The Role of Quercetin, Flavonols and Flavones in Modulating Inflammatory Cell Function

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Abstract: Flavonoids are polyphenolic substances derived from plants that play several pharmacological activities. They possess anti-viral, anti-microbial, anti-inflammatory and anti-allergic potential that can be expressed on different cell types, both in animal and human models. Many of these properties prove inhibitory to a huge panoply of molecular targets in the micromolar concentration range, either by down-regulating or suppressing many inflammatory pathways and functions. Flavonoids exert their properties both as purified aglycone molecules and as plant extracts. Depending on little changes in the flavone-backbone and on subtle mechanisms of cell behavior and responsiveness, flavonoids can play a modulating, biphasic and regulatory action on immunity and inflammation; in this context only few flavones and flavonols have been assayed, mainly because of their chemical similarity with quercetin, so evidence reported in the literature about the action of flavonoids is limited to a restricted group of molecules. Many of the effects reported about flavonoids regard quercetin, as probably the most diffused and known nature-derived flavonol. Quercetin has shown a biphasic behavior in basophils at nanomolar doses and hence its action on cells involved in allergic inflammation is here described. Like many other molecules sharing a flavone ring, quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins often crucial for a cellular specific function. This overview collects and discusses the role of flavonoids as anti-infectious and anti-inflammatory compounds, trying to focus on the complex and modulating interaction of these polyphenolic substances with cell function. However, the wide group of intracellular targets and the elevated number of natural compounds potentially effective as anti-inflammatory therapeutical agents, asks for further insights and evidence to comprehend the role of these substances in animal cell biology.

Keywords: Aryl hydrocarbon receptors, basophils, biphasic modulation, flavonoids, inflammation, quercetin.

INTRODUCTION

Dietary flavonoids represent the principal anti-microbial and anti-inflammatory component provided by edible plants that have an impact on human health [1-4]. Quercetin ($C_{15}H_{10}O_7$, 3,3',4',5,7-penthahydroxy-2-phenyl-chromen-4-one) is a member of these naturally occurring polyphenolic compounds, which share a common flavone nucleus made up of two benzene rings linked through a heterocyclic pyrone one. Quercetin is synthesized in plants starting from the aminoacid phenylalanine. Phenylalanine (1) is converted to 4-coumaroyl-CoA (2) in a series of steps known as the general phenylpropanoid pathway using phenyl ammonia-lyase (EC 4.3.1.5), cinnamate-4-hydroxylase (EC 1.14.13. 11), and 4-coumaroyl-CoA ligase (EC 6.2.1.12) [5]. The metabolite 4-coumaroyl-CoA (2) is added to three molecules of malonyl-CoA (3) to form tetrahydroxychalcone (4) using 7,2'-dihydroxy, 4'-methoxyisoflavanol synthase. Tetrahydroxychalcone is then converted into naringenin (5) using chalcone isomerase. Naringenin is then converted into eriodictyol (6) using flavanoid 3' hydroxylase and eriodictyol is then converted into dihydroquercetin (7) with flavanone 3-hydroxylase which is then converted into quercetin using flavanol synthase [6] (Fig. **1**). The amount, classification and ratio between certain species of polyphenolic compounds in plants depend on several environmental factors [7]; for example in *Arnica montana* L. cv. ARBO, a pronounced increase in the ratio of B-ring ortho-diphenolic (quercetin) compared to B-ring monophenolic (kaempherol) flavonols resulted from a decrease in temperature by 5 degrees °C in the applied climate regime [8]. Generally, the role of flavonoids as the main chemical actors in red, yellow, purple and blue pigments in plants and as potential preventive and therapeutical substances in human health, has gained these compounds a great deal of attention over the past years. A huge wealth of information has been collected on the structures, chemical properties, biological activities, biosynthesis and metabolism of these molecules; however, the wide spectrum of molecular targets and the bimodal behavior taken on by these substances depending on cell type and on functional condition, have made it very difficult to unravel the skein of their biological effect. Flavonoids constitute a relatively heterogeneous family of aromatic molecules including six major groups that can be found in most higher plants: the chalcones, flavones, flavonols, flavandiols, anthocyanins and condensed tannins or proto-anthocyanidins, collecting more than 4,000 different chemical molecules; a seventh group, the aurones, is widespread but not ubiquitous [6,9]. Quercetin is the most represented polyphenolic derivative of flavonols. Plants rich in quercetin are onions, apples, broccoli, berries, capers, where quercetin is usually present as a glucoside-conjugate or as a methyl-glucoside-conjugate [10,11]. Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone (IUPAC name : 3-hydroxy-2-phenyl-1-benzopyran-4-one). Their diversity stems from the different positions of the

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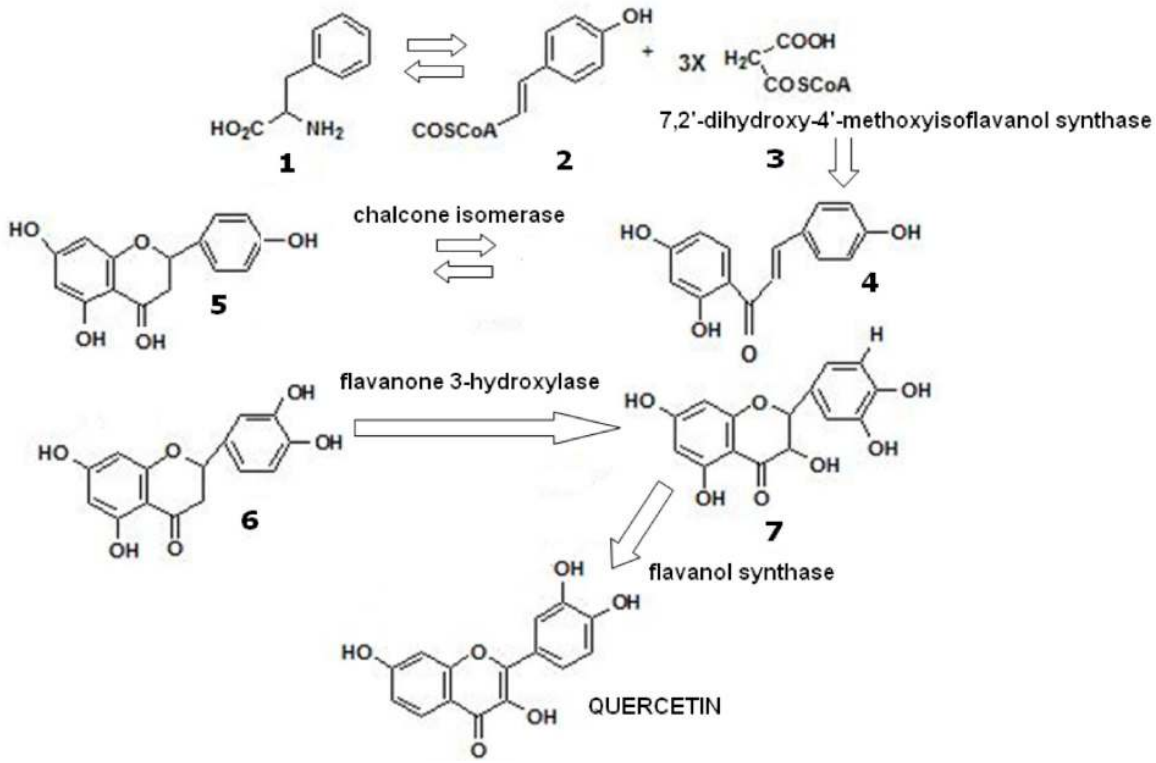

Fig. (1). A summary of main pathways of quercetin biosynthesis in plants.

phenolic -OH groups and/or from the presence of methoxyl radicals; Fig. (**2**) lists the most important and diffused flavonoids in their aglycone form. In plants flavonoids exist almost entirely as glyco-conjugated compounds. As far as quercetin is concerned, its glycosilated forms are represented by the aglycone form conjugated with different monosaccharides, such as hyperoside (+ galactose), isoquercitin (+ glucose), quercitrin (+ rhamnose), rutin (+ rutinose), all linked as 3-O-glycosides while spiraeoside is a quercetin-4-O-glucose and troxerutin is a rutin having three hydroethyl groups [12,13]. However, many other methyl-glycosides and methyl-rhamnosides of quercetin have been discovered. In humans, when digested conjugated quercetin undergoes radical transformation inside the gut due to intestinal microflora, biochemical pathways such as methylation and hydroxylation lead to the formation of other flavonoids: for example, 3'-methylation of quercetin leads to isorhamnetin and 5'-hydroxylation of quercetin to myricetin [14]. The first step of metabolism is characterized by hydrolysis of the sugar link, which is common to most polyphenolics. In humans this may occur in the gut by the action of lactase (a glycoside hydrolase) along the brush border membrane of enterocytes lining the villi of the small intestine, or after the transport into the enterocyte by a cytosolic *β*-glucosidase. Phenolic glycosides that reach the colon will be hydrolysed by the gut microflora, but may also undergo further metabolism and degradation. Once absorbed, dietary phenolics can be methylated (mainly at position 3,3',4',7) by methyl-transferases of tissues to a varying degree, and will be conjugated to sulphate and/or glucuronic acid prior to excretion. These phenolic metabolites will be the flavonols responsible for many biological activity within the body together with other well known flavones-derived compounds and should be the subject of any further investigation [15-17].

CHEMICAL KINSHIP OF QUERCETIN WITH OTHER FLAVONOIDS AS ANTI-INFLAMMATORY AGENTS

Several reports have described a role for flavonoids as agents able to affect immunity [18] and inflammation [19,20], both *in vitro* [18,21-28], and *in vivo* or in animal models [29-37], even as natural inhibitors of HIV proteases [38] and against antibiotic-resistant bacteria [39]; the popular proverb "an apple a day keeps the doctor away" seems to have met its scientific evidence concerning inflammation [40]. However, to check possible links between biological effects of quercetin on immunity and inflammation and flavonol chemical likeness with other polyphenols [24], biochemical research should identify which chemical groups might be strategic for the anti-inflammatory properties of different flavonols [41], as the anti-oxidant and anti-inflammatory behavior of flavonoids seem to be structure-related [42], as well as their ability to interact with cell membrane and to enter the cytoplasm [43]. Table **1** summarizes the main properties of flavones and flavonols in immunity and inflammation. Cheong and colleagues have studied the structure-activity relationship of flavonoids as anti-allergic compounds in rat basophilic leukaemia cell line RBL-2H3: among 22 flavone-derived compounds tested, luteolin, apigenin, fisetin, and quercetin were found to be most active with IC_{50} values less than 10 μM [44].

This reasoning limits greatly the number of flavonoids related to quercetin that might be evaluated as potential anti-inflammatory compounds. Among most studied flavone-derived compounds in immunology, quercetin differs from

other cognate molecules for the position of –OH group in A and B rings (quercetin: –OH 3,4,5,7). Within the same family of flavonoids, closest kindred could be substances that share the same polyphenolic (flavonic) backbone but which possess different amount and displacement of hydroxyl groups. Main flavone (2-phenyl-1-benzopyran-4-one) derivatives having 3-OH and different number of hydroxyl radicals in different position, are 3-hydroxyflavone, 6-hydroxyflavone, fisetin (3,3',4',7-tetrahydroxy-flavone), galangin (3,5,7-trihydroxy-flavone), kaempherol (3,4',5,7-tetrahydroxy-flavone), quercetin (3,3',4',5,7-penthahydroxy-flavone), myricetin (3,3',4',5',5,7-hexahydroxy-flavone), morin (2-(2,4-dihydroxyphenyl)3,5,7-trihydroxychromen-4-one) and gossypetin (2-(3,4-dihydroxyphenyl)-3,5,7,8-tetrahydroxychromen-4-one), to which are commonly associated other flavones such as luteolin (3',4',5,7-tetrahydroxyflavone), apigenin (4',5,7-trihydroxyflavone), chrysin (5,7-dihydroxyflavone) baicalein (5,6,7-trihydroxyflavone) and scutellarein (5,6,7,4'-tetrahydroxyflavone) (Fig. **2**). Most of the evidence reporting an effect of flavonoids towards cells involved in immunity and inflammatory response concerns the above listed compounds, while many O-methylated flavones, except for wogonin, are less considered (Fig. **2**).

FLAVONES AND FLAVONOLS AND THEIR ANTI-INFLAMMATORY ACTION

Some of these flavone-related substances, for example 3-hydroxyflavone, are not commonly found in natural sources; 3-hydroxyflavone is the chemical product of the Algar-Flynn-Oyamada reaction [45], whereby a chalcone undergoes an oxidative cyclization in the presence of hydrogen peroxide to form the flavonol. The flavonoid 6-hydroxyflavone was found in the porcupine flower, an indian plant of Acantaceae family (*Barleria prionitis*, L.) [46] and it was reported as a noncompetitive inhibitor of cytochrome P450 2C9 [47]. Many of the anti-inflammatory effects reported *in vitro* concern the inhibitory activity of those flavonoids on cytokine production or on other inflammatory mediators release.

Like many other flavonoids, fisetin can be found in fruits, such as berries but it is commonly represented in plants belonging to the genus *Acacia* (*A. berlandieri*, *A. greggi*) or *Rhus* (*R. cotinus*). Fisetin was reported to have an antiviral effect [48]; more recently, this flavonol has been found to be neuroprotective and to inhibit the aggregation of the amyloid beta protein that may cause the progressive neuronal loss in Alzheimer's disease, an evidence that may lead to recognize this compound as a new type of therapeutic drug for this neurological pathology [49]. Fisetin can be easily methylated by microorganisms [50] and even by human liver [51].

Quercetin has also an immunosuppressive effect on dendritic cells function [52]. Baicalein, isolated from the roots of *Scutellaria baicalensis* Georgi, has an anti-inflammatory activity [53] and moreover it inhibits plasminogen activator inhibitor 1 (PAI-1), the principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), both activators of plasminogen and hence fibrinolysis (the physiological breakdown of blood clots), induced in cultured human umbilical vein endothelial cells (HUVEC) by IL-1 and TNF-α, probably through an action on protein kinase C (PKC) [54]. On the other hand, some flavonoids such as quercetin, kaempherol and apigenin are able to inhibit matrix metalloproteinases [55], which are normally

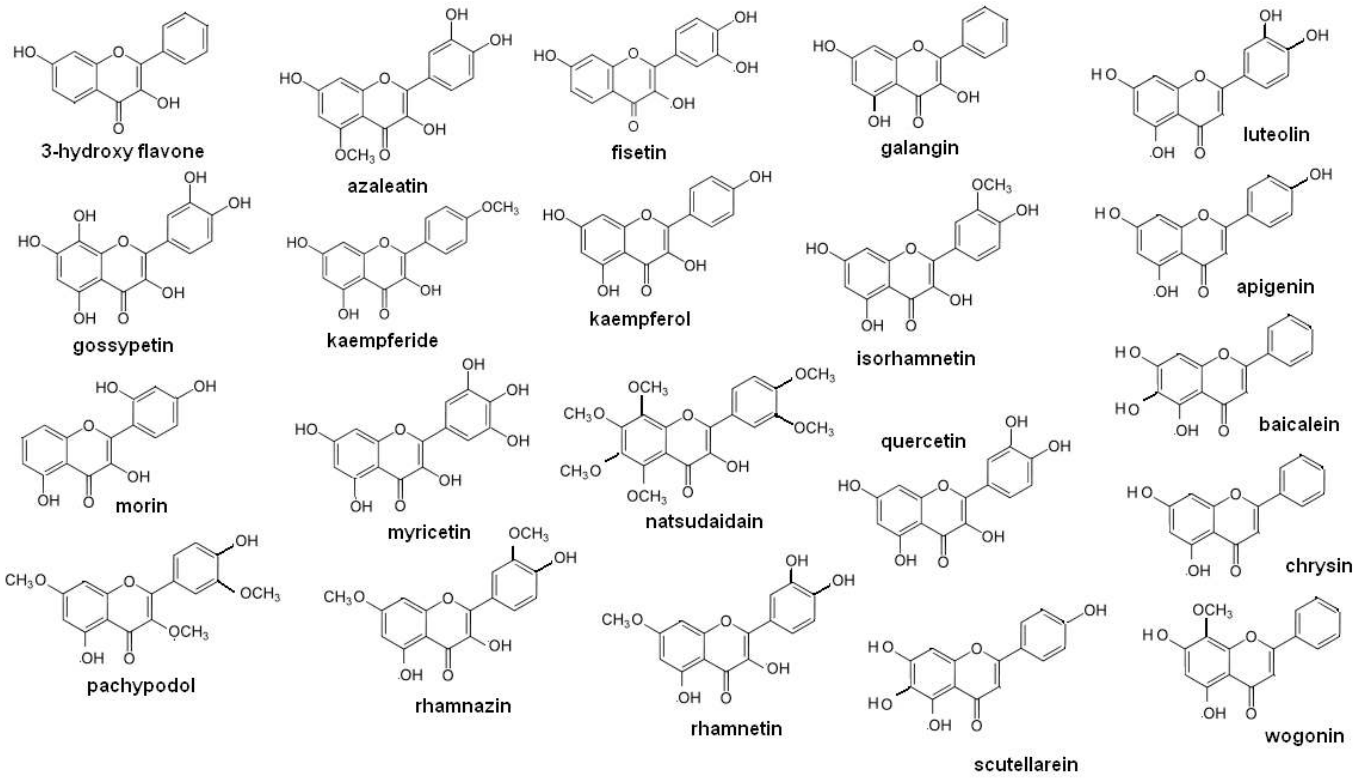

Fig. (2). Principal flavones and flavonoids studied as anti-inflammatory agents. Main O-methyl-derived flavonoids are also reported.

Table 1. Summary of the Main Targets and Effects of Flavones and Flavonols on Inflammation

IUPAC Name	Common Name	Action		Reference
4',5,7-trihydroxyflavone	Apigenin	Anti-allergic		[218]
		↓ ↓ ↓	RBL-2H3 degranulation, human mast cells ICAM-1 E-selectins in HUVECs	[44,59,146,148].
		↓ ↓ ↓ ↓	TNF-α expression, IL-4, IL-13 basophils RBL-2H3: TNF-α, IL-4 IL-2 macrophages Pro-inflammatory cytokines in PBMC	[136,108,141,143, 304]
		↓ ↓	TNF-α expression, matrix metalloproteinases NFκB	[55,155, 304]
5,6,7-trihydroxyflavone	Baicalein	Anti-inflammatory, anti-asthmatic		[53, 56]
		↓ ↓	Basophil histamine release, RBL-2H3 degranulation Histamine, leukotrienes, PGD2, GM-CSF in HCMC	[57,58,117]
		↓ ↓	plasminogen activator inhibitor 1 NFκB	[54,155]
5,7-dihydroxyflavone	Chrysin	Anti-viral: ↓ herpes virus replication, ↓ human neutrophil		[66, 118]
		↓	ICAM-1	[146]
		↑ ↓ ↓	IL-2 macrophages Pro-inflammatory cytokines in PBMC NFκB	[141,143,155]
3,3',4',7-tetrahydroxy-flavone	Fisetin	Antiviral, ↓ aggregation of beta-amyloid proteins		[48, 49]
		↓ ↓ ↓	RBL-2H3 degranulation CD40, ICAM-1 in HMC-1 E-selectins in HUVECs	[44,145,148].
		↓ ↓ ↓ ↓ ↓ ↓ ↓	IL-4, IL-13 basophils RBL-2H3: TNF-α, IL-4 IL-8 gene expression HEK-293 Gene expression pro-inflammatory cytokines TNF-α, MCP-1, IL-6, IL-8, VEGF in fibroblasts IL-4, IL-13, IL-5 in KU812 IL-2 macrophages	[108, 136-141]
		↓ ↓	PARP NFκB	[135,155]
3,5,7-trihydroxy-flavone	Galangin	↓ human neutrophil. Adenosine receptor antagonist, anti-bacterial activity, anti-viral activity		[118, 126, 131, 132]
		↑	IL-2 macrophages	[141]
2-(3,4-dihydroxyphenyl)-3,5,7,8-tetrahydroxychromen-4-one	Gossypetin	↓	PARP	[135]
3,4',5,7-tetrahydroxy-flavone	Kaempherol	Anti-inflammatory, anti-allergic, ↓ herpes virus replication, ↓ cytomegalovirus, ↓ H. pylori, anti-bacterial, anti-parasites,		[64, 66, 71, 73, 76, 77]
		↓↑ ↓ ↑ ↓ ↓ ↓ ↓ ↓	Human mast cells: biphasic action on histamine release Neutrophil function Tryptase and histamine content in mast cells and in hCBMCs FcεRI expression and histamine release in KU812F CD23 mRNA and p38MAPK in Caco-2 Chemokine release in RBL-2H3 ICAM-1 E-selectins in HUVECs	[59,62, 74,82,84, 144, 146, 148, 155].

(Table 1) contd…..

IUPAC Name	Common Name	Action		Reference
		↓ ↓ ↓	Beta-glucuronidase, lysozime in rat neutrophil IL-8 gene expression HEK-293 TNF-α, IL-6, IL-8 in hCBMCs	[72, 137, 144].
		↓ ↓ ↓	matrix metalloproteinases Nitric oxide synthase, cyclooxygenase NFκB	[55, 85, 155]
3',4',5,7-tetrahydroxy-flavone	Luteolin	Anti-allergic, Anti-ashtmatic, Anti-viral against SARS, anti-Leishmania, neuro-protective, anti-sclerosis, protective in liver fibrosis. ↓ Complement activity		[68, 78, 89, 90-93, 100-102]
		↓ ↓ ↓ ↑ ↓	RBL-2H3 degranulation, histamine release in human mast cells, neutrophil function CD40 expression, AP1 activation in basophil ICAM-1 IL-10 E-selectins in HUVECs	[44, 59, 62, 97, 98, 146-148].
		↓ ↓ ↑ ↓ ↓	IL-4, IL-13 basophils RBL-2H3: TNF-α, IL-4 IL-2 macrophages Pro-inflammatory cytokines in PBMC NFκB	[108, 136,141,143,155]
2-(2,4-dihydroxyphenyl)3,5,7-trihydroxychromen-4-one	Morin	↓ ↓	Neutrophil function Tryptase and histamine in hCBMCs	[62, 144]
		↓	TNF-α, IL-6, IL-8 in hCBMCs	[144]
3,3',4',5',5,7-hexahydroxy-flavone	Myricetin	Analgesic activity, anti-inflammatory, anti-allergic		[112, 113, 115]
		↓ ↑ ↓	Mast cell histamine release Protection against DNA damage in human lymphocytes Histamine and tryptase in hCBMCs	[114] [116] [144]
		↓	TNF-α, IL-6, IL-8 in hCBMCs	[144]
3,3',4',5,7-penthahydroxy-flavone	Quercetin	Antiviral, immunosuppressive in dendritic cells, anti-allergic, ↓ circulating HMGB-1		[48, 67, 52, 65, 151]
		↓ ↑ ↓ ↓ ↓ ↑ ↑ ↓ ↓↑	RBL-2H3 degranulation, neutophil function Tryptase and histamine content in mast cells FcεRI expression and histamine release in KU812F CD23 mRNA and p38MAPK in Caco-2 Chemokine release in RBL-2H3 Protection against DNA damage in human lymphocytes IL-10 E-selectins in HUVECs Biphasic effects on basophil	[44, 62, 63, 74, 82, 84, 116, 147, 148, 261]
		↓ ↓ ↑ ↓	RBL-2H3: TNF-α, IL-4 IL-8 gene expression HEK-293 IL-2 macrophages TNF-α, IL-6, IL-8 in hCBMCs	[108, 137, 141, 144]
		↓ ↓ ↓	matrix metalloproteinases PARP NFκB	[55,135,155]
5,6,7,4'-tetrahydroxyflavone	Scutellarein	↓	RBL-2H3 degranulation	[58]

inhibited by PAI-1. The anti-allergic substance baicalein was used in the treatment of bronchial asthma, and in other cellular systems [56], as a natural inhibitor of basophil histamine release [57]. In rat basophilic leukemia cell lines (RBL-2H3) baicalein and scutellarein showed an inhibitory activity on cell degranulatory response but baicalein showed also a relevant cytotoxicity compared to the other flavonoid [58].

Biphasic and modulatory effects were observed with these flavonoids in inflammation. Apigenin and cromoglycate inhibited the secretion elicited by compound 48/80 in human mast cells but did not modify the calcium ionophore A23187-induced secretion [59]. The effect of kaempherol on the histamine release induced by compound 48/80 was biphasic. Low doses (1.0-10 μM) of the compound potentiated secretion whereas higher doses inhibited histamine secretion [59]. Some of the tested drugs revealed a higher potency when compared to quercetin, while luteolin exhibited the highest inhibitory effects of mast cell histamine secretion [59]. Wogonin inhibits inducible PGE_2 in macrophages [60] and inhibits their lipopolysaccharide-induced nitric oxide production [61]. Flavonoids impairment on cell function was observed also on neutrophils [62], to which many flavonoids exert their anti-inflammatory action [63]. The effect of kaempherol, quercetin, morin and fisetin on neutrophils showed a complex response pattern, as each compound differed and cell depolarization was enhanced by some and inhibited by others, while hydrogen peroxidase generation was inhibited by each, supporting some previous findings indicating that membrane potential depolarization and respiratory burst are dissociable events. None of the flavonoids affected the resting potential, while all perturbed the stimulus-coupled response, the direction and extent of the perturbation depending upon the stimulus and the function assessed [62]. Hence, in inflammatory models, the effects of flavonoids appear complex and suggest several sites of action depending upon the flavonoid subcellular distribution and pathway of stimulation. Kaempherol and quercetin were reported as long lasting anti-inflammatory substances [64], besides being well known anti-allergic compounds [65]. As antivirals chrysine and kaempherol in propolis cause a concentration-dependent reduction of intracellular replication of herpes-virus strains when monolayers are infected and subsequently cultured in a drug-containing medium but are unable to reduce viral infectivity [66]. Even anti-viral effects attributed to quercetin have been reported in literature [67]. Luteolin in Chinese multiherb remedy against SARS possesses anti-complement properties [68]. Kaempherol glycosides were usually tested as anti-viral substances [69,70]. Seven flavonoid glycosides of the kaempherol series were evaluated for their ability to inhibit cytomegalovirus and flavonoids bearing an acyl group were found to be the most active molecules [71]: this strengthens the role that a single substituent has on the specific property of a flavonoid when the latter is compared to its chemically closest phenolic compound.

As an anti-inflammatory substance kaempherol is able to affect both leukocyte function and microorganisms. In rat neutrophils kaempherol from *Rhamnus* species is able to inhibit β-glucuronidase and lysozime release following stimulation with formylated peptides/cytochalasin B [72]. Oral administration of kaempherol significantly decreased the numbers of *Helicobacter pylori* in gerbils' stomachs, as well as *in vitro* inhibiting colonies in a dose-dependent manner [73]. In mast cells kaempherol at 100 μM inhibited cell proliferation by over 80% on either day 3, 4, or 5 of culture. Other flavonoids showed a typical pattern of action. Quercetin showed this level of inhibition only on day 5, myricetin inhibited by 50% at days 3-5, whereas inhibition by morin was less than 20%. All flavonoids (except for morin) at 100 μM increased histamine and tryptase but not β-hexosaminidase content; equally, at days 3 and 4 of culture quercetin also increased the development of secretory granules [74]. So, different actions have been reported by flavonoids in mast cells. Mast cells participate in allergies, and also in immunity and inflammation by secreting proinflammatory cytokines. A recent study performed with 40 different flavonoids in rat basophilic leukaemia cell line (RBL-2H3) reported that flavonoid aglycones showed a stronger activity for histamine release-inhibition and cytotoxicity than glycosides, and both activities were almost in parallel; baicalein behave as a good inhibitory agent but having a potent cytotoxicity, whereas micromolar doses of scutellarein inhibited significantly histamine release from mast cells without affecting their viability [58]. Extracts obtained from the leaves and branches of various *Cistus* species have been used worldwide as folk remedy for the treatment of various inflammatory ailments including rheumatism and renal inflammations. 3-O-methylquercetin, 3,7-O-dimethylquercetin and 3,7-O-dimethylkaempherol were isolated as the main active ingredients from the ethanol extract of laurel leaved cistus *Cistus laurifolius* L. (Cistaceae). In *in vivo* animal models these flavonoids were shown to possess a strong anti-nociceptive and anti-inflammatory activities, without inducing any apparent acute toxicity as well as gastric damage [75]. Kaempherol in crazyweed *Oxytropis falcata* Bunge, a wild growing Leguminosae plant, exhibits a strong anti-bacterial activity [76] but the same flavonol showed also anti-parasites activity [77], as well as luteolin and other flavones [78-81]. Extracts rich in flavonoids (quercetin, kaempherol) from hearthleaf or lizard tail *Houttuynia cordata* Thumb (Saururaceae) suppress FcεRI expression and the IgE binding activity in human basophilic cell line KU812F: reverse transcription-polymerase chain reaction analysis showed that levels of the mRNAs for FcεRI-α and γ-chains, were decreased by the treatment of *H. cordata* extract [82]. Addition of *H. cordata* extract to culture medium was also observed to result in a reduction in the release of histamine from the cells [82]. Kaempherol was the main component able to induce this suppression activity, as reported also in KU812F with the purified flavonol from lotus *Nelumbo nucifera* stamens [83]. Kaempherol and quercetin inhibited the secretion of allergic mediators in RBL-2H3 cells and suppressed the CD23 mRNA expression and p38MAPK activation in IL-4 stimulated Caco-2 cells; the flavonols also suppressed IgE-OVA induced extra signal-regulated protein kinase (ERK) activation and chemokine release [84]. Kaempherol has been reported to inhibit nitric oxide synthase and cyclooxygenase enzymes in animal models, so in turn contributing to its anti-inflammatory activity [85].

Luteolin is a flavone with a well-known anti-inflammatory [86-88], anti-allergic and neuroprotective

activity [89,90], tested successfully in multiple sclerosis models [91,92] and recently showing even a protective role in carbon-tetrachloride induced liver fibrosis [93]. It is the main component in lemongrass (*Cymbopogon citratus*) having an anti-inflammatory activity [94]. Its role as anti-allergic substance depends on several factors [95,96]. In basophils luteolin inhibits CD40 expression and AP-1 activation [97,98]. Luteolin is able to inhibit niacin-induced flush in rats by acting on PGD_2 and 5-HT plasma levels [99] and exerts an anti-asthmatic action [100,101]. Luteolin from *Olea europea* L. leaves, together with apigenin, has shown to exert anti-complementary activity [102]; together with quercetin and apigenin it plays a protective role towards pancreatic beta-cells injured by cytokines during inflammation [103]. The same flavone from *Begonia malabarica* L. or from Argentin tagetes (Asteraceae) showed anti-microbic properties [104,105]. The structure of luteolin, 3',4',5,7-tetrahydroxyflavone, seems to be most suitable for the oral anti-inflammatory activity and that existence or disappearance of a hydroxyl group may cause a loss of efficiency [106]. Stem bark extracts from *Cassia siamea* Lam., a widespread medicinal plant traditionally used in sub-Saharian Africa, contain kaempherol, luteolin and apigenin and have anti-inflammatory activity [107]. In mast cells, luteolin (IC_{50} = 3.0 μM), diosmetin (2.1 μM), and fisetin (3.0 μM) have potent inhibitory activity on β-exosaminidase release assay, an evidence that has suggested the authors the following structural requirements of flavonoids: (a) the 2-3 double bond of flavones and flavonols is essential for the activity; (b) the 3- or 7-glycoside moiety reduce the activity; (c) as the hydroxyl groups at the 3', 4', 5, 6, and 7-positions increased in number, the inhibitory activities become stronger; (d) the flavonols with a pyrogallol type moiety (the 3',4',5'-trihydroxyl groups) at the B ring exhibited less activity than those with a phenol type moiety (the 4'-hydroxyl group) or catechol type moiety (the 3',4'-dihydroxyl groups) at the B ring; (e) the activities of flavones were stronger than those of flavonols; and (7) methylation of flavonols at the 3-position reduced the activity, although several flavones and flavonols with the 4'- and/or 7-methoxyl groups did not obey rules (c), (d), and (e); in this context flavonoids such as apigenin, luteolin, diosmetin, fisetin, and quercetin, inhibit the antigen-IgE-mediated TNF-α and IL-4 production from RBL-2H3 cells, both of which participate in the late phase of type I allergic reactions [108]. Anti-inflammatory property of luteolin was assayed also *in vivo*: topical application of nanoparticular solubilisate of *Reseda luteola* extract, highly rich in luteolin, reduced inflammation following skin irradiation with ultraviolet B light, resulting as effective as hydrocortisone [109]. Serotonin and quercetin target the same region of the active site of the 5-hydroxytriptamine type 2 receptor with a similar binding energy but quercetin having a much bigger inhibition constant, so suggesting that quercetin may act as a natural inhibitor of the receptor blocking the acute inflammation generated by serotonin [110]. An interesting bimodal and synergistic mechanism has been reported recently concerning interaction between flavonoids and pro-vitamins in the inflammatory response [111]. Myricetin from *Myrica rubra* has an analgesic activity [112] and it is a potent anti-inflammatory agent [113]; pure myricetin, as well as other cognate flavones, is able to inhibit mast cell histamine release [114]. In ovoalbumin (OVA)-sensitized mice myricetin inhibited pulmonary cell migration and IgE and IgG_1-OVA specific production, so ameliorating the allergic reaction [115]. Myricetin and quercetin are able to protect peroxide-induced DNA damage in human lymphocytes at higher doses than 10 μM, while silymarin, a mixture of flavonolignans extracted from blessed milk thistle (*Sylibum marianum*), are not [116]. Baicalein, a flavone sharing its structure with apigenin, luteolin and chrysin, inhibits histamine, leukotrienes, prostaglandin D_2 and granulocyte-macrophage colony stimulating factor (GM-CSF) release in human cultured mast cells (HCMC) activated with anti-IgE [117]. Several flavonols having a structure very close to quercetin such as galangin, chrysin or scutellarein have poorly studied regarding their possible action as anti-inflammatory polyphenolic substances, if we except the evidence reported by Limasset and colleagues on human neutrophils, in which chrysin and galangin inhibited the release of reactive oxygen species in cells stimulated by bacterial formylated peptides, phorbol esters or opsonized zymosan [118]. Chrysin and galangin are common flavonoid of *Apis mellifera* beeswax [119], propolis and honey [120-122] and propolis suppresses leukotrienes and prostaglandins production in macrophages [123] or induces production of tumor growth factor-beta (TGF-β) [124]; galangin is a chemopreventive, anti-genotoxic natural compound [125] and an adenosine-receptor antagonist [126] but although a close relationship between adenosine and inflammation has been traced [127], no work has yet been reported on the effect of galangin on basophils or mast cells, even if galangin has shown anti-bacterial [128-131] and anti-viral properties [132]. Gossypetin was discovered as an anti-inflammatory flavonol in rat carrageenin induced-paw oedema model [133,134] but it is also, together with fisetin and quercetin, an inhibitor of poly (ADP-ribose) polymerase 1 (PARP-1) [135]. This wide panoply of actions on inflammation and immunity by flavonoids is really amazing but their mechanism of action is yet rather puzzling.

FLAVONES AND FLAVONOLS: THEIR ACTION ON CELL MEMBRANE MARKERS AND CYTOKINES

Together with luteolin and apigenin, fisetin was reported to be a strong inhibitor of IL-4 and IL-13 but not of LTC_4 release in purified human peripheral blood basophils stimulated with anti-IgE or with IL-3+anti-IgE for 2 days [136]. The same flavonol is able, together with kaempherol and quercetin, at concentration of over 20 μM, to block IL-8 promoter activation and gene expression induced by tumor necrosis factor-alpha (TNF-α) in human embryonic kidney cell culture (HEK293) but affected cell viability at doses higher than 40 μM, while kaempherol did not [137]. The ability of fisetin to reduce gene-expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, MIP-1α, IL-6 and MIP-2, was confirmed in mouse models [138]. In a rheumatoid arthritis (RA) model, hexane-extracts of lacquer tree highly rich in fisetin *Rhus verniciflua* Stokes (RVHxR), whose leaves are used in Chinese medicine as anti-helminth therapy, significantly inhibited IL-1β-induced fibroblast-like synovial cells (FLS) proliferation in a dose-dependent manner. Flavonol-rich RVHxR and fisetin significantly decreased IL-1β-induced inflammatory cytokines (TNF-α,

IL-6/chemokines (IL-8, monocyte chemoattractant protein MCP-1), and vascular endothelial growth factor (VEGF) of RA FLS. At a molecular level, flavonol-rich RVHxR dose dependently diminished the phophorylation of extracellular signal regulated kinase (ERK) and phospho-Jun NH(2)-terminal kinase (JNK), and also its down regulation induced by RVHxR at nontoxic concentrations, while it activated the phosphorylation of p38MAPK in IL-1β-stimulated RA FLS; pure fisetin inhibited in a dose-response manner VEGF production (at doses higher than 1.0 μg/ml) and revealed a stronger ability in inhibiting cell proliferation without IL1-β induction than RVHxR [139]. This different behavior between toxic and non-toxic doses as well as between pure flavonoids and plant extracts, represents one of the most critical points of the investigation about the biological action of flavonoids in inflammation. As a matter of fact, most of the works carried out *in vitro* used purified aglycone flavonoids at micromolar doses, as plant extracts may have a complex and synergistic behavior when evaluated on cell function, due to the presence of different polyphenolic compounds, most of them in conjugated form and because a biochemical insight on the role of flavonoids in medicine needs to evaluate their action as purified substances. However, research on plant extracts is absolutely useful to focus onto the role of nature derived phytochemical complexes used in therapy and of nutraceuticals, as well as of human daily diet. Purified fisetin suppresses the induction of IL-4, IL-13, and IL-5 mRNA expression following stimulation with A23187 in erythroid/basophilic cell lines KU812 and in basophils following cross-linkage of the IgE receptor. Fisetin reduced IL-4, IL-13, and IL-5 synthesis but not IL-6 and IL-8 production by KU812 cells. In addition, fisetin inhibited IL-4 and IL-13 synthesis by anti-IgE antibody-stimulated human basophils and IL-4 synthesis by allergen-stimulated basophils from allergic patients [140]. Among the flavonoids examined, kaempherol and quercetin showed substantial inhibitory activities in cytokine expression but to a lesser extent than fisetin, which suppresses the expression of Th2-type cytokines (IL-4, IL-13, and IL-5) by basophils [140].

Apigenin and fisetin inhibit IL-2 secretion in macrophages while luteolin, galangin, quercetin and chrysin increased the same [141]. Pretreatment of macrophages with luteolin, luteolin-7-glucoside, quercetin, and the isoflavonoid genistein inhibited both the LPS-stimulated TNF-α and interleukin-6 release, whereas eriodictyol and hesperetin only inhibited TNF-α release. From the compounds tested luteolin and quercetin were the most potent in inhibiting cytokine production with an IC_{50} of less than 1.0 and 5.0 μM for TNF-α release, respectively [142]. In a recently reported work apigenin and its structural analogues chrysin and luteolin were used to evaluate their capacity to inhibit the production of pro-inflammatory cytokines by lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells (PBMC). Apigenin, chrysin and luteolin dose-dependently inhibited both pro-inflammatory cytokine production and metabolic activity of LPS-stimulated PBMC. With increasing concentration of apigenin, chrysin or luteolin, the monocytes/macrophages disappeared as measured by flow cytometry. This also appeared to occur in the non-LPS-stimulated PBMC. At the same time there was an increase in dead cells. T- and B-lymphocytes were not affected. Quercetin and naringenin had virtually no effects on cytokines, metabolic activity or on the number of cells in the studied cell populations. In conclusion, monocytes were specifically eliminated in PBMC by apigenin, chrysin or luteolin treatment *in vitro* at relatively low concentrations (around 8.0 μM), in which apigenin appeared to be the most potent [143]. Human umbilical cord blood-derived cultured mast cells (hCBMCs) grown in the presence of stem cell factor (SCF) and IL-6 were preincubated for 15 min with the flavonols quercetin, kaempherol, myricetin and morin (0.01, 0.1, 1.0, 10 or 100 μM), followed by activation with anti-IgE antibodies. Secretion was quantitated for IL-6, IL-8, TNF-α, histamine and tryptase levels. Release of IL-6, IL-8 and TNF-α was inhibited by 82-93% at 100 μM quercetin and kaempherol, and by 31-70% by myricetin and morin. Tryptase release was inhibited by 79-96% at 100 μM quercetin, kampferol and myricetin, but only by 39% with morin; histamine release was inhibited by 52-77% by the first three flavonols, but only by 28% with morin. Moreover, these flavonols suppressed intracellular calcium ion elevations in a dose-response manner, with morin being the weakest, they also inhibited phosphorylation of the calcium-insensitive protein kinase C theta (PKCθ) [144]. Flavonoids are also able to reduce the expression of several membrane markers related with cell activation during an inflammatory process. In human mast cells (HMC-1) lines, fisetin suppresses cell spreading and gene expression in cells stimulated by activated T cell membrane, as well as granzyme B expression and the induced activation of NFκB and MAPKs; moreover, fisetin also reduces the amount of cell surface antigen CD40 and intercellular adhesion molecule-1 (ICAM-1) on activated HMC-1 cells [145]. ICAM-1 expression has been implicated in the processes of inflammation and carcinogenesis. Kaempherol, chrysin, apigenin, and luteolin are reported as active inhibitors of ICAM-1 expression [146]. In macrophages luteolin and quercetin at low concentration (less than 10 μM) are able to induce the production of anti-inflammatory and regulatory cytokine IL-10 [147].

A study by Takano Ishikawa and coll. investigated the suppressive effect of flavonoids on TNF-α-stimulated E-selectin expression on HUVECs by carrying out a comparative examination of 37 flavonoids. Several flavonoids, namely fisetin, luteolin and apigenin (subclass of flavones), kaempherol and quercetin (flavonols), eriodictyol (flavanones), genistein (isoflavones) and butein (chalcones) exhibited an inhibitory effects. Considerations on the structure of flavonoids, the C2-C3 double bond of C-ring and 4-keto functional group, were reported as essential for their inhibition activities [148]. Luteolin and quercetin not only modulated the expression of astrocytes specific molecules such as glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), and ceruloplasmin (CP) both in the presence and absence of IL-1β but also decreased the elevated levels of pro-inflammatory cytokine IL-6 and chemokine IL-8, interferon-inducible protein (IP-10), monocyte-chemoattractant protein-1 (MCP-1), and RANTES from IL-1β activated astrocytes [149]. In PMA-stimulated monocyte/macrophage-like HL-60 cells, preincubation with 20 μM β-carotene significantly enhanced the release of two pro-inflammatory mediators, IL-8 and TNF-α and slightly increased the DNA-damaging ability of these cells. By

contrast, 2 μM β-carotene had an inhibitory effect on the inflammatory reaction in PMA-stimulated cells [150]. The higher dose of β-carotene also exerted pro-inflammatory effects in lipopolysaccharide-stimulated RAW264.7 macrophagic cells. Furthermore, quercetin, naringenin, and α-tocopherol partly suppressed the pro-inflammatory effects of 20 μM of β-carotene on PMA-stimulated HL-60 cells, and the suppressing effects of quercetin and naringenin were better than or similar to those of α-tocopherol. In these experiments quercetin also additively or synergistically enhanced the observed inhibitory effects of 2 μM β-carotene on the secretion of pro-inflammatory mediators and the DNA-damaging ability of PMA-stimulated HL-60 cells [150]. In the inflammatory mechanism the pathogenesis of sepsis is mediated in part by the pathogen associated molecular pattern molecule (PAMP), bacterial endotoxin, which stimulates macrophages to sequentially release early (e.g., TNF-α, IL-1β) and late (for example high mobility group box 1 protein (HMGB1) pro-inflammatory mediators: the latter, which has been reported as a late mediator of lethal sepsis, has prompted investigation into development of several new experimental therapeutics either limiting release, blocking HMGB1 itself or its nominal receptors. Quercetin reduces circulating levels of HMGB1 in animals with established endotoxemia. In macrophage cultures, quercetin inhibited release as well as the cytokine activities of HMGB1, including limiting the activation of MAPK and NFκB, two signaling pathways that are critical for HMGB1-induced subsequent cytokine release [151].

FLAVONES AND FLAVONOLS: THEIR ACTION ON SIGNALING PROTEINS AND ENZYME ACTIVITY

The role of intracellular signaling, kinases, proteases, lipid rafts and transcription factors represents another critical aspect in finding a deeper comprehension of anti-inflammatory effects of flavonoids [152,153]. Moreover, the presence or absence of strategic substituents in the flavonic ring may play a diriment role for flavonoid action in inflammation, as it has been shown before [154]. Flavonoids are nature-derived NFκB inhibitors [155]. They inhibit TNF-α induced ICAM-1 expression through an effect on this transcription factor [146]. Quercetin attenuates IL-1β-induced expression of ICAM-1 mRNA and protein in a dose-dependent manner; the flavonoid actively inhibits inhibitory protein of nuclear factor-kappa B (IκB) degradation, and NFκB activity [156]. In the inflammatory signaling the c-fos and c-jun, components of activator protein-1 (AP-1), were mediated by the MAPK pathway. While ERK and p38 were involved in the c-fos mRNA expression, JNK was involved in the c-jun mRNA expression. In this context the inhibitory effect of quercetin on ICAM-1 expression was mediated by the sequential attenuation of the c-fos and c-jun mRNA expressions. These effects by the flavonol were partially inhibited by a specific inhibitor of p38MAPK (SB203580), but not by PD98059, a specific inhibitor of extracellular signal-regulated kinase (ERK), and by SP600125, a specific inhibitor of c-Jun-N-terminal kinase (JNK), an evidence that suggests that quercetin negatively modulating ICAM-1 is partly dependent on MAPK pathways [156].

The anti-inflammatory effect of apigenin and luteolin were reported as inhibiting the IκB kinase (IKK) activity, the IκBα degradation, the nuclear factor-kappaB (NFκB) DNA-protein binding, and the NFκB luciferase activity [146]. In this context the transcription factor activator protein-1 (AP-1) is involved in ICAM-1 expression. Extracellular signal-regulated kinase (ERK) and p38 were involved in the c-fos mRNA expression, and c-Jun NH(2)-terminal kinase (JNK) was involved in the c-jun mRNA expression. All three mitogen-activated protein kinase (MAPK) activities were inhibited by apigenin and luteolin, while kaempherol and chrysin only inhibited the JNK activity: in this picture the inhibitory effects of apigenin and luteolin on ICAM-1 expression are mediated by the sequential attenuation of the three MAPKs activities, the c-fos and c-jun mRNA expressions, and the AP-1 transcriptional activity, whereas IKK/NFκB pathway is also involved; however, kaempherol- and chrysin-mediated inhibitions are primarily executed through the attenuation of JNK activity, c-jun mRNA expression, and AP-1 activity; in this scenario, the role of -OH group at positions 5 and 7 of A ring and at position 4 of B ring has been reported as relevant [146].

According to Lotito *et al*. the 5,7-dihydroxyl substitution of a flavonoid A-ring and 2,3-double bond and 4-keto group of the C-ring were the main structural requirements for inhibition of adhesion molecule expression [157].

Flavonoids are able to interact with adenosine receptors [158] and this property is intriguing for evaluating the role of these compounds in purinergic signaling. However, a flavonoid has often a wide spectrum of targets and pleiotropic actions. Quercetin inhibits cytotoxic lymphocyte function [159], inhibits IL-6 production in LPS-stimulated neutrophils [160], regulates leukocyte biology [161] with a stimulus-specific action [162], affects the balance Th1/Th2 in a murine model of asthma [163], and inhibits anaphylactic contraction in guinea pig ileum smooth muscle [164]. Luteolin suppresses adipocyte-induced macrophage activation by inhibiting phosphorylation of JNK [165]. Luteolin pretreatment attenuated LPS-induced extracellular signal-regulated kinase, p38, and Akt phosphorylation. LPS treatment of human gingival fibroblasts resulted in NFκB translocation but cell pretreatment with luteolin abolished LPS effects on NFκB translocation. In addition, luteolin treatment blocked LPS-induced cellular proliferation inhibition without affecting genetic material integrity [166]. In mouse skin epidermal kaempherol attenuated the UVB-induced phosphorylation of several mitogen-activated protein kinases (MAPKs), including ERKs, p38, and JNKs, but had no effect on the phosphorylation of the upstream MAPK regulator Src [167]. Expression of the majority of lipopolysaccharide-induced TLR4 target genes is mediated through a MyD88-independent (TRIF-dependent) signaling pathway. Luteolin suppresses activation of interferon regulatory factor 3 (IRF3) and NFκB induced by TLR3 and TLR4 agonists resulting in the decreased expression of target genes such as TNF-α, IL-6, IL-12, IP-10, IFNβ, CXCL9, and IL-27 in macrophages. The flavonoid, as like as other flavone-related compound (quercetin, chrysin), inhibits also TBK1-kinase activity and IRF3 dimerization and phosphorylation, leading to the reduction of TBK1-dependent gene expression [168]. Many intracellular proteins and enzymes involved in cell function are usual targets for flavonoids, such as protein kinase C (PKC) [169], cyclic AMP phosphodiesterase [170], xanthine oxidase

[171,172], nitric oxide sinthase [173], 1-alkil-2-lyso-sn-glycero-3-phosphocholine (lyso PAF) acetyltransferase [174], 5'-nucleotidase [175], metallopeptidases [176], AMP-activated protein kinase [177], butyrylcholinesterase [178], phosphoinositide-3-kinase (PI3K) of which fisetin and quercetin are well known inhibitors but myricetin has shown to be the most potent (IC_{50} = 1.8 μM), while luteolin and apigenin were also effective inhibitors with IC_{50} values of 8 and 12 μM, respectively; a structure-activity study indicated that the position, number and substitution of the hydroxyl group of the B ring, and saturation of the C2-C3 bond are important factors affecting flavonoid inhibition of PI3K [179]. Flavonoids from *Achillea millefolium* L., traditionally used not only in the treatment of gastro-intestinal and hepatic disorders, are natural inhibitors of proteases such as human neutrophil elastase and matrix metalloproteinases 2 and 9 [180].

ARYL HYDROCARBON RECEPTORS, FLAVONOIDS AND INFLAMMATION

The aryl hydrocarbon receptor (AhR), is a cytosolic protein belonging to the family of nuclear receptors, which controls transcription of a wide range of structurally unrelated genes. Chemoprotective phytochemicals exhibit multiple activities and interact with different receptors, such as aryl hydrocarbon receptors. To date, the most potent AhR ligand is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The aryl hydrocarbon receptor (AhR), a basic helix-loop-helix (bHLH) protein belonging to the Per-Arnt-Sim (PAS) family, is a ligand-activated transcription factor known to mediate biological and toxicological effects by binding polycyclic aromatic hydrocarbons [181]. The unliganded AhR forms a complex with two molecules of heat shock protein 90 (hsp90), hepatitis B virus X-associated protein 2 (XAP2) and p23, and exists in the citosol [182]. In response to the binding of a ligand, the AhR dissociates from these partner proteins, translocates into the nucleus, and forms a heterodimer with the AhR nuclear translocator (Arnt) [183]. The AhR/Arnt heterodimer binds to a DNA sequence called the dioxin response element (DRE) and regulates the expression of a battery of genes including those for the cytochrome P450 (CYP) 1A subfamily and other responsive proteins [184]. Exposure to TCDD leads to a number of toxic effects, in particular to tumor promotion and immunosuppression. The function of AhR in cells and living organisms therefore seems to be of paramount importance. Its absence in AhR null mice, results in severe phenotype abnormalities, such as liver half size with fibrosis, accumulation of retinoic acid and immune system insufficiency. For phytochemicals as flavonoids this point addresses to the role of polyphenolic dietary compounds in drug interactions [47,185]. An important role of AhR inheres in its transcriptional control of several biotransformation enzymes (CYP1A1/2,1B1). Hence AhR is the crucial factor in the regulation of xenobiotic metabolism. Under pathophysiological conditions, such as inflammation, the level and activity of the AhR target gene CYP1A is decreased. Thus it is likely, that mediators and/or products of inflammation affect AhR function. Many flavonoids act as agonists/antagonists of AhR [186-189]. Polychlorinated biphenyls (PCBs) are widespread environmental contaminants, and co-planar PCBs can induce oxidative stress and activation of pro-inflammatory signaling cascades which are associated with atherosclerosis. The majority of the toxicological effects elicited by the co-planar PCB exposure are associated to the activation of the aryl hydrocarbon receptor (AHR) and subsequent induction of responsive genes. Quercetin, can significantly reduce PCB induction of oxidative stress and expression of the AHR responsive gene cytochrome P450 1A1 (CYP1A1), so suggesting that inflammatory pathways induced by co-planar PCBs can be down-regulated by the dietary flavonoid quercetin through mechanisms associated with functional caveolae [190]. The action of quercetin on CYP1A1 was assessed also *in vivo* [191,192]. Galangin inhibits CYP1A1 expression induced by TCDD in Caco-2 cells [193] and generally flavonoids are recognized as natural anti-dioxin toxicity substances [194]. Anyway, along with some criticism about plant polyphenolic toxicity and health benefits [195,196], some flavones or flavonols, by interacting with the AhR, behave as pro-cancerogenic molecules, for example luteolin enhances the availability of benzo(a)pyrene in colon carcinoma cells [197,198]. Aromatic hydrocarbons such as flavonoids interacting with the AhR induce changes in actin cytoskeleton through an action on glyceraldehydes-3-phosphate [199]. Flavones and flavonols were stronger suppressive agents (antagonists) of AhR than flavanones and catechins in cell-free systems [200]. The interaction between these flavonoids and the AhR complex differs among the subclasses [201]. Actually, the mechanism by which flavonoids suppress the AhR-mediated signal transduction is not yet fully understood. In this mechanism might be involved PKC but not PI3K: LY294002, an inhibitor of PI3K suppressed the DNA-binding action of the AhR in an independently from its effect on PI3K, while wortmannin did not suppress DNA-binding activity [200]. The flavonoid chrysin, phloretin, kaempherol, galangin, naringenin, genistein, quercetin, myricetin, luteolin, baicalein, daidzein, apigenin, and diosmin were investigated for their the AhR-dependent activities in Ah-responsive MCF-7 human breast cells, HepG2 human liver cancer cells, and mouse hepatoma Hepa-1 cells transiently or stably transfected with plasmids expressing a luciferase reporter gene linked to multiple copies of a consensus dioxin-responsive element. The AhR agonist activities of the above listed flavonoids (1 and 10 micro M) were as high as 25% of the maximal response induced by 5 nM TCDD, and their potencies were dependent on cell context. Galangin was active only in Hepa-1 cells, and cantharidin induced activity only in human HepG2 and MCF-7 cells, while luteolin was an AhR antagonist in both cell lines [187]. Substituted flavones have a significant effect on AhR function: the halogenated flavones exhibited competitive Ah receptor binding affinities (IC_{50} = 0.79 to 2.28 nM) that were comparable to that observed for TCDD (1.78 nM) [202]. The compounds also induced transformation of the rat cytosolic Ah receptor and induced CYP1A1 gene expression in MCF-7 human breast cancer cells. Transcriptional activation of the human CYP1A1 gene (coding for cytochrome P450 1A1) is, infact, mediated by the aryl hydrocarbon receptor. Quercetin caused a time- and concentration-dependent increase in the amount of CYP1A1 mRNA and CYP1A1 enzyme activity in MCF-7 cells, through an involvement of AhR. Together with

kaempherol the flavonol is able to compete with TCDD for binding to a cytosolic extract of MCF-7 cells [203].

As a general view, the interest in aryl hydrocarbon receptors from researchers devoted to flavonoid investigation is mainly related to chemoprevention and cancer progress, as flavonoids, as agonists/antagonists of AhR, are also able to affect cell cycle [204]. Moreover, in the inflammatory process AhR participates to type 1 regulatory T-cells differentiation induced by IL-27 [205] and links autoimmune disorders mediated by TH17 cells to environmental toxins [206], as AhR controls T(reg) and TH17 differentiation [207], so becoming a major point at issue in immunology [208,209]. Aryl hydrocarbon receptor plays a role in innate immunity to *Listeria monocytogenes* infection in mice; however, using the RAW264.7 and J774 murine macrophage cell lines and bone marrow-derived macrophages, no significant difference between flavonoid-treated and control macrophages were found and macrophages incubated with flavonoids alone did not exhibit a significant increase in release of tumor necrosis factor TNF-α, a crucial cytokine in anti-Listeria resistance [210]. Hence, a clear relationship between the AhR, immunity (inflammation) and flavonoids, has yet to be traced. Ashida and colleagues has recently reviewed the dietary ligands of the AhR, by introducing recent articles that have demonstrated the molecular mechanisms by which food factors regulate the AhR transformation and downstream drug-metabolizing enzymes, namely that food factors act as antagonists because they basically suppress the AhR transformation by different mechanisms [211]. Mukai and colleagues have investigated the effect of apigenin, luteolin, galangin and kaempherol on the DNA-binding activity of AhR. They reported that these flavonoids suppressed the nuclear translocation of the AhR and dissociation of its partner proteins in mouse hepatoma cells Hepa-1c1c7 [200].

So, which role for AhR in the anti-inflammatory activity of flavones and flavonols? A role for the AhR activation has been recently addressed concerning dendritic cells [212,213] and regarding the evidence that the AhR is a modulator of anti-viral immunity [214]. This may lead to an interesting debate about the relationship between pollutants, environmental toxins and AhR-interacting polyphenols [215], in which a more complex picture of innate and adaptive immunity, involving antigen presenting cells and Th17, including autoimmunity disorders, should be forwarded [216,217].

EFFECT OF QUERCETIN ON BASOPHILS AND MAST CELLS

The anti-allergic role of quercetin and other flavonoids was reviewed recently by Kawai and colleagues [218] but this property has been known for many years [219]. Basophils are circulating blood granulocytes involved in hypersensitivity (atopic) and anaphylactic reactions [220,221], which are able to promote chronic allergy inflammation [221,222], to regulate Th2 cell function [223,224] and immune cell memory [225,226] and even to behave as antigen presenting cells [227]. When activated, basophils degranulate to release histamine, proteoglycans (e.g. heparin and chondroitin), and proteolytic enzymes (e.g. elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes (LTC_4, PGD_2), express cysteinyl leukotrienes (LTD_4, LTE_4) receptors [228], produce several important cytokines (IL4, IL6, IL13, IL3) [229-232] and express activation-related membrane markers [233], many of them useful to focus onto basophil cell function and to allow the diagnosis of allergy [234,235]. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation [236]. Since the first description by Paul Ehrlich [237] basophils have been recognized as unique, white-blood cells with metachromatic-staining properties. The outstanding characteristics of basophils, including expression of high-affinity receptors for IgE, histamine content, and metachromatic staining, are also prerogative of tissue-dwelling mast cells, with which basophils share a common hematopoietic lineage [238]. Mast cells and basophils are hence granulated metachromatic cells which possess complex and partially overlapping roles in acquired and innate immunity, including both effector and regulatory activities, but more recently basophil has gained new consideration about its strategic role in immunity [222]. Like other granulocytes basophils are motile cells; along with the progression of allergic reactions, basophils migrate from the blood compartment to inflamed tissues and function as allergic, inflammatory cells. Mast cells and basophils cooperate in exacerbating [239] and/or modulating inflammation as well as in mediating subsequent tissue repair [240]. During inflammation mast cells release a series of potent pro-angiogenic molecules that stimulate both vessel sprouting and new vessel formation. Recently reported data suggest that basophils may also play a role in inflammation-related angiogenesis, mainly through the expression of several forms of vascular endothelial growth factors and their receptors [240]. This evidence is interesting as quercetin exerts anti-angiogenic actions [241-243] and due to this property it has a therapeutic effect in atopic dermatitis [244] and works in asthma models [245,246]. Some authors reported that the effects of circulating quercetin conjugates on angiogenesis were significantly different depending on the nature of the conjugate, as quercetin-3'-sulphate was pro-angiogenic while quercetin-3-glucuronide was anti, so that inhibition or activation of angiogenesis could be subtly shifted as a result of metabolism *in vivo* [243].

Research on basophil biology is hampered, nevertheless, by their paucity in peripheral blood, which results in scant yields and leads to a little feasibility of their isolation, often forcing the researcher to time consuming and quite expensive approaches for their purification; moreover, the lack of genetic homogeneous cell lines and of knock out animal models and finally the existence of a relatively high response variability among healthy population, have rendered it very difficult to study these leukocytes. These facts had shifted *de facto* the attention mainly towards rather more easily to hand mast cell lines [247].

Generally, most of the *in vitro* study about the action of quercetin on leukocytes and other cells involved in inflammation was performed by using pure aglycone-dihydrate quercetin. Quercetin aglycone is able to inhibit histamine release in human basophils when they are activated with various agonists [248-253] and to inhibit peptide presentation in antigen presenting cells [254].

Histamine release of basophils from allergic subjects reporting a history of ragweed hay fever and positive prick test with 1:20 ragweed extract was inhibited by doses of quercetin ranging from 5,0 to 50 μM: the highest inhibition was observed on basophils stimulated with an IgE-mediated agonist, such as anti-IgE, allergens or concanavalin A (ConA), whereas a lower inhibitory effect was shown on basophils triggered with non-IgE mediated agonists, such as calcium ionophore A23187, phorbol esters (PMA) or bacterial formylated peptides (fMLP) [251]. This first evidence of a stimulus-dependent action of the aglycone flavonoid on basophilic cells was consistent with the inhibitory activity of quercetin on rat mast cells and on the release of the granule-associated enzyme β-glucuronidase in rabbit polymorphonuclear leucocytes [255]. The use of basophil histamine release to study the effect of quercetin and other flavonic compounds, was reported as the simplest and more reliable way to evaluate basophil degranulating response to agonists [256,257], though this approach has yet several technical limitations [258]. In the whole blood medium basophil response depends on the presence of other leukocytes and some author has postulated the hypothesis that the effect of flavonoids on basophils in cell buffered suspensions containing other leukocytes might be due to their antioxidant property or to an action towards hydrogen peroxide stimulation of histamine induced by neutrophils [259]. Flow cytometry has changed dramatically the technical approach by which basophil activation could be evaluated [234,260]; however very few works used flow cytometry to study the effect of flavonoids on basophils. In basophils quercetin inhibits the expression of many membrane molecules that are up-regulated following cell activation, as like as CD63 and CD203c; this effect is more pronounced with anti-IgE than with the calcium ionophore A23187 and when the granule-associated marker CD63 is considered [261]. In basophils quercetin has shown a biphasic stimulus-dependent behavior [261]; actually, some agents are able to induce an hormetic mechanisms in leukocytes. For example low concentration of hydrogen peroxide appears to augment and high concentration to inhibit histamine release induced by anti-IgE in human basophils [259] and that hydrogen peroxide impairs inflammatory mediator release in RBL-2H3 basophilic/mast cell line [262].

Quercetin is known as a natural inhibitor of mast cell exocytosis since many years [263]; quercetin from plant extracts is able to induce downregulation of the degranulatory response in mast cells as like as the purified compound [264,265]. Although, in the past, it was chemically and functionally related to cromoglycate [95] its inhibitory function did not always correlate with this anti-allergic compound [266]; while quercetin is able to inhibit RBL-2H3 degranulation, as assayed by the β-exosaminidase release, cromoglycate and ketotifen are uneffective on the degranulatory event stimulated by anti-IgE or A23187 [267]. Martin and coll. using cromoglycate and quercetin as inhibitors of mast cell, showed that cromoglycate suppressed its target nucleoside diphosphate kinase (NDPK) at millimolar dose range, while quercetin at micromolar concentration, leading to the consequent failure to generate GTP [268]. Inhibition by quercetin is reversed by using an inhibitor of heme-oxygenase 1 (HO-1), an evidence that has suggested a role for this enzyme in rat mast cell degranulatory response: HO-1 activity is upregulated following short exposure to the flavonoid, while a longer incubation of RBL-2H3 with quercetin leads also to an increase in HO-1 expression [269]. Stimulus-coupled response of quercetin towards mast cell function has been reported for many years: quercetin inhibits significantly mast cell degranulation and histamine release when cells are stimulated with antigens, anti-IgE, ATP and concanavalin A but very little when stimulated with calcium ionophores [263]. This stimulus-related response was reported also for quercetin in feverfew (*Tanacetum parthenium*) [270]. Some experiments of our laboratory have shown that in human basophils activated with calcium ionophore A23187 quercetin is able to decrease only CD63 membrane up-regulation, which is associated with degranulation, but not that of CD203c (unpublished data). In a work of Kurose and coll. when histamine release was elicited by adding Ca^{++} at various times after antigen-stimulation of sensitized cells in Ca^{++}-free medium, the drugs to be tested were added shortly before each Ca^{++} addition. Quercetin was effective only when added before or immediately after antigen challenge, theophylline and disodium cromoglycate (DSCG) were active irrespective of the time interval between antigen and Ca++ addition while verapamil was more effective when added before or simultaneously with antigen than when added later. This evidence, together with other results after performing two-stage experiments, led to the suggestion that quercetin selectively and verapamil primarily act to block calcium-gate opening resulting from antigen-antibody interaction on the mast cell membrane, while theophylline and DSCG selectively inhibit the passage of calcium through open calcium channels [271,272]. This mechanism is compatible also with the hypothesis that quercetin may act on phosphoinositide-3 kinase [273]. However, the role of calcium was questionable as quercetin was able to inhibit histamine release even in a calcium-free medium, so suggesting that an alternative explanation for its action should be sought [274-276]. A question whether A23187, the main calcium-related segretagogue of basophil or mast cell function, could by-pass inhibition by calcium flux blockers also arose [277]. The role of calcium, therefore, is still a very hot topic. Another great issue dealing with the molecular action of quercetin is phosphoinositide-3-kinase target. Previously published reports have shown that quercetin is able to inhibit PI3K by binding to the catalytic pocket of the enzyme: as for instance, LY294002, a synthetic inhibitor of PI3K, has actually a chemical kinship with the flavonoid quercetin [278]. Quercetin can interact with the phosphatidyl inositol signaling system, leading to the blockage of membrane Ca^{++}-channels (see Fig. **3**). The IC_{50} for quercetin as an inhibitor of PI3K is around 1,8-20 μM [179], which corresponds to the inhibitory range observed in previously reported results [261]. Fig. (**3**) describes in a cartoon the possible effects of quercetin on an IgE-mediated stimulation pathway and on a non-IgE-mediated stimulation one, mainly through an action towards PI3K and the phosphatidyl inositol signaling system.

Previous reports have shown that sub-micromolar concentrations of quercetin inhibit IgE-mediated activation of basophil and primes a G-coupled receptor signaling, such as formyl pepides receptors [261].

Taking into account the downstream signaling pathway of FcεRI-anti-IgE complex, a suggestion would come that the inhibition of PI3K leads to the loss of phosphorylation of downstream kinases such as Bruton's tyrosine kinase (BTK) [279] which in turn is able to phosphorylate PLCγ, thus leading to the production of inositol-1,4,5-triphosphate (IP_3) and to diacylglycerol (DAG) from the precursor phosphatidylinosytol-4,5-biphosphate (PtdIns 4,5-P_2 or PIP_2) (Fig. **3A**). While DAG remains to the membrane, IP_3 diffuses to the cytosol and binds to and activates the $InsP_3$ receptor on the membrane of the endoplasmic reticulum (ER), opening a calcium channel, resulting in the release of Ca^{2+} into the cytoplasm. Inhibition of IP_3 leads to suppression of $[Ca^{++}]_{int}$ signaling, necessary for the degranulatory event. DAG is able to activate protein kinase C (PKC), which in turn activates membrane markers up-regulation and histamine release (Fig. **3B**) [280]: inhibition of DAG suppresses this function. Warner JA and coll. observed that the amount of histamine release associated with activation of basophils through IgE receptor aggregation, among different preparations of basophils, was found to be correlated to an increase in membrane bound PKC-like activity [281]. These results also suggested that PKC activation may have a role in IgE-mediated histamine release in human basophils and that quercetin might inhibit basophil function by blocking DAG precursor for PKC in the upstream signaling pathways. The inhibition of PI3K by quercetin would also prevent the formation of phosphatidylinositol 3,4,5-triphosphate (PtdIns3,4,5-P_3) which activates extracellular calcium influx by membrane Ca^{++} channels (Fig. **3C**) [282]. The effect on PI3K signaling has an effect also on other intracellular signaling systems

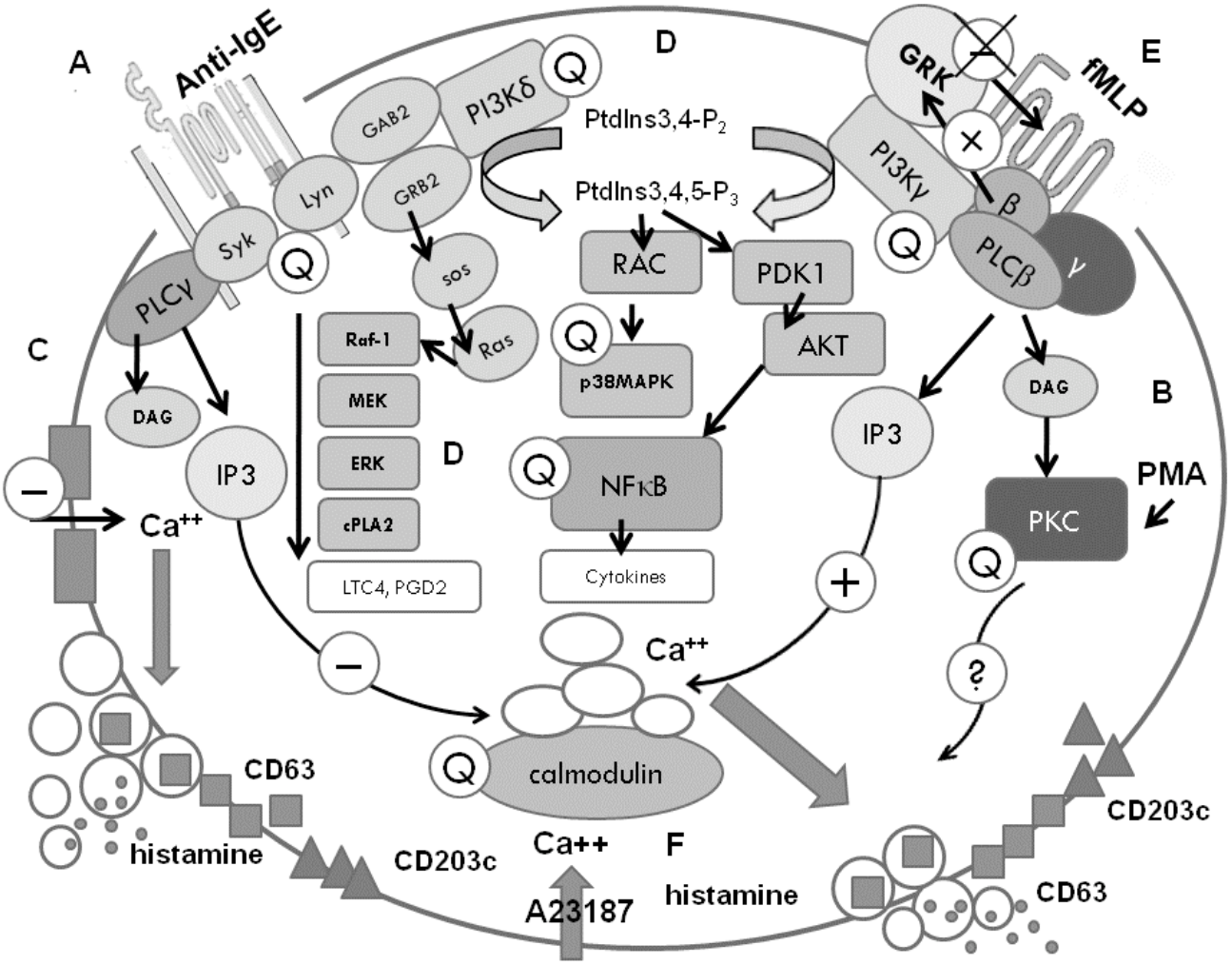

Fig. (3). Cartoon describing the possible targets of quercetin in human basophils leading to its biphasic effect on cell function. Pathways of inhibition (-) or priming (+) by quercetin in the basophil. (**A**) inhibition at the level of IgE-FcεRI, through suppression of Syk and receptor-related tyrosine kinases or of PI3K, leading to the inhibition of PLCγ and of IP3-induced [Ca^{++}] from intracellular stores and blockage of degranulation, histamine release, CD63 ed CD203c up-membrane expression; (**B**) the involvement of DAG and PKC may be biphasic, as in a G-protein coupled response degranulation and activation markers membrane expression are upregulated (primed) through a probable activation of DAG. However, quercetin is insensitive on PMA triggering; (**C**) PI3K inhibition may lead to blockage of extracellular calcium entry from calcium channels and (**D**) to intracellular kinases signaling leading to gene expression and byosynthesis of eicosanoids; (**E**) effect on fMLP-G-protein-coupled seven spanning receptor explained in the text. Inhibition of G-related PI3K may lead to a disfunction in G-protein receptor kinase (GRK) which cannot desensitize G-coupled receptor, leading to a sustained response (for example of PLCβ), (**F**) effect on calcium-calmodulin signaling pathway. See text for further explanation. Q = possible consensus targets of quercetin.

leading to gene transcription, such as the PDK1-AKT-NFκB pathway and the RAC-MKK3/6-p38-MAPK pathway, which lead to many other intracellular function, such as cytoskeleton and actin modelling, intracellular transport of molecules to membrane, granules transport and gene transcription (Fig. **3**). Quercetin acts also on the upstream FcεRI-signaling pathway by interacting with src-kinases and spleen tyrosine kinase (Syk) [283], so affecting also the Grb2-SOS-Ras-Raf-1-MAPK signalling pathway leading to LTC_4 and PGD_2 production (Fig. **3D**).

Previous unpublished data from our laboratory have shown that the effects of quercetin were superimposable to those of wortmannin (a potent PI3K inhibitor [284,278]); this evidence strongly suggests a role for PI3K in the dual effects performed by the flavonoid. G-protein coupled receptors, such as the fMLP receptor, activate the PI3Kγ isoform through interactions with Gβγ of the PI3K p101 and p110γ subunits [285]. Increasing evidence suggests that monomeric p110γ may function as a downstream regulator of G-protein coupled receptor dependent signal transduction [285]: Gβγ is able to activate a G-coupled receptor kinase (GRK) which desensitizes the receptor. Interactions of quercetin with this Gβγ-p101/p110γ might exert an action leading to these possible results: a) the inability of Gβγ sequestered by p101/p110γ complex to activate G-coupled receptors kinases (GRKs) and to desensitize the receptor, leading to a priming mechanism for example by inducing a sustained activation of downstream protein kinases involved in membrane molecule up-displacement and intracellular signaling, such as p38-MAPK [286]; b) the long-lasting activation of Gβγ-associated PLCβ due to a defect in the Gβγ/PI3K dissociation, leading to an increase in signaling mediators able to trigger the degranulation event (by IP_3-calcium signaling or by the activation of DAG-PKC pathway), so resulting in a priming effect (Fig. **3E**).

Previous reports have shown that the flavonol proved insensitive to target protein kinase C (PKC), as resulted from the use of PMA as basophil stimulant [251]: quercetin was unable to inhibit CD63 and CD203c membrane up-regulation in basophils stimulated with phorbol esters and no dissociation between the two markers investigated was actually observed by using PMA [233]. So, PKC pathway triggered by PMA, and presumably by other physiologic stimulants, is a quercetin-indipendent route to basophil activation (see also Fig. **3B**).

Previous works has reported that CD203c and CD63 upregulation in response to calcium signal by A23187 showed different kinetics [235], an evidence that probably suggests different pathways of calcium involvement in the expression of the two markers [287]. These results indicate that the calcium-mediated signaling is essential both for the LAMP-3 CD63 and for the ENPP-3 CD203c upregulation, as A23187-mediated calcium influx stimulates both the expression of basophil activation markers and histamine release, but on the same time they suggest also that the transduction pathway diverges in two distal branches, one of which (LAMP-3) is sensitive to quercetin and is related to the degranulatory event [258], the other is much more resistant to this inhibition. It is well known that A23187 promotes the activation of Ca^{++}/calmodulin pathway [288], which is inhibited by quercetin [289]. Calmodulin constitutes an obligate link in signal transduction pathways leading to human leukocyte histamine release if the trigger is a calcium ionophore but not when responses are induced by anti-IgE, fMLP or PMA [288]. Quercetin ability to target calmodulin drives to the suggestion that those events inhibited by the flavonoid, i.e. the histamine release and CD63 membrane up-regulation, were presumably related to a Ca^{++}/calmodulin dependent pathway in basophils activated with A23187, while the expression of CD203c, which was not significantly affected by the flavonoid even at its highest dose, might be a calmodulin-independent event. This marker is probably translocated to the membrane by other calcium dependent vesicular-transport mechanisms [290] (Fig. **3F**).

Quercetin can stabilize mast cell membrane [291], so being able to attenuate epithelial responses of guinea pigs colonic mucosa to *T. spiralis* [292]. A role for mast cell in neurological disorders has been also traced. Among mast cell products, the protease tryptase could be associated with neurodegenerative processes through the activation of specific receptors (PARs) expressed in the brain, while interleukin IL-6 likely causes neurodegeneration and exacerbates dysfunction induced by other cytokines; or it could have a protective effect against demyelinisation. Quercetin causes a decrease in the release of tryptase [293] and IL-6 and the down-regulation of histidine decarboxylase (HDC) mRNA from human mast cell (HMC)-1 cells, suggesting a role for quercetin as a therapeutical molecule for neurological diseases mediated by mast cell degranulation [294].

Rat basophilic leukemia (RBL) cells are similar to bone-marrow derived mast cells and to mucosal mast cells (MMC), the latter of which may be involved in inflammatory bowel diseases. RBL cell lines are not able to accumulate histamine and secretory granules under regular growing conditions; the flavonoid quercetin inhibits mast cell secretion of histamine and cell proliferation and constitutive histamine release while it induced synthesis of rat mast cell protease (RMCP) II [295] and triggered processes leading to accumulation of secretory granules; moreover, cell viability was retained in the presence of quercetin, whereas untreated cells did not survive after 6 days of growth [296]. In these cells quercetin did not affect the expression of mRNA for alpha-subunit of immunoglobulin E (IgE) receptor, but led to increased expression of mRNA for, and synthesis of RMCP II, which is a marker protein for MMC [296]. Other evidence reported that in RBL-2H3 cell line quercetin induces differentiation and maturation [297]. Sagawa and coll. have studied hop water extract (*Humulus lupulus* L.) (HWE) by evaluating histamine release from human basophilic KU812 cells induced by calcium ionophore A23187. HWE significantly inhibited histamine release, but boiling water extract and chloroform-methanol extract did not show any inhibitory effect on it. A 50% methanol-eluted fraction separated from HWE by XAD-4 column chromatography (MFH) had a strong inhibitory effect as compared with HWE. Quercetin glycosides and kaempherol glycosides were identified in MFH, of which quercetin glycosides contributed to the inhibition of histamine release [298]. Quercetin in Brazilian herbal medicine from *Cissus sicyoides* aerial parts inhibits mast cell degranulation and histamine release in RBL-2H3 cell line [299]. Secretagogues such as mastoparan, compound 48/80, substance P, and somatostatin stimulate

secretion in rat peritoneal mast cells through direct activation of the heterotrimeric G protein, $G_{(i-3)}$. Cultured RBL-2H3 mast cells do not normally respond to these secretagogues, but they can do so after prolonged exposure to quercetin [300]. This flavonol, causes phenotypic changes in RBL-2H3 cells and induces a substantial increase (more than sevenfold) in the expression of alpha subunits of the pertussis toxin-sensitive G proteins, $G_{(i-2)}$ and $G_{(i-3)}$ [300]. Long term incubation of rat mast cells with quercetin markedly stimulates Ca^{++}-dependent exocytosis and release of arachidonic acid; in these cells undergoing prolonged quercetin treatment a reduction of membrane GTPase compared to ATPase activity was also observed [301], while quercetin + compound 48/80 induced an increase in GTPase and phospholypase D activity and augmented transiently also phospholypase C function [302].

Along with other flavones, quercetin was considered as a transport ATPase inhibitor [263]. In rat peritoneal mast cells, quercetin showed an immediate inhibitory effect at micromolar concentrations on histamine release mediated by antigen or ConA cross-link of FcεRI, and by ATP but it had little effect on release induced by calcium ionophores A23187 and X537A [263]. The role of membrane Ca^{2+}/Mg^{2+} activated ATPase for histamine release in mast cells, together with the differential action of flavones on calcium ionophores activated cells, havegiven hope about the balancing role of calcium in modulating flavonoid response as anti-inflammatory agents [303]. The activity of quercetin in absence of exogenous calcium could not be simply explained in terms of its postulated ability to block movement of the cation from the external environment into the cell and so, alternative modes of action had to be considered [275]. In human cultured mast cells (HCMCs) Kimata and coll. showed at 1-100 μM a concentration-dependent inhibition of IgE-mediated histamine release by quercetin and other flavonoids: also in this work cells activated by the calcium ionophore A23187 showed a lower inhibitory dose-dependent response to quercetin than IgE [117].

CONCLUSIONS

Flavones and flavonols appear to be the main anti-inflammatory plant-derived compounds worth considering as potential anti-inflammatory drugs in medicine. An outline of these compounds in inflammation has not yet been traced, despite the enormous amount of evidence reported in the literature. The wide heterogeneity of molecular targets, even if it is possible actually to define few strategic components of cell signaling and function thoroughly targeted by most of flavonoids, and the frequently complex, synergistic, or biphasic action played by these compounds, makes quite difficult to address an application of flavonoids in drug therapy and pharmacology. Furthermore, some evidence showing a comparable or greater effectiveness of phyto-extracts compared with compounds in the form of aglycones, has lately moved the attention to the action of flavonoids in the natural preparations. Almost all the effects reported for flavones and flavonols in inflammation are related to micromolar (sub-millimolar) concentration ranges of the polyphenolic compounds: this issue raises criticism about the possible genotoxic or cytotoxic action of these substances as well as their effective dose from conjugated forms in bioavailability assay. Biphasic and modulatory action of quercetin observed at nanomolar doses in basophils, as well as other biphasic mechanisms reported at low micromolar concentrations, which are very close to many reported issues on flavonoid dietary plasma levels, suggest that a role for these compounds in human health can be traced by further investigating their balancing and complex regulatory action in cell function, rather than their inhibitory property. The study of a few simple cellular models such as basophils and mast cells and of few chemically close-related flavonoids, may perhaps contribute in the next future to a clearer explanation of the role of these natural substances in human biology.

ACKNOWLEDGMENTS

This work was supported by grants from Ministero Istruzione Università e Ricerca (MIUR, ex 60%).

CONFLICT OF INTEREST

The author declares that has not any conflict of interest

ABBREVIATIONS

Akt = A family of protein kinases known also as protein kinases B (PKB)

ENPP = Endo-nucleotidase-pyrophosphatase-phoshpodiesterase

FcεRI = High affinity receptor for IgE

IFN-β = Interferon-beta

IL = Interleukin

LAMP = Lysosome-associated membrane protein

MFH = Metformin hydrochloride

MIP-1α = Macrophage inflammatory protein-1alpha

MIP-2 = Macrophage inflammatory protein-2

MyD88 = Myeloid differentiation primary response gene 88, a protein used as adaptator in mice of Toll-like receptors

PGD_2 = Prostaglandin D2

PGE_2 = Prostaglandin E2

RANTES = A chemokine, namely regulated upon activation normal T cell expressed and secreted

RAW 264.7 = A mouse macrophage cell line

TBK-1 = Tank binding kinase-1, a serine/threonine protein kinase

TLR = Toll-like receptor

TRIF = TIR-domain containing adapter inducing interferon-beta, is an adapter in responding to activation of toll-like receptors

XAD-4 = Amberlyte XAD-4 is a commercial polymeric resin for chromatography of phenolic substances

REFERENCES

[1] Hollman, P.C.; Katan, M.B. Health effects and bioavailability of dietary flavonols. *Free Radic. Res.*, **1999**, *31* (Suppl), S75-S80.

[2] Hollman, P.C.; Katan, M.B. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.*, **1999**, *37*, 937-942.

[3] Yang, R.Y.; Lin, S.; Kuo, G. Content and distribution of flavonoids among 91 edible plant species. *Asia Pac. J. Clin. Nutr.*, **2008**, *17*(Suppl 1), 275-279.

[4] Ross, J.A.; Kasum, C.M. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr.*, **2002**, *22*, 19-34.

[5] Lu, S.; Zhou, Y.; Li, L.; Chiang, V.L Distinct roles of cinnamate-4-hydrolase genes in Populus. *Plant Cell Physiol.*, **2006**, *47*, 905-914.

[6] Winkel-Shirley B. Flavonoids biosynthesis. a colourful model for genetics, biochemistry, cell biology and biotechnology. *Plant Physiol.*, **2001**, *126*, 485-493.

[7] Olsen, K.M.; Slimestad, R.; Lea, U.S.; Brede, C.; Lovdal, T.; Ruoff, P.; Verheul, M.; Lillo, C. Temperature and nitrogen effects on regulators and products of the flavonoid pathway: experimental and kinetic model studies. *Plant Cell Environ.*, **2009**, *32*, 286-299.

[8] Albert, A.; Sareedenchai, V.; Heller, W.; Seidlitz, H.K.; Zidorn, C. Temperature is the key to altitudinal variation of phenolics in Arnica montana L. cv. ARBO. *Oecologia.*, **2009**, *160*, 1-8.

[9] Cazarolli, L.H.; Zanatta, L.; Alberton, E.H.; Figueiredo, M.S.; Folador, P.; Damazio, R.G.; Pizzolatti, M.G.; Silva, F.R. Flavonoids: prospective drug candidates. *Mini Rev. Med. Chem.*, **2008**, *8*, 1429-1440.

[10] Yang, X.H.; Liu, Y.Y.; Liu, L.J.; Zhou, X.P.; Toshio, S. A new flavonoid glycoside from Allium cepa L. var agrogatum Don. *Yao. Xue. Xue. Bao.*, **2000**, *35*, 752-755.

[11] Rodriguez, G.B.; Rodriguez, E.M.; Diaz, R.C. Flavonoids in onion cultivars (Allium cepa L.). *J. Food Sci.*, **2008**, *73*, C599-C605.

[12] Crozier, A.; Jaganath, I.B.; Clifford, M.N. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.*, **2009**, *26*, 1001-1043.

[13] Veitch, N.C.; Grayer, R.J. Flavonoids and their glycosides, including anthocyanins. *Nat. Prod. Rep.*, **2008**, *25*, 555-611.

[14] Gutzeit, H.O.; Tokalov, S.V.; Ludwig-Muller, J.; Rusak, G. Monitoring flavonoid metabolism in human cells by exploiting fluorescence elicited upon quercetin/protein interactions. *Croat. Chem. Acta.*, **2005**, *78*, 337-342.

[15] Williamson, G.; Day, J.; Plumb, G.W.; Couteau, G. Human metabolic pathways of dietary flavonoids and cinnamates. *Biochem. Soc. Trans.*, **2000**, *28*, 16-22.

[16] Day, A.J.; Mellon, F.; Barron, D.; Sarrazin, G.; Morgan, M.R.; Williamson, G. Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. *Free Radic. Res.*, **2001**, *35*, 941-952.

[17] Loke, W.M.; Jenner, A.M.; Proudfoot, J.M.; McKinley, A.J.; Hodgson, J.M.; Halliwell, B.; Croft, K.D. A metabolite profiling approach to identify biomarkers of flavonoid intake in humans. *J. Nutr.*, **2009**, *139*, 2309-2314.

[18] Berg, P.A.; Daniel, P.T. Effects of flavonoid compounds on the immune response. *Prog. Clin. Biol. Res.*, **1988**, *280*, 157-171.

[19] Serafini, M.; Peluso, I.; Raguzzini, A. Flavonoids as anti-inflammatory agents. *Proc. Nutr. Soc.*, **2010**, *69*, 273-278.

[20] Garcia-Lafuente, A.; Guillamon, E.; Villares, A.; Rostagno, M.A.; Martinez, J.A. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm. Res.*, **2009**, *58*, 537-552.

[21] Middleton, E. Jr. Effect of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.*, **1998**, *439*, 175-182.

[22] Guardia, T.; Rotelli, A.E.; Juarez, A.O.; Pelzer, L.E. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco.*, **2001**, *56*, 683-687.

[23] Borrelli, F.; Maffia, P.; Pinto, L.; Ianaro, A.; Russo, A.; Capasso, F.; Ialenti, A. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia.*, **2002**, *73*, S53-S63.

[24] Rotelli, A.E.; Guardia, T.; Juarez, A.O.; de la Rocha, N.E.; Pelzer, L.E. Comparative study of flavonoids in experimental models of inflammation. *Pharmacol. Res.*, **2003**, *48*, 601-606.

[25] Chiang, L.C.; Ng, L.T.; Chiang, W.; Chang, M.Y.; Lin, C.C. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. *Planta Med.*, **2003**, *69*, 600-604.

[26] Wu, C.H.; Wu, C.F.; Huang, H.W.; Jao, Y.C.; Yen, G.C. Naturally occurring flavonoids attenuate high glucose-induced expression of proinflammatory cytokines in human monocytic THP-1 cells. *Mol. Nutr. Food Res.*, **2009**, *53*, 984-995.

[27] Du Toit, K.; Buthelezi, S.; Bodenstein, J. Anti-inflammatory and antibacterial profiles of selected compounds found in South African propolis. *S. Afr. J. Sci.*, **2009**, *105*, 470-472.

[28] el-Gammal, A.A.; Mansour, R.M. Antimicrobial activities of some flavonoid compounds. *Zentralbl. Mikrobiol.*, **1986**, *141*, 561-565.

[29] Song, Y.; Manson, J.E.; Buring, J.E.; Sesso, H.D.; Liu, S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *J. Am. Coll. Nutr.*, **2005**, *24*, 376-384.

[30] Tormakangas, L.; Vuorela, P.; Saario, E.; Leinonen, M.; Saikku, P.; Vuorela, H. *In vivo* treatment of acute Chlamydia pneumoniae infection with the flavonoids quercetin and luteolin and an alkyl gallate, octyl gallate, in a mouse model. *Biochem. Pharmacol.*, **2005**, *70*, 1222-1230.

[31] Kumazawa, Y.; Kawaguchi, K.; Takimoto, H. Immunomodulating effects of flavonoids on acute and chronic inflammatory responses caused by tumor necrosis factor alpha. *Curr. Pharm. Des.*, **2006**, *12*, 4271-4279.

[32] Hamalainen, M.; Nieminen, R.; Vuorela, P.; Heinonen, M.; Moilanen, E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators Inflamm.*, **2007**, *2007*, 45673

[33] Gonzalez-Segovia, R.; Quintanar, J.L.; Salinas, E.; Ceballos-Salazar, R.; Viles-Jimenez, F.; Torres-Lopez, J. Effect of the flavonoid quercetin on inflammation and lipid peroxidation induced by Helicobacter pylori in gastric mucosa of guinea pig. *J. Gastroenterol.*, **2008**, *43*, 441-447.

[34] Loke, W.M.; Proudfoot, J.M.; Hodgson, J.M.; McKinley, A.J.; Hime, N.; Magat, M.; Stocker, R.; Croft, K.D. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler. Thromb. Vasc. Biol.*, **2010**, *30*, 749-757.

[35] Heinz, S.A.; Henson, D.A.; Nieman, D.C.; Austin, M.D.; Jin, F. A 12-week supplementation with quercetin does not affect natural killer cell activity, granulocyte oxidative burst activity or granulocyte phagocytosis in female human subjects. *Br. J. Nutr.*, **2010**, 1-9.

[36] Heinz, S.A.; Henson, D.A.; Austin, M.D.; Jin, F.; Nieman, D.C. Quercetin supplementation and upper respiratory tract infection: a randomized community clinical trial. *Pharmacol. Res.*, **2010**, *62*, 237-242.

[37] Comalada, M.; Camuesco, D.; Sierra, S.; Ballester, I.; Xaus, J.; Galvez, J.; Zarzuelo, A. *In vivo* quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur. J. Immunol.*, **2005**, *35*, 584-592.

[38] Xu, H.X.; Wan, M.; Dong, H.; But, P.P.; Foo, L.Y. Inhibitory activity of flavonoids and tannins against HIV-1 protease. *Biol. Pharm. Bull.*, **2000**, *23*, 1072-1076.

[39] Xu, H.X.; Lee, S.F. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother. Res.*, **2001**, *15*, 39-43.

[40] Jung, M.; Triebel, S.; Anke, T.; Richling, E.; Erkel, G. Influence of apple polyphenols on inflammatory gene expression. *Mol. Nutr. Food Res.*, **2009**, *53*, 1263-1280.

[41] Wleklik, M.; Luczak, M.; Panasiak, W.; Kobus, M.; Lammer-Zarawska, E. Structural basis for antiviral activity of flavonoids-naturally occurring compounds. *Acta. Virol.*, **1988**, *32*, 522-525.

[42] Wang, L.; Tu, Y.C.; Lian, T.W.; Hung, J.T.; Yen, J.H.; Wu, M.J. Distinctive antioxidant and antiinflammatory effects of flavonols. *J. Agric. Food Chem.*, **2006**, *54*, 9798-9804.

[43] Tsuchiya, H. Structure-dependent membrane interaction of flavonoids associated with their bioactivity. *Food Chem.,* **2010**, *120*, 1089-1096.

[44] Cheong, H.; Ryu, S.Y.; Oak, M.H.; Cheon, S.H.; Yoo, G.S.; Kim, K.M. Studies of structure activity relationship of flavonoids for the anti-allergic actions. *Arch. Pharm. Res.*, **1998**, *21*, 478-480.

[45] Li, J.J. Algar-Flynn-Oyamada reaction. In: *Name Reactions*, Springer, Berlin Heidelberg, **2009**, 6-7.

[46] Daniel, M. *Medicinal plants-chemistry and properties*. Science, Publishers, Enfield New Hampshire, USA, **2006**, 78-

[47] Si, D.; Wang, Y.; Zhou, Y.H.; Guo, Y.; Wang, J.; Zhou, H.; Li, Z.S.; Fawcett, J.P. Mechanism of CYP2C9 inhibition by flavones and flavonols. *Drug Metab. Dispos.*, **2009**, *37*, 629-634.

[48] Bakay, M.; Mucsi, I.; Beladi, I.; Gabor, M. Effect of flavonoids and related substances. II. Antiviral effect of quercetin, dihydroquercetin and dihydrofisetin. *Acta. Microbiol. Acad. Sci. Hung.*, **1968**, *15*, 223-227.

[49] Akaishi, T.; Morimoto, T.; Shibao, M.; Watanabe, S.; Sakai-Kato, K.; Utsunomiya-Tate, N.; Abe, K. Structural requirements for the flavonoid fisetin in inhibiting fibril formation of amyloid beta protein. *Neurosci. Lett.*, **2008**, *444*, 280-285.

[50] Hosny, M.; Dhar, K.; Rosazza, J.P. Hydroxylations and methylations of quercetin, fisetin, and catechin by Streptomyces griseus. *J. Nat. Prod.*, **2001**, *64*, 462-465.

[51] De Santi, C.; Pietrabissa, A.; Mosca, F.; Pacifici, G.M. Methylation of quercetin and fisetin, flavonoids widely distributed in edible vegetables, fruits and wine, by human liver. *Int. J. Clin. Pharmacol. Ther.*, **2002**, *40*, 207-212.

[52] Huang, R.Y.; Yu, Y.L.; Cheng, W.C.; OuYang, C.N.; Fu, E.; Chu, C.L. Immunosuppressive effect of quercetin on dendritic cell activation and function. *J. Immunol.*, **2010**, *184*, 6815-6821.

[53] Butenko, I.G.; Gladtchenko, S.V.; Galushko, S.V. Antiinflammatory properties and inhibition of leukotriene C4 biosynthesis in-vitro by flavonoid baicalein from Scutellaria baicalensis Georgy roots. *Agents Actions*, **1993**, *39*, C49-C51.

[54] Kimura, Y.; Okuda, H.; Hayashi, K.Y.; Matsushita, N. Effects of baicalein isolated from roots of Scutellaria baicalensis Georgi on interleukin 1 beta- and tumour necrosis factor alpha-induced tissue-type plasminogen activator and plasminogen activator inhibitor-1 production in cultured human umbilical vein endothelial cells. *Phytother. Res.*, **1997**, *11*, 363-367.

[55] Lim, H.; Kim, H.P. Inhibition of mammalian collagenase, matrix metalloproteinase-1, by naturally-occurring flavonoids. *Planta Med.*, **2007**, *73*, 1267-1274.

[56] Kakegawa, H.; Matsumoto, H.; Satoh, T. Inhibitory effects of some natural-products on the activation of hyaluronidase and their antiallergic actions. *Chem. Pharm. Bull.*, **1992**, *40*, 1439-1442.

[57] Tanno, Y.; Shindoh, Y.; Takishima, T. Modulation of human basophil growth *in vitro* by xiao-qing-long-tang (syo-seiryu-to), chai-pu-tang (saiboku-to), qing-fei-tang (seihai-to), baicalein and ketotifen. *Am. J. Chin. Med.*, **1989**, *17*, 45-50.

[58] Kawasaki, M.; Toyoda, M.; Teshima, R.; Sawada, J.; Hayashi, T.; Arisawa, M.; Shimizu, M.; Morita, N.; Inoue, S.; Saito, Y. In-vitro antiallergic activity of flavonoids in histamine-release assay using rat basophilic leukemia (RBL-2H3) cells. *Shokuhin. Eiseigaku. Zasshi.,* **1994**, *35*, 497-503.

[59] Amellal, M.; Bronner, C.; Briancon, F.; Haag, M.; Anton, R.; Landry, Y. Inhibition of mast cell histamine release by flavonoids and biflavonoids. *Planta Med.*, **1985**, *51*, 16-20.

[60] Wakabayashi, I.; Yasui, K. Wogonin inhibits inducible prostaglandin E-2 production in macrophages. *Eur. J. Pharmacol.,* **2000**, *406*, 477-481.

[61] Wakabayashi, I. Inhibitory effects of baicalein and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacol. Toxicol*., **1999**, *84*, 288-291.

[62] Pagonis, C.; Tauber, A.I.; Pavlotsky, N.; Simons, E.R. Flavonoid impairment of neutrophil response. *Biochem. Pharmacol.*, **1986**, *35*, 237-245.

[63] Busse, W.W.; Kopp, D.E.; Middleton, E. Jr. Flavonoid modulation of human neutrophil function. *J. Allergy Clin. Immunol.*, **1984**, *73*, 801-809.

[64] Della, L.R.; Ragazzi, E.; Tubaro, A.; Fassina, G.; Vertua, R. Anti-inflammatory activity of benzopyrones that are inhibitors of cyclo- and lipo-oxygenase. *Pharmacol. Res. Commun.*, **1988**, *20* (Suppl 5), 91-94.

[65] Medeiros, K.C.; Faustino, L.; Borduchi, E.; Nascimento, R.J.; Silva, T.M.; Gomes, E.; Piuvezam, M.R.; Russo, M. Preventive and curative glycoside kaempferol treatments attenuate the TH2-driven allergic airway disease. *Int. Immunopharmacol.*, **2009**, *9*, 1540-1548.

[66] Debiaggi, M.; Tateo, F.; Pagani, L.; Luini, M.; Romero, E. Effects of propolis flavonoids on virus infectivity and replication. *Microbiologica*, **1990**, *13*, 207-213.

[67] Choi, H.J.; Kim, J.H.; Lee, C.H.; Ahn, Y.J.; Song, J.H.; Baek, S.H.; Kwon, D.H. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antiviral Res.*, **2009**, *81*, 77-81.

[68] Zhang, T.; Chen, D. Anticomplementary principles of a Chinese multiherb remedy for the treatment and prevention of SARS. *J. Ethnopharmacol.*, **2008**, *117*, 351-361.

[69] Min, B.S.; Tomiyama, M.; Ma, C.M.; Nakamura, N.; Hattori, M. Kaempferol acetylrhamnosides from the rhizome of Dryopteris crassirhizoma and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase. *Chem. Pharm. Bull. (Tokyo)*, **2001**, *49*, 546-550.

[70] Robin, V.; Irurzun, A.; Amoros, M.; Boustie, J.; Carrasco, L. Antipoliovirus flavonoids from Psiadia dentata. *Antivir. Chem. Chemother.*, **2001**, *12*, 283-291.

[71] Mitrocotsa, D.; Mitaku, S.; Axarlis, S.; Harvala, C.; Malamas, M. Evaluation of the antiviral activity of kaempferol and its glycosides against human cytomegalovirus. *Planta Med.*, **2000**, *66*, 377-379.

[72] Wei, B.L.; Lu, C.M.; Tsao, L.T.; Wang, J.P.; Lin, C.N. *In vitro* anti-inflammatory effects of quercetin 3-O-methyl ether and other constituents from Rhamnus species. *Planta Med.*, **2001**, *67*, 745-747.

[73] Kataoka, M.; Hirata, K.; Kunikata, T.; Ushio, S.; Iwaki, K.; Ohashi, K.; Ikeda, M.; Kurimoto, M. Antibacterial action of tryptanthrin and kaempferol, isolated from the indigo plant (Polygonum tinctorium Lour.), against Helicobacter pylori-infected Mongolian gerbils. *J. Gastroenterol.*, **2001**, *36*, 5-9.

[74] Alexandrakis, M.; Letourneau, R.; Kempuraj, D.; Kandere-Grzybowska, K.; Huang, M.; Christodoulou, S.; Boucher, W.; Seretakis, D.; Theoharides, T.C. Flavones inhibit proliferation and increase mediator content in human leukemic mast cells (HMC-1). *Eur. J. Haematol.*, **2003**, *71*, 448-454.

[75] Kupeli, E.; Yesilada, E. Flavonoids with anti-inflammatory and antinociceptive activity from Cistus laurifolius L. leaves through bioassay-guided procedures. *J. Ethnopharmacol.*, **2007**, *112*, 524-530.

[76] Jiang, H.; Hu, J.R.; Zhan, W.Q.; Liu, X. Screening for fractions of Oxytropis falcata Bunge with antibacterial activity. *Nat. Prod. Res.*, **2009**, *23*, 953-959.

[77] Marin, C.; Boutaleb-Charki, S.; Diaz, J.G.; Huertas, O.; Rosales, M.J.; Perez-Cordon, G.; Guitierrez-Sanchez, R.; Sanchez-Moreno, M. Antileishmaniasis activity of flavonoids from Consolida oliveriana. *J. Nat. Prod*., **2009**, *72*, 1069-1074.

[78] Mittra, B.; Saha, A.; Chowdhury, A.R.; Pal, C.; Mandal, S.; Mukhopadhyay, S.; Bandyopadhyay, S.; Majumder, H.K. Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Mol. Med.*, **2000**, *6*, 527-541.

[79] Cimanga, R.K.; Kambu, K.; Tona, L.; Hermans, N.; Apers, S.; Totte, J.; Pieters, L.; Vlietinck, A.J. Cytotoxicity and *in vitro* susceptibility of Entamoeba histolytica to Morinda morindoides leaf extracts and its isolated constituents. *J. Ethnopharmacol.*, **2006**, *107*, 83-90.

[80] Tasdemir, D.; Kaiser, M.; Brun, R.; Yardley, V.; Schmidt, T.J.; Tosun, F.; Ruedi, P. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrob. Agents Chemother.*, **2006**, *50*, 1352-1364.

[81] Das, B.B.; Sen, N.; Roy, A.; Dasgupta, S.B.; Ganguly, A.; Mohanta, B.C.; Dinda, B.; Majumder, H.K. Differential induction of Leishmania donovani bi-subunit topoisomerase I-DNA cleavage complex by selected flavones and camptothecin: activity of flavones against camptothecin-resistant topoisomerase I. *Nucleic Acids Res.*, **2006**, *34*, 1121-1132.

[82] Shim, S.Y.; Seo, Y.K.; Park, J.R. Down-regulation of FcepsilonRI expression by Houttuynia cordata Thunb extract in human basophilic KU812F cells. *J. Med. Food*, **2009**, *12*, 383-388.

[83] Shim, S.Y.; Choi, J.S.; Byun, D.S. Kaempferol isolated from Nelumbo nucifera stamens negatively regulates FcepsilonRI expression in human basophilic KU812F cells. *J. Microbiol. Biotechnol.*, **2009**, *19*, 155-160.

[84] Lee, E.J.; Ji, G.E.; Sung, M.K. Quercetin and kaempferol suppress immunoglobulin E-mediated allergic inflammation in RBL-2H3 and Caco-2 cells. *Inflamm. Res.*, **2010**,

[85] Mahat, M.Y.; Kulkarni, N.M.; Vishwakarma, S.L.; Khan, F.R.; Thippeswamy, B.S.; Hebballi, V.; Adhyapak, A.A.; Benade, V.S.; Ashfaque, S.M.; Tubachi, S.; Patil, B.M. Modulation of the cyclooxygenase pathway *via* inhibition of nitric oxide production contributes to the anti-inflammatory activity of kaempferol. *Eur. J. Pharmacol.*, **2010**,

[86] Seelinger, G.; Merfort, I.; Schempp, C.M. Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. *Planta Med.*, **2008**, *74*, 1667-1677.

[87] Tordera, M.; Ferrandiz, M.L.; Alcaraz, M.J. Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. *Z. Naturforsch. C.*, **1994**, *49*, 235-240.

[88] Kang, O.H.; Choi, J.G.; Lee, J.H.; Kwon, D.Y. Luteolin isolated from the flowers of Lonicera japonica suppresses inflammatory mediator release by blocking NF-kappaB and MAPKs activation pathways in HMC-1 cells. *Molecules*, **2010**, *15*, 385-398.

[89] Kimata, M.; Inagaki, N.; Nagai, H. Effects of luteolin and other flavonoids on IgE-mediated allergic reactions. *Planta Med.*, **2000**, *66*, 25-29.

[90] Dirscherl, K.; Karlstetter, M.; Ebert, S.; Kraus, D.; Hlawatsch, J.; Walczak, Y.; Moehle, C.; Fuchshofer, R.; Langmann, T. Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J. Neuroinflammation*, **2010**, *7*, 3.

[91] Theoharides, T.C. Luteolin as a therapeutic option for multiple sclerosis. *J. Neuroinflammation*, **2009**, *6*, 29.

[92] Sternberg, Z.; Chadha, K.; Lieberman, A.; Drake, A.; Hojnacki, D.; Weinstock-Guttman, B.; Munschauer, F. Immunomodulatory responses of peripheral blood mononuclear cells from multiple sclerosis patients upon *in vitro* incubation with the flavonoid luteolin: additive effects of IFN-beta. *J. Neuroinflammation*, **2009**, *6*, 28-

[93] Domitrovic, R.; Jakovac, H.; Tomac, J.; Sain, I. Liver fibrosis in mice induced by carbon tetrachloride and its reversion by luteolin. *Toxicol. Appl. Pharmacol.*, **2009**, *241*, 311-321.

[94] Figueirinha, A.; Cruz, M.T.; Francisco, V.; Lopes, M.C.; Batista, M.T. Anti-inflammatory activity of Cymbopogon citratus leaf infusion in lipopolysaccharide-stimulated dendritic cells: contribution of the polyphenols. *J. Med. Food*, **2010**, *13*, 681-690.

[95] Bronner, C. and Landry, Y. Kinetics of the inhibitory effect of flavonoids on histamine secretion from mast cells. *Agents Actions*, **1985**, *16*, 147-151.

[96] Middleton E Jr; Fujiki, H.; Savliwala, M.; Drzewiecki, G. Tumor promoter-induced basophil histamine release: effect of selected flavonoids. *Biochem. Pharmacol.*, **1987**, *36*, 2048-2052.

[97] Hirano, T.; Arimitsu, J.; Higa, S.; Naka, T.; Ogata, A.; Shima, Y.; Fujimoto, M.; Yamadori, T.; Ohkawara, T.; Kuwabara, Y.; Kawai, M.; Kawase, I.; Tanaka, T. Luteolin, a flavonoid, inhibits CD40 ligand expression by activated human basophils. *Int. Arch. Allergy Immunol.*, **2006**, *140*, 150-156.

[98] Hirano, T.; Higa, S.; Arimitsu, J.; Naka, T.; Ogata, A.; Shima, Y.; Fujimoto, M.; Yamadori, T.; Ohkawara, T.; Kuwabara, Y.; Kawai, M.; Matsuda, H.; Yoshikawa, M.; Maezaki, N.; Tanaka, T.; Kawase, I.; Tanaka, T. Luteolin, a flavonoid, inhibits AP-1 activation by basophils. *Biochem. Biophys. Res. Commun.*, **2006**, *340*, 1-7.

[99] Papaliodis, D.; Boucher, W.; Kempuraj, D.; Theoharides, T.C. The flavonoid luteolin inhibits niacin-induced flush. *Br. J. Pharmacol.*, **2008**, *153*, 1382-1387.

[100] Jin, M.; Yang, J.H.; Lee, E.; Lu, Y.; Kwon, S.; Son, K.H.; Son, J.K.; Chang, H.W. Antiasthmatic activity of luteolin-7-O-glucoside from Ailanthus altissima through the downregulation of T helper 2 cytokine expression and inhibition of prostaglandin E2 production in an ovalbumin-induced asthma model. *Biol. Pharm. Bull.*, **2009**, *32*, 1500-1503.

[101] Leemans, J.; Cambier, C.; Chandler, T.; Billen, F.; Clercx, C.; Kirschvink, N.; Gustin, P. Prophylactic effects of omega-3 polyunsaturated fatty acids and luteolin on airway hyperresponsiveness and inflammation in cats with experimentally-induced asthma. *Vet. J.*, **2010**, *184*, 111-114.

[102] Pieroni, A.; Heimler, D.; Pieters, L.; Van, P.B.; Vlietinck, A.J. *In vitro* anti-complementary activity of flavonoids from olive (Olea europaea L.) leaves. *Pharmazie*, **1996**, *51*, 765-768.

[103] Kim, E.K.; Kwon, K.B.; Song, M.Y.; Han, M.J.; Lee, J.H.; Lee, Y.R.; Lee, J.H.; Ryu, D.G.; Park, B.H.; Park, J.W. Flavonoids protect against cytokine-induced pancreatic beta-cell damage through suppression of nuclear factor kappaB activation. *Pancreas*, **2007**, *35*, e1-e9.

[104] Ramesh, N.; Viswanathan, M.B.; Saraswathy, A.; Balakrishna, K.; Brindha, P.; Lakshmanaperumalsamy, P. Phytochemical and antimicrobial studies of Begonia malabarica. *J. Ethnopharmacol.*, **2002**, *79*, 129-132.

[105] Tereschuk, M.L.; Baigori, M.D.; De Figueroa, L.I.; Abdala, L.R. Flavonoids from Argentine Tagetes (Asteraceae) with antimicrobial activity. *Methods Mol. Biol.*, **2004**, *268*, 317-330.

[106] Ueda, H.; Yamazaki, C.; Yamazaki, M. A hydroxyl group of flavonoids affects oral anti-inflammatory activity and inhibition of systemic tumor necrosis factor-alpha production. *Biosci. Biotechnol. Biochem.*, **2004**, *68*, 119-125.

[107] Nsonde Ntandou, G.F.; Banzouzi, J.T.; Mbatchi, B.; Elion-Itou, R.D.; Etou-Ossibi, A.W.; Ramos, S.; oit-Vical, F.; Abena, A.A.; Ouamba, J.M. Analgesic and anti-inflammatory effects of Cassia siamea Lam. stem bark extracts. *J. Ethnopharmacol.*, **2010**, *127*, 108-111.

[108] Mastuda, H.; Morikawa, T.; Ueda, K.; Managi, H.; Yoshikawa, M. Structural requirements of flavonoids for inhibition of antigen-Induced degranulation, TNF-alpha and IL-4 production from RBL-2H3 cells. *Bioorg. Med. Chem.*, **2002**, *10*, 3123-3128.

[109] Casetti, F.; Jung, W.; Wolfle, U.; Reuter, J.; Neumann, K.; Gilb, B.; Wahling, A.; Wagner, S.; Merfort, I.; Schempp, C.M. Topical application of solubilized Reseda luteola extract reduces ultraviolet B-induced inflammation *in vivo*. *J. Photochem. Photobiol. B.*, **2009**, *96*, 260-265.

[110] Rotelli, A.E.; Aguilar, C.F.; Pelzer, L.E. Structural basis of the anti-inflammatory activity of quercetin: inhibition of the 5-Hydroxytryptamine Type 2 Receptor. *Eur. Biophys. J.*, **2009**, *38(7)*, 865-871

[111] Harasstani, O.A.; Moin, S.; Tham, C.L.; Liew, C.Y.; Ismail, N.; Rajajendram, R.; Harith, H.H.; Zakaria, Z.A.; Mohamad, A.S.; Sulaiman, M.R.; Israf, D.A. Flavonoid combinations cause synergistic inhibition of proinflammatory mediator secretion from lipopolysaccharide-induced RAW 264.7 cells. *Inflamm. Res.*, **2010**, in press

[112] Tong, Y.; Zhou, X.M.; Wang, S.J.; Yang, Y.; Cao, Y.L. Analgesic activity of myricetin isolated from Myrica rubra Sieb. et Zucc. leaves. *Arch. Pharm. Res.*, **2009**, *32*, 527-533.

[113] Wang, S.J.; Tong, Y.; Lu, S.; Yang, R.; Liao, X.; Xu, Y.F.; Li, X. Anti-inflammatory activity of myricetin isolated from myrica rubra Sieb. et Zucc. leaves. *Planta Med.*, **2010**, in press

[114] Park, H.H.; Lee, S.; Son, H.Y.; Park, S.B.; Kim, M.S.; Choi, E.J.; Singh, T.S.; Ha, J.H.; Lee, M.G.; Kim, J.E.; Hyun, M.C.; Kwon, T.K.; Kim, Y.H.; Kim, S.H. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch. Pharm. Res.*, **2008**, *31*, 1303-1311.

[115] Medeiros, K.C.; Figueiredo, C.A.; Figueredo, T.B.; Freire, K.R.; Santos, F.A.; Cantara-Neves, N.M.; Silva, T.M.; Piuvezam, M.R. Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice. *J. Ethnopharmacol.*, **2008**, *119*, 41-46.

[116] Duthie, S.J.; Collins, A.R.; Duthie, G.G.; Dobson, V.L. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes. *Mutat. Res.*, **1997**, *393*, 223-231.

[117] Kimata, M.; Shichijo, M.; Miura, T.; Serizawa, I.; Inagaki, N.; Nagai, H. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. *Clin. Exp. Allergy*, **2000**, *30*, 501-508.

[118] Limasset, B.; Ledoucen, C.; Dore, J.C.; Ojasoo, T.; Damon, M.; Depaulet, A.C. effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils - multivariate-analysis of structure-activity-relationships (Sar). *Biochem. Pharmacol.*, **1993**, *46*, 1257-1271.

[119] Tomasbarberan, F.A.; Ferreres, F.; Tomaslorente, F.; Ortiz, A. Flavonoids from apis-mellifera beeswax. *Z. Naturforsch. C.*, **1993**, *48*, 68-72.

[120] Siess, M.H.; Lebon, A.M.; CanivencLavier, M.C.; Amiot, M.J.; Sabatier, S.; Aubert, S.Y.; Suschetet, M. Flavonoids of honey and propolis: characterization and effects on hepatic drug-metabolizing enzymes and benzo[a]pyrene-DNA binding in rats. *J. Agric. Food Chem.*, **1996**, *44*, 2297-2301.

[121] Ferreres, F.; Andrade, P.; Tomasbarberan, F.A. Flavonoids from Portuguese Heather Honey. *Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung*, **1994**, *199*, 32-37.

[122] Soler, C.; Gil, M.; Garciaviguera, C.; Tomasbarberan, F.A. Flavonoid Patterns of French Honeys with Different Floral Origin. *Apidologie*, **1995**, *26*, 53-60.

[123] Mirzoeva, O.K. and Calder, P.C. The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukot. Essent. Fatty Acids*, **1996**, *55*, 441-449.

[124] Ansorge, S.; Reinhold, D.; Lendeckel, U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-beta1 production of human immune cells. *Z. Naturforsch. C.*, **2003**, *58*, 580-589.

[125] Heo, M.Y.; Sohn, S.J.; Au, W.W. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. *Mutat. Res.*, **2001**, *488*, 135-150.

[126] Ji, X.D.; Melman, N.; Jacobson, K.A. Interactions of flavonoids and other phytochemicals with adenosine receptors. *J. Med. Chem.*, **1996**, *39*, 781-788.

[127] Livingston, M.; Heaney, L.G.; Ennis, M. Adenosine, inflammation and asthma--a review 566. *Inflamm.Res.*, **2004**, *53*, 171-178.

[128] Park, Y.K.; Koo, M.H.; Ikegaki, M.; Cury, J.A.; Rosalen, P.L. Effects of propolis on Streptococcus mutans, Actinomyces naeslundii and Staphylococcus aureus. *Revista de Microbiologia*, **1998**, *29*, 143-148.

[129] Afolayan, A.J.; Meyer, J.J.M. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of Helichrysum aureonitens. *J. Ethnopharmacol.*, **1997**, *57*, 177-181.

[130] Cushnie, T.P.T.; Hamilton, V.E.S.; Lamb, A.J. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. *Microbiol. Res.*, **2003**, *158*, 281-289.

[131] Pepeljnjak, S.; Kosalec, I. Galangin expresses bactericidal activity against multiple-resistant bacteria: MRSA, Enterococcus spp. and Pseudomonas aeruginosa. *Fems. Microbiol. Lett.*, **2004**, *240*, 111-116.

[132] Meyer, J.J.M.; Afolayan, A.J.; Taylor, M.B.; Erasmus, D. Antiviral activity of galangin isolated from the aerial parts of Helichrysum aureonitens. *J. Ethnopharmacol.*, **1997**, *56*, 165-169.

[133] Ferrandiz, M.L.; Alcaraz, M.J. Antiinflammatory activity and inhibition of arachidonic-acid metabolism by flavonoids. *Agents Actions*, **1991**, *32*, 283-288.

[134] Mounnissamy, V.M.; Gopal, V.; Gunasegaran, R.; Saraswathy, A. Antiinflammatory activity of gossypetin isolated from Hibiscus sabdariffa. *Indian J. Heterocycl. Chem.*, **2002**, *12*, 85-86.

[135] Geraets, L.; Moonen, H.J.J.; Brauers, K.; Wouters, E.F.M.; Bast, A.; Hageman, G.J. Dietary flavones and flavonoles are inhibitors of poly(ADP-ribose)polymerase-1 in pulmonary epithelial cells. *J. Nutr.*, **2007**, *137*, 2190-2195.

[136] Hirano, T.; Higa, S.; Arimitsu, J.; Naka, T.; Shima, Y.; Ohshima, S.; Fujimoto, M.; Yamadori, T.; Kawase, I.; Tanaka, T. Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. *Int. Arch. Allergy Immunol.*, **2004**, *134*, 135-140.

[137] Lee, S.; Kim, Y.J.; Kwon, S.; Lee, Y.; Choi, S.Y.; Park, J.; Kwon, H.J. Inhibitory effects of flavonoids on TNF-alpha-induced IL-8 gene expression in HEK 293 cells. *BMB Rep.*, **2009**, *42*, 265-270.

[138] Geraets, L.; Haegens, A.; Brauers, K.; Haydock, J.A.; Vernooy, J.H.; Wouters, E.F.; Bast, A.; Hageman, G.J. Inhibition of LPS-induced pulmonary inflammation by specific flavonoids. *Biochem. Biophys. Res. Commun.*, **2009**, *382*, 598-603.

[139] Lee, J.D.; Huh, J.E.; Jeon, G.; Yang, H.R.; Woo, H.S.; Choi, D.Y.; Park, D.S. Flavonol-rich RVHxR from Rhus verniciflua Stokes and its major compound fisetin inhibits inflammation-related cytokines and angiogenic factor in rheumatoid arthritic fibroblast-like synovial cells and *in vivo* models. *Int. Immunopharmacol.*, **2009**, *9*, 268-276.

[140] Higa, S.; Hirano, T.; Kotani, M.; Matsumoto, M.; Fujita, A.; Suemura, M.; Kawase, I.; Tanaka, T. Fisetin, a flavonol, inhibits TH2-type cytokine production by activated human basophils. *J. Allergy Clin. Immunol.*, **2003**, *111*, 1299-1306.

[141] Lyu, S.Y.; Park, W.B. Production of cytokine and NO by RAW 264.7 macrophages and PBMC *in vitro* incubation with flavonoids. *Arch. Pharm. Res.*, **2005**, *28*, 573-581.

[142] Xagorari, A.; Papapetropoulos, A.; Mauromatis, A.; Economou, M.; Fotsis, T.; Roussos, C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 181-187.

[143] Hougee, S.; Sanders, A.; Faber, J.; Graus, Y.M.; van den Berg, W.B.; Garssen, J.; Smit, H.F.; Hoijer, M.A. Decreased pro-inflammatory cytokine production by LPS-stimulated PBMC upon *in vitro* incubation with the flavonoids apigenin, luteolin or chrysin, due to selective elimination of monocytes/macrophages. *Biochem. Pharmacol.*, **2005**, *69*, 241-248.

[144] Kempuraj, D.; Madhappan, B.; Christodoulou, S.; Boucher, W.; Cao, J.; Papadopoulou, N.; Cetrulo, C.L.; Theoharides, T.C. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br. J. Pharmacol.*, **2005**, *145*, 934-944.

[145] Nagai, K.; Takahashi, Y.; Mikami, I.; Fukusima, T.; Oike, H.; Kobori, M. The hydroxyflavone, fisetin, suppresses mast cell activation induced by interaction with activated T cell membranes. *Br. J. Pharmacol.*, **2009**, *158*, 907-919.

[146] Chen, C.C.; Chow, M.P.; Huang, W.C.; Lin, Y.C.; Chang, Y.J. Flavonoids inhibit tumor necrosis factor-alpha-induced up-regulation of intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells through activator protein-1 and nuclear factor-kappaB: structure-activity relationships. *Mol. Pharmacol.*, **2004**, *66*, 683-693.

[147] Comalada, M.; Ballester, I.; Bailon, E.; Sierra, S.; Xaus, J.; Galvez, J.; de Medina, F.S.; Zarzuelo, A. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. *Biochem. Pharmacol.*, **2006**, *72*, 1010-1021.

[148] Takano-Ishikawa, Y.; Goto, M.; Yamaki, K. Inhibitory effects of several flavonoids on E-selectin expression on human umbilical vein endothelial cells stimulated by tumor necrosis factor-alpha. *Phytother. Res.*, **2003**, *17*, 1224-1227.

[149] Sharma, V.; Mishra, M.; Ghosh, S.; Tewari, R.; Basu, A.; Seth, P.; Sen, E. Modulation of interleukin-1beta mediated inflammatory response in human astrocytes by flavonoids: implications in neuroprotection. *Brain Res. Bull.*, **2007**, *73*, 55-63.

[150] Yeh, S.L.; Wang, H.M.; Chen, P.Y.; Wu, T.C. Interactions of beta-carotene and flavonoids on the secretion of pro-inflammatory mediators in an *in vitro* system. *Chem. Biol. Interact.*, **2009**, *179*, 386-393.

[151] Tang, D.; Kang, R.; Xiao, W.; Zhang, H.; Lotze, M.T.; Wang, H.; Xiao, X. Quercetin prevents lipopolysaccharide-induced hmgb1 release and proinflammatory function. *Am. J. Respir. Cell Mol. Biol.*, **2009**,

[152] Williams, R.J.; Spencer, J.P.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med.*, **2004**, *36*, 838-849.

[153] Kaneko, M.; Takimoto, H.; Sugiyama, T.; Seki, Y.; Kawaguchi, K.; Kumazawa, Y. Suppressive effects of the flavonoids quercetin and luteolin on the accumulation of lipid rafts after signal transduction *via* receptors. *Immunopharmacol. Immunotoxicol.*, **2008**, *30*, 867-882.

[154] Sugiyama, T.; Kawaguchi, K.; Dobashi, H.; Miyake, R.; Kaneko, M.; Kumazawa, Y. Quercetin but not luteolin suppresses the induction of lethal shock upon infection of mice with Salmonella typhimurium. *FEMS Immunol. Med. Microbiol.*, **2008**, *53*, 306-313.

[155] Nam, N.H. Naturally occurring NF-kappaB inhibitors. *Mini Rev. Med. Chem.*, **2006**, *6*, 945-951.

[156] Ying, B.; Yang, T.; Song, X.; Hu, X.; Fan, H.; Lu, X.; Chen, L.; Cheng, D.; Wang, T.; Liu, D.; Xu, D.; Wei, Y.; Wen, F. Quercetin inhibits IL-1 beta-induced ICAM-1 expression in pulmonary epithelial cell line A549 through the MAPK pathways. *Mol. Biol. Rep.*, **2009**, *36*, 1825-1832.

[157] Lotito, S.B.; Frei, B. Dietary flavonoids attenuate tumor necrosis factor alpha-induced adhesion molecule expression in human aortic endothelial cells. Structure-function relationships and activity after first pass metabolism. *J. Biol. Chem.*, **2006**, *281*, 37102-37110.

[158] Jacobson, K.A.; Moro, S.; Manthey, J.A.; West, P.L.; Ji, X.D. Interactions of flavones and other phytochemicals with adenosine receptors. *Flavonoids in Cell Function*, **2002**, *505*, 163-171.

[159] Schwartz, A.; Sutton, S.L.; Middleton, E. Jr. Quercetin inhibition of the induction and function of cytotoxic T lymphocytes. *Immunopharmacology*, **1982**, *4*, 125-138.

[160] Liu, J.; Li, X.; Yue, Y.; Li, J.; He, T.; He, Y. The inhibitory effect of quercetin on IL-6 production by LPS-stimulated neutrophils. *Cell Mol. Immunol.*, **2005**, *2*, 455-460.

[161] Schneider, C.; Berton, G.; Spisani, S.; Traniello, S.; Romeo, D. Quercetin, a regulator of polymorphonuclear leukocyte (PMNL) functions. *Adv. Exp. Med. Biol.*, **1979**, *121*, 371-379.

[162] Berton, G.; Schneider, C.; Romeo, D. Inhibition by quercetin of activation of polymorphonuclear leucocyte functions. Stimulus-specific effects. *Biochim. Biophys. Acta.*, **1980**, *595*, 47-55.

[163] Park, H.J.; Lee, C.M.; Jung, I.D.; Lee, J.S.; Jeong, Y.I.; Chang, J.H.; Chun, S.H.; Kim, M.J.; Choi, I.W.; Ahn, S.C.; Shin, Y.K.; Yeom, S.R.; Park, Y.M. Quercetin regulates Th1/Th2 balance in a murine model of asthma. *Int. Immunopharmacol.*, **2009**, *9*, 261-267.

[164] Fanning, M.J.; Macander, P.; Drzewiecki, G.; Middleton, E. Jr. Quercetin inhibits anaphylactic contraction of guinea pig ileum smooth muscle 4983. *Int. Arch. Allergy Appl. Immunol.*, **1983**, *71*, 371-373.

[165] Ando, C.; Takahashi, N.; Hirai, S.; Nishimura, K.; Lin, S.; Uemura, T.; Goto, T.; Yu, R.; Nakagami, J.; Murakami, S.; Kawada, T. Luteolin, a food-derived flavonoid, suppresses adipocyte-dependent activation of macrophages by inhibiting JNK activation. *FEBS Lett.*, **2009**, *583*, 3649-3654.

[166] Gutierrez-Venegas, G.; Kawasaki-Cardenas, P.; rroyo-Cruz, S.R.; Maldonado-Frias, S. Luteolin inhibits lipopolysaccharide actions on human gingival fibroblasts. *Eur. J. Pharmacol.*, **2006**, *541*, 95-105.

[167] Mi, L.K.; Lee, K.W.; Jung, S.K.; Lee, E.J.; Heo, Y.S.; Bode, A.M.; Lubet, R.A.; Lee, H.J.; Dong, Z. Kaempferol inhibits UVB-induced COX-2 expression by suppressing Src kinase activity. *Biochem. Pharmacol.*, **2010**,

[168] Lee, J.K.; Kim, S.Y.; Kim, Y.S.; Lee, W.H.; Hwang, D.H.; Lee, J.Y. Suppression of the TRIF-dependent signaling pathway of Toll-like receptors by luteolin. *Biochem. Pharmacol.*, **2009**, *77*, 1391-1400.

[169] Ferriola, P.C.; Cody, V.; Middleton, E. Jr. Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem. Pharmacol.*, **1989**, *38*, 1617-1624.

[170] Kuppusamy, U.R.; Das, N.P. Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem. Pharmacol.*, **1992**, *44*, 1307-1315.

[171] Nagao, A.; Seki, M.; Kobayashi, H. Inhibition of xanthine oxidase by flavonoids. *Biosci. Biotechnol. Biochem.*, **1999**, *63*, 1787-1790.

[172] Pauff, J.M.; Hille, R. Inhibition studies of bovine xanthine oxidase by luteolin, silibinin, quercetin, and curcumin. *J. Nat. Prod.*, **2009**, *72*, 725-731.

[173] Olszanecki, R.; Gebska, A.; Kozlovski, V.I.; Gryglewski, R.J. Flavonoids and nitric oxide synthase. *J. Physiol. Pharmacol.*, **2002**, *53*, 571-584.

[174] Yanoshita, R.; Chang, H.W.; Son, K.H.; Kudo, I.; Samejima, Y. Inhibition of lysoPAF acetyltransferase activity by flavonoids. *Inflamm. Res.*, **1996**, *45*, 546-549.

[175] Kavutcu, M.; Melzig, M.F. *In vitro* effects of selected flavonoids on the 5'-nucleotidase activity. *Pharmazie*, **1999**, *54*, 457-459.

[176] Bormann, H.; Melzig, M.F. Inhibition of metallopeptidases by flavonoids and related compounds. *Pharmazie*, **2000**, *55*, 129-132.

[177] Hwang, J.T.; Kwon, D.Y.; Yoon, S.H. AMP-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. *N. Biotechnol.*, **2009**, *26*, 17-22.

[178] Katalinic, M.; Rusak, G.; Domacinovic, B.J.; Sinko, G.; Jelic, D.; Antolovic, R.; Kovarik, Z. Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. *Eur. J. Med. Chem.*, **2009**,

[179] Agullo, G.; Gamet-Payrastre, L.; Manenti, S.; Viala, C.; Remesy, C.; Chap, H.; Payrastre, B. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. *Biochem. Pharmacol.*, **1997**, *53*, 1649-1657.

[180] Benedek, B.; Kopp, B.; Melzig, M.F. Achillea millefolium L. s.l. -- is the anti-inflammatory activity mediated by protease inhibition? *J. Ethnopharmacol.*, **2007**, *113*, 312-317.

[181] Fernandez-Salguero, P.M.; Hilbert, D.M.; Rudikoff, S.; Ward, J.M.; Gonzalez, F.J. Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. *Toxicol. Appl. Pharmacol.*, **1996**, *140*, 173-179.

[182] Carlson, D.B.; Perdew, G.H. A dynamic role for the Ah receptor in cell signaling? Insights from a diverse group of Ah receptor interacting proteins. *J. Biochem. Mol. Toxicol.*, **2002**, *16*, 317-325.

[183] Elferink, C.J.; Gasiewicz, T.A.; Whitlock, J.P., Jr. Protein-DNA interactions at a dioxin-responsive enhancer. Evidence that the transformed Ah receptor is heteromeric. *J. Biol. Chem.*, **1990**, *265*, 20708-20712.

[184] Schrenk, D. Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. *Biochem. Pharmacol.*, **1998**, *55*, 1155-1162.

[185] Kimura, Y.; Ito, H.; Ohnishi, R.; Hatano, T. Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity. *Food Chem. Toxicol.*, **2010**, *48*, 429-435.

[186] Chan, H.Y.; Chen, Z.Y.; Tsang, D.S.; Leung, L.K. Baicalein inhibits DMBA-DNA adduct formation by modulating CYP1A1 and CYP1B1 activities. *Biomed. Pharmacother.*., **2002**, *56*, 269-275.

[187] Zhang, S.; Qin, C.; Safe, S.H. Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. *Environ. Health Perspect.*, **2003**, *111*, 1877-1882.

[188] Amakura, Y.; Tsutsumi, T.; Sasaki, K.; Nakamura, M.; Yoshida, T.; Maitani, T. Influence of food polyphenols on aryl hydrocarbon receptor-signaling pathway estimated by *in vitro* bioassay. *Phytochemistry*, **2008**, *69*, 3117-3130.

[189] Sergent, T.; Dupont, I.; Van der, H.E.; Scippo, M.L.; Pussemier, L.; Larondelle, Y.; Schneider, Y.J. CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2 cells. *Toxicol. Lett.*, **2009**, *191*, 216-222.

[190] Choi, Y.J.; Arzuaga, X.; Kluemper, C.T.; Caraballo, A.; Toborek, M.; Hennig, B. Quercetin blocks caveolae-dependent pro-inflammatory responses induced by co-planar PCBs. *Environ. Int.*, **2009**,

[191] Chen, Y.; Xiao, P.; Ou-Yang, D.S.; Fan, L.; Guo, D.; Wang, Y.N.; Han, Y.; Tu, J.H.; Zhou, G.; Huang, Y.F.; Zhou, H.H. The simultaneous effects of flavonoid quercetin on the activities of CYP1A2, CYP2A6, NAT2 and XO in healthy volunteers. *Clin. Exp. Pharmacol. Physiol.*, **2009**,

[192] Chen, Y.; Xiao, P.; Ou-Yang, D.S.; Fan, L.; Guo, D.; Wang, Y.N.; Han, Y.; Tu, J.H.; Zhou, G.; Huang, Y.F.; Zhou, H.H. Simultaneous action of the flavonoid quercetin on cytochrome P450 (CYP) 1A2, CYP2A6, N-acetyltransferase and xanthine oxidase activity in healthy volunteers. *Clin. Exp. Pharmacol. Physiol.*, **2009**, *36*, 828-833.

[193] Hamada, M.; Satsu, H.; Ashida, H.; Sugita-Konishi, Y.; Shimizu, M. Metabolites of Galangin by 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Inducible Cytochrome P450 1A1 in Human Intestinal Epithelial Caco-2 Cells and Their Antagonistic Activity toward Aryl Hydrocarbon Receptor. *J. Agric. Food Chem.*, **2010**.

[194] Ashida, H. Suppressive effects of flavonoids on dioxin toxicity. *Biofactors*, **2000**, *12*, 201-206.

[195] Stopper, H.; Schmitt, E.; Kobras, K. Genotoxicity of phytoestrogens. *Mutat.Res.*, **2005**, *574*, 139-155.

[196] Crinnion, W.J. Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Altern. Med. Rev.*, **2010**, *15*, 4-12.

[197] Dertinger, S.D.; Nazarenko, D.A.; Silverstone, A.E.; Gasiewicz, T.A. Aryl hydrocarbon receptor signaling plays a significant role in mediating benzo[a]pyrene- and cigarette smoke condensate-induced cytogenetic damage *in vivo*. *Carcinogenesis*, **2001**, *22*, 171-177.

[198] Bothe, H.; Gotz, C.; Stobbe-Maicherski, N.; Fritsche, E.; Abel, J.; Haarmann-Stemmann, T. Luteolin enhances the bioavailability of benzo(a)pyrene in human colon carcinoma cells. *Arch. Biochem. Biophy.*, **2010**, *498*, 111-118.

[199] Reyes-Hernandez, O.D.; Mejia-Garcia, A.; Sanchez-Ocampo, E.M.; Castro-Munozledo, F.; Hernandez-Munoz, R.; Elizondo, G. Aromatic hydrocarbons upregulate glyceraldehyde-3-phosphate dehydrogenase and induce changes in actin cytoskeleton. Role of the aryl hydrocarbon receptor (AhR). *Toxicology*, **2009**, *266*, 30-37.

[200] Mukai, R.; Shirai, Y.; Saito, N.; Fukuda, I.; Nishiumi, S.; Yoshida, K.I.; Ashida, H. Suppression mechanisms of flavonoids on aryl hydrocarbon receptor-mediated signal transduction. *Arch. Biochem. Biophys.*, **2010**, in press

[201] Fukuda, I.; Mukai, R.; Kawase, M.; Yoshida, K.; Ashida, H. Interaction between the aryl hydrocarbon receptor and its antagonists, flavonoids. *Biochem. Biophys. Res. Commun.*, **2007**, *359*, 822-827.

[202] Lu, Y.F.; Santostefano, M.; Cunningham, B.D.; Threadgill, M.D.; Safe, S. Substituted flavones as aryl hydrocarbon (Ah) receptor agonists and antagonists. *Biochem. Pharmacol.*, **1996**, *51*, 1077-1087.

[203] Ciolino, H.P.; Daschner, P.J.; Yeh, G.C. Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochem. J.*, **1999**, *340* (Pt. 3), 715-722.

[204] Reiners, J.J. Jr.; Clift, R.; Mathieu, P. Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor. *Carcinogenesis*, **1999**, *20*, 1561-1566.

[205] Apetoh, L.; Quintana, F.J.; Pot, C.; Joller, N.; Xiao, S.; Kumar, D.; Burns, E.J.; Sherr, D.H.; Weiner, H.L.; Kuchroo, V.K. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat. Immunol.*, **2010**, in press

[206] Veldhoen, M.; Hirota, K.; Westendorf, A.M.; Buer, J.; Dumoutier, L.; Renauld, J.C.; Stockinger, B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature*, **2008**, *453*, 106-109.

[207] Quintana, F.J.; Basso, A.S.; Iglesias, A.H.; Korn, T.; Farez, M.F.; Bettelli, E.; Caccamo, M.; Oukka, M.; Weiner, H.L. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature*, **2008**, *453*, 65-71.

[208] Esser, C.; Rannug, A.; Stockinger, B. The aryl hydrocarbon receptor in immunity. *Trends. Immunol.*, **2009**, *30*, 447-454.

[209] Stevens, E.A.; Mezrich, J.D.; Bradfield, C.A. The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. *Immunology*, **2009**, *127*, 299-311.

[210] Berryman, L. and Czuprynski, C.J. Flavonoid treatment of murine macrophages does not alter ingestion or intracellular survival of Listeria monocytogenes. *Immunopharmacol. Immunotoxicol.*, **2008**, *30*, 217-226.

[211] Ashida, H.; Nishiumi, S.; Fukuda, I. An update on the dietary ligands of the AhR. *Expert Opin. Drug. Metab. Toxicol.*, **2008**, *4*, 1429-1447.

[212] Bankoti, J.; Burnett, A.; Navarro, S.; Miller, A.K.; Rase, B.; Shepherd, D.M. Effects of TCDD on the fate of naive dendritic cells. *Toxicol. Sci.*, **2010**, *115*, 422-434.

[213] Bankoti, J.; Rase, B.; Simones, T.; Shepherd, D.M. Functional and phenotypic effects of AhR activation in inflammatory dendritic cells. *Toxicol. Appl. Pharmacol.*, **2010**, *246*, 18-28.

[214] Head, J.L.; Lawrence, B.P. The aryl hydrocarbon receptor is a modulator of anti-viral immunity. *Biochem. Pharmacol.*, **2009**, *77*, 642-653.

[215] Lawrence, B.P. Environmental toxins as modulators of antiviral immune responses. *Viral Immunol.*, **2007**, *20*, 231-242.

[216] Cua, D.J.; Tato, C.M. Innate IL-17-producing cells: the sentinels of the immune system. *Na.t Rev. Immunol.*, **2010**, *10*, 479-489.

[217] Kang, H.K.; Ecklund, D.; Liu, M.; Datta, S.K. Apigenin, a non-mutagenic dietary flavonoid, suppresses lupus by inhibiting autoantigen presentation for expansion of autoreactive Th1 and Th17 cells. *Arthritis. Res. Ther.*, **2009**, *11*, R59-

[218] Kawai, M.; Hirano, T.; Higa, S.; Arimitsu, J.; Maruta, M.; Kuwahara, Y.; Ohkawara, T.; Hagihara, K.; Yamadori, T.; Shima, Y.; Ogata, A.; Kawase, I.; Tanaka, T. Flavonoids and related compounds as anti-allergic substances. *Allergol. Int.*, **2007**, *56*, 113-123.

[219] Saraf, A.S.; Oganesyan, E.T. Flavonoids As Potential Antiallergic Agents. *Khimiko-Farmatsevticheskii Zhurnal*, **1991**, *25*, 4-8.

[220] Simons, F.E. 9. Anaphylaxis. *J. Allergy Clin. Immunol.*, **2008**, *121*, S402-S407.

[221] Mukai, K.; Obata, K.; Tsujimura, Y.; Karasuyama, H. New Insights into the Roles for Basophils in Acute and Chronic Allergy. *Allergol. Int.*, **2009**, *58*, 11-19.

[222] Karasuyama, H.; Mukai, K.; Tsujimura, Y.; Obata, K. Newly discovered roles for basophils: a neglected minority gains new respect. *Nat. Rev. Immunol.*, **2009**, *9*, 9-13.

[223] Gibbs, B.F.; Streatfield, C.; Falcone, F.H. Basophils as critical orchestrators of Th2-type immune responses. *Expert. Rev. Clin. Immunol.*, **2009**, *5*, 725-734.

[224] Sokol, C.L.; Medzhitov, R. Role of basophils in the initiation of Th2 responses. *Curr. Opin. Immunol.*, **2010**, *22*, 73-77.

[225] Kawakami, T. Basophils now enhance memory. *Nat. Immunol.*, **2008**, *9*, 720-721.

[226] Denzel A; Maus UA; Gomez MR; Moll C; Niedermeier M; Winter C; Maus R; Hollingshead S; Briles D.E.; Kunz-Schughart Leoni A; Talke Y; Mack M. Basophils enhance immunological memory responses. *Nature*, **2008**, *9*, 733-742.

[227] Maddur, M.S.; Kaveri, S.V.; Bayry, J. Basophils as antigen presenting cells. *Trends. Immunol.*, **2010**, *31*, 45-48.

[228] Gauvreau, G.M.; Plitt, J.R.; Baatjes, A.; MacGlashan, D.W. Expression of functional cysteinyl leukotriene receptors by human basophils. *J. Allergy Clin. Immunol.*, **2005**, *116*, 80-87.

[229] Min, B.; Paul, W.E. Basophils and type 2 immunity. *Curr. Opin. Hematol.*, **2008**, *15*, 59-63.

[230] Stone, K.D.; Prussin, C.; Metcalfe, D.D. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.*, **2010**, *125*, S73-S80.

[231] Yamaguchi, M.; Koketsu, R.; Suzukawa, M.; Kawakami, A.; Iikura, M. Human Basophils and Cytokines/Chemokines. *Allergol. Int.*, **2009**, *58*, 1-10.

[232] Schroeder, J.T.; Chichester, K.L.; Bieneman, A.P. Human basophils secrete IL-3: evidence of autocrine priming for phenotypic and functional responses in allergic disease. *J. Immunol.*, **2009**, *182*, 2432-2438.

[233] Hennersdorf, F.; Florian, S.; Jakob, A.; Baumgartner, K.; Sonneck, K.; Nordheim, A.; Biedermann, T.; Valent, P.; Buhring, H.J. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of ige-dependent upregulation. *Cell Res.*, **2005**, *15*, 325-335.

[234] de Weck, A.L.; Sanz, M.L.; Gamboa, P.M.; Aberer, W.; Bienvenu, J.; Blanca, M.; Demoly, P.; Ebo, D.G.; Mayorga, L.; Monneret, G.; Sainte-Laudy, J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int. Arch. Allergy Immunol.*, **2008**, *146*, 177-189.

[235] Chirumbolo, S.; Vella, A.; Ortolani, R.; De Gironcoli M.; Solero, P.; Tridente, G.; Bellavite, P. Differential response of human basophil activation markers: a multi-parameter flow cytometry approach. *Clin. Mol. Allergy*, **2008**, *6*, 12-

[236] Schroeder, J.T. Basophils beyond effector cells of allergic inflammation. *Adv. Immunol.*, **2009**, *101*, 123-161.

[237] Ehrlich, P. Beitra¨ge zur Kenntnis der granulierten Bindegewebszellen und der eosinophilen Leukocythen. *Arch. Anat. Physiol.*, **1879**, 166-169.

[238] Arinobu, Y.; Iwasaki, H.; Akashi, K. Origin of Basophils and Mast Cells. *Allergol. Int.*, **2009**, *58*, 21-28.

[239] Marone, G.; Triggiani, M.; de, P.A. Mast cells and basophils: friends as well as foes in bronchial asthma? *Trends. Immunol.*, **2005**, *26*, 25-31.

[240] Crivellato, E.; Travan, L.; Ribatti, D. Mast cells and basophils: a potential link in promoting angiogenesis during allergic inflammation. *Int. Arch. Allergy Immunol.*, **2010**, *151*, 89-97.

[241] Igura, K.; Ohta, T.; Kuroda, Y.; Kaji, K. Resveratrol and quercetin inhibit angiogenesis *in vitro*. *Cancer Lett.*, **2001**, *171*, 11-16.

[242] Kong L; Wu K; Lin H Inhibitory effects of quercetin on angiogenesis of experimental mammary carcinoma. *Chin. J. Clin. Oncol.*, **2005**, *2*, 631-636.

[243] Donnini, S.; Finetti, F.; Lusini, L.; Morbidelli, L.; Cheynier, V.; Barron, D.; Williamson, G.; Waltenberger, J.; Ziche, M. Divergent effects of quercetin conjugates on angiogenesis. *Br. J. Nutr.*, **2006**, *95*, 1016-1023.

[244] Jung, M.K.; Hur, D.Y.; Song, S.B.; Park, Y.; Kim, T.S.; Bang, S.I.; Kim, S.; Song, H.K.; Park, H.; Cho, D.H. Tannic acid and quercetin display a therapeutic effect in atopic dermatitis *via* suppression of angiogenesis and TARC expression in Nc/Nga mice. *J. Invest. Dermatol.*, **2010**, *130*, 1459-1463.

[245] Miller, A.L. The etiologies, pathophysiology, and alternative/complementary treatment of asthma. *Altern. Med. Rev.*, **2001**, *6*, 20-47.

[246] Rogerio, A.P.; Dora, C.L.; Andrade, E.L.; Chaves, J.S.; Silva, L.F.; Lemos-Senna, E.; Calixto, J.B. Anti-inflammatory effect of quercetin-loaded microemulsion in the airways allergic inflammatory model in mice. *Pharmacol. Res.*, **2010**, *61*, 288-297.

[247] Foreman, J.C. Mast cells and the actions of flavonoids. *J. Allergy Clin. Immunol.*, **1984**, *73*, 769-774.

[248] Middleton, E. Jr.; Drzewiecki, G.; Krishnarao, D. Quercetin: an inhibitor of antigen-induced human basophil histamine release. *J. Immunol.*, **1981**, *127*, 546-550.

[249] Middleton, E. Jr.; Krishnarao, D.G.; Atkins, D.; Drzewiecki, G. The flavonoids: a brief review and study of effects on antigen-induced histamine release from human basophils. *Trans. Am. Clin. Climatol. Assoc.*, **1981**, *92*, 234-252.

[250] Middleton, E. Jr.; Drzewiecki, G. Effects of flavonoids and transitional metal cations on antigen-induced histamine release from human basophils. *Biochem. Pharmacol.*, **1982**, *31*, 1449-1453.

[251] Middleton, E. Jr.; Drzewiecki, G. Flavonoid inhibition of human basophil histamine release stimulated by various agents. *Biochem. Pharmacol.*, **1984**, *33*, 3333-3338.

[252] Middleton, E. Jr. Effect of flavonoids on basophil histamine release and other secretory systems. *Prog. Clin. Biol. Res.*, **1986**, *213*, 493-506.

[253] Ezeamuzie, I.C.; Assem, E.S. Modulation of the effect of histamine-releasing lymphokine on human basophils. *Agents Actions*, **1984**, *14*, 501-505.

[254] Gong, J.; Chen, S.S. Polyphenolic antioxidants inhibit peptide presentation by antigen-presenting cells. *Int. Immunopharmacol.*, **2003**, *3*, 1841-1852.

[255] Bennett, J.P.; Gomperts, B.D.; Wollenweber, E. Inhibitory effects of natural flavonoids on secretion from mast cells and neutrophils. *Arzneimittelforschung.*, **1981**, *31*, 433-437.

[256] Pruzansky, J.J.; Zeiss, C.R.; Patterson, R. A linear correlation between histamine release and degranulation of human basophils by specific antigen or the ionophore A23187. *Immunology*, **1980**, *40*, 411-416.

[257] Dvorak, A.M.; Newball, H.H.; Dvorak, H.F.; Lichtenstein, L.M. Antigen-induced IgE-mediated degranulation of human basophils. *Lab. Invest.*, **1980**, *43*, 126-139.

[258] de Weck, A.L.; Sanz, M.L.; Gamboa, P.M.; Aberer, W.; Bienvenu, J.; Bianca, M.; Demoly, P.; Ebo, D.G.; Mayorga, L.; Monneret, G.; Sainte-Laudy, J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls: II Technical issues. *J. Invest. Allergol. Clin. Immunol.*, **2008**, *18*, 143-155.

[259] Ogasawara, H.; Fujitani, T.; Drzewiecki, G.; Middleton E Jr The role of hydrogen peroxide in basophil histamine release and the effect of selected flavonoids. *J. Allergy Clin. Immunol.*, **1986**, *78*, 321-328.

[260] Ebo, D.G.; Bridts, C.H.; Hagendorens, M.M.; Aerts, N.E.; De Clerck, L.S.; Stevens, W.J. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B. Clin. Cytom.*, **2008**, *74*, 201-210.

[261] Chirumbolo, S.; Conforti, A.; Ortolani, R.; Vella, A.; Marzotto, M.; Bellavite, P. Stimulus-specific regulation of CD63 and CD203c membrane expression in human basophils by the flavonoid quercetin. *Int. Immunopharmacol.*, **2010**, *10*, 183-192.

[262] Guerin-Marchand, C.; Senechal, H.; Pelletier, C.; Fuher, H.; Olivier, R.; David, B.; Berthon, B. Blank U H2O2 impairs inflammatory mediator release from immunologically stimulated RBL-2H3 cells through a redox-sensitive, calcium-dependent mechanism. *Inflamm. Res.*, **2001**, *50*, 341-349.

[263] Fewtrell, C.M.; Gomperts, B.D. Quercetin: a novel inhibitor of Ca2+ influx and exocytosis in rat peritoneal mast cells. *Biochim. Biophys. Acta.*, **1977**, *469*, 52-60.

[264] Lee, E.; Choi, E.J.; Cheong, H.; Kim, Y.R.; Ryu, S.Y.; Kim, K.M. Anti-allergic actions of the leaves of Castanea crenata and isolation of an active component responsible for the inhibition of mast cell degranulation. *Arch. Pharm. Res.*, **1999**, *22*, 320-323.

[265] Haggag, E.G.; bou-Moustafa, M.A.; Boucher, W.; Theoharides, T.C. The effect of a herbal water-extract on histamine release from mast cells and on allergic asthma. *J. Herb. Pharmacother.*, **2003**, *3*, 41-54.

[266] Pearce, F.L.; Befus, A.D.; Bienenstock, J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J. Allergy Clin. Immunol.*, **1984**, *73*, 819-823.

[267] Passante, E.; Ehrhardt, C.; Sheridan, H.; Frankish, N. RBL-2H3 cells are an imprecise model for mast cell mediator release. *Inflamm. Res.*, **2009**, *58*, 611-618.

[268] Martin, M.W.; O'Sullivan, A.J.; Gomperts, B.D. Inhibition by cromoglycate and some flavonoids of nucleoside diphosphate kinase and of exocytosis from permeabilized mast cells. *Br. J. Pharmacol.*, **1995**, *115*, 1080-1086.

[269] Matsushima, M.; Takagi, K.; Ogawa, M.; Hirose, E.; Ota, Y.; Abe, F.; Baba, K.; Hasegawa, T.; Hasegawa, Y.; Kawabe, T. Heme oxygenase-1 mediates the anti-allergic actions of quercetin in rodent mast cells. *Inflamm. Res.*, **2009**, *58*, 705-715.

[270] Hayes, N.A.; Foreman, J.C. The activity of compounds extracted from feverfew on histamine release from rat mast cells. *J. Pharm. Pharmacol.*, **1987**, *39*, 466-470.

[271] Kurose, M. Inhibition of anaphylactic histamine release from heterologously sensitized mast cells: differential effects of drugs which interfere with calcium influx. *Acta. Med. Okayama.*, **1981**, *35*, 307-317.

[272] Saeki, K.; Kurose, M. Histamine release from rat mast cells sensitized with mouse antiserum. *Agents. Actions.*, **1981**, *11*, 98-100.

[273] Walker, E.H.; Pacold, M.E.; Perisic, O.; Stephens, L.; Hawkins, P.T.; Wymann, M.P.; Williams, R.L. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol. Cell.*, **2000**, *6*, 909-919.

[274] Ennis, M.; Truneh, A.; White, J.R.; Pearce, F.L. Inhibition of histamine secretion from mast cells. *Nature*, **1981**, *289*, 186-187.

[275] White, J.R.; Pearce, F.L. Effect of anti-allergic compounds on anaphylactic histamine secretion from rat peritoneal mast cells in the presence and absence of exogenous calcium. *Immunology*, **1982**, *46*, 361-367.

[276] White, J.R.; Pearce, F.L. Effect of anti-allergic and cyclic AMP-active drugs on histamine secretion from rat mast cells treated with the novel calcium ionophore chlortetracycline. *Int. Arch. Allergy Appl. Immunol.*, **1983**, *71*, 352-356.

[277] Parker, W.L.; Martz, E. Calcium ionophore A23187 as a secretagogue for rat mast cells: does it bypass inhibition by calcium flux blockers? *Agents Actions*, **1982**, *12*, 276-284.

[278] Walker, E.H.; Pacold, M.E.; Perisic, O.; Stephens, L.; Hawkins, P.T.; Wymann, M.P.; Williams, R.L. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, ly294002, quercetin, myricetin, and staurosporine. *Mol. Cell*, **2000**, *6*, 909-919.

[279] Rommel, C.; Camps, M.; Ji, H. PI3Kd and PI3Kg: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nature Rev. Immunol.*, **2007**, *7*, 191-201.

[280] Miura, K.; Macglashan, D.W.Jr. Expression of protein kinase C isozymes in human basophils: regulation by physiological and nonphysiological stimuli. *Blood*, **1998**, *92*, 1206-1218.

[281] Warner, J.A.; Macglashan, D.W. Jr. Protein kinase C (PKC) changes in human basophils. IgE-mediated activation is accompanied by an increase in total PKC activity. *J. Immunol.*, **1989**, *142*, 1669-1677.

[282] Viard, P.; Butcher, A.J.; Halet, G.; Davies, A.; Nurnberg, B.; Heblich, F.; Doplhin, A.C. PI3K promotes voltage-dependent calcium channel trafficking to the plasma membrane. *Nat. Neurosci.*, **2004**, *7*, 939-946.

[283] Navarro-Nunez, L.; Lozano, M.L.; Martinez, C.; Vicente, V.; Rivera, J. Effect of quercetin on platelet spreading on collagen and fibrinogen and on multiple platelet kinases. *Fitoterapia*, **2010**, *81*, 75-80.

[284] Arcaro, A.; Wymann, M.P. Wortmannin is a potent phosphoinositide-3 kinase inhibitor: the role of phosphatidylinositol-3,4,5 triphosphate in neutrophil responses. *Biochem. J.*, **1993**, *296*, 297-301.

[285] Brock, C.; Schaefer, M.; Reusch, H.P.; Czupalla, C.; Michalke, M.; Spicher, K.; Schultz, G.; Nurnberg, B. Roles of Gbetagamma in membrane recruitment and activation of p110gamma/p101 phosphoinositide 3-kinase gamma. *J. Cell. Biol.*, **2003**, *160*, 89-99.

[286] Mocsai, A.; Jakus, Z.; Vantus, T.; Berton, G.; Lowell, C.A.; Ligeti, E. Kinase pathways in chemoattractant-induced degranulation of neutrophils: the role of p38 mitogen-activated protein kinase activated by Src family kinases. *J. Immunol.*, **2000**, *164*, 4321-4331.

[287] Woo, C.S.; Chan, W.; Sampson, H.A.; Shreffler, W.G. Cell-by-cell characterization of signaling and activation marker upregulation during basophil stimulation and non specific desensitization. *J. All. Clin. Immunol.*, **2009**, *123*, S199-post 764.

[288] Bergstrand, H.; Lundquist, B. Human basophil histamine release is differently affected by inhibitors of calmodulin, diacylglycerol

kinase and peptidyl prolyl cis-trans isomerase in a secretagogue specific manner. *Allergy*, **2007**, *47*, 353-361.

[289] Nishino, H.; Naitoh, E.; Iwashima, A.; Umezawa, K. Quercetin interacts with calmodulin, a calcium regulatory protein. *Experientia*, **1984**, *40*, 184-185.

[290] Dvorak, A.M. Ultrastructural studies of human basophils and mast cells. *J. Histochem. Cytochem.*, **2005**, *53*, 1043-1070.

[291] Johri, R.K.; Zutshi, U.; Kameshwaran, L.; Atal, C.K. Effect of quercetin and Albizzia saponins on rat mast cell. *Indian J. Physiol. Pharmacol.*, **1985**, *29*, 43-46.

[292] Wang, Y.Z.; Palmer, J.M.; Cooke, H.J. Neuroimmune regulation of colonic secretion in guinea pigs. *Am. J. Physiol.*, **1991**, *260*, G307-G314.

[293] Castellani, M.L.; Kempuraj, D.; Frydas, S.; Theoharides, T.C.; Simeonidou, I.; Conti, P.; Vecchiet, J. Inhibitory effect of quercetin on tryptase and MCP-1 chemokine release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line. *Neuroimmunomodulation.*, **2006**, *13*, 179-186.

[294] Kempuraj, D.; Castellani, M.L.; Petrarca, C.; Frydas, S.; Conti, P.; Theoharides, T.C.; Vecchiet, J. Inhibitory effect of quercetin on tryptase and interleukin-6 release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line. *Clin. Exp. Med.*, **2006**, *6*, 150-156.

[295] Alexandrakis, M.; Singh, L.; Boucher, W.; Letourneau, R.; Theofilopoulos, P.; Theoharides, T.C. Differential effect of flavonoids on inhibition of secretion and accumulation of secretory granules in rat basophilic leukemia cells. *Int. J. Immunopharmacol.*, **1999**, *21*, 379-390.

[296] Trnovsky, J.; Letourneau, R.; Haggag, E.; Boucher, W.; Theoharides, T.C. Quercetin-induced expression of rat mast cell protease II and accumulation of secretory granules in rat basophilic leukemia cells. *Biochem. Pharmacol.*, **1993**, *46*, 2315-2326.

[297] Trnovsky, J.; Letourneau, R.; Theoharides, T.C. Quercetin Induces Differentiation Maturation of Rat Basophilic Leukemia (Rbl) Cells. *Faseb. J.*, **1991**, *5*, A1006-A1006.

[298] Segawa, S.; Yasui, K.; Takata, Y.; Kurihara, T.; Kaneda, H.; Watari, J. Flavonoid glycosides extracted from hop (Humulus lupulus L.) as inhibitors of chemical mediator release from human basophilic KU812 cells. *Biosci. Biotechnol. Biochem.*, **2006**, *70*, 2990-2997.

[299] Xu, F.; Matsuda, H.; Hata, H.; Sugawara, K.; Nakamura, S.; Yoshikawa, M. Structures of new flavonoids and benzofuran-type stilbene and degranulation inhibitors of rat basophilic leukemia cells from the Brazilian herbal medicine Cissus sicyoides. *Chem. Pharm. Bull. (Tokyo)*, **2009**, *57*, 1089-1095.

[300] Senyshyn, J.; Baumgartner, R.A.; Beaven, M.A. Quercetin sensitizes RBL-2H3 cells to polybasic mast cell secretagogues through increased expression of Gi GTP-binding proteins linked to a phospholipase C signaling pathway. *J. Immunol.*, **1998**, *160*, 5136-5144.

[301] Zussman, A.; Sagi-Eisenberg, R. Stimulation of Ca(2+)-dependent exocytosis and release of arachidonic acid in cultured mast cells (RBL-2H3) by quercetin. *Int. J. Immunopharmacol.*, **2000**, *22*, 747-754.

[302] Chahdi, A.; Fraundorfer, P.F.; Beaven, M.A. Compound 48/80 activates mast cell phospholipase D *via* heterotrimeric GTP-binding proteins. *J. Pharmacol. Exp. Ther.*, **2000**, *292*, 122-130.

[303] Chakravarty, N. The role of plasma membrane Ca++-Mg++ activated adenosine triphosphatase of rat mast cells on histamine release. *Acta. Pharmacol. Toxicol. (Copenh)*, **1980**, *47*, 223-235.

[304] Chandler, D.; Woldu, A.; Rahmadi, A.; Shanmugam, K.; Steiner, N.; Wright, E.; avente-Garcia, O.; Schulz, O.; Castillo, J.; Munch, G. Effects of plant-derived polyphenols on TNF-alpha and nitric oxide production induced by advanced glycation endproducts. *Mol. Nutr. Food Res.*, **2010**, *54 Suppl 2*, S141-S150.

[305] Harrison, A.P.; Cooper, R.G. Quercetin: health benefits with relevance to TNF-alpha linked inflammatory diseases. *J. Preclin. Clin. Res*., **2008**, *2 (2)*, 135-139.

Received: July 13, 2009 **Revised: August 12, 2010** **Accepted: August 18, 2010**