	OH	OH	R

R=β-glucopyranuronosyl



Flavonol

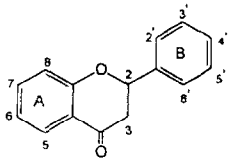
	5	7	3'	4'	5'
Quercetin	OH	OH	OH	OH	-
Kaempferol	OH	OH	-	OH	-
Myricetin	OH	OH	OH	OH	OH



Flavonol glycosides

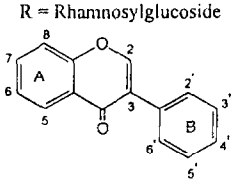
	3	5	7	3'	4'
Rutin	R	OH	OH	OH	OH

R = Rhamnosylglucoside



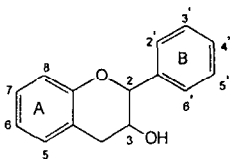
Flavanone

	5	7	4'
Naringenin	OH	OH	OH



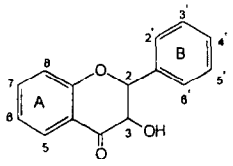
Isoflavone

	5	7	4'
Genistein	OH	OH	OH



Flavan-3-ol

	5	7	3'	4'
Catechin	OH	OH	OH	OH



Flavanolol

	5	7	3'	4'
Taxifolin	OH	OH	OH	OH

FIG. 2

Chemical structure of some flavonoids.

methylated derivatives. *Anthocyanidines* are red-blue pigments in plants closely related to flavonoids, differing only in that the C-ring is open. Their biological activities would appear to be similar to those of flavonoids (1). *Aurones* and *anthochlors* are yellow pigments found in flowers. Flavonols and flavones are the most widely occurring flavonoids; of these quercetin, kaempferol, myricetin, chrysin and apigenin are widely distributed. Flavanones and flavanols (catechin, epigallocatechin), but also dihydroflavones and dihydrochalcones, are considered minor flavonoids because of their limited natural distribution. Some flavanols are termed *pycnogenols* because they tend to form dimers

TABLE 1

Some Natural Sources of Flavonoids

Flavonoid	Source: dietary products and medicinal plants
Flavones	
Apigenin	*Petroselinum sativum, Apium graveolens*
Flavone glycosides	
Baicalin	*Scutellaria baicalensis*
Flavonols	
Quercetin	*Allium cepa, Solanum lycopersicum, Vaccinium macrocarpon Vitis vinifera, Olea europea, Thea sinensis, Crataegus cuneata, Glycirriza glabra, Pueraria thumbergiana, Morus alba*
Kaempferol	*Cichorea endivia, Vitis vinifera, Thea sinensis, Raphanus sativus*
Myricetin	*Vaccinium macrocarpon, Thea sinensis, Vitis vinifera*
Flavonol glycosides	
Rutin	*Sophora japonica, Fagopyrum esculentum, Eucaliptus macrohyncha, Stellaria media*
Flavan-3-ol	
Catechin	*Thea sinensis, Vitis vinifera*
Flavanones	
Naringenin	*Eucalyptus globulus*
Flavanonols	
Taxifolin	*Citrus* fruits sp. (*aurantium, limon*, etc.)
Isoflavones	
Genistein	*Soya hispida, Stellaria media, Pueraria lobata, Sophora japonica*

From Rich-Evans modified (7)

(condensation of two identical compounds): proanthocyanidines are examples of flavanol dimers. Considering that all of these compounds have survived in plant tissue throughout evolution (6), it is easy to imagine that these structurally diverse compounds have some important purpose in nature. In any case, their distribution varies between plant species (Table 1); for example berries, grapes and red or blue fruits contain high levels of anthocyanins, citrus fruit flavanones, green tea catechins, etc (7). Also some flavonoids (having small molecular weight) are responsible for the tartness and bitterness of many fruits, whereas others (having larger molecular weight) are responsible for their astringency (especially tannins).

Pharmacokinetics of Flavonoids

The extent of absorption of flavonoids is an important unsolved problem because of the limited data available about them, particularly in humans. Animal studies (4) show that flavonoids present in foods are to be considered non-absorbable because they are bound to sugars as β-glycosides (with the exception of catechins). In fact, only free flavonoids, without a sugar molecule (so-called aglycones), are thought to be able to pass through the gut wall. The hydrolysis of the β-glycosidic bonds occurs only in the colon by micro-organisms that at the same time degrade dietary flavonoids. This is because no enzymes capable of splitting the bond are present or secreted into the gut . After absorption, the subsequent metabolism of flavonoids is quite well known from animal studies, while no data for humans are available. The liver is largely responsible for the metabolism of absorbed flavonoids (8). The intestinal wall and kidney are the secondary sites of the metabolism of the absorbed flavonoids. However, this depends on the flavonoids: those found in citrus fruits are poorly metabolized by the intestinal microflora, quercetin is not absorbed in humans, rutin is poorly absorbed, whereas procyanidolignanes are readily absorbed in mice. Flavonoids metabolized by intestinal bacteria are converted to hormone-like compounds with weak estrogenic and antifecondative activities. Hydroxy groups are conjugated with glucuronic acid or sulphate and, in addition, methylation may occur. The glucuronides and sulphates are excreted in the bile.

The possibility of an enterohepatic cycle has been postulated: micro-organisms in the colon can hydrolyse glucuronides and sulphates, which then presumably enables absorption of the liberated aglycones.

Flavonoids, once absorbed, influence many biological functions including protein synthesis, cell proliferation differentiation and angiogenesis, making them beneficial in a variety of human disorders.

Biological Activities of Flavonoids

The great prevalence of flavonoids and anthocyanidines in the vegetal kingdom is not accidental; not only do they act as the coloured pigments of flowers but also as enzyme inhibitors, precursors of toxic substances, a defence against ultraviolet radiation exposure, chelating agents of metals noxious for plants, and reducing agents. In addition, flavonoids are involved in photosensitization and energy transfer, morphogenesis and sex determination, levels of respiration and photosynthesis, action of plant growth hormones and regulators (9-13), gene expression and behavior. Through the food chain animals and humans ingest flavonoids, and there is much data concerning a wide range of biological activities of these compounds in humans. For example, they were utilized in medicine as protection for vascular integrity (14), as antiosteoporotic agents (15) and for their antihepatotoxic properties (16). Some flavonoids were examined for their activity in experimental tumor model systems both *in vitro* (17) and *in vivo* (18). Certain flavonoids were shown to inhibit the activity of enzymes such as aldoso-reductase (19) and xanthine-oxidase (20). Flavonoids were also reported to act in the gastrointestinal tract as either antiulcer (21), antispasmodic (22, 23), antisecretory or antidiarrhoeal (24) agents.

TABLE 2

Potential Effects of Flavonoids in Animals and Human

Analgesic
Hesperidin (67)

Antiallergic
Khellin (5)
Quercetin (44)

Antianginous
Isoflavone (33)

Antiatherogenic
Quercetin (85)

Anticancer
Baicalein (50)
Catechin (17)
Epigallocatechin(51)
Kaempferol-3-O-β-D-glucopiranoside(60)
Nobiletin (48)
Quercetin (18, 51, 53, 58)
Rutin (18)
Tangeretin (49)
Tricin (60)
Woogonin (50)

Antidiabetic
Quercetin (1)

Antidiarrhoeal

Apigenin (24)
Flavone(24)
Kaempferol (24)
Morin (24)
Myricetin (24)
Naringenin (24)
Quercetin (24, 84)
Quercitrin (80, 81)

Antihepatotoxic
Gossypin (38)
Hispidulin (16)
Hydroxyetilrutoside(38)
Kolaviron (35)
Quercetin (42
Silymarin (34, 36, 37)

Antiinflammatory
Apigenin (25, 63)
Chrysin (25)
Gossypin (64)
Hibrifolin (64)
Hypolaetin-8-β-D glucoside (64)
Luteolin (63)
Myricetin (25)
Nepetin (62)
Quercetin (25, 65)
Quercitrin (65, 66)
Sidertoflavanone (64)

Antiosteoporotic
Ipriflavone (15, 45, 46)

Antispasmotic
Apigenin (22, 23)
Catechin (22)
Chrysin (22, 23)
Flavone (22,23)
Kaempferol (22)
Quercetin (22, 23)

Antiulcer
Flavanone (74)
Flavone (74)
Hypolaetin-8-glucoside (69,70)
Kaempferol (73)
Quercetin (73, 74)
Rutin (73)
Solon (68)

Vascular protection
Anthocyanidine (31)
Citrin (28)
Rutoside (29)

Finally, some flavonoids were found to possess a good antinflammatory activity (20, 25),which this was related mainly to their inhibiting production of inflammatory mediators such as prostaglandins, leukotrienes (26) or nitric oxide (27). Potential effects of different flavonoids in animals and human are listed in Table 2.

Vascular Protection by Flavonoids and Anthocyanosides

Flavonoids and anthocyanidines were found to act on blood vessels in the form of vitamin P. These compounds are necessary for the maintenance of a normal vascular permeability and, in fact, are used for certain pathological states in which there is a defect in vascular wall permeability. The first studies to describe such properties of flavonoids were reported in 1936 with the isolation from lemon juice of a substance (citrin, a mixture of hesperidin and eriodictyol glucoside) that normalized capillary resistance in patients with certain pathologies involving a low vascular resistance (28) More recently, the effects of O-(β-hydroxyethyl)-rutoside (HR) were examined in patients with chronic venous insufficiency: the infusion with HR was reported to have restored the altered haemorheological parameters (29). Other flavonoids were shown to increase skin capillary resistance. The exact mechanism of this action is still unclear but it has been postulated that this activity could be linked with the effects that some flavonoids have on platelets, leukocytes and on the enzymes of blood coagulation (14,30). Much data have also been reported on the significant activity of anthocyanidines in the treatment of vascular damage: it was demonstrated that the anthocyanosides of *Vaccinium myrtillus*, when given orally to rats, were capable of preventing experimentally-induced vascular damage such as thrombosis without exibiting toxicity even at doses of 100 mg/kg. The activity of these anthocyanosides could be related to an increase in the PGI_2-like activity released from arterial tissue (31).

Mian et al. (32) reported that the protection of the capillary wall was mediated through a dual mechanism, in particular by an increase in the endothelium barrier-effect through a stabilization of the membrane phospholipids, caused by an increase in the biosynthetic processing of the acid mucopolysaccharides of the connective tissue ground substances and by a restoration of the affected mucopolysaccharidic pericapillary sheath. Data exist which also suggest a very promising effect of flavonoids, particularly isoflavone, in angina pectoris (33).

Antihepatotoxic Properties

Flavonoids extracted from *Silybum marianum* have been used for centuries in folk medicine for the treatment of liver disorders. The active constituents of this extract reside in a mixture termed silymarin, which in experimental animal models was demonstrated to exert not only a positive effect on intact liver cells, or cells not yet irreversibly damaged (acting on the cell membranes to prevent the entry of toxic substances), but also to stimulate their regenerative capacity after partial hepatectomy (34). Other investigations were carried out on the antihepatotoxic activity of seeds of the Nigerian plant *Garcinia kola*, and evidence was found for a potent antihepatotoxic effect of kolaviron (a fraction of the defatted alchoolic extract) and of Garcinia biflavanones in mice that were intoxicated with phalloidin (35). Flavonoids extracted from *Baccaris trimera* were reported to protect mice from hepatic damage: hispidulin appeared to be the most active flavonoid in this plant and protected mice intoxicated with phalloidin (80% of survival rate) while other flavonoids in this extract (quercetin, luteolin, nepetin and apigenin) were less active or even inactive (16). The hepatoprotective activity of silybin, the main flavolignan occurring in the mixture silymarin, was investigated in mice intoxicated with nontherapeutic (i.e.hepatotoxic) doses of acetaminophen: the flavonoid was also effective in this instance and protected the liver, but the exact mechanism of this protection was not determined (36). However, it seems that silybinin binds a subunit of the DNA-dependent RNA

polymerase I that activates this enzyme. As a consequence, protein synthesis is increased, leading to an accelerated regeneration and production of hepatocytes (37). More recently the influence of dermal applications of sulphur mustard on hepatic lipid peroxidation and the protective activity of flavonoids were investigated. Administration of gossypin and hydroxyethyl rutoside significantly increased the survival rate of mice thus treated, suggesting that flavonoids may be benefical in reducing the toxic effect of sulphur mustard (38). Actually silybin and other flavonoids are widely utilized in the treatment of liver diseases and diseases associated with increased vascular permeability and capillary fragility. Some flavonoid compounds also exert a protective effect against the increased capillary permeability induced by x-irradiation (39). An antitoxic activity has been then reported for quercetin, a compound able to exert some ameliorative effects on tissue damage provoked by cigarette smoke (40) and to reduce the cytotoxic effect of T-2 mycotoxin (41).

Recently Gilani et al. (42) reported that quercetin extracted from *Artemisia scoparia* possesses some protective activity on paracetamol-induced hepatotoxicity in mice and rats.

Antiallergic Properties

Flavonoids are also known for their anti-allergic effects. These effects are in part attributed to the influence of flavonoids on the production of histamine (43). In fact flavonoids inhibit enzymes that increase histamine release from mast cells and basophils: cyclic AMP phosphodiesterase and calcium-dependent ATPase. Cyclic AMP phosphodiesterase degrades cAMP: large amounts of cAMP act by blocking intracellular reservoirs of histamine. Also calcium-dependent ATPase degrades ATP to release energy to facilitate the gating of calcium across the cell membrane: high intracellular calcium levels also cause histamine release from cellular storage granules. Khellin, a flavonoid isolated from fruits of the Egyptian plant *Ammi visnaga*, was used for the treatment of asthma and other disturbances (5). It is currently little used because of side effects like nausea and vomiting. Studies to find better substances led to the development of sodium cromoglycate, a drug that prevents degranulation of the mast cells and consequently the release of histamine and other endogenous substances causing asthma. This drug has no effect on acute asthmatic attacks, but it acts prophylactically. It is now known that quercetin and other substances are more potent than sodium cromoglycate in inhibiting histamine release from mast cells (44).

Antiosteoporotic Properties

Osteoporosis is a pathology characterized by a low bone mineral density or content that is particularly common in older women. Dietary factors, such as vitamin, (especially vitamin D), protein and calcium are important for the maintenance of bone quantity and strength. Data suggest that flavonoids may play a role in preventing osteoporosis (15,33). It was shown, for example, that treatment with ipriflavone, an isoflavone derivative (7-isopropoxyisoflavone) for a period of one year (3 x 200 mg daily doses) reduced pain and increased mobility in 73% of the patients with osteoporosis. Ipriflavone increased the density of distal metaphysis in a dose-dependent manner and also tended to increase the density of the diaphysis in rats with glucocorticoid-induced osteoporosis (45). Experimental studies showed that this compound acts by inhibiting osteoclastic bone resorption both *in vitro* and *in vivo*. Although the mechanism of this inhibition is unclear, it was demonstrated that ipriflavone directly inhibited osteoclastic activity by the modulation of intracellular free calcium (46). Much published evidences support the antiosteoporotic activity of ipriflavone both *in vitro* and *in vivo*. For example, it was reported that ipriflavone's major metabolites inhibited parathyroid hormone-induced bone resorption in foetal rat long bones *in vitro*, which suggested that these metabolites may play an important role in the pharmacological effects of ipriflavone (47).

Antitumor Effect

Many researchers have conducted *in vitro* studies on the potential antitumor activity of flavonoids. For example, the antitumor activity of catechin, a flavanol present in green tea, *Areca catechu, Crataegus oxyacantha, Cinnamomum cassia, Polygonum multiflorum, Rheum palmatum,* was examined using tumor invasion models (17). This particular flavonoid inhibited such invasiveness and it was suggested that the activity of catechin may be related to its ability to bind tissue-type plasminogen activator (t-PA) to laminin, a molecule of extracellular matrix that play an important role during tumor cell adhesion, leading to partial inactivation of t-PA (48). *Citrus* flavonoids (tangeretin) also exhibit anti-invasive activity, but appear to act by a different mechanism. They show a poor affinity for the extracellular matrix and do not bind enzymes to laminin (48). *In vitro* studies have been made on the antiproliferative effect of *Citrus* flavonoids quercetin, taxifolin, nobletin and tangeretin on human squamous cell carcinoma (49) . Whereas nobiletin and tageretin, which are both polymethoxylated flavonoids, markedly inhibited carcinoma cell growth at all concentrations tested, quercetin and taxifolin were ineffective. The difference in action may be due to the greater uptake by cell membrane of the less hydrophhilic polymethoxylated flavonoids However, ascorbic acid is able to potentiate the cytostatic effect of quercetin.

Flavonoids extracted from *Scutellaria baicalensis* (baicalein, baicalin and woogonin) exerted *in vitro* a concentration-dependent inhibition (10^{-6} M- 10^{-4} M) of the proliferative response of cultured rabbit vascular smooth muscle cells upon exposure to 5% calf serum (50). Aside from quercetin and baicalein, epigallocatechin gallate and green tea extract were also reported to inhibit tumor growth by inhibiting mitosis (51). Some flavonoids (quercetin, epigallocatechin) and green tea extract inhibit tumor growth both by inhibiting some phase of the cell cycle and by blocking or competing for hormone receptor sites (52-54). Rutin, on the contrary, does not bind to estrogen binding sites and does not inhibit the growth of hormone-depending tumors. Quercetin, but not rutin, was also reported to be effective in inhibiting of *in vitro* bromodeoxyuridine incorporation by cells from transitional cell carcinoma of the bladder (53). Other mechanisms by which flavonoids may inhibit tumor growth include: stabilizing collagen, altering gene expression and reducing free radicals. It is known that agents that inhibit collagen breackdown may inhibit invasion and metastasis. Catechin and its derivatives anthocyanins and proanthocyanidins promote collagen synthesis (14,55), increase collagen resistance (56) and inhibit collagenase activity (57). Quercetin inhibits human breast cancer cells *in vitro* by inhibiting the expression of the mutated P_{53} plasma membrane protein: the P_{53} protein is a tumor suppressor protein and plays a key role in apoptosis (58). Flavonoids also possess free radical scavenging effect and therefore inhibit tumor invasion and metastasis (52,59). The free radical scavenging effect of flavonoids is greater than that of vitamins C and E. Flavonoids were determined to have *in vivo* activity on experimental tumors. For example, quercetin and rutin administered by diet were reported as inhibiting azoxymethanol-induced colonic neoplasia in mice (18). The flavonoids tricin and kaempferol-3-O-β-D-glucopyranoside derived from the traditional Chinese medicinal plant *Wikstroemia indica,* demonstrated antileukaemic activity in the P-388 leukemic mice (60). Furthermore, epidemiological studies indicated that diets containing linseed and soy (rich in isoflavonoids and lignans) may protect against colon, breast and prostatic cancer (61).

Antinflammatory Activity

Anti-inflammatory properties of flavonoids have been studied both *in vitro* and *in vivo.* Many groups carried out *in vitro* research. Landolfi et al. (25), for example, showed that many of the flavonoids studied were capable of modifying the metabolism of platelet arachidonic acid. This group also reported that some flavonoids, such as myricetin and quercetin, blocked both the cyclooxygenase and lipooxygenase pathways at relatively high concentrations, while at lower concentrations the lipooxygenase pathway was the primary target of inhibitory activity. In contrast, the inhibition of

cyclooxygenase and the consequent increase in intracellular cyclic AMP appeared to be the major mechanism involved in the antiaggregating effect of flavonoids such as flavone, apigenin, chrysin and phloretin. The mechanism appeared to be different for myricetin and quercetin (which primarly inhibited lipooxygenase). Their antiaggregating activity was relatively low but they were able to inhibit the first wave of cyclic AMP-induced aggregation and increased the cyclic AMP response to PGI_2. The studies of Middleton and Kandaswami (26) suggested that flavonoids may have significant *in vivo* effects on homeostasis of the immune system and on the behaviour of secondary cell systems involved in the inflammatory response. However, they concluded that more work is required to strengthen this hypothesis.

Flavonoids were demonstrated as possessing *in vivo* anti-inflammatory properties. Some reports suggested that they had good anti-inflammatory activity without the ulcerogenic side-effects of other anti-inflammatory drugs. In contrast, flavonoids were described as having anti-ulcer effects (See below).

Nepetin, a flavonoid obtained from *Nepeta hindostana*, was investigated in both acute and chronic models of inflammation in rats and found to possess significant activity in both proliferative and exudative phases of inflammation (62). Apigenin and luteolin from *Chamomilla recutita* were found to significantly inhibit the oedema caused by croton oil. This activity may have been due to a direct inhibition of arachidonic acid metabolism or to other mechanisms such as inhibition of histamine release or promotion of scavenging activity (63). Studies on the *in vivo* anti-inflammatory activity of the flavonoids, particularly hypolaetin-8-β-D-glucoside, sidertoflavone (isolated from the Spanish *Sideritis mugronensis*) and the flavonol glycosides gossypin and hibifolin (isolated from traditional Indian medicinal plants), showed a dose-dependent inhibition of both paw oedema and leucocyte accumulation in the peritoneum in the carrageenan-induced models of oedema and peritonitis (64).

Other studies were carried out on the anti-inflammatory activities of quercetin and quercitrin. In an *ex vivo* system utilizing the carrageenan-induced pleurisy model in rats, quercetin reduced in a dose-related manner the induced contractions of both prostaglandin E_2 and leukotriene B_4 and leukocyte migration in the exudate. Quercetin also reduced LTB_4 synthesis in cells stimulated with ionophore A23187 both *ex-vivo* and *in vitro*. Quercitrin exhibited similar but less activity than quercetin (65). Moreover, more recently it was reported that quercitrin decreased colonic damage and the incidence of diarrhoea and normalized colonic fluid transport in rats with chronic experimental colitis (66). Other *in vivo* studies have been carried out on the anti-inflammatory and analgesic activities of flavonoids, such as those extracted from *Citrus aurantium* (hesperidin), *Machaerium villosum* (duartin) and from *Cyclolobium clausseni* (claussequinone). The experimental models included the rat paw oedema induced by carrageenan and dextran, the carrageenan-induced pleurisy, the acetic acid-induced writhing and the tail-flick method. When administered subcutaneously hesperidin, although inactive *per os*, exhibited significant anti-inflammatory activity on rat paw oedema induced by both carrageenan and dextran and on carrageenan pleurisy, without producing the adverse side effects of other classes of anti-inflammatory drugs. Hesperidin also exhibited analgesic activity in mice. The effect was obtained in the acetic acid-induced writhings but not on tail-flick response, indicating that hesperidin exerts an analgesic effect through peripheral, but not central mechanisms. Duartin and claussequinone produced no detectable effects (67).

Gastrointestinal Activity

Antiulcer Effect

One of the most important side effects of conventional anti-inflammatory drugs is their ulcerogenic activity. As referred to above, flavonoids were found to be good anti-inflammatory compounds and

were also able to protect the gastric mucosa against a variety of ulcerogenic agents (39). Many studies were performed examining the antiulcerogenic actions of flavonoids using both naturally derived and synthetic compounds.

Solon is a synthetic isoprenyl flavonoid derived from sophoradin, itself isolated from the traditional Chinese medicinal plant *Sophora subprostata.* This compound showed good antiulcerogenic and gastroprotective effects. The exact mechanism of its actions remains unclear, but it was proposed that it influences the formation and metabolism of prostaglandins in the gastric mucosa (68). A significant antiulcerogenic activity by hypolaetin-8-glucoside, a flavonoid present in several species of the genus *Sideritis,* was reported (69,70).

Other flavonoids that appear to exert antiulcerogenic activity are naringin and quercetin. In experiments using a rat model of ethanol-induced gastric ulcers, both of these flavonoids displayed significant antiulcerogenic effects. In particular, naringin (400 mg/kg, 60 min. before absolute ethanol) had a gastroprotective effect and significantly reduced ulceration. The authors suggested that the gastroprotective action of naringin could be explained in part through a complex non-prostaglandin-dependent mechanism that involved an increase in the glycoprotein content and viscosity of the gastric mucosal gel. They further postulated that free-radical scavenging may be involved (71). Treatment with quercetin (200 mg/kg) had protective effect in that it significantly reduced the severity of ulcers and increased the amount of glycoprotein content of gastric mucus. It was suggested that quercetin exerts its cytoprotective effect through a complex mechanism involving stimulation of prostaglandin and inhibition of leukotriene production, via mucus production and antioxidant properties (72). The antiulcerogenic effects of flavone, quercetin, naringin, rutin and kaempferol were also investigated in relation to the production of platelet activating factor (PAF), a potent ulcerogenic agent (73), in a rat model of acidified ethanol-induced gastric damage. Intraperitoneally administered quercetin, rutin and kaempferol reduced damage in a dose-dependent manner (25-50 mg/kg), while naringin reduced gastric damage only at high dose levels (200-400 mg/kg) and flavone was inactive. Relatively large amounts of PAF were present in the gastric mucosa of rats treated with acidified ethanol, and those flavonoids found to have protective properties against the induced gastric damage were active when the doses utilized were sufficient to reduce PAF formation. The authors concluded that although the degree of PAF inhibition produced by flavonoids was probably one of the main reasons for the reduction in gastric damage, other mechanisms could also be involved (21).

The actions of flavonoids on parietal cell acid secretion, gastric mucosal prostaglandin production and *Helicobacter pylori* growth were also investigated by Beil et al. (74). They found that the flavonoids flavone, flavanone and quercetin inhibited *Helicobacter pylori* growth and the formation of acid by parietal cells in response to stimulation by histamine and dibutyryl cyclic AMP. All flavonoids tested also inhibited the gastric proton pump (H^+/K^+). Their actions on the formation of gastric mucosal cell prostaglandin E_2 was, in contrast, not inhibitory: flavone and flavanone increased PGE_2 release, whereas quercetin was found to be inactive in this respect. It was concluded, in relation to their low toxicity and to the properties reported (antisecretory action, stimulation of prostaglandins, inhibition of *H. pylori*), that flavonoids could have a therapeutic potential ideal for treatment of gastrointestinal diseases associated with *H. pylori* infection, i.e. type B gastritis and duodenal ulcer.

Inhibition of Intestinal Motility and Secretion

Flavonoids were demonstrated to have effects on intestinal motility both *in vitro* and *in vivo.* For example, it was reported that quercetin and other flavonoids inhibited guinea-pig ileum induced contractions (75,76). Capasso et al. (22) screened 13 flavonoids (apigenin, catechin, crysin, flavone, hesperetin, kaempferol, morin, myricetin, naringenin, naringin, phloridzin, quercetin and taxifolin) on contractions in guinea-pig ileum induced by PGE_2, LTD_4, acetylcholine and $BaCl_2$. With respect to PGE_2 induced contractions, inhibition was seen with several flavonoids: apigenin (the most active),

quercetin, kaempferol and crysin. In the experiments using LTD_4-induced contractions, apigenin, quercetin and kaempferol were again active inhibitors, although to a lesser extent than in the PGE_2 system. These spasmolytic effects of flavonoids may have been due to a nonspecific action since they were also found to inhibit acetylcholine and $BaCl_2$ induced contractions. The authors proposed that calcium was involved since they found that pretreatment with a calcium channel blocker (verapamil) augmented the inhibitory actions of the flavonoids.

Flavonoids were shown to inhibit electrically-induced contractions (transmural electrical stimulations) and those that are induced by a variety of agonists, such as acetylcholine, 5-hydroxytriptamine, histamine and certain prostaglandins. Capasso et al. (23) examined the effects of flavone, apigenin, kaempferol, quercetin and crysin in this type of system and found that flavone was the most active in inhibiting the contractions. The inhibition was observed at low concentrations (10^{-5} M) whereas the other flavonoids were inhibitory only at higher concentrations ($3x10^{-5}$ to $1x\ 10^{-4}$ M). These authors concluded that the spasmolytic effects displayed by the flavonoids were not related to an inhibition of cyclooxygenase but rather to interference with calcium influx and/or calcium release from intracellular stores. Compounds that inhibit intestinal contractions *in vitro* may also inhibit intestinal motility *in vivo* (77). *In vivo* studies were performed on the effect of quercetin on gastrointestinal transit in mice using the charcoal method. Quercetin (25-50 mg/kg) significantly and dose-dependently inhibited small intestinal transit (SIT). Pretreatment of animals with verapamil potentiated the effects of quercetin and hence suggested a calcium-mediated mechanism, although the exact mechanism was difficult to clarify (78). Silymarin and silibinin (flavolignans from *Silybum marianum*) which have antihepatotoxic activity (see above), dose-dependently inhibited SIT at higer deses (100-200 mg/kg). They also increased the large intestinal transit (LIT) time, suggesting that they either stimulated nonperistaltic contractions or reduced peristaltic contractions (79).

More recently structure-activity relationship of the flavonoids on inhibition of intestinal motility have been performed. Intraperitoneal administration of some flavonoids (apigenin, flavone, kaempferol, morin, myricetin , naringin and rutin) significantly reduced both SIT and LIT as well as inhibiting intestinal fluid accumulation and castor oil-induced diarrhoea. Other flavonoids (naringenin, silibinin, silymarin and taxifolin), tested only for an effect on SIT, were found to be active at relatively high doses (100-200 mg/kg), whereas hesperitin, catechin and phloridzin were inactive even at doses higher than 200 mg/kg. The authors concluded that the potency of those active flavonoids was greately influenced by the structure of the molecules and that an α-2-adrenergic mechanism may be involved, since the effects of these flavonoids on gastrointestinal functions were antagonized by yohimbine and phentolamine (24).

Flavonoids also seem to inhibit diarrhoea that ensues from an increased intestinal motility and secretion. It has been reported that quercitrin (3-rhamnosyl quercetin), derived from *Euphorbia hirta,* inhibited diarrhoea induced by castor oil, arachidonic acid and prostaglandin E_2 in mice (80,81). Quercetin, previously shown to inhibit intestinal motility and secretion, has also been demonstrated to inhibit castor oil-induced diarrhoea (24).

Recently nitric oxide was proposed as a mediator of intestinal secretion associated with the administration of laxatives (82,83), and Di Carlo et al. (84) postulated that nitric oxide may be involved in the activity of quercetin on the intestine.

Flavonoids and Heart Disease

Current epidemiological evidence suggest that inadequate intake of certain nutrients predispose humans to chronic degenerative diseases (85). In particular it was demonstrated that intake of an adequate diet rich in vegetable and fruit reduces the likelihood of cardiovascular diseases, but the

exact mechanisms for this protective effect are inadequately understood. However, increased circulating antioxidants are believed to be important. This is supported by recent trials reporting that the intake of antioxidant flavonols predict a reduced rate of coronary-heart disease mortality in elderly male: in particular, those epidemiological studies show that dietary intake of flavonoids (quercetin, catechin and epicatechin, notably present in red wine but also in fruits and vegetables) is inversely associated with subsequent coronary heart disease (86).

This effect seems to be in part related to their antioxidant activity: in fact oxidized low density lipoproteins are atherogenic and are considered to be a crucial intermediate in the formation of atherosclerotic plaques and, recently, much attention has been paid to the antioxidant properties of flavonoids that seem to prevent the oxidation of low density lipoproteins (87). However, not all of the actions of flavonoids are thought to be due to their antioxidant activity. In particular their activity in reducing platelet aggregation or damage from ischemia and reperfusion should also be considered (88). In addition they also promote nitric oxide production by vascular endothelium, inhibit the synthesis of thromboxane in platelets and leukotriene in neutrophils, and modulate the production of lipoproteins (89).

Other Biological Effects

Flavonoids may prevent diabetic cataracts by inhibiting lens aldose reductase (90). Ong and Khoo (91) showed that myricetin possesses both hypoglycemic and hypotriglyceridemic effects in diabetic animals. The consumption of myricetin and other flavonoids might prevent atherosclerosis and thus reduce the risk of coronary heart disease (92). Several flavonoids exert anti-aggregatory effects through the inhibition of phosphodiesterase (25) as well as the inhibition of thromboxane B_2 formation (93). Antiviral and antimicrobial properties of flavonoids against different bacterial strains have also been demonstrated (94,95). The effect of flavonoids on the immune system is complex and still unclear. In high concentrations they inhibit lymphocyte functions ,but in lower concentrations they may act as immunostimulants in immunodeficient individuals (96). The immunomodulating activities of flavonoids depend in part on their capacity to inhibit the formation of both eicosanoids and histamine and because they are free radical scavengers.

Flavonoids and Nitric Oxide

The actions of some flavonoids may be correlated with their capacity to interact with nitric oxide (NO), which is a mediator of various biological systems (97). For example, flavonoids are well known scavengers of oxygen free radicals and recent studies suggest that they may also be very potent scavengers of nitric oxide free radical, raising the speculation that this ability plays a role in the putative therapeutic effects of flavonoids (98). Some flavonoids, as previously discussed, are inhibitors of lipooxygenase, and this may be the reason why quercetin blocked the vasodilatation due to endothelium derived relaxing factor (NO). However there may be another mechanism of action for the inhibition of NO production independent of the activity on lipoxygenase (99).

Nitric oxide is formed by an enzyme called nitric oxide synthase (NOS), which is a type of diaphorase enzyme. Recent data suggest that flavonoids could inhibit *in vitro* brain NADPH diaphorase activity. These experiments showed that quercetin and apigenin markedly inhibited this enzyme's activity in a concentration-dependent fashion, suggesting that these flavonoids may also be able to inhibit the production of nitric oxide in the brain (100). Chiesi and Shwaller (101) studied the action of tannins and the flavonoid compound quercetin on constitutive endothelial NOS activity and found that both tannins and quercetin inhibited NOS activity, although the tannins were the more potent inhibitors.The flavonoids flavone, 3'-amino-4'-hydroxyflavone and genistein were investigated for their inhibitory

activity of nitric oxide production in murine macrophages:3'-amino-4'-hydroxyflavone showed the greatest potency. These data supported the suggestion that flavones could modulate the immune response and inflammatory reactions by controlling the production of nitric oxide (27). Di Carlo et al. (84) examined the gastrointestinal activities of quercetin in animals pretreated with two inhibitors of NOS, N^G-nitro-L-arginine methyl ester and N^G-monomethyl-L-arginine (L-NAME and L-NMMA). This pretreatment potentiated the delay in transit, intestinal secretion and diarrhoea caused by quercetin, and it was thus suggested that nitric oxide was involved in the gastrointestinal activities of quercetin.

Furthermore, the reported antitumor properties of flavonoids were in part related to their activity against nitric oxide, which was reported to be carcinogenic. In particular it was shown that epigallocatechin gallate is able to inhibit the inducible nitric oxide synthase gene expression and enzyme activity (102).

Conclusions

There is much evidence that flavonoids have important effects on various biological systems. These effects may have therapeutic uses, but this potential of the flavonoids has not yet been realized even though a large body of research supports the possible utilization of these compounds in medicine. The reasons could be: (i). flavonoids have not shown such a prominent and significant biological activity; (ii) flavonoids (catechin in particular) have shown severe side effects such as acute intravascular hemolysis, acute renal failure, trombocytopenia; (iii) flavonoids (quercetin, kaempferol, galangin) have produced genotoxic effects: (iv) the marketing regulations are very severe. In spite of this the derivatives of rutin are used to increase capillary resistance, these compounds are also recommended for the treatment of circulatory disorders and inflammations. Flavonolignan complex is used to protect liver, and ipriflavone, a synthetic isoflavone, is useful in osteoporosis.

On the other hand the use of plants containing flavonoids, either alone or in combination, has increased due to both the increasing demand by the consumer for compounds of natural origin and by the attention given to dietary plants containing this class of molecule as natural cancer chemopreventive compounds.

It is important that research on the different activities of flavonoid compounds be continued, especially since more recent studies described herein point to a growing number of new areas for the therapeutic uses of flavonoids, considered by Middleton "as natural biologic response modifiers".

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