# The Role of Flavonoids as Modulators of Inflammation and on Cell Signaling Pathways


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## Abbreviations

| | |
|---|---|
| 4′-HW | 4′-hydroxywogonin |
| 5B | (E)-3-(3,4-dimethoxyphenyl)-1-(5-hydroxy-2,2-dimethyl-2H-chromen-6-yl) prop-2-en-1-one |
| 67LR | 67-kDa laminin receptor |
| AA | Arachidonic acid |
| Afla | Amentoflavone |
| Akt | Protein kinase B |
| Alp | Alpinetin |
| Amp | Ampelopsin |
| AMPK | Adenosine monophosphate-activated protein kinase |
| AP-1 | Activator protein-1 |
| Api | Apigenin |
| Ast | Astragalin |
| BBB | Blood-brain barrier |
| BMDM | Bone marrow-derived macrophages |
| C3G | Cyanidin-3-O-glucoside |
| CAMs | Cell surface adhesion molecules |
| CAT | Catalase |
| Cat | Catechin |
| Chr | Chrysin |
| CNS | Central nervous system |
| COMT | Catechol-O-methyltransferase |
| COX | Cyclooxygenase |

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|---|---|
| Dai | Daidzein |
| DMH | 1,2-dimethyl hydrazine |
| DNA | Deoxyribonucleic acid |
| EGCG | Epigallocatechin-3-gallate |
| EGF | Epidermal growth factor |
| eNOS | Endothelial nitric oxide synthase |
| EpRE | Electrophile-responsive element |
| ERK | Extracellular signal-regulated kinases |
| Eup | Eupatilin |
| Fis | Fisetin |
| FlkA | Flavokawain A |
| Gen | Genisteína |
| GEN-27 | 5-hydroxy-7-[2-hydroxy-3-(piperidin-1-yl) propoxy]-3-{4-[2-hydroxy-3-(piperidin-1-yl) propoxy] phenyl}-4H-chromen-4-one |
| GPx | Glutathione peroxidase |
| HaCaT cells | Human keratinocytes |
| hAs | Human astrocytes |
| hBMEC | Injured human brain microvascular endothelial cell |
| HCT116 | Human colon tumour |
| HGF | Human gingival fibroblasts |
| HIF-1α | Hypoxia-inducible factor 1-α |
| HMGB | High-mobility group box |
| HMGB1 | High-mobility group box 1 protein |
| HO-1 | Heme oxygenase-1 |
| hPBMCs | Human peripheral blood mononuclear cells |
| HUVEC | Human umbilical vein endothelial cell |
| Ibc | Isobavachalcone |
| Ica | Icariin |
| ICAM | Intercellular adhesion molecule |
| ICT | 3,5,7-trihydroxy-4′-methoxy-8-(3-hydroxy-3-methylbutyl)-flavone |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IKK | IκB kinase |
| IL | Interleukin |
| iNOS | Inducible nitric oxide synthase |
| IRAK | IL-1 receptor-associated kinase |
| IκB | Inhibitor of kappa-B |
| JAK | Janus kinase |
| JNK | c-Jun N-terminal kinases |
| L2H17 | 1-(3,4-Dihydroxyphenyl)-3-(2-methoxyphenyl)prop-2-en-1-one |
| LicoC | Licochalcone C |
| LOX | Lypooxygenase |
| LPH | Lactase phlorizin hydrolase |
| LPS | Lipopolysaccharide |
| LT | Leukotriene |

| | |
|---|---|
| Lut | Luteolin |
| Mal | Malvidin |
| Mal3OG | Malvidin-3-O-glucoside |
| MALP-2 | Macrophage-activating lipopeptide 2-kDa |
| MAPK | Mitogen-activated protein kinase |
| MCAO | Middle cerebral artery occlusion |
| MCP | Monocyte chemoattractant protein |
| MIP | Macrophage inflammatory protein |
| mMEC | Mouse mammary epithelial cell |
| MMP | Matrix metalloproteinase |
| MPO | Myeloperoxidase |
| mRNA | Messenger ribonucleic acid |
| Nag | Naringin |
| Nar | Naringenin |
| NF-κB | Nuclear factor kappa B |
| nNOS | Neuronal NOS |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| Nrf2 | Nuclear factor-erythroid-related factor 2 |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| Ono | Ononin |
| OroA | Oroxylin A |
| OVA | Ovalbumin |
| PAI-1 | Plasminogen activator inhibitor 1 |
| PCB | Polychlorinated biphenyl |
| PDGF | Platelet-derived growth factor |
| Pel | Pelargonidin |
| Peo | Peonidin |
| PG | Prostaglandin |
| Phl | Phloretin |
| PI3K | Phosphatidylinositol-3 kinase |
| Pin | Pinocembrin |
| PKC | Protein kinase C |
| poly[I:C] | Polyriboinosinic polyribocytidylic acid |
| PPAR | Peroxisome proliferator-activated receptor |
| Pru | Prunetin |
| Pue | Puerarin |
| Quer | Quercetin |
| RAGE | Receptor for advanced glycation end products |
| RANTES | Regulated upon activation normal T-cell expressed and secreted |
| ROS | Reactive oxygen species |
| Rut | Rutin |
| SG | Sophoraflavanone |
| SIRT | Sirtuin |
| SOCS | Suppressors of cytokine signaling |

| | |
|---|---|
| SOD | Superoxide dismutase |
| STATs | Signal transducer and activator of transcription |
| SULTs | Sulfotransferases |
| TACR-1 | Tachykinin receptor 1 |
| Tax | Taxifolin |
| TBARS | Thiobarbituric acid reactive substances |
| TGF | Tumour growth factor |
| TLR | Toll-like receptor |
| TNF-α | Tumour necrosis factor-α |
| Tollip | Toll-interacting protein |
| Tri | Tricin |
| TX | Thromboxane |
| UgoM | Ugonin M |
| UGTs | Uridine 5′-diphospho-glucuronosyltransferases |
| UV | Ultraviolet |
| VCAM | Vascular cell adhesion molecule |
| VEGF | Vascular endothelial growth factor |
| Vel | Velutin |
| Vix | Vitexin |
| Won | Wogonin |

## 1 Introduction

Flavonoids are naturally occurring polyphenolic compounds widely distributed in the plant kingdom and found in all vascular plants. Not only are they present in plant organs such as flowers, fruits, barks, roots and seeds but also in different products including tea and wine (Middleton and Kandaswami 1994).

These compounds give the flowers the yellow, orange, blue and red colours. Flavonoids play a role in the plant growth; they act as visual attractors for pollination and protect plants against stressor factors such as ultraviolet radiation and the attack of insects and microorganisms (Hassan and Mathesius 2012). Flavonoids are low molecular weight compounds having a benzo-γ-pyrone moiety in their structure and are synthetised through the phenylpropanoid pathway. Their function has been demonstrated to be highly structure-dependent (Bakhtiari et al. 2017).

The chemical structure of flavonoids is based on a 15-carbon skeleton constituted by two benzene rings (A and B) which are linked via a heterocyclic pyrane ring (C). Flavonoids are mainly found either as aglycones (their basic structure), as glycosides or as methylated derivatives. Based on the different substitution and the oxidation pattern of ring C, flavonoids are classified into different subclasses: flavones, flavonols, flavanols, flavanones, isoflavones, anthocyanidins, chalcones and flavanonols (Fig. 1). The hydroxyl group substitution often occurs at C-3, C-5, C-7, C-3′, C-4′ and C-5′. When glycosides are formed, the glycosidic linkage is normally located at positions 3 or 7, and the carbohydrate can be rhamnose, glucose,

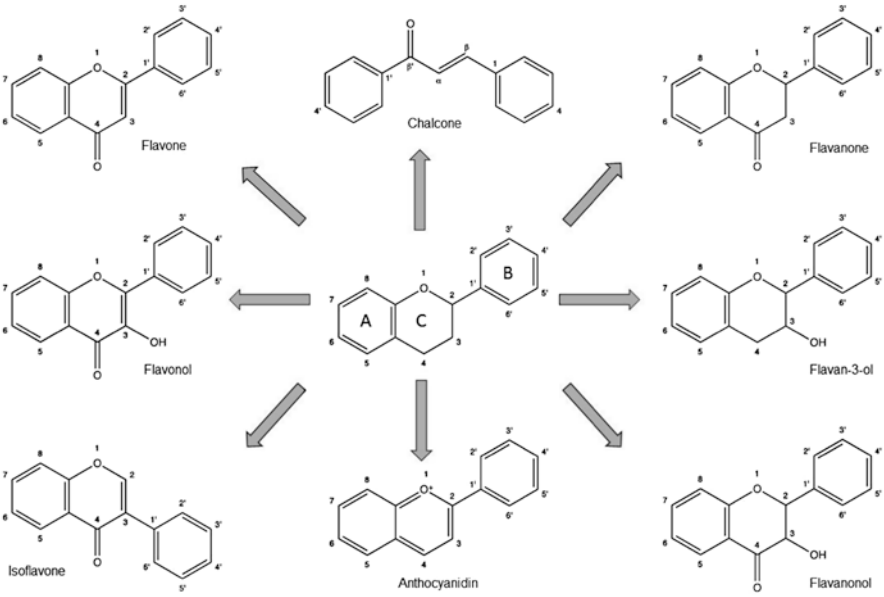


**Fig. 1** Chemical structures of the main classes of flavonoids

glucorhamnose, galactose or arabinose (Xiao 2017). Although they are not regarded as nutrients, flavonoids are important constituents of the human diet, being flavonols, flavones, anthocyanidins, catechins, flavanones and isoflavones the major subgroups. Flavonols are mainly present in leafy vegetables, apples, onions, broccoli and berries. Flavones and anthocyanidins are found in relatively small quantities in grains, leafy vegetables and herbs. Catechins are abundant in tea, apples, grapes, chocolate and red wine. Flavanones are found in citrus fruit, and isoflavones are mainly found in soybeans (Wang et al. 2009). It is estimated that the total number of flavonoids known is around 8000 (Bode and Dong 2013), and this number is increasing considering their great structural diversity.

The interest in the biological activities of these compounds arose around 1930 when a mixture of the flavonoids eriodictyol and hesperidin called citrin (isolated from *Citrus* spp. juice) was found to have vitamin-like activity and was designated as 'vitamin P'. This term was coined to indicate that this mixture decreased capillary permeability, prolonged the life span of Guinea pigs and reduced the signs of hypovitaminosis C in scorbutic experimental animals. Later on, the term vitamin P was abandoned because these compounds did not meet the requirements to be considered a vitamin (Middleton and Kandaswami 1994). When flavonoids were determined as the compounds responsible for these biological activities, research studies were undertaken in order to isolate such compounds and to study their mechanism of action.

In the late 1980s, the research on flavonoids received an additional impulse with the discovery of a phenomenon known as the 'French paradox'. Epidemiological studies indicate that French people have a relatively low incidence of cardiovascular disease and increased longevity while having a diet rich in saturated fats. This finding correlated with a diet replete in flavonoid-rich foods in association with red wine consumption. It has been suggested that the flavonoid intake is inversely correlated with mortality due to coronary heart disease (Formica and Regelson 1995; Knekt et al. 1996).

Flavonoids have long been recognized to possess a broad spectrum of biological activities such as antioxidant, anti-inflammatory, hepatoprotector, antibacterial, antiviral, antidiabetic, antiproliferative and anticarcinogenic (Chen et al. 2017). Epidemiological studies have indicated that a high dietary intake of flavonoids is associated with a decreased risk of a wide range of diseases including cardiovascular disease (Kuriyama et al. 2006). In this sense, flavonoids may influence lipid metabolism by inhibiting low-density lipoprotein oxidation, thus reducing atherosclerotic lesion formation. They are also known to inhibit platelet aggregation, to decrease vascular cell adhesion molecule expression, to improve endothelial function and to reduce blood pressure (Vauzour et al. 2010). Their consumption has also been associated with a reduced risk of lung cancer, breast cancer, renal cancer, non-Hodgkin's lymphoma and colorectal cancer (Fink et al. 2007; Frankenfeld et al. 2008; Gerd et al. 2008; Tang et al. 2009), better cognitive outcomes and with a reduced risk of dementia (Letenneur et al. 2007; Commenges et al. 2000). According to Williamson (2017), in the 1990s, the antioxidant activity of polyphenols was considered a panacea. However, over the last two decades, the attention has been focused on the concept of flavonoids as potential modulators of intracellular signaling cascades that are vital for cell functioning.

## 2 Absorption and Metabolism of Flavonoids

It is estimated that the daily intake of flavonoids contributed by the diet ranges from 50 to 800 mg/day (Pietta 1998, 2000), though some authors state that it can be up to 1 g (Middleton and Kandaswami 1994). However, the amount of polyphenols that should be consumed to derive maximum benefit is difficult to estimate. A cup of green tea or a glass of red wine can provide up to 200 mg of total flavonoids: one onion, 40 mg/100 g; a green salad, 1 mg/100 g; one apple, 6–10 mg; a peach, 1–2 mg; and an orange, 10 mg (Pietta 1998). In the UK, the mean intake of flavonols per day (mainly present in tea, cocoa, apples and broad beans) is 590 mg/day, and the intake of flavanones (citrus fruit) and flavonols (tea, apples or onions) is 25 and 61 mg/day, respectively. However, the intakes are dependent on individual diets and are highly variable (Williamson 2017).

The absorption of dietary flavonoids may depend upon the structure of the flavonoid (i.e. glycosides or aglycones), molecular size, molecular configuration, lipophilicity, solubility and pKa (Kumar and Pandey 2013). The absorption of these compounds may take place either in the small intestine, which is an efficient route that leads to high plasma levels or in the colon. Aglycones can be absorbed by the small intestine, while glycosides are considered too hydrophilic to be absorbed by passive diffusion in this site. Some flavonoid glycosides are enzymatically hydrolysed by either lactase-phlorizin hydrolase (LPH) or by β-glucosidase, and then the aglycones enter epithelial cells by passive diffusion.

However, those glycosides which are not substrates for these enzymes (e.g. flavonoids linked to a rhamnose moiety) are transported to the colon where the intestinal microflora degrade them to simple phenolic acids, which may be absorbed and further metabolized in the liver. The enzymatic deglycosylation driven by LPH and β-glucosidase is recognized as the first and determinant step in the absorption of flavonoids (known as phase I deglycosylation). In this sense, pharmacokinetic data suggest that quercetin (*Quer*) glucoside is absorbed in the small intestine, whereas quercetin rutinoside is absorbed in the colon after deglycosylation, showing that the presence of the sugar moiety determines the site of absorption (Day et al. 2000; Marín et al. 2015). However, barely 5–10% of total flavonoids may be absorbed in the small intestine, while unabsorbed flavonoids reach the colon to be excreted in the faeces (Gleichenhagen and Schieber 2016).

Taking into account that the absorption capacity of the colon is far less efficient than that of the small intestine, only a minimum absorption of these glycosides is to be expected. According to Hollmann (2004), two compartments are to be considered in the metabolism of flavonoids: the first one comprising the small intestine, the liver and the kidneys, and the other one, the colon. Flavonoids that are unabsorbable in the small intestine and flavonoids that have been absorbed and then secreted with bile will ultimately reach the colon.

Once absorbed (in either the small intestine or the colon), the metabolism of flavonoids is dominated by phase II enzymes, such as catechol-O-methyltransferase (COMT),sulfotransferases(SULTs)anduridine5′-diphospho-glucuronosyltransferases (UGTs). UGTs are the major contributors, followed by SULTs and COMT (Chen et al. 2014). Flavonoid metabolites enter the bloodstream by the portal vein and are transported to the liver, where they may undergo further phase II transformations, and then are transported back to the bloodstream to be secreted in urine (Kumar and Pandey 2013; Marín et al. 2015) (Fig. 2). The complexity of the flavonoid metabolism implies that after their consumption, a wide variety of metabolites can be generated. Thus, the bioactive forms of flavonoids are not those found in plants, such as the glycosides or aglycones. Instead, circulating glucuronides, sulphates and O-methylated derivatives (formed only with flavonoids bearing a catechol B-ring) are believed to be those most likely to exert the biological effects and express beneficial effects in humans and animals (Spencer et al. 2001, 2003, 2004).

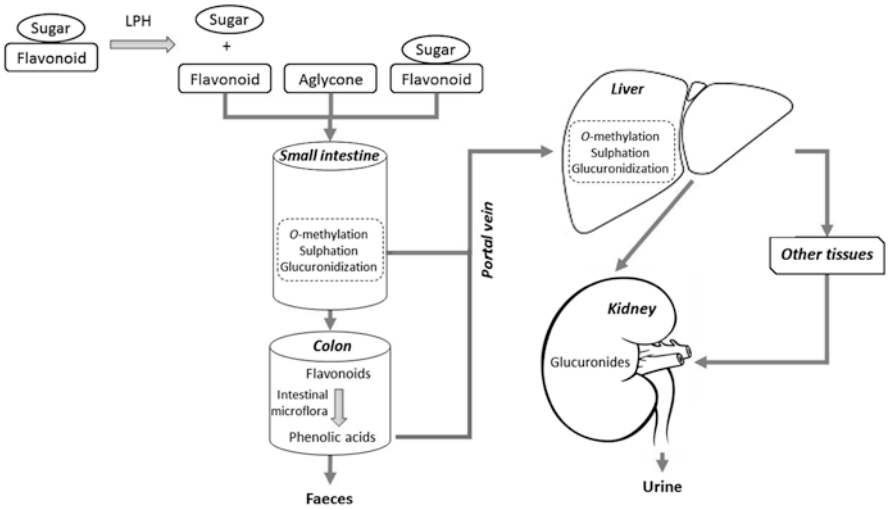


**Fig. 2** Schematic diagram of the absorption and metabolism of flavonoids in humans. Aglycones can be absorbed in the small intestine. Flavonoids glycosides may be deglycosilated by LPH, β-glucosidase or intestinal microflora to aglycones and simple phenolic acids, respectively. The aglycones and phenolic acids enter the portal vein and are further metabolized in the liver

In this sense, Jaeger et al. (2017) have stated that it is important to use flavonoid metabolites when the mechanisms of action are studied in vitro. They also stated that the limited concentration of dietary flavonoid metabolites present in the circulation following ingestion and the key role played by the gut microbiota in the biotransformation of flavonoids in humans should also be taken into consideration.

## 3 Inflammation

Inflammation is a complex host response of body tissues to harmful stimuli, such as pathogens (bacteria, fungi and viruses), trauma or toxic compounds. It is a protective response involving host's cells, blood vessels, proteins and other molecular mediators (Kumar et al. 2013). The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and damaged tissues and initiate tissue repair. The inflammatory status involves endothelial and epithelial cells, neutrophils, monocytes, macrophages and lymphocytes. The local and recruited cells are stimulated to release numerous mediators that amplify the inflammatory response and recruit additional cells (Firestein 2012). There are two types of inflammation, i.e. acute and chronic. Acute inflammation is the initial response of the body to harmful stimuli. It has a rapid onset

and is short-lived (few hours or a few days). It is characterized by the release of numerous chemical mediators, fluid and plasma protein exudation and the migration of leukocytes (Kumar et al. 2013). When the stimulus persists, chronic inflammation may develop, which may be more insidious and long-lasting (weeks to months).

Chronic inflammation is characterized by simultaneous tissue destruction, mainly induced by the products secreted by inflammatory cells, and tissue repair involving vessel proliferation. A wide range of progressive diseases, including rheumatoid arthritis, asthma, atherosclerosis, neurological diseases and cancer, are related to chronic inflammation (Ribeiro et al. 2015).

The inflammatory response is characterized by the coordinated activation of various signaling pathways that regulate the expression of both pro- and anti-inflammatory mediators in resident tissue cells and leukocytes recruited from the blood. During the inflammatory process, mediators such as histamine, serotonin, prostaglandins (PGs), leukotrienes (LTs), platelet-derived growth factor (PDGF), reactive oxygen species (ROS), nitric oxide (NO), cytokines and chemokines may either be produced locally by cells (tissue macrophages, mast cells, endothelial cells or leucocytes) at the site of inflammation or may be derived from circulating inactive precursors that become activated in situ (complement proteins and kinins) (Kumar et al. 2013; Agati et al. 2012).

During inflammation, macrophages are activated by interferon gamma (IFN-γ), complement, immune complexes, lipopolysaccharide (LPS) and cytokines, such as interleukin (IL)-1β, tumour necrosis factor alpha (TNF-α) and IL-6. LPS initiates a signaling cascade through its interaction with Toll-like receptor 4 (TLR4) (Lu et al. 2008). Activated macrophages also produce inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-12 and chemokines, such as IL-8, monocyte chemoattractant proteins (MCP-1 and MCP-2), complement cascade proteins, $PGE_2$, thromboxane (TX) $A_2$ and leukotrienes (LTB4) that contribute to the propagation of inflammation (Ribeiro et al. 2015). The activation of the signaling pathway leads to the release of the nuclear factor kappa B (NF-κB), which activates genes associated with the transcription of proteins related to the inflammatory process, such as inducible nitric oxide synthase (iNOS), which is the enzyme involved in NO synthesis, cyclooxygenases (COXs) and cytokines such as TNF-α, IL-6 and IL-1β.

The activator protein 1 (AP-1) is another transcription factor that responds to a wide variety of stimuli, such as bacterial and virus infection, stress and growth factors. This factor is important during the inflammatory response since it regulates gene expression of pro-inflammatory mediators, including cytokines (Shaulian and Karin 2001). Thus, the suppression of the expression of these pro-inflammatory mediators allows the amelioration and serves as a key mechanism to prevent and control inflammation (Agati et al. 2012; Fan et al. 2017).

## *3.1 Inflammatory Mediators*

### 3.1.1 Nitric Oxide

NO is a highly reactive free radical produced by many cell types which is involved in the regulation of the inflammatory cascade. Such regulation includes not only its own production by immunocompetent cells but also the recruitment of leukocytes. NO is synthesized from L-arginine by nitric oxide synthase (NOS), which exists in three different isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and iNOS. While a small amount of NO, synthesized by nNOS and eNOS, is essential for the normal functioning of the organism, when NO is synthesized in considerable amounts by iNOS, it participates in inflammatory processes acting synergistically with other inflammatory mediators (Nathan 1992; Tuñón et al. 2009). The activity of iNOS is induced by IL-1β, TNF-α, IFN-α, viral antigens, bacteria, protozoa and fungi, as well as by a low oxygen tension and a low environmental pH.

### 3.1.2 Arachidonic Acid Metabolites

Eicosanoids derive from the metabolism of arachidonic acid (AA) and comprise PGs, LTs, TXs and lipoxins. They play a vital role in physiologic and pathologic processes in immunity and inflammation (Zurier 2013). AA metabolites can mediate every step of inflammation, and agents that inhibit their synthesis diminish inflammation. The arachidonate metabolism is mediated by COX isoenzymes and by lipoxygenases (LOXs). Products of the COX pathway include PGs and TXA and are produced by COX-1 and COX-2. The former is produced in response to inflammatory stimuli and is expressed in many tissues (endothelium, monocytes, platelets, renal collecting tubules and seminal vesicles) and participates in the synthesis of PGs, which regulate physiological processes in response to hormones and other stimuli (Smith and Langenbach 2001). COX-2 is expressed primarily in cells involved in inflammation (macrophages, fibroblasts and endothelial cells), and its expression is induced by various stimuli, including PDGF and epidermal growth factor (EGF) and pro-inflammatory cytokines (IL-1β and TNF-α) (Ribeiro et al. 2015). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen inhibit COX activity, thereby blocking the PGs synthesis.

The synthesis of LTs involves multiple steps and is produced by 5-LOX, the major AA-metabolizing enzyme in neutrophils (Kumar et al. 2013). LOXs are responsible for the generation of hydroxyl acids and LTs from AA. There are three distinct LOX isozymes, namely, 5-LOX, 12-LOX and 15-LOX. $LTB_4$ is produced by neutrophils and is a potent chemotactic agent for neutrophils. $LTC_4$, $LTD_4$ and $LTE_4$ are produced mainly in mast cells. These mediators cause bronchoconstriction and increase vascular permeability (Kumar et al. 2013).

### 3.1.3 Cytokines

Cytokines are proteins that are mainly produced by activated lymphocytes and macrophages. They are the major mediators of local and intercellular communications that are required for an integrated response to a variety of stimuli (Tuñón et al. 2009). The production and secretion of cytokines are transcriptionally regulated. Their major role is the regulation of the intensity and duration of the inflammatory response. The expression of cytokines may be triggered by different stimuli such as trauma, stress, ischemia, ultraviolet light, microbes, local complement activation, ROS and nitrogen species and cytokines themselves working in autocrine loops (Ribeiro et al. 2015). The main cytokines involved in the inflammatory response are TNF-α and IL-1. These cytokines induce the expression of adhesion molecules in endothelial tissue and participate in the synthesis of other cytokines, such as IL-6, chemokines (IL-8 and MCP-1), growth factors, eicosanoids and NO (Kumar et al. 2013).

Cytokines related to acute inflammation are IL-1, TNF-α, IL-6, IL-11, IL-8, IL-16 and IL-17, among others. These cytokines usually act locally, and they mediate multiple effects, mainly leukocyte recruitment and migration. Cytokines involved in chronic inflammation are those mediating humoral responses, like IL-4, IL-5, IL-6, IL-7 and IL-13, whereas cellular responses are usually governed by IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, IFN-γ, transforming growth factor (TGF)-β and TNF-α (Feghali and Wright 1997). Among these mediators, IL-1, TNF-α and IL-6 are the most studied cytokines involved in chronic inflammation-related diseases. There are different cytokine structurally related receptors that mediate cytokine communication, i.e. type I and type II cytokine receptors, TNF receptor, chemokine receptors, TGF-$\beta$ receptor and a Toll/IL-1 receptor. After binding to the receptors, cytokines mediate their effects through the activation of several intracellular signaling pathways, such as the Janus kinases (JAK) and their downstream transcriptional factors, including the signal transducers and activators of transcription (STATs), phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinases (MAPKs) signaling cascades and the NF-κB pathway. Once the signaling cascade is initiated, several transcription factors such as NF-κB, AP-1 and nuclear factor of activated T cells can be recruited to the cytokine promoter region (Ribeiro et al. 2015). There are evidences suggesting that inflammatory cytokines have potential as therapeutic targets to treat inflammatory diseases. In this sense, several drugs such as etanercept and infliximab and anakinra have been developed as inhibitors of TNF-α and IL-1β, respectively (Agati et al. 2012).

### 3.1.4 Chemokines

Chemokines are a family of small (8–10 kDa) proteins that act primarily as leukocyte chemoattractants. The major roles of chemokines are to recruit leukocytes to the site of the inflammation and to control the normal anatomic organization of cells in different tissues. They exert their biological effects by binding to specific G

protein-coupled receptors on target cells. Chemokines are divided into four groups, being the CXC and CC chemokines the two major ones. The former act primarily on neutrophils. IL-8 is the main representative of this group, and it is produced mainly in response to microbial products and other cytokines, such as IL-1 and TNF-α. CC chemokines include MCP-1, macrophage inflammatory protein (MIP)-1α and MIP-1β among others (Kumar et al. 2013). Some chemokines and their receptors are up-regulated in both acute and chronic inflammatory diseases. This finding provided the pharmaceutical industry with new targets for therapeutic intervention against different diseases. There are several approaches that are being developed to block the effects of chemokines, including small-molecule antagonists of chemokine receptors, modified chemokines and antibodies directed against chemokine receptors (Wells et al. 2006).

### 3.1.5 Cell Adhesion Molecules

Cell surface adhesion molecules (CAMs) are proteins involved in cell-cell and cell-extracellular matrix contact in a process named cell adhesion.

CAMs play vital roles in numerous physiological and pathological processes (Cines et al. 1998) including cell growth, differentiation, embryogenesis, immune cell transmigration and response and metastasis. Adhesion molecules are also capable of transmitting information from the extracellular matrix into the cell. The endothelial dysfunction is closely related to inflammatory processes, in which the adhesion of circulating monocytes to vascular endothelial cells is a critical step in both inflammation and atherosclerosis (Tuñon et al. 2009). Endothelial cells respond to pro-inflammatory stimuli such as TNF-α, LPS and IL-1β and recruit leucocytes by selectively expressing adhesion molecules on the surface (Iiyama et al. 1999). CAMs are grouped into four families: immunoglobulin (Ig) superfamily, integrins, cadherins and selectins. Adhesion molecules include members of the Ig superfamily such as the intercellular adhesion molecules (ICAMs), the vascular-cell adhesion molecule (VCAM-1) and endothelial cell selectin (E-selectin), among others (Tuñón et al. 2009).

## *3.2 Inflammation-Associated Intracellular Signaling Pathways*

The set of processes by which a cell converts a signal or external stimulus into another specific signal or response is known as the biochemical pathway of signal transduction. LPS is an inflammatory stimulator of macrophages that triggers the production of pro-inflammatory mediators. The stimulation of TLR4 receptors with LPS leads to the activation of various intracellular signaling pathways such as those involving the inhibitor of κB (IκB) kinase (IKK), PI3K, protein kinase B (Akt) and MAPKs. These molecules eventually lead to the activation of transcription factors such as NF-κB, AP-1 or signal transducers and STATs, whose deoxyribonucleic acid (DNA)-binding capacity is modified by the various protein kinases involved in signal transduction, including MAPKs (Kim et al. 2004; Komatsu et al. 2017).

Inhibitory or stimulatory effects on these biochemical pathways profoundly affect cellular functions, altering the state of phosphorylation of target molecules and modulating gene expression (Williams et al. 2004). Inflammatory cells also produce soluble mediators, such as metabolites of the arachidonic acid, cytokines and chemokines, which act by further recruiting inflammatory cells to the site of damage producing more reactive species.

### 3.2.1 Nuclear Transcription Factor Kappa-B (NF-kB) Pathway

The NF-κB pathway is the main pathway when inflammatory responses develop. This factor plays a central role in the expression of more than 150 genes involved in immune and inflammatory responses. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized LDL and bacterial or viral antigens. NF-κB can occur as either a homo- or a heterodimer consisting of five different transcription factor proteins: (RelA), c-Rel, Rel-B, p50 and p52 (Fan et al. 2017), the most common association is that between p50 and p65.

In an inactivated state, NF-κB is located in the cytosol complexed with the inhibitory protein IκB. Five IκB-like proteins have already been identified: IκBα, IκBβ, IκBγ, IκBε and Bcl-3. The binding of inflammatory mediators to their respective receptors triggers a signaling cascade that leads to the phosphorylation and activation of the IKK complex (IKK α,β,γ). IKK, in turn, phosphorylates the IκB-α protein, which results in ubiquitination, dissociation of IκB-α from NF-κB and eventual degradation of IκB-α by the proteosome (Rabinovich et al. 2011). The activated NF-κB then translocates into the nucleus where it binds to specific sequences of DNA and induces the expression of pro-inflammatory mediators. NF-κB has been reported as one of the most remarkable pro-inflammatory gene expression regulators which mediates the synthesis of several cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, as well as COX-2 (Lawrence 2009; Bertics et al. 2014) (Fig. 3).

### 3.2.2 Signal Transducer and Activator of Transcription (STAT) Protein Family

The STATs proteins are intracellular transcription factors that mediate many aspects of cellular immunity, proliferation, apoptosis and differentiation, taking part in the regulation of cellular responses to cytokines, chemoattractants and growth factors. In unstimulated cells, STAT proteins are inactive in the cytosol. After their association with activated receptors, STAT proteins are phosphorylated by members of the JAK family of non-receptor protein-tyrosine kinases, which are associated with cytokine receptors. The tyrosine phosphorylation promotes the dimerisation of STAT proteins, which then translocate to the nucleus, where they stimulate the transcription of their target genes. Further studies have shown that STAT proteins are also activated downstream of receptor protein-tyrosine kinases, where their

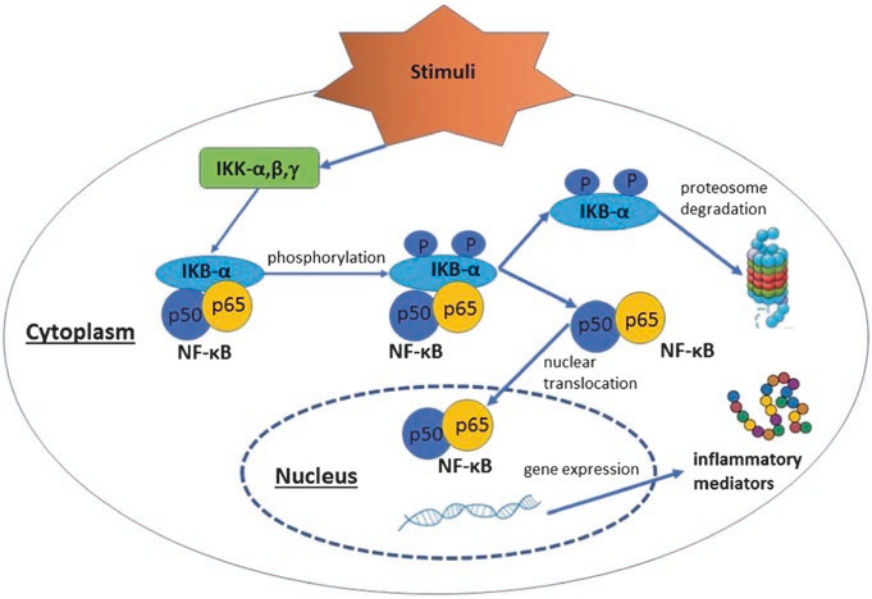


**Fig. 3** Nuclear transcription factor kappa-B (NF-κB) pathway

phosphorylation may be catalyzed either by the receptors themselves or by associated non-receptor kinases. The STAT transcription factors thus serve as direct links between both cytokine and growth factor receptors on the cell surface and regulation of gene expression in the nucleus (Cooper 2000).

It has been demonstrated that the activation of the STAT3/5 pathways leads to subsequent COX-2 expression, while the activation of STAT1 correlates with the expression of iNOS and adhesion molecules (Kretzmann et al. 2008).

### 3.2.3 Activator Protein 1 (AP-1) Pathway

One of the most important signaling targets in the activation of T cells is the transcription factor AP-1. It is constituted by a set of structurally related dimers and formed by proteins of the Fos, Jun and ATF subfamilies (Rabinovich et al. 2011), which all have to dimerise before binding to their DNA target sites. AP-1 regulates many aspects of cell physiology in response to environmental changes, such as stress and radiation or to growth factor signals thereby acting like an environmental biosensor (Wagner 2001). In addition to the common regulation and activation of c-Jun by MAPKs, there are several other signaling pathways and interactions leading to c-Jun protein expression and thus AP-1 activation (Kappelmann et al. 2014).

### 3.2.4 Mitogen-Activated Protein Kinases (MAPKs) Pathway

Several studies have shown that the activation of NF-κB is triggered by MAPKs. There are three main subgroups of MAPKs: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. These kinases play a key role in the regulation of numerous cellular functions, including gene expression, mitosis, differentiation, apoptosis and cellular responses to inflammation (Cargnello and Roux 2011). It has also been demonstrated that they are involved in the signal transduction pathways that lead to the induction of pro-inflammatory mediators (Owuor and Kong 2002; Kaminska 2005; Kim et al. 2008; Xu et al. 2010). Several studies have shown that MAPKs play critical roles for the activation of NF-κB. MAPKs are important signaling components in the conversion of extracellular signals into intracellular responses through serial phosphorylation cascades. Upon stimulation, MAPKs are phosphorylated and activate the downstream protein kinases and transcription factors leading to the expression of pro-inflammatory mediators such as TNF-α, IL-6 and iNOS (Komatsu et al. 2017). Among the MAPK family members, the ERK route is frequently activated by mitogens and growth factors, while inflammation is a main trigger for JNK and p38 (Santangelo et al. 2007). Hence, the inhibition of MAPKs blocks inflammation through the modulation of the levels of pro- and anti-inflammatory mediators (Chen et al. 2017).

## 4 Flavonoids in the Inflammatory Response

In recent years, there has been an increasing progress in the elucidation of the mechanisms by which flavonoids exert their biological activities. A high intake of flavonoids has been associated with a reduced risk of cardiovascular disease, cancer and neurodegenerative disorders. In addition to their already known free radical scavenger effect, it has been demonstrated that flavonoids exert these beneficial effects through the interaction with cellular signaling pathways that mediate cell function under both normal and pathological conditions (Vauzour et al. 2010). It has been demonstrated that flavonoids are able to inhibit the expression of NOS, COX and LOX, which are responsible for the production of NO, PGs and LTs, respectively (Tuñón et al. 2009). Thus, the inhibition of these enzymes by flavonoids may be one of the most important mechanisms governing their anti-inflammatory activity (García-Lafuente et al. 2009) (Fig. 4). In a study carried out by Hämäläinen et al. (2007), the authors investigated the effects of 36 natural phenolic compounds on NO production in macrophages exposed to an inflammatory stimulus and evaluated their mechanisms of action. The most effective compounds were daidzein, genistein, isorhamnetin, kaempferol, quercetin, naringenin and pelargonidin, which

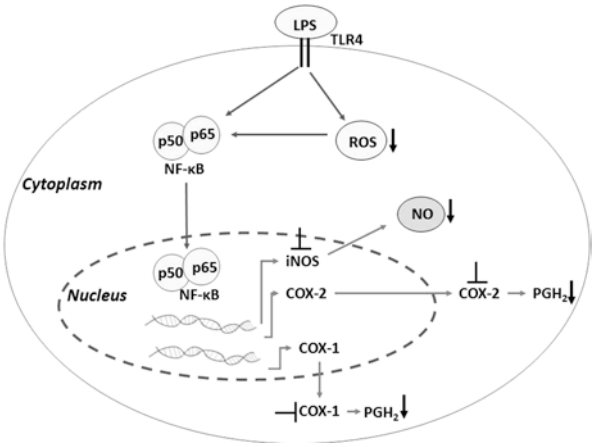


**Fig. 4** Inhibitory effect of flavonoids on ROS, NO and PG. LPS binds to TLR4 and triggers the generation of ROS that activate the nuclear translocation of NF-κB. The NF-κB activation mediates iNOS and COX expression. These enzymes synthetise NO and PG, respectively. Black arrows represent a suppressive effect of flavonoids, and the T-shaped symbol represents the inhibitory activity. (Adapted from Leyva-López et al. 2016)

inhibited iNOS expression and NO production in a dose-dependent manner. The structural requirements for the inhibition of NO production were found to be the presence of a C-2,3 double bond, whereas the presence of sugar substituents either decreased or abolished the inhibitory effect. Hydroxyl groups in positions 7 and 4′ were found in all active compounds; such substitutions were not essential for the activity of the compound.

Flavonoids have been reported to act on the protein kinase and lipid kinase signaling cascades such as PI3K, Akt/PKB, tyrosine kinases, protein kinase C (PKC) and MAPKs (Spencer 2010; Park et al. 2011), inhibiting the transcription of factors as AP-1 or NF-κB. The inhibitory activity exerted on kinases is due to the competition with ATP for the binding to the catalytic sites on these enzymes, thus blocking signal transduction and cell activation processes in cells of the immune system (Ribeiro et al. 2015). Either the inhibitory or the stimulatory effects exerted on these pathways are likely to affect cell functioning by altering the phosphorylation state of target molecules and by modulating gene expression (Williams et al. 2004).

As anti-inflammatory agents, flavonoids have a similar mechanism of action to NSAIDs, since they inhibit the COXs responsible for the synthesis of PGs, which are also involved in physiological processes. The in vitro activity of flavonoids in the inflammatory response also involves other inflammatory mediators such as cytokines, adhesion molecules and chemokines (Agati et al. 2012; Leyva-López et al. 2016). Various flavonoids have been described as good modulators of cytokine production. The structural requirements for a flavonoid to exert a good inhibition of LPS-stimulated TNF-α release are the presence of a double bond at position C2-C3, with an ‘oxo’ function at position C4 and the presence of OH groups at positions 3′

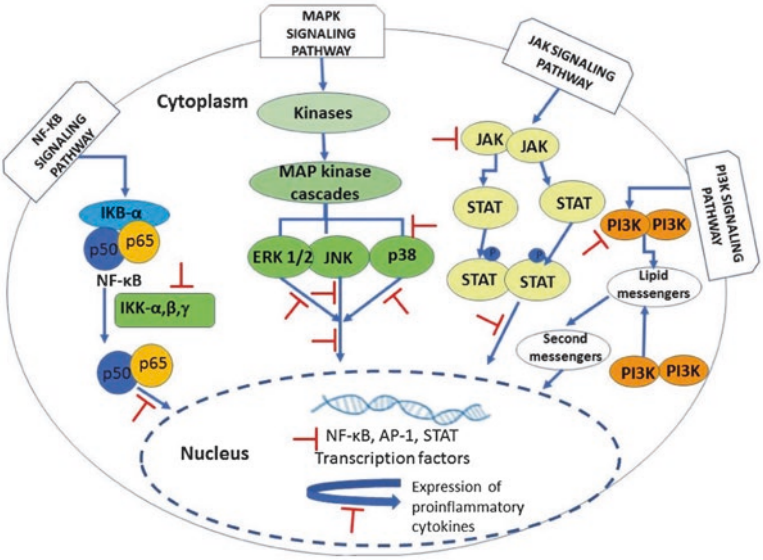


**Fig. 5** Mechanism of action by which flavonoids block inflammation through inhibition of the function of NF-κB, MAPK, JAK and PI3K signaling pathways. The red T-shaped symbol indicates inhibition

and 4´ (Ribeiro et al. 2015). Molecular activities of flavonoids include the inhibition of transcription factors such as NF-κB and AP-1, as well as the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) (Tuñón et al. 2009; Serafini et al. 2010; Chen et al. 2017) (Fig. 5).

This chapter focuses on the results of recent studies assessing the role of the different subclasses of flavonoids as modulators of inflammatory mediators and on cell signaling pathways.

## *4.1 Flavones*

In a mouse model of middle cerebral artery occlusion (MCAO), the pretreatment with chrysin (5,7-dihydroxyflavone, *Chr*) successfully decreased neurological deficit scores and infarct volumes, as compared with the control group. In this context, the up-regulation of NF-κB, COX-2 and iNOS caused by MCAO was inhibited by *Chr*. The increases in glial cell numbers and pro-inflammatory cytokine (IL-1β, IL-6, IL-12, IL-1α, IL-17A, IFN-γ and TNF-α) secretion usually caused by ischemia/reperfusion were significantly ameliorated by the pretreatment with *Chr* (Yao et al. 2014). Additionally, *Chr* prevents the increase in the number of inflammatory cells, IL-4 and IL-12 in an experimental model of asthma, which is a chronic airway inflammatory disorder. The decreased levels of IFN-γ were up-regulated, and the

phosphorylation of Akt and ERK was decreased by *Chr*. Therefore, the authors hypothetised that *Chr* might have beneficial effects on chronic asthma (Yao et al. 2016). *Chr* significantly ameliorated the cardiac dysfunction in an induced myocardial injury model in diabetic rats that presented an up-regulated peroxisome proliferator-activated receptor (PPAR)-γ expression and a downregulation of receptor for advanced glycation end products (RAGE). In this model, inflammation was reduced through the inhibition of NF-κB p65/IKK-β and reduction of TNF-α levels. In addition, *Chr* inhibited the nitro-oxidative stress, as assessed by the levels of glutathione, thiobarbituric acid reactive substances (TBARS), NO and expression of superoxide dismutase (SOD) and eNOS, among others (Rani et al. 2016).

To evaluate the effect of flavones on diabetes mellitus, Wang et al. (2017) studied the effects of vitexin (8-D-glucosyl-4′,5,7-trihydroxyflavone, *Vix*) on pancreatic β-cell function in a model of LPS-stimulated rat islet tissue and in INS-1 cells. The authors demonstrated that both cell damage and apoptosis were decreased in cells treated with *Vix*. The pretreatment of cells with *Vix* reduced the production of TNF-α and attenuated the production of high-mobility group box (HMGB) in response to LPS stimulation.

It has been demonstrated that the treatment of ulcerative colitis with amentoflavone (3′,8′-biapigenin; *Afla*) decreases the levels of the inflammatory cytokines TNF-α, IL-1β and IL-6 together with the expression of iNOS and COX-2. It has also been observed that this flavone was able to inhibit the activation and nuclear translocation of NF-κB (p65/p50). These results allow postulating *Afla* as a potential protective compound in acetic acid-induced ulcerative colitis (Sakthivel and Guruvayoorappan 2013).

The neuroprotective effect of wogonin (5,7-dihydroxy-8-methoxyflavone, *Won*), a potent anti-inflammatory flavonoid, has been demonstrated through the reduction of the inflammatory response mediated by TLR4/NF-κB signaling pathway in mice with traumatic brain injury. A marked reduction in leukocyte infiltration, microglial activation, expression of TLR4, translocation of NF-κB to the nucleus and its DNA-binding activity, matrix metalloproteinase (MMP)-9 activity and expression of IL-1β, IL-6, inflammatory protein of macrophages-2 and COX-2 was observed after treatment with *Won* (Chen et al. 2012). The anti-inflammatory activity of 4′-hydroxywogonin (4′,5,7-trihydroxy-8-methoxyflavone, *4′-HW*) has also been demonstrated in vivo (Fan et al. 2017). In LPS-stimulated RAW 264.7 macrophages, *4′-HW* blocked the expression of COX-2 and iNOS, thus decreasing the levels of their products $PGE_2$ and NO, respectively. Moreover, in the same model, *4′-HW* suppressed the activation of TAK1 and TAB1, suggesting that TAK1/IKK/NF-κB signaling pathways were inhibited and downregulated the phosphorylation of MAPKs and PI3/Akt. This methoxyflavone also decreased the production of intracellular ROS. Furthermore, *4′-HW* also proved to have anti-inflammatory effects in a model of LPS-induced inflammation in an acute lung injury mice model (Fan et al. 2017).

Luteolin (3′,4′,5,7-tetrahydroxyflavone, *Lut*) has been demonstrated to inhibit the ROS increase, lipid peroxidation and glutathione depletion induced by short-term exposure of human bronchial epithelial cells (BEAS-2B) to Cr(VI). In these cells, the treatment with *Lut* decreased the Cr (VI)-induced promoter activity of

AP-1, hypoxia-inducible factor 1-α (HIF-1α), COX-2 and iNOS. An inhibition of the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) and vascular endothelial growth factor (VEGF) was also observed. *Lut* inhibited multiple gene products including those related to inflammation: MAPK, NF-κB, COX-2, STAT-3, iNOS and TNF-α. *Lut* has been postulated as a potential chemopreventive agent against Cr (VI)-induced carcinogenesis (Pratheeshkumar et al. 2014). After TNF-α stimulation, *Lut* inhibited the adhesion of monocytes to endothelial cells and suppressed the expression of MCP-1, ICAM-1 and VCAM-1, which enhances the endothelial cell-monocyte interaction. In endothelial cells, inflammation is apparently prevented by suppression of the NF-κB pathway, since *Lut* decreased the NF-κB transcriptional activity, IκBα degradation, expression of IκB kinase β and subsequent NF-κB p65 nuclear translocation. *Lut* also proved to have anti-inflammatory effects in vivo, as assessed by histologic studies and chemokine levels (Jia et al. 2015). Besides, *Lut* has been evaluated as a potential therapeutic agent in the prevention and/or treatment of Alzheimer's disease in a human blood-brain barrier (BBB) model. In this model, the p38 MAPK-mediated NF-κB signaling pathway was examined by coculturing human brain microvascular endothelial cells (hBMECs) and human astrocytes (hAs) under fAβ1-40-damaged conditions (Zhang et al. 2017). *Lut* suppressed the production of inflammatory mediators and cytokines, such as COX-2, TNF-α, IL-1β, IL-6 and IL-8. However *Lut* did not display any scavenging effect on intracellular ROS in hBMECs and hAs.

Palmieri et al. (2012) have determined the effects of apigenin (4′,5,7-trihydroxyflavone, *Api*) on the TNF-α-induced endothelial dysfunction by evaluating the expression of eNOS and MMP-9. In this case, *Api* blocked the TNF-α-induced expression of eNOS and MMP-9 and the TNF-α-triggered activation of Akt, p38 MAPK and JNK signaling on endothelial. The use of specific Akt inhibitors, which presented Api-like effects on eNOS and MMP-9 expression, allowed demonstrating that the induction of eNOS and MMP-9 caused by TNF-α depends on Akt activation. The main mechanism of inhibition of Akt signaling involved 'classical' and 'nonclassical' ERs. A recent study has demonstrated that *Api* up-regulates the gene expression of inflammatory IL-17 cytokine family and LTA and the expression of the interferon beta 1 gene in BxPC-3 human pancreatic cancer cells (Johnson and De Mejia 2013). The effect of *Api* in a rodent model of diabetic nephropathy has also been evaluated (Malik et al. 2017). The administration of *Api* to streptozotocin-induced diabetic rats reduced ROS generation and restored the antioxidant status. Moreover, an anti-apoptotic effect was also demonstrated, since *Api* inhibited the MAPK/NF-κB/TNF-α and TGF-β1/MAPK/fibronectin pathways.

Another interesting methoxyflavone is velutin (3′,5-dihydroxy-4′,7′-dimethoxyflavone, *Vel*), isolated from the pulp of açaí fruit (*Euterpe oleracea* Mart.). This compound caused a significant reduction in the production of TNF-α and IL-6 in RAW 264.7 macrophages and in mouse peritoneal macrophages. *Vel* effectively inhibited the expression of pro-inflammatory cytokines through a significant reduction in the TNF-α and IL-6 messenger ribonucleic acid (mRNA) levels by inactivating NF-κB and by inhibiting p38 and JNK phosphorylation in the two macrophage models. In these cells, *Vel* displayed an inhibitory capacity on NF-kB activation that was higher than that of *Lut* and *Api* (Xie et al. 2012).

Tricin (4′,5,7-trihydroxy-3′,5′-dimethoxyflavone, *Tri*) isolated from Njavara rice (*Oryza sativa* L.) has been demonstrated to cause a significant downregulation of pro-inflammatory markers in human peripheral blood mononuclear cells (hPBMCs) stimulated with LPS. In that study, *Tri* reduced the NO production and iNOS expression; it attenuated LPS-induced COX-2 activity and $PGE_2$ production and blocked LPS-induced TNF-α and IL-6 production. Furthermore, *Tri* reduced the LPS-induced production of MMPs by hPBMCs and suppressed the LPS-induced activation of NF-κB and nuclear translocation of p65 (Shalini et al. 2012).

A flavone isolated from *Artemisia asiatica* Nakai (Asteraceae), eupatilin (5,7-dihydroxy-3′,4′,6′-trimethoxyflavone, *Eup*), has proved to have an anti-inflammatory effect in human bronchial epithelial cells affecting cell functionality and inflammatory cell adhesion in response to stimulation with TNF-α. In the study conducted by Jung et al. (2012), the authors demonstrated that *Eup* suppressed the expression of ICAM-1 and VCAM-1 mRNA in bronchial BEAS-2B epithelial cells stimulated with TNF-α. This effect was achieved by blocking the Akt-NF-κB signaling pathway, since a blockage of the IKK activity was detected. However, in BEAS-2B cells, the signaling of AP-1 was not affected, since no variations were detected in the levels of c-fos. These results established that, in bronchial epithelial cells, *Eup* caused a decrease in the adhesion of both monocytes and eosinophils to these cells due to the inhibition of Akt, thus suggesting that this flavone could modulate the pathogenesis of asthma as regards the generation of the inflammatory infiltrate.

Oroxylin A (5,7-dihydroxy-8-methoxyflavone, *OroA*) is the major flavonoid isolated from the roots of *Scutellaria baicalensis* Georgi. This compound is known as a potential anti-inflammatory agent. Song et al. (2012) have determined the action of *OroA* on LPS-induced angiogenesis in vitro and in ovo models. *OroA* affected negatively the expression of the LPS acceptor TLR4 and the activation of MAPKs, as well as the phosphorylation of JNK, p38 and ERK. Besides, the translocation of NF-κB dimers to the nucleus was limited after treatment with *OroA*. Kim et al. (2012) have evaluated the modulatory capacity of 5,6,7-trimethoxy- and 5,6,7-trihydroxyflavone derivatives on NO and $PGE_2$ production in LPS-stimulated RAW 264.7 cells. Thus, in this experimental model, 4′-bromo-5,6,7-trimethoxyflavone suppressed the expression of iNOS and COX-2. Furthermore, this compound downregulated the release of TNF-α, IL-6 and IL-1β as well as the expression of NO, $PGE_2$, TNF-α, IL-6 and IL-1β. These results suggested that the modulation exerted on the NF-κB signaling pathway would generate an anti-inflammatory response through the decrease in the degradation and phosphorylation rates of IκB-α.

The 3,5,7-trihydroxy-4′-methoxy-8-(3-hydroxy-3-methylbutyl)-flavone (*ICT*), a new derivative of the flavonol icariin, suppressed the LPS-induced TNF-α production in the human monocytic cell line THP-1, PBMCs and human monocytes in a dose-dependent manner. The pretreatment with *ICT* produced the downregulation of CD14/TLR4 by blocking the NF-κB and MAPK signaling pathways (Wu et al. 2012).

The modulation of the intestinal inflammatory response by flavones (*Chr*, *3′,4′-Dihydroxyflavone*, *Api*, *Lut* and *Quer*) and their unmethylated analogues have been evaluated by During and Larondelle (2013). The production of soluble pro-inflammatory mediators, such as IL-8, IL-6, MCP-1 and COX-2-derived $PGE_2$, and the activation of NF-κB in 3d-confluent and 21d-differentiated Caco-2 cells stimulated with IL-1β were evaluated after treatments with these flavones. The Caco-2 cell model allowed demonstrating that the O-methylation of *Chr* enhances its anti-inflammatory properties. Of all flavones, the demethylated form of *Chr* has displayed the highest anti-inflammatory activity. The effect of this derivative was achieved by a reduction of IL-8, IL-6, MCP-1 and COX-2-derived $PGE_2$ levels. The presence of hydroxyl groups on ring A (positions 5 and 7), the absence of methoxylation of the 3′-hydroxyl group on ring B and the methoxylation of the 3-hydroxyl group on ring C seemed to be responsible for the intestinal anti-inflammatory activity.

## *4.2 Flavonols*

Lee et al. (2013) have studied the possible barrier protective effects of rutin (quercetin-3-O-rutinoside, *Rut*) on the secretion of pro-inflammatory mediators as well as the signaling pathways activated in human umbilical vein endothelial cells (HUVEC) stimulated with LPS. *Rut* blocked the disruption of the vascular barrier induced by LPS, the expression of CAM as well as the adhesion/transendothelial migration of monocytes to human endothelial cells. In addition, in the same model, *Rut* abrogated the permeability increase induced by acetic acid and the leukocyte migration induced by carboxymethyl cellulose. In addition, *Rut* reduced the expression of TNF-α and the activation of NF-κB induced by LPS. These findings allowed postulating *Rut* as a protective agent against inflammatory vascular diseases. In another study, Yoo et al. (2013) have observed that the treatment with *Rut* inhibited the up-regulation of VCAM-1, ICAM-1 and E-selectin caused by high-mobility group box 1 protein (HMGB1), and apparently this effect is mediated through attenuation of the HMGB1 signaling pathway. According to this study, *Rut* resulted in the reduction of HMGB1-induced mortality. *Rut* was also found to suppress the production of TNF-α and IL-6 and the activation of NF-κB and ERK1/2 by HMGB1.

*Fisetin* (3,3′,4′,7-tetrahydroxyflavone, *Fis*) has been demonstrated to be active in a mouse model of ultraviolet (UV) B-induced inflammation. In mice exposed to UV B radiation and then treated with *Fis* applied topically, a reduction of the hyperplasia and the infiltration of inflammatory cells as well as the levels of inflammatory mediators, such as TNF-α, IL-1β, IL-6 and $PGE_2$, and its receptors, and decreased COX-2 and myeloperoxidase (MPO) activities were observed. *Fis* inhibited UV B-induced expression of PI3K and Akt phosphorylation. The activation of the NF-κB signaling pathway was also inhibited in *Fis*-treated mice. *Fis* reduced the UV B-induced expression of IKKα/β and IκBα protein phosphorylation, thus restoring the IκBα protein levels. *Fis* also inhibited the activation of the p65 transcription

factor and its nuclear translocation in UV B-exposed skin (Pal et al. 2015). The biological activity of *Fis* has also been evaluated in a murine model of acute pancreatitis where both pre- and post-treatment with this flavonol reduced the severity of acute pancreatitis and pancreatitis-associated lung injury. The pretreatment with *Fis* caused a decrease in pancreatic levels of TNF-α, IL-1β and IL-6. In vivo, *Fis* suppressed IκBα degradation and NF-κB activation, as well as activation of JNK, with similar in vitro effects on acinar pancreatic cells. In contrast, *Fis* did not affect the activation of ERK 1/2 and p38. Accordingly, the pretreatment with *Fis* inhibited the activation of JNK and the degradation of IκBα on pancreatic acinar cells. As observed in vivo, the treatment with *Fis* inhibited the production of TNF-α, IL-1β and IL-6 (Jo et al. 2014). In human gingival fibroblasts (HGFs) treated with *Porphyromonas gingivalis* LPS, *Fis* caused a significant reduction in the synthesis of $PGE_2$ and the expression of COX-2 without affecting cell viability. In this model, the treatment with the flavonoid inhibited the activation of ERK, JNK and p38 of the MAPK pathway, which is induced upon LPS treatment (Gutiérrez-Venegas et al. 2014). In a murine model of early brain injury after subarachnoid haemorrhage, high doses (50 mg/kg) of *Fis* improved neurological function parameters and reduced brain edema. TLR4 expression and NF-κB translocation to the nucleus were significantly reduced, as was the production of inflammatory cytokines such as TNF-α and IL-1β (Zhou et al. 2015a). In a study evaluating the antiseptic effects of *Fis* on HMGB1-mediated inflammation, this flavonoid proved to modulate pro-inflammatory responses. In HUVECs, HMGB1 augmented the phosphorylation of NF-κB, ERK1/2 and Akt, in addition to increasing TNF-α and IL-β production. These effects were significantly reduced by *Fis,* as was NF-κB p65 translocation to the nucleus (Yoo et al. 2014). In her 2015 review, Maher summarizes the effect of *Fis* on the central nervous system (CNS) functions. As regards inflammation, *Fis* proved to reduce LPS-induced microglial activation and neurotoxicity. Accordingly, the levels of TNF-α, $PGE_2$, iNOS and COX-2 were reduced after treatment with the flavonoid, and these effects seemed to be mediated by the inhibition of activation of NF-κB. *Fis* also suppressed other pro-inflammatory signaling pathways, such as JNK and p38 MAPK, in microglia in the temporary middle cerebral artery occlusion stroke model in mice. Results indicated that *Fis* has both in vitro and in vivo anti-inflammatory activity on the CNS immune system (Maher 2015). Other studies on microglial activation have shown that *Fis* inhibits cell migration and ROS production. Moreover, the expression of iNOS along with NO production was also reduced in cells stimulated with LPS plus IFN-γ and with peptidoglycan. The LPS/IFN-γ- or peptidoglycan-enhanced production of IL-1β was inhibited by *Fis*. This flavonol generated an endogenous increase in the anti-oxidative heme oxygenase-1 (HO-1) expression through the PI-3 kinase/Akt and the p38 signaling pathways, but not through ERK and JNK in microglia. *Fis* also significantly attenuated inflammation-related microglial activation and coordination deficit in mice in vivo (Chuang et al. 2014).

Icariin (4′-O-methyl-8-γ,γ-dimethylallyl kaempferol-3-rhamnoside-7-glucoside, *Ica*), a prenyl flavonoid glycoside, is the major active compound of *Herba epimedii*, which is a centuries-old traditional medicine herb. Formulations prepared with this

herb are the most frequently prescribed ones (Zhang et al. 2014; Kong et al. 2015). The anti-inflammatory activity of *Ica* has been evaluated in a TNF-α/IFN-γ-induced inflammatory response in human keratinocytes (HaCaT cells). In HaCaT cells, the TNF-α/IFN-γ-induced production of IL-6, IL-8, IL-1β and MCP-1 and gene expression of IL-8, IL-1β, ICAM-1 and tachykinin receptor 1 (TACR1) were inhibited by *Ica*. The treatment with *Ica* produced a reduction in the phosphorylation of p38 MAPK and ERK that was augmented upon stimulation with TNF-α/IFN-γ. The abnormal expression of TNF-α-R1 and IFN-γ-R1 found in HaCaT cells after TNF-α/IFN-γ stimulation was modified by *Ica*, which downregulated the levels of the former and up-regulated the levels of the latter. These effects were mediated, at least partially, via the inhibition of the p38-MAPK signaling pathway, as well as by the regulation of the TNF-α-R1 and IFN-γ-R1-related signals (Kong et al. 2015). In an unpredictable chronic mild stress model of depression in rats, the chronic treatment with *Ica*, which can freely cross the BBB, reverted the increased levels of oxidative-nitrosative stress markers and inflammatory mediators like TNF-α and IL-1β. The activation of the NF-κB signaling pathway and increased iNOS mRNA expression in the hippocampus was also reverted by *Ica* (Liu et al. 2015). *Ica* modulates the activity of the histone deacetylase sirtuin (SIRT)6, with a maximum activating effect at 10 M. After treatment with *Ica*, the up-regulation of SIRT6 protein expression was observed, while the expression of NF-κB (p65) was downregulated in heart tissue and in aortic endothelial cells. An inhibitory effect of *Ica* on NF-κB inflammatory signaling pathways, as evidenced by decreased mRNA TNF-α, ICAM-1, IL-2, and IL-6 levels, was observed (Chen et al. 2015).

Astragalin (kaempferol-3-glucoside, *Ast*) is found in several plants, such as *Podophyllum peltatum*, *Paeonia lactiflora*, *Phytolacca americana*, *Cicer arietinum*, *Onobrychis arenorie*, *Phaseolus vulgaris*, *Rosa agrestis* and *Glycyrrhiza macedonica* (Li et al. 2014a; You et al. 2017). In primary-cultured mouse mammary epithelial cells (mMECs), *Ast* inhibited the production of TNF-α, IL-6 and NO, as well as expression of iNOS and COX-2 after LPS stimulation. The treatment of mMECs with *Ast* decreased the LPS-induced TLR4 expression, NF-kB activation, IκBα degradation and the phosphorylation of p38 and ERK (Li et al. 2014a) (Fig. 6).

### *4.3 Flavanones*

The anti-inflammatory effect of alpinetin (7-hydroxy-5-methoxyflavanone, *Alp*), which is the main flavonoid of *Alpinia katsumadai* Hayata, has been investigated to find that *Alp* blocks the inflammatory process both in vitro, in LPS-stimulated RAW 264.7 cells, and in vivo in a LPS-induced acute lung injury model (Huo et al. 2012). The pretreatment with *Alp* induced a strong blockage of the production of TNF-α, IL-6 and IL-1β induced by LPS. In addition, in the in vitro model, *Alp* inhibited IκBα, p65, p38 and ERK phosphorylation. Besides, in the in vivo model, histopathologic studies demonstrated that the changes in the mouse lungs were minimal. Several findings suggest that *Alp* would act through the NF-κB and MAPK

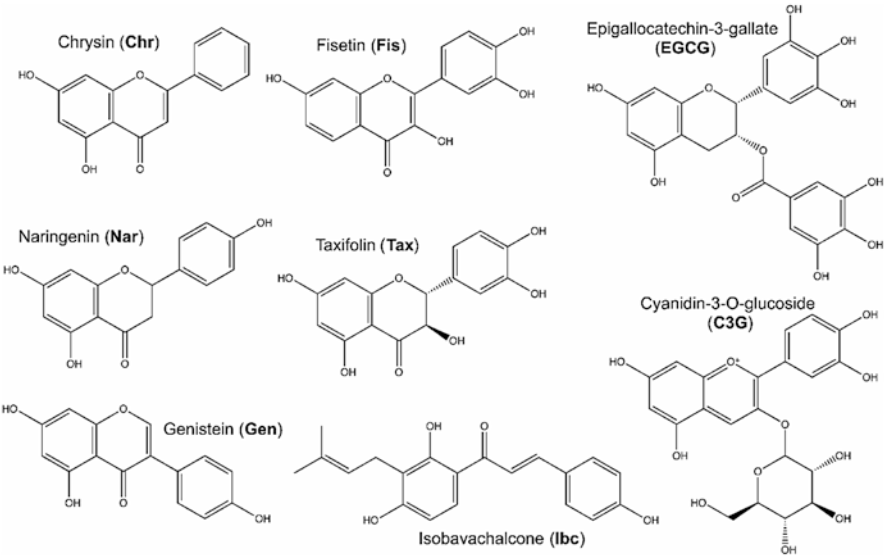


**Fig. 6** Chemical structures of some flavonoids that act as modulators of inflammatory mediators and on cell signaling pathways

signaling pathways and that *Alp* would act as a potential protective agent in the acute lung injury model. Furthermore, the effect and mechanism of action of *Alp* was evaluated in a LPS-induced mouse mastitis model. In this in vivo study, *Alp* prevented the infiltration with neutrophils and the activation of myeloperoxidase and downregulated the expression of TNF-α, IL-1β and IL-6. Likewise, the phosphorylation of IκB-α and NF-κB p65 and the expression of TLR4, induced by LPS, were inhibited by the flavonoid. Additionally, in the in vitro model, *Alp* inhibited the expression of TLR4 and the production of TNF-α, IL-1β and IL-6 in LPS-stimulated primary mouse mammary epithelial cells. These results indicate that *Alp* could be considered a potential therapeutic agent for the treatment of mastitis, since it modulates the activation of the NF-κB signaling pathway mediated by the activation of TLR4 (Chen et al. 2013). Furthermore, Hu et al. (2013) have evaluated the signaling pathways involved in the anti-inflammatory activity of *Alp* in human THP-1 macrophages stimulated by LPS. In this case, *Alp* prevented the synthesis of TNF-α, IL-6 and IL-1β. *Alp* inhibited the activation of NF-κB, the degradation of IκBα and the phosphorylation of ERK, JNK and p38. Moreover, it was observed that the activation of PPAR-γ caused by *Alp* led to the decrease in the expression of TLR4 and the consequent inhibition of TLR4-dependent activation of NF-κB and MAPK. In turn, these events led to an inhibition of the release of pro-inflammatory cytokines.

Naringenin (4′,5,7-trihydroxyflavanone, *Nar*), a flavonoid derived from grapefruit and related citrus species, proved to have a protective effect in a model of LPS-induced human bronchial epithelium injury by suppressing the secretion of TNF-α, IL-6, SOD, NOS, MPO and NO. The LPS-induced up-regulation of NF-κB p65

mRNA expression was also reduced by *Nar*, and this flavonoid effectively suppressed NF-κB activation by inhibiting the degradation of IκB-α and the translocation of p65. The reduction in the secretion of TNF-α and IL-6 is possibly mediated by a blockage in the activation of the NF-κB and MAPK signaling pathways, since *Nar* inhibited the phosphorylation of ERK1/2, JNK and p38 MAPK (Yu et al. 2014). Furthermore, the suppressors of cytokine signaling (SOCS)-3 expression and the anti-inflammatory effects of *Nar* in microglial cells are regulated by adenosine monophosphate-activated protein kinase (AMPK) α and PKC δ. *Nar* downregulates the expression of iNOS and COX-2 and inhibits the release of NO. *Nar* has also displayed significant protective effects on microglial activation and improved the motor coordination function in a murine model (Wu et al. 2016).

Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside, *Nag*), a flavanone-7-O-glycoside formed between naringenin and the disaccharide neohesperidose, is found as the major flavonoid glycoside in grapefruit. This flavone gives grapefruit juice its bitter taste (Pubchem 2017). In HaCaT cells, the pretreatment with *Nag* prevented UV B-induced apoptosis and the production of ROS and decreased the levels of inflammatory cytokines, such as IL-1β, IL-6, IL-8 and COX-2, as compared to UV B-exposed and non-treated cells. *Nag* inhibited the activation of p38 and JNK upon exposure of these cells to UV B. In a mouse model, the topical treatment prevented epidermal thickening, IL-6 production, apoptosis and the over expression of COX-2 caused by UV B irradiation. *Nag* also blocked the UV B-induced activation of p38. *Nag* would confer protection against UV B both in vitro and in vivo through inhibition of MAPK/p38 activation (Ren et al. 2016). Cisplatin, an effective chemotherapeutic agent, is known to cause a decline in the concentrations of reduced glutathione and ascorbic acid, a decrease in membrane-bound ATPases and glutathione peroxidase (GPx) activities and an increase in the activity of catalase (CAT) and SOD in striatum tissue of aged rats. The deterioration of striatum tissue was prevented by the treatment with *Nag*; the change in antioxidant enzymes was revoked, and the increase in malondialdehyde, protein carbonyls, NO and TNF-α levels was suppressed. Accordingly, *Nag* inhibited p53-, NF-κB- and TNF-α-mediated inflammation. Thus, *Nag* proved to have neuroprotective effects in this model (Chtourou et al. 2015). In an experimental diabetes mellitus rat model, the treatment with *Nag* improved the condition of the animals. In the cerebral cortex and hippocampus, the glucoside reduced the levels of oxidative stress markers and pro-inflammatory factors, such as TNF-α and IL-6. *Nag* also activated the expression of PPARγ, which inhibits the inflammatory response. The cognitive deficit in diabetic rats was also ameliorated by *Nag* through a decrease of oxidative stress marker levels and pro-inflammatory factors and activation of the PPARγ signaling pathway (Qi et al. 2015). The pretreatment with *Nag* of murine splenocytes exposed to ionizing radiation prevented intracellular ROS generation, thus preventing lipid peroxidation and nitrite production. A reduction in nuclear DNA damage and a recovery of cell viability were also observed after treatment with the flavonoid. *Nag* blocked the p38 phosphorylation and the downstream cascade of events involving inhibition of the NF-κB pathway (Manna et al. 2015).

In injured hBMECs, pinocembrin (5,7-dihydroxyflavanone, *Pin*), a flavonoid abundant in propolis, *Pinus* heartwood and *Eucalyptus*, reverts the cytotoxicity of β-amyloid peptides, which are known to be involved in Alzheimer's disease pathogenesis. In this model, the flavonoid increases cell viability and attenuates nuclear damage, and lower levels of LDH are released. *Pin* inhibits the inflammatory response through various mechanisms, including inhibition of MAPK activation, downregulation of IKK, a decrease in IκBα degradation, inhibition of NF-κB p65 nuclear translocation and the consequent reduction in the release of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6). The anti-inflammatory effects of *Pin* in hBMECs are probably related to the inhibition of the MAPK and the NF-κB signaling pathways (Liu et al. 2014b). In LPS-stimulated BV2 microglial cells, *Pin* inhibited the production of TNF-α, IL-1β, NO and $PGE_2$ and the expression of iNOS and COX-2. PI3K and Akt phosphorylation and NF-κB activation were inhibited by *Pin*. Induction of nuclear translocation of Nrf2 and expression of HO-1 have also been observed after treatment with this flavonoid (Zhou et al. 2015b).

Sophoraflavanone G (5,7,D,2′,4′-tetrahydroxy-8-lavandulylflavanone, *SG*), isolated from *Sophora flavescens*, has been evaluated as a potential anti-inflammatory agent in LPS-stimulated RAW 264.7 macrophages. In these cells, *SG* blocked the expression of iNOS and COX-2, with the consequent decrease of NO and $PGE_2$. *SG* also reduced the production of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α. *SG* inhibited the phosphorylation of the p65 subunit of NF-κB, thus preventing its translocation to the nucleus. Although *SG* stimulated the synthesis of HO-1, it was observed that the activation of MAPK was downregulated, since the phosphorylation of ERK1/2, JNK and p38 did not occur. When cells were cocultured with *SG* and MAPK, lower activation levels of iNOS and COX-2 were observed. These results confirm the anti-inflammatory effect of *SG* evidenced by the negative modulation of NF-kB and MAPK signaling pathways (Wun et al. 2013).

Ugonin M (5,4′-dihydroxy-4″,4′-dimethyl-5′-methyl-5″H-dihydrofuran [2″,3″:6,7] flavanone, *UgoM*) has been isolated from *Helminthostachys zeylanica* (L.) Hook, which is a traditional Chinese medicine plant popularly used for the treatment of inflammation, among other applications. This flavanone suppresses the production of pro-inflammatory mediators such as NO, TNF-α, IL-1β and IL-6 and decreases cell counts and the protein content in the bronchoalveolar lavage fluid in LPS-induced acute lung injury in mice. In this context, *UgoM* attenuated pulmonary edema. Likewise, *UgoM* prevented the activation of iNOS and COX-2 in LPS-induced inflammation. *UgoM* blocked the translocation of NF-κB and the activation of MAPK through the degradation of NF-κB and IκB-α, as well as through the promotion of phosphorylation of ERK and p38 MAPK. In addition, in this model, the expression of TLR4 was blocked. On the other hand, in the same model, it was demonstrated that *UgoM* inhibited the expression of MPO and stimulated the expression of HO-1 and the antioxidant enzymes SOD, GPx and CAT (Wu et al. 2017).

### 4.4 Flavanonols

Taxifolin (2R,3R)-3,3′,4′,5,7-pentahydroxyflavanone, *Tax*) reverted the increase in mast cell infiltration caused by 1,2-dimethyl hydrazine (DMH) in a mouse colon cancer model. *Tax* also favoured the activation of antioxidant pathways through the increase in the levels of Nrf2, which activates the expression of cytoprotective genes in response to ROS. *Tax* downregulated the NF-κB and Wnt signaling pathways. The expression of NF-κB, TNF-α and COX-2 were reduced when compared to the group treated only with DMH. *Tax* would exert chemopreventive effects by modulating inflammatory, Wnt and antioxidant response pathways (Manigandan et al. 2015). The flavanonols 2′-hydroxy yokovanol and 2′-hydroxy neophellamuretin, isolated from the leaves and stems of *Desmodium caudatum*, along with other flavonoids, inhibited the production of IL-6, IL-12 and TNF-α in LPS-stimulated bone marrow-derived dendritic cells (Li et al. 2014c).

*Ampelopsis grossedentata* (Hand-Mazz) W.T. Wang, known as rattan tea, is popularly used in China for its anti-inflammatory and other pharmacological properties. It has been demonstrated that one of its main compounds is ampelopsin (3,5,7,3′,4′,5′-hexahydroxyflavanone, *Amp*). To understand the molecular mechanisms involved in the anti-inflammatory effects exerted by this flavonoid, the production of NO by RAW264.7 macrophages stimulated with LPS was evaluated. The pretreatment with *Amp* blocked the production of NO and the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. *Amp* blocked the activation of iNOS with the consequent inhibition in the translocation of NF-κB due to the inhibition of KKα/β and IκBα phosphorylation and nuclear translocation NF-κB p65. In addition, *Amp* inhibited the release of Akt without affecting MAPK phosphorylation. *Amp* also interfered with ROS-mediated PI3K/Akt phosphorylation. Thus, the anti-inflammatory effects of *Amp* are related to the inhibition of the Akt, IKK and NF-κB signaling pathways (Qi et al. 2012).

### 4.5 Flavan-3-ols

Epigallocatechin-3-gallate [(−)-cis-3,3′,4′,5,5′,7-hexahydroxy-flavane-3-gallate, *EGCG*)], the most abundant catechin in green tea infusions and one of the most active molecules known for its antioxidant properties, is known to downregulate the TLR4 signal transduction in LPS-stimulated endothelial cells. This downregulation is mediated by the 67-kDa laminin receptor (67LR) and by an up-regulation of the Toll-interacting protein (Tollip), which is a negative regulator of TLR signaling (Byun et al. 2014; Legeay et al. 2015). *EGCG* also modulates inflammatory responses in adipocytes through the 67LR, leading to a reduction of inflammatory mediator and cytokine levels (IKKβ, p-NF-κB, TNF-α and IL-6) after LPS stimulation. These data suggest that *EGCG* suppresses TLR4 signaling in LPS-stimulated adipocytes via 67LR (Bao et al. 2015). In an in vivo model of crescentic

glomerulonephritis, the treatment with *EGCG* reduced mortality and markedly improved renal function and histology, when compared with vehicle-treated mice. More importantly, *EGCG* caused a decrease in p-Akt, p-JNK, p-ERK1/2 and p-P38 as well as restoration of PPARγ and SIRT1 levels. The Nrf2 signaling, which was impaired in vehicle-treated mice, was restored by *EGCG* (Ye et al. 2015). After stimulation of human hepatocytes with LPS, an increase in the production of TNF-α, regulated upon activation normal T-cell expressed and secreted (RANTES), MCP-1, ICAM-1, NO, VEGF and MMP-2 was observed. This effect was reduced by the pretreatment of cells with *EGCG*. The effects observed were related to the inhibition of NF-κB and MAPK signaling pathways through a downregulation of p-IκBα, p65, p-p65, p-p38, p-ERK1/2 and p-Akt, thus indicating that *EGCG* suppresses LPS-induced inflammatory response and oxidant stress and exerts hepatocyte-protective activity (Liu et al. 2014a). Besides, the exposure of human endothelial cells to environmental pollutants such as polychlorinated biphenyls (PCBs) increases the expression of vascular inflammatory mediators, including IL-6, CRP, ICAM-1, VCAM-1 and IL-1α/β. The pretreatment with *EGCG* prevents such increase together with an inhibition of nuclear import of p65, a decreased p65 NF-κB subunit and histone acetyltransferase p300 chromatin binding, as well as an increased chromatin binding of histone deacetylase HDAC1/2 and hypoacetylation of histone H3. Therefore, *EGCG* decreases PCB-induced vascular toxicity through epigenetic modifications (Liu et al. 2016). It has been postulated that *EGCG* might have renoprotective effects in a unilateral ureteral obstruction mice model. In the obstructed kidney, the induced oxidative stress and inflammatory response, as represented by inflammatory cytokines such as TNF-α, IL-6 and IL-1β, was prevented by *EGCG*, which was able to inhibit NF-κB, to enhance Nrf2 nuclear translocation and to promote HO-1 production (Wang et al. 2015b). In human HUVEC cells, *EGCG* suppressed the expression of IL-6, ICAM-1, TNF-α, and MCP-1 and the generation of ROS induced by uric acid. This suppression was achieved through the inhibition of Notch-1 signaling pathways (Xie et al. 2015). In a non-alcoholic fatty liver disease murine model, the treatment with *EGCG* caused downregulation in the expression of key pathological oxidative (e.g. nitrotyrosine formation) and pro-inflammatory markers (e.g. iNOS, COX-2 and TNF-α). *EGCG* inhibited the activity of TGF/SMAD, PI3K/Akt/FoxO1 and NF-κB pathways, thus reducing the severity of liver injury (Xiao et al. 2014).

(+)-Catechin [(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol, *Cat*] reduced the levels of iNOS and COX-2 and the production of NO and ROS after stimulation of BV-2 (a mouse microglial cell line) with LPS. Even though the production of TNF-α and IL-6 was suppressed, IL-4 levels were increased. *Cat* inhibited IκB-α phosphorylation, thus inhibiting the nuclear translocation of NF-κB p65. On the other hand, the activation of Akt was inhibited and so was the phosphorylation of ERK1/2 and p38 MAPK. *Cat* also suppressed AMPK activity. It has been postulated that the anti-inflammatory activity exerted on this cell type was related to the suppression of pro-inflammatory mediators and inhibition of NF-κB activity through Akt, ERK, p38 MAPK and AMPK pathways (Hussein et al. 2015).

### *4.6 Anthocyanidins*

Cyanidin-3-O-glucoside (3,3′,4,5,7-pentahydroxyflavylium-3-O-glucoside, *C3G*) is an anthocyanin commonly present in food and vegetables in the human diet. *C3G* has been demonstrated to have inhibitory capacity on the production of TNF-α, IL-6 and IL-1β both in vitro on HUVECs and in vivo in an acute respiratory distress syndrome model. The pretreatment with *C3G* improved histopathologic and clinical parameters in vivo. In the lung tissue, *C3G* has proved to suppress the LPS-induced NF-kB and MAPK signaling pathways activation by blocking the phosphorylation of IκB-α, NF-κB/P65, ERK, p38 and JNK (Ma et al. 2015). When HUVECs were exposed to palmitic acid, a significant increase in the levels of free radicals and oxidative stress markers occurred; however, this status was reverted upon treatment with *C3G*. The activation of NF-κB pro-inflammatory pathway and the expression of adhesion molecules induced by palmitic acid were inhibited by *C3G* possibly through the activation of the Nrf2/electrophile-responsive element (EpRE) pathway, since *C3G* induced Nrf2 nuclear localisation and activation of cellular antioxidant and cytoprotective genes (Fratantonio et al. 2015). Recent evidences have shown how, in the presence of *C3G*, TNF-α-stimulated intestinal cells can modify the physiological functioning of endothelial cells. The protective effects exerted by the anthocyanidin have also been demonstrated. In this in vitro non-contact coculture system with TNF-α-activated Caco-2 intestinal cells, E-selectin and VCAM-1 mRNA levels were increased as were leukocyte adhesion and NF-κB levels, which were inhibited by *C3G*. It has been observed that TNF-α stimulates the nuclear translocation of NF-κB and the expression of the genes encoding TNF-α and IL-8, whereas the pretreatment with *C3G* significantly reduces these effects by preventing the p38 translocation. In addition, *C3G* blocked the activation of TNF-α-stimulated HUVECs, in which the expression of E-selectin and VCAM-1 mRNA and increased levels of NF-κB were observed. This study has demonstrated that the main protective mechanism against chronic intestinal inflammatory diseases is related to the selective inhibition of the NF-κB pathway, making anthocyanidins important therapeutic agents to treat this disease (Ferrari et al. 2017). He et al. (2017) have evaluated the protective effects of *C3G* from sunlight UV radiation. In that study, *C3G* prevented apoptosis, the morphological changes and increased the viability of HaCaT cells exposed to UV B irradiation. In the same model, *C3G* also displayed a great ROS scavenging capacity. The expression of COX-2 in irradiated cells was also blocked by *C3G*. This flavonoid was also found to decrease the activation of EGF receptor in HaCaT, and this effect was mediated through the inhibition of Akt phosphorylation. It has been suggested that the photoprotective effects exerted by the flavonoid in UV B-irradiated keratinocytes were due to the interaction of the MAPK and Akt signaling pathways, since the nuclear translocation of p38, ERK and JNK were abrogated.

Taking into account that the evolution of atherosclerosis is related to the activation of the NF-κB pathway that leads to endothelial dysfunction and vascular inflammation and that anthocyanins are natural compounds with an important antioxidant

activity, Paixão et al. (2012) have evaluated the effect of malvidin-3-O-glucoside (3,5,7-trihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl) chromeno-3O-glucoside, *Mal3OG*) on the biosynthesis of NO and on the activation of NF-κB induced by peroxynitrite in bovine arterial endothelial cells. The treatment with *Mal3OG* increased the release of NO by endothelial cells. In addition, *Mal3OG* facilitated both the phosphorylation of Akt and eNOS and decreased peroxynitrite-induced iNOS expression. Upon evaluating the activity of NF-κB in treated cells, a decrease in the nitration of IkB was observed. Moreover, a decrease in the peroxynitrite-induced expression of COX-2 and IL-6 production was also observed. These results allow postulating anthocyanidins as potential protective agents against cardiovascular diseases; and therefore, they are considered useful in the development of functional and nutraceutical foods.

The anti-inflammatory activity of malvidin (3,4′,5,7-tetrahydroxy-3′,5′-dimethoxyflavylium, *Mal*), the main constituent of wine, has been evaluated in LPS-stimulated RAW 264.7 macrophages. In these cells, the treatment with *Mal* blocked the activation of NF-κB induced by LPS and the ROS production. Besides *Mal* downregulated the activation of MAPK, stimulated the expression of MKP-1 and activated the PI-3-kinase-Akt pathway. Moreover, *Mal* maintained the mitochondrial membrane potential after LPS-induced depolarization in RAW 264.7 macrophages and reduced the nuclear translocation and the binding of NF-κB to DNA (Bognar et al. 2013). Furthermore, neither peonidin (3,4′,5,7-tetrahydroxy-3′-methoxyflavylium, *Peo*) nor *Mal* decreased the expression of inflammatory genes when added alone; however, the treatment of adipocytes with a combination of *Mal* and *Peo* (1:1) followed by LPS decreased the mRNA levels of IL-6, IL-1β, IL-8, MCP-1, TLR2, TNF-α, COX-2 and INF-γ-induced protein-10 (Mackert and McIntosh 2016).

Pelargonidin (3,4′,5,7-tetrahydroxyflavylium, *Pel*) and its glucoside form pelargonidin-3-glucoside (*P3G*), which are found in blue, purple and red fruits and vegetables, have antioxidant and antidiabetic activities in vivo. *Pel* inhibits the LPS-mediated secretion of HMGB1 by endothelial cells. HMGB1, a nucleosomal protein, mediates the production of TNF-α, IL-1α, IL-1β and IL-6 and activates NF-κB and ERK1/2 in HUVECs. In these cells, these effects are prevented by *Pel* (Min et al. 2016).

Byun et al. (2013) have evaluated the anti-inflammatory potential of procyanidin trimer *C1* in LPS-stimulated primary bone marrow-derived macrophages (BMDM) as an alternative to the tumorigenic RAW 264.7 cell line. The pretreatment with *C1* prevented the production of iNOS-derived NO and the pro-inflammatory cytokines IL-6 and TNF-α. Concurrently, in BMDM, it was observed that *C1* inhibited the release of $PGE_2$ and COX-2 and the expression of cell surface molecules (CD80, CD86 and MHC class II). It is believed that the downregulation of TLR4 would be responsible for the inhibition of MAPK and NF-κB signaling induced by LPS.

## *4.7 Isoflavonoids*

Genistein (4′,5,7-trihydroxyisoflavone, *Gen*), an isoflavone derivative found in soy, has proved to reduce the secretion of IL-1β, IL-6 and IL-8 from TNF-α-stimulated MH7A cells (human synoviocytes). Upon TNF-α stimulation, NF-κB translocation to the nucleus and IκB kinase-α/β and IκBα phosphorylation were suppressed by *Gen*, and AMPK activity was inhibited. The inhibitory effect of *Gen* on TNF-α-induced pro-inflammatory cytokine production is dependent on AMPK activation. Data suggest that *Gen* would suppress TNF-α-induced inflammation through the inhibition of the ROS/Akt/NF-κB pathway and the promotion of AMPK activation in these cells (Li et al. 2014b). *Gen* also has anti-inflammatory effects on BV-2 microglia cells stimulated with the β-amyloid peptide 25–35 (Ab25–35). *Gen* has been demonstrated to revert the up-regulation of the mRNA and protein expression of IL-1β and iNOS and the downregulation of the expression of IL-10 caused by Ab25–35. This flavonoid also reverted the upregulation of TLR4 and NF-κB (p65 and p50) and inhibited the DNA binding and transcriptional activities of NF-kB (Zhou et al. 2014).

*GEN-27* [5-hydroxy-7-[2-hydroxy-3-(piperidin-1-yl) propoxy]-3-{4-[2-hydroxy-3-(piperidin-1-yl) propoxy] phenyl}-4H-chromen-4-one] is a newly synthesized *Gen* derivative which reduces the secretion of pro-inflammatory cytokines IL-6 and IL-1 in THP-1 (human monocytes) and inhibits the nuclear translocation of NF-κB and phosphorylation of IκB and IKKα/β in both HCT116 (human colon tumour) and THP-1 cells. *GEN-27* modulates the NF-κB signaling pathway involved in inflammation-induced cancer cell proliferation (Wang et al. 2016).

Puerarin 8-(β-D-glucopyranosyl-7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, *Pue*) is an isoflavonoid isolated from the roots of *Pueraria lobate*, a plant used in the traditional Chinese medicine. *Pue* has been demonstrated to improve the histologic parameters of ovalbumin (OVA)-induced allergic inflammation in a murine asthma model. The increase in eosinophil counts and IL-4, IL-5 and IL-13 caused by OVA were prevented by the administration of *Pue*. On the other hand, IFN-γ levels, which were reduced after OVA induction, were restored by the flavonoid. *Pue* substantially inhibited eotaxin-3 levels, as compared with controls (Wang et al. 2015a).

Daidzein 4′,7-dihydroxyisoflavone (*Dai*) is an isoflavone found in soy. It has been demonstrated that *Dai* has effects on the adipocyte-macrophage crosstalk. When 3 T3-L1 adipocytes were cocultured with RAW 264.7 macrophages and treated with *Dai*, the increased mRNA levels of MCP1 and IL-6 were reduced. This phenomenon was also observed in RAW 264.7 macrophages cultured alone with *Dai*. *Dai* induced a significant inhibition of the palmitate-induced phosphorylation of JNK; however, no effects were observed on NF-κB activation after treatment with the flavonoid. *Dai* probably regulates pro-inflammatory gene expression by activating PPAR-α and PPAR-γ and by inhibiting the JNK pathway in adipocyte-macrophage cocultures (Sakamoto et al. 2016).

Dong et al. (2017) have determined the anti-inflammatory effects and molecular mechanisms of ononin (formononetin-7-glucoside, *Ono*) in LPS-stimulated RAW 264.7 macrophages. *Ono* has been isolated from the roots of *Astragalus membranaceus* (Fisch.) Bunge. This flavonoid did not alter cell viability. *Ono* downregulated mRNA expression of COX-2 and iNOS and inhibited the synthesis of $PGE_2$ and NO and the production of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6. In addition, in LPS-treated cells, the phosphorylation of IκB-α, ERK, JNK and MAPKs proteins was significantly increased by *Ono*. This finding suggests that the anti-inflammatory activity is exerted through the modulation of the translocation of NF-κB and MAPK pathway-related proteins. Yang et al. (2013) have evaluated the anti-inflammatory activity of Prunetin (4′,5-dihydroxy-7-methoxyisoflavone, *Pru*) and elucidated its molecular mechanism of action. *Pru* effects were evaluated in LPS-stimulated murine macrophages. In vitro assays have demonstrated that *Pru* inhibits LPS-induced NO and $PGE_2$ production through the suppression of iNOS and COX-2 at the transcriptional level. Besides *Pru* avoided the activation of NF-κB and the subsequent downstream induction of pro-inflammatory mediators such as TNF-α, IL-6 and IL-1β by the negative modulation of phosphorylation of IKK-IκBα-NF-κB signaling. The treatment of RAW 264.7 macrophages with *Pru* decreased the expression of iNOS and COX-2 and pro-inflammatory mediators (NO and $PGE_2$). As a consequence, MAPK and NF-κB signaling pathways were affected by *Pru*.

## *4.8 Chalcones*

1-(3,4-Dihydroxyphenyl)-3-(2-methoxyphenyl)prop-2-en-1-one (*L2H17*), a synthetic chalcone derivative, inhibits the expression of pro-inflammatory cytokines (TNF-α and IL-6), cell adhesion molecules (VCAM-1 and ICAM-1), chemokines and macrophage adhesion via modulation of the MAPK/NF-κB pathway in peritoneal macrophages in a hyperglycemia-induced inflammation murine model. Similar effects were observed in vivo, which contributed to a reduction of key markers for renal and cardiac dysfunction. In fact, in diabetic mice treated with *L2H17*, less fibrosis and pathological changes in both renal and cardiac tissues were observed (Fang et al. 2015b). In obesity-related glomerulopathy, it has been observed that *L2H17* protects against renal injury also by modulating the MAPK/NF-κB pathways and decreasing the expression of pro-inflammatory cytokines and cell adhesion molecules (Fang et al. 2015a).

Structure-activity relationship studies have shown that α-X-substituted 2′,3,4,4′-tetramethoxychalcones enhance the transcriptional activity of Nrf2 while inhibiting NF-κB. Inflammatory signaling pathways are known to be modulated by compounds that alkylate cysteinyl thiols. A positive correlation has been found between the anti-inflammatory and the thiol alkylating activity, that is, stronger electrophiles (X = $CF_3$, Br and Cl) are more potent. Nonetheless, the strongest electrophiles (X = CN and $NO_2$) have been found to be ineffective (Rücker et al. 2015).

In brain endothelial cells, isobavachalcone (2′,4,4′-trihydroxy-3′-(3-methyl-2-butenyl)-chalcone, *Ibc*), which is a flavonoid present in *Psoralea corylifolia*, downregulates ICAM-1 expression and arrests NF-κB activity upon LPS stimulation, as well as after macrophage-activating lipopeptide 2-kDa (MALP-2) or polyriboinosinic polyribocytidylic acid (poly[I:C]) exposure. *Ibc* also downregulates LPS or poly[I:C]-induced expression of IFN-β, indicating that it can modulate both MyD88-dependent and TRIF-dependent signaling of TLR4 (Lee et al. 2015). *Ibc*, isolated from *Angelica keiskei*, has been demonstrated to modulate the inflammatory response. The modulation of iNOS expression by *Ibc* in murine macrophages stimulated with TLR agonists has been evaluated. *Ibc* suppressed the iNOS expression induced by MALP-2 (TLR2 and TLR6), poly [I:C] (TLR3) and LPS (TLR4). As *Ibc* was able to regulate the TLR signaling pathways, and considering that these receptors are known to be directly related to the induction of the innate immune response, *Ibc* could be considered a potential anti-inflammatory drug (Shin et al. 2013).

Chalcone glycosides are 4′-glycosidised-3′-oxychalcones and have been reported in *Brassica rapa* L. 'hidabeni', a popular Japanese turnip mainly cultivated and consumed as a traditional vegetable. The activities of various synthetic 'hidabeni' chalcones have been studied. Two compounds (3′,3,4,5-tetramethoxy-4′-hydroxychalcone and 3′,3,4,5-tetramethoxychalcone) have proved to inhibit NO production. The suppression of the LPS-induced iNOS expression caused by these compounds was due to the inhibition of STAT1, but not NF-κB, JNK or p38, pathways. 3′,3,4,5-tetramethoxychalcone also inhibited the activation of the MEK/ERK pathway (Hara et al. 2014).

Phloretin (2′,4′,6′-trihydroxy-3-(4-hydroxyphenyl)propiophenone, *Phl*) is a dihydrochalcone isolated from the apple tree and the pear tree. This flavonoid inhibits the release of $PGE_2$, the expression of COX-2 and the production of IL-8, MCP-1 and IL-6 in IL-1β-stimulated human lung epithelial A549 cells. ICAM-1 gene and protein expression along with monocyte adhesion to inflammatory A549 cells were suppressed by the flavonoid. *Phl* modified different signaling cascades causing inhibition of phosphorylation of Akt and MAPK and a reduction in nuclear translocation of NF-κB p65. *Phl* might exert an anti-inflammatory effect by inhibiting the synthesis of pro-inflammatory cytokines and COX-2 and ICAM-1 expression through the blockage of NF-κB and MAPK signaling pathways (Huang et al. 2015).

The chalcone (E)-3-(3,4-dimethoxyphenyl)-1-(5-hydroxy-2,2-dimethyl-2H-chromen-6-yl) prop-2-en-1-one (*5B*) has been demonstrated to reduce carrageenan-induced mouse foot edema and adjuvant-induced arthritis. In addition, the antiarthritic effects of *5B* have been evaluated in a collagen-induced arthritis in vivo model, while to investigate molecular mechanisms involved in the anti-inflammatory effects, the RAW 264.7 cell line was used. The pretreatment with *5B* prevented the advance of arthritis together with the blockade of the recruitment of $CD68^+$ cells in the knee joint. Moreover, a decrease in the secretion of TNF-α, IL-1β and IL-6 was observed. In LPS-stimulated macrophages, *5B* suppressed the expression of iNOS, COX-2, TNF-α, IL-6, IL-1β, NO and $PGE_2$. Besides, *5B* suppressed the activation of NF-κB induced by LPS; the latter effect was achieved by a modulation of IκB

phosphorylation, the degradation of IκB and the nuclear translocation of p65 and p50. Likewise, *5B* suppressed the expression of TLR4 induced by LPS, the degradation of IL-1 receptor-associated kinase (IRAK) and the phosphorylation of JNK and ERK, but it had little positive effect on the activation of p38 kinase. Thus, *5B* could be a potential agent against rheumatoid arthritis, since its anti-inflammatory effect was found to be mediated by the TLR4, NF-κB and ERK/JNK signaling pathways in monocytes (Li et al. 2013).

Flavokawain A (2′-hydroxy-4,4′,6′-trimethoxychalcone, *FlkA*) is a chalcone derivative isolated from kava (*Piper methysticum*) extracts, which have been used as popular beverage in the Pacific islands. The suppressive effect of *FlkA* on the expression of pro-inflammatory mediators in LPS-stimulated macrophages and the molecular mechanisms responsible for these activities have been evaluated. *FlkA* inhibited the expression of iNOS and COX-2, together with the production of NO and $PGE_2$ in LPS-stimulated RAW 264.7 cells. The activation of the NF-κB and AP-1 signaling pathways were negatively affected when the cells were treated with *FlkA*. In the same experimental model, this flavonoid also attenuated the activation of JNK and p38 MAPK, which are responsible for the expression of iNOS and COX-2. In addition, *FlkA* blocked the expression of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6. These findings allowed concluding that *FlkA* modulates the expression of pro-inflammatory mediators through NF-kB, AP-1 and JNK/p38 MAPK signaling pathways (Kwon et al. 2013). Another natural chalcone, *licochalcone C* ((2E)-3- (4-hydroxy-2-methoxy-3- (3-methyl-2-butenyl) phenyl)-1-(4-hydroxyphenyl) -2-propen-1-one, *LicoC*), has been found to inhibit NF-κB translocation and the generation of pro-inflammatory mediators, such as iNOS, ICAM-1 and VCAM-1. Furthermore, *LicoC* stimulated the phosphorylation of PI3K/Akt/eNOS with the consequent activation of the signaling pathway. As the protective effect of *LicoC* could be blocked with a specific PI3K inhibitor, the presence of this compound would be essential in the sepsis-induced inflammation (Franceschelli et al. 2017). The effects of flavonoids on intracellular signaling pathways and mediators associated with inflammation are summarized in Table 1.

## 5 Studies Performed in Humans

Studies assessing the evaluation of the effects of flavonoids in inflammation performed in either healthy human volunteers or in patients are scarce, as compared to in vitro and in vivo assays. Most of the studies have consisted in the administration of foods such as tea, fruit juices, grape extracts and red wine containing a mixture of flavonoids. Other studies evaluate the activity of pure polyphenolic compounds. Ribeiro et al. (2015) have reviewed the studies published before 2014. More recent research works include a systematic review carried out by Rangel-Huerta et al. (2015). In that review, authors examine the efficacy of phenolic compounds in cardiovascular diseases. Seventy-two articles were selected in which randomized

**Table 1** Effects of flavonoids on intracellular signaling pathways and mediators associated with inflammation

| Compound | Mechanism of action | | Reference |
|---|---|---|---|
| | Signaling pathway | Mediators | |
| *Flavones* | | | |
| Chrisin | ↓ NF-κB | ↓ COX-2; ↓ iNOS; ↓ IL-1β; ↓ IL-6; ↓ IL-12; ↓ IL-1α; ↓ IL-17A; ↓ IFN-γ; ↓ TNF-α | Yao et al. (2014) |
| | ↓ phosphorylation of Akt and ERK | ↓ IL-4; ↓ IL-12; ↑ IFN-γ | Yao et al. (2016) |
| | ↓ NF-κB | ↓ TNF-α; ↓ SOD; ↓ eNOS; ↓ NO | Rani et al. (2016) |
| | ↓ NF-κB | ↓ IL-8; ↓ IL-6; ↓ MCP-1; ↓ $PGE_2$ | During and Larondelle (2013) |
| Luteolin | ↓ AP-1; ↓ NF-κB; ↓ MAPK; ↓ STAT-3 | ↓ HIF-1α; ↓ COX-2; ↓ iNOS; ↓ IL-1β; ↓ IL-6; ↓ IL-8; ↓ TNF-α; ↓ VEGF | Pratheeshkumar et al. (2014) |
| | ↓ NF-κB | ↓ MCP-1; ↓ VCAM-1; ↓ ICAM-1 | Jia et al. (2015) |
| | ↓ NF-κB; ↓ MAPK | ↓ COX-2; ↓ TNF-α; ↓ IL-1β; ↓ IL-6; ↓ IL-8 | Zhang et al. (2017) |
| Vitexin | ↓HMGB1; ↓(p38) MAPK | ↓ TNF-α | Wang et al. (2017) |
| Amentoflavone | ↓ NF-κB (p65–50) | ↓TNF-α; ↓ IL-1β; ↓ IL-6; ↓ COX-2; ↓ iNOS | Sakthivel and Guruvayoorappan (2013) |
| Wogonin | ↓TLR4, ↓ NF-κB | ↓MMP-9; ↓IL-1β; ↓IL-6; ↓IPM-2; ↓COX-2 | Chen et al. (2012) |
| Velutin | ↓ NF-κB; ↓ (p38) MAPK; ↓ JNK | ↓TNF-α; ↓ IL-6 | Xie et al. (2012) |
| Apigenin | ↓Akt; ↓p38 MAPK | ↓ TNF-α; ↓ MMP-9; ↓ eNOS | Palmieri et al. (2012) |
| | – | ↓ IL17; ↓ LTA | Johnson and De Mejia (2013) |
| | ↓MAPK; ↓NF-κB | ↓ TNF-α; ↓ TGF-β1 | Malik et al. (2017) |
| Tricin | – | ↓TNF-α; ↓IL-6; ↓iNOS; ↓COX-2 | Shalini et al. (2012) |
| Eupatilin | ↓Akt; ↓NF-κB | ↓ TNF-α; ↓ VCAM-1; ↓ ICAM-1 | Jung et al. (2012) |

(continued)

**Table 1** (continued)

| Compound | Mechanism of action | | Reference |
|---|---|---|---|
| | Signaling pathway | Mediators | |
| 3,5,7-trihydroxy-4′-methoxy-8-(3-hydroxy-3-methylbutyl)-flavone | ↓ CD14 / TLR4 | – | Wu et al. (2012) |
| 4'-Hydroxywogonin | ↓ TAK1 / IKK / NF-κB;<br>↓ PI3K / Akt | ↓ iNOS; ↓ PGE2; ↓ TAK1;<br>↓ TAB1 | Fan et al. (2017) |
| 4′-bromo-5,6,7-trimethoxyflavone | ↓NF-κB | ↓ iNOS; ↓ COX-2;<br>↓ TNF-α, ↓ IL-6, ↓ IL-1β;<br>↓ NO, ↓ $PGE_2$ | Kim et al. (2012) |
| Oroxylin A | ↓TLR4; ↓ MAPKs;<br>↓ NF-κB | ↓ JNK; ↓ p38 NF-κB;<br>↓ ERK | Song et al. (2012) |
| *Flavonols* | | | |
| Fisetin | ↓ PI3K; ↓ phosphorylation of Akt;<br>↓ NF-κB | ↓ COX-2; ↓ $PGE_2$; ↓ MPO;<br>↓ IL-1β; ↓ IL-6; ↓ TNF-α | Pal et al. (2015) |
| | ↓ NF-κB; ↓ JNK | ↓ IL-1β; ↓ IL-6; ↓ TNF-α | Jo et al. (2014) |
| | ↓ ERK; ↓ JNK; ↓ MAPK (p38) | ↓ COX-2; ↓ $PGE_2$ | Gutiérrez-Venegas et al. (2014) |
| | ↓ NF-κB; ↓ TLR-4 | ↓ IL-1β; ↓ TNF-α | Zhou et al. (2015a) |
| | ↓ NF-κB; ↓ ERK; ↓ Akt | ↓ IL-1β; ↓ TNF-α | Yoo et al. (2014) |
| | ↓ NF-κB; ↓ JNK;<br>↓ MAPK (p38) | ↓ COX-2; ↓ iNOS; ↓ $PGE_2$;<br>↓ TNF-α | Maher (2015) |
| | ↑ PI3K/Akt; ↑ MAPK | ↓ iNOS; ↓ NO; ↓ IL-1β;<br>↑ HO-1 | Chuang et al. (2014) |
| Icariin | ↓ ERK; ↓ MAPK (p38) | ↓ IL-1β; ↓ IL-6; ↓ IL-8;<br>↓ MCP-1; ↓ ICAM-1 | Kong et al. (2015) |
| | ↓ NF-κB | ↓ IL-1β; ↓ TNF-α; ↓ iNOS | Liu et al. (2015) |
| | ↓ NF-κB | ↓ IL-2; ↓ IL-6; ↓ TNF-α;<br>↓ ICAM-1 | Chen et al. (2015) |

| | | | |
|---|---|---|---|
| Astragalin | ↓ NF-κB; ↓ ERK; ↓ TLR-4 | ↓ IL-6; ↓ TNF-α; ↓ NO;<br>↓ COX-2; ↓ iNOS | Li et al. (2014a) |
| Rutin | ↓ NF-κB | ↓ TNF-α | Lee et al. (2013) |
| | ↓ NF-κB | ↓ TNF-α; ↓ IL-6 | Yoo et al. (2013) |
| *Flavanones* | | | |
| Alpinetin | ↓ NF-κB; ↓ MAPK | ↓ TNF-α; ↓ IL-6; ↓ IL-1β;<br>↓ IκB-α; ↓ NF-κB p65;<br>↓ p38 | Huo et al. (2012) |
| | ↓ NF-κB | ↓ TNF-α; ↓ IL-6; ↓ IL-1β;<br>↓ IκB-α; ↓ NF-κB p65;<br>↓ TLR4 | Chen et al. (2013) |
| | ↓ NF-κB; ↓ MAPK | ↓ TNF-α; ↓ IL-6; ↓ IL-1β;<br>↓ IκBα; ↓ ERK; ↓ JNK;<br>↓ p38; ↓ p65 | Hu et al. (2013) |
| Sophoraflavanone G | ↓ NF-κB; ↓ MAPK; | ↓ IL-1β, ↓ IL-6; ↓ TNF-α; ↓iNOS; ↓COX-2; ↓ ERK;<br>↓ JNK; ↓ p38 | Wun et al. (2013) |
| Ugonin M | ↓ NF-κB; ↓ MAPK (p38) | ↓NO, ↓TNF-α, ↓ IL-1β;<br>↓ IL-6; ↓ iNOS; ↓ COX-2;<br>↓ IκB-α; ↓ ERK; ↓ TLR | Wu et al. (2017) |
| Naringenin | ↓ NF-κB; ↓ ERK;<br>↓ JNK; ↓ MAPK (p38) | ↓ IL-6; ↓ TNF-α; ↓ NO;<br>↓ SOD; ↓ NOS; ↓ MPO | Yu et al. (2014) |
| | ↑ AMPK; ↑ PKCδ | ↓ COX-2; ↓ iNOS; ↓ NO;<br>↑ SOCS-3 | Wu et al. (2016) |

(continued)

**Table 1** (continued)

| Compound | Mechanism of action | | Reference |
|---|---|---|---|
| | Signaling pathway | Mediators | |
| Naringin | ↓ JNK; ↓ MAPK (p38) | ↓ IL-1β; ↓ IL-6; ↓ IL-8; ↓ COX-2 | Ren et al. (2016) |
| | ↓ p53; ↓ NF-κB | ↓ SOD; ↓ CAT; ↓ NO; ↓ TNF-α | Chtourou et al. (2015) |
| | ↑ PPARγ | ↓ IL-6; ↓ TNF-α | Qi et al. (2015) |
| | ↓ MAPK (p38); ↓ NF-κB | – | Manna et al. (2015) |
| Pinocembrin | ↓ MAPK; ↓ NF-κB | ↓ IL-1β; ↓ IL-6; ↓ TNF-α | Liu et al. (2014b) |
| | ↓ PI3K; ↓ phosphorylation of Akt; ↓ NF-κB; ↑ Nrf2 | ↓ IL-1β; ↓ TNF-α; ↓ NO; ↓ $PGE_2$; ↓ COX-2; ↓ iNOS; ↑ HO-1 | Zhou et al. (2015b) |
| *Flavanonols* | | | |
| Taxifolin | ↑ Nrf2; ↓ NF-κB; ↓ Wnt | ↓ TNF-α; ↓ COX-2 | Manigandan et al. (2015) |
| 2′-hydroxy yokovanol / 2′-hydroxy neophellamuretin | – | ↓ IL-12; ↓ IL-6; ↓ TNF-α | Li et al. (2014c) |
| Ampelopsin | ↓ NF-κB; ↓ MAPK; ↓ROS; ↓Akt; ↓IKK | ↓NO, ↓TNF -α, ↓ IL-1β; ↓ IL-6; ↓ iNOS; ↓ NF-κB p65; ↓KKα / β; ↓IκBα | Qi et al. (2012) |

| | | | |
|---|---|---|---|
| *Flavan-3-ols* | | | |
| Epigallocatechin-3-gallate | ↓ TLR-4; ↑ Tollip | – | Byun et al. (2014a) |
| | ↓ NF-κB | ↓ IL-6; ↓ TNF-α | Bao et al. (2015) |
| | ↓ phosphorylation of Akt; ↓ ERK; ↓ JNK;<br>↓ MAPK (p38);<br>↑ PPARγ; ↑ Nrf2 | – | Ye et al. (2015) |
| | ↓ MAPK; ↓ NF-κB | ↓ TNF-α; ↓ RANTES;<br>↓ MCP-1; ↓ ICAM-1; ↓ NO; ↓ VEGF; ↓ MMP-2 | Liu et al. (2014a) |
| | ↓ NF-κB | ↓ IL-6; ↓ CRP; ↓ ICAM-1;<br>↓ VCAM-1; ↓ IL-1 α/β | Liu et al. (2016) |
| | ↑ Nrf2; ↓ NF-κB | ↓ IL-1β; ↓ IL-6; ↓ TNF-α;<br>↑ HO-1 | Wang et al. (2015b) |
| | Notch-1 | ↓ IL-6; ↓ TNF-α; ↓ ICAM-1; ↓ MCP-1 | Xie et al. (2015) |
| | ↓ NF-κB;<br>↓ TGF/SMAD;<br>↓ PI3K/Akt | ↓ TNF-α; ↓ COX-2; ↓ iNOS | Xiao et al. (2014) |
| Catechin | ↓ Akt; ↓ ERK;<br>↓ NF-κB; ↓ MAPK (p38); ↓ AMPK | ↓ COX-2; ↓ iNOS; ↓ ROS;<br>↓ NO; ↓ IL-6; ↓ TNF-α;<br>↑ IL-4 | Hussein et al. (2015) |
| *Anthocyanidins* | | | |
| Cyanidin-3-glucoside | ↓ NF-κB; ↓ ERK;<br>↓ JNK; ↓ MAPK (p38) | ↓ IL-1β; ↓ IL-6; ↓ TNF-α | Ma et al. (2015) |
| | ↓ NF-κB; ↑ Nrf2 | – | Fratantonio et al. (2015) |
| | ↓ NF-κB | ↓ TNF-α | Ferrari et al. (2017) |
| | ↓ MAPK (p38); ↓ Akt | ↓ p38, ↓ ERK; ↓ JNK | He et al. (2017) |

(continued)

**Table 1** (continued)

| Compound | Mechanism of action | | Reference |
|---|---|---|---|
| | Signaling pathway | Mediators | |
| Peonidin<br>Malvidin | ↓ TLR-2 | ↓ IL-1β; ↓ IL-6; ↓ IL-8;<br>↓ TNF-α; ↓ MCP-1; ↓ COX-2 | Mackert and McIntosh (2016) |
| Pelargonidin | ↓ NF-κB; ↓ ERK | ↓ IL-1α; ↓ IL-1β; ↓ IL-6;<br>↓ TNF-α | Min et al. (2016) |
| Malvidin | ↓ NF-κB; ↓ MAPK; ↓ROS | ↓ MKP-1; ↓ Akt | Bognar et al. (2013) |
| Malvidin 3O-Glucoside | ↓ NF-κB; ↓Akt | ↓ iNOS; ↓ COX-2; ↓ IL-6 | Paixão et al. (2012) |
| Procyanidin trimer *C1* | ↓ NF-κB; ↓ MAPK | ↓ IL-6; ↓ TNF-α; ↓ $PGE_2$;<br>↓ COX-2; ↓ TLR4 | Byun et al. (2013) |
| *Isoflavonoids* | | | |
| Genistein | ↓ NF-κB; ↑ AMPK | ↓ IL-1β; ↓ IL-6; ↓ IL-8;<br>↓ TNF-α | Li et al. (2014b) |
| | ↓ NF-κB; ↓ TLR4 | ↓ IL-1β; ↓ iNOS; ↑ IL-10 | Zhou et al. (2014) |
| GEN-27 | ↓ NF-κB | ↓ IL-1; ↓ IL-6 | Wang et al. (2016) |
| Puerarin | – | ↓ IL-4; ↓ IL-5; ↓ IL-13;<br>↑ IFN-γ | Wang et al. (2015a) |
| Daidzein | ↓ JNK; ↑ PPAR-α/−γ | ↓ IL-6; ↓ MCP-1 | Sakamoto et al. (2016) |
| Ononin | ↓ NF-κB; ↓ MAPK | ↓ COX-2; ↓ iNOS; ↓ IL-6;<br>↓ TNF-α; ↓ IL-1β | Dong et al. (2017) |
| Prunetin | ↓ NF-κB; ↓ MAPK | ↓ COX-2; ↓ iNOS; ↓ IL-6;<br>↓ TNF-α; ↓ IL-1β | Yang et al. (2013) |
| *Chalcones* | | | |
| L2H17 | ↓ MAPK; ↓ NF-κB | ↓ IL-6; ↓ TNF-α; ↓ ICAM-1; ↓ VCAM-1 | Fang et al. (2015a) |
| | ↓ MAPK; ↓ NF-κB | ↓ IL-6; ↓ TNF-α; ↓ ICAM-1; ↓ VCAM-1; ↓ IL-1β; ↓ IL-2; ↓ IL-12; ↓ IFN-γ | Fang et al. (2015b) |

| | | | |
|---|---|---|---|
| Isobavachalcone | ↓ NF-κB | ↓ ICAM-1; ↓ IFN-β | Lee et al. (2015) |
| | ↓TLR4 | ↓ iNOS; ↓ MALP-2 (TLR2 and TLR6); ↓ poly [I: C] | Shin et al. (2013) |
| 3′,3,4,5-tetramethoxy-4′-hydroxychalcone | ↓ STAT-1 | ↓ NO | Hara et al. (2014) |
| 3′,3,4,5-tetramethoxychalcone | ↓ STAT-1; ↓ ERK | ↓ NO | Hara et al. (2014) |
| Phloretin | ↓ MAPK; ↓ NF-κB;<br>↓ phosphorylation of Akt | ↓ IL-6; ↓ IL-8; ↓ IL-1β;<br>↓ MCP-1; ↓ COX-2;<br>↓ ICAM-1; ↓ $PGE_2$ | Huang et al. (2015) |
| (E) -3- (3,4-Dimethoxyphenyl) -1-(5-hydroxy-2,2-dimethyl-2H-chromen-6-yl) prop-2-en-1-one | ↓ NF-κB; ↓ MAPK | ↓ IL-6; ↓ TNF-α; ↓ IL-1β;<br>↓ $PGE_2$; ↓ COX-2; ↓ iNOS;<br>↓ ERK; ↓ JNK | Li et al. (2013) |
| Flavokawain A | ↓ NF-κB; ↓ AP-1; ↓JNK; ↓ p38 MAPK | ↓ COX-2; ↓ iNOS; ↓ IL-6;<br>↓ TNF-α; ↓ IL-1β | Kwon et al. (2013) |
| Licochalcone C | ↓ NF-κB | ↓ iNOS, ↓ ICAM-1,<br>↓ VCAM-1 | Franceschelli et al. (2017) |

↓ reduces, inhibits or downregulates, ↑ increases, stimulates or up-regulates

controlled trials with prospective, parallel or crossover designs in humans were included. Evidences indicate that flavonols are helpful in decreasing risk factors of cardiovascular disease, although further rigorous works are necessary to support that hypothesis. Cassidy et al. (2015) have conducted a study in a population of adults in the United States to assess if higher dietary flavonoid (anthocyanins, flavonols, flavanones, flavan-3-ols, polymers and flavones) intakes are associated with anti-inflammatory effects. The authors used an inflammation score that integrated 12 individual inflammatory biomarkers, which included CRP, TNF-α, IL-6, MCP-1 and MPO, among others. The authors concluded that there are evidences suggesting that the anti-inflammatory effect may be the central component underlying the reduction of risk of certain chronic diseases associated with higher intakes of anthocyanins and flavonols. The effects of (-)-epicatechin and quercetin-3-glucoside on some biomarkers of endothelial dysfunction and inflammation have been evaluated in a randomized double-blind, placebo-controlled, crossover trial in (pre)hypertensive adults. Results have shown that diet supplementation with pure epicatechin (100 mg/d) for a period of 4 weeks decreased soluble E-selectin levels, which is a marker of endothelial dysfunction. Supplementation with quercetin-3-glucoside (160 mg/d), during the same period, significantly decreased the levels of soluble E-selectin and IL-1β and the z score for inflammation (Dower et al. 2015). Recently, Javadi et al. (2017) have assessed the effects of *Quer* supplementation (500 mg/day, 8 weeks) on inflammatory factors and clinical symptoms. The study was a randomized, double-blind, placebo-controlled clinical trial of women with rheumatoid arthritis. The authors concluded that symptoms, including pain, early morning stiffness, disease activity and health assessment questionnaire score, were improved following *Quer* supplementation and demonstrated that *Quer* decreased TNF-α levels, possibly through suppression of cytokine gene expression. Kokkou et al. (2016) have carried out a study to evaluate the impact of grape juice supplementation on smoking-induced inflammatory processes and fibrinolytic impairment. The study has had a randomized, placebo-controlled, double-blind, cross-over design in which 26 healthy smokers received a 2-week oral treatment. Serum levels of ICAM-1 and plasminogen activator inhibitor 1 (PAI-1) were measured as markers of inflammatory and fibrinolytic status, respectively. The treatment with grape juice improved inflammatory and fibrinolytic status in healthy smokers and attenuated the acute smoking-induced increase of ICAM-1 and PAI-1 levels.

## 6 Conclusions

A high dietary flavonoid intake has been associated with a reduced risk and prevalence of cardiovascular and other inflammation-related diseases. Thus, over the past 10–15 years, research on flavonoids has received much attention in order to investigate their potential as new therapeutic drugs to treat these inflammatory disorders. Flavonoids have many advantages, as compared to synthetic drugs. These include fewer side effects and the fact that they are widely distributed in foods. Besides,

they are readily absorbed in the intestine. As shown herein, the anti-inflammatory activity of flavonoids involves modulation of pro-inflammatory mediators through different intracellular pathways displaying a multitarget anti-inflammatory action. Research on this type of natural compounds has been carried out with the different classes of flavonoids; however, most of the studies are in vitro assays or animal models. According to the presented data, flavonoids could be considered candidates to proceed to the next phase in the drug development process. To date, human studies are scarce, but they provide some evidence of the efficacy of flavonoids as potential anti-inflammatory agents. Therefore, further well-designed in vivo experiments, along with good quality clinical studies, are needed to obtain conclusive results to determine if the findings obtained in vitro can be extrapolated to in vivo systems.

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