Flavonoids Health Benefits and Their Molecular Mechanism

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Abstract: Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plants. Until now, more than 9000 different flavonoid compounds were described in plants, where they play important biological roles by affecting several developmental processes. There has been increasing interest in the research of flavonoids from dietary sources, due to growing evidence of the versatile health benefits of flavonoids including anti-inflammatory, antioxidant, antiproliferative and anticancer activity, freeradical scavenging capacity, antihypertensive effects, coronary heart disease prevention and anti-human immunodeficiency virus functions. This paper reviews the current advances in flavonoids in food with emphasis on mechanism aspects on the basis of the published literature, which may provide some guidance for researchers in further investigations and for industries in developing practical health agents.

Keywords: Flavonoids, mechanism, health benefits, review.

INTRODUCTION

Flavonoids are a large group of phenolic plant constituents. Approximately 9000 different flavonoids from different plant sources have been described so far, and each year, hundreds of newly identified flavonoids are being recorded in the literature [1]. They occur mainly in fruit, vegetables, nuts, seeds, flowers, and bark and are generally present in plants as glycosides [2, 3].

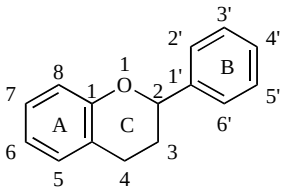

Fig. (1). Chemical structure of the flavonoid nuclear.

In general, all flavonoids are derivatives of the 2-phenylchromone parent compound composed of three phenolic rings referred to as A, B and C rings (Fig. **1**). Flavonoids are classified according to their chemical structure. The major flavonoid classes include flavones, flavonols, flavanones, catechins (or flavanols), anthocyanidins, isoflavones, dihydroflavonols, and chalcones (Fig. **2**). As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity in both *in vivo* and *in vitro* systems [4-7]. The functionality in human health is supported by the ability of the flavonoids to induce human protective enzyme systems [8, 9], and by a number of epidemiological studies suggesting protective effects against cardiovascular diseases, cancers, and other age-related diseases [10-12]. Further studies are necessary for confirmation of the beneficial effects, identification of dose-response relationships, and identification the most bioactive flavonoids. Several reviews have been written on dietary sources [11, 13-15], bioactivities [13, 16-19] and biosynthesis [20-23] of flavonoids previously and so they will not be covered in detail here. This review surveys work done since 2005 in exploring the acting mechanism of flavonoids.

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INDUCERS OF HEME OXYGENASE-1

The anti-inflammatory properties of chalcones in murine macrophages were initially reported to involve a direct scavenging effect on superoxide anion production and inhibition of inducible nitric oxide synthase (iNOS) expression [24, 25]. However, more recent data reveal that the anti-inflammatory and protective effects of chalcones are strongly associated with and depend on the expression and activity of heme oxygenase-1 (HO-1) [26-27]. This inducible protein is the limiting step in the catabolism of heme to biliverdin and carbon monoxide (CO), two endogenous by-products that have been demonstrated to participate directly in the cellular defense against oxidative stress and inflammatory processes [28-30].

Sawle *et al.* [31] show that heme oxygenase activity and HO-1 expression markedly increased in macrophages exposed to 5-25 μM chalcones possessing one, three, or six methoxy substituents present on the aromatic rings. The increased heme oxygenase activity was directly associated with an augmented anti-inflammatory effect, which can be maximally achieved within a range of concentrations of

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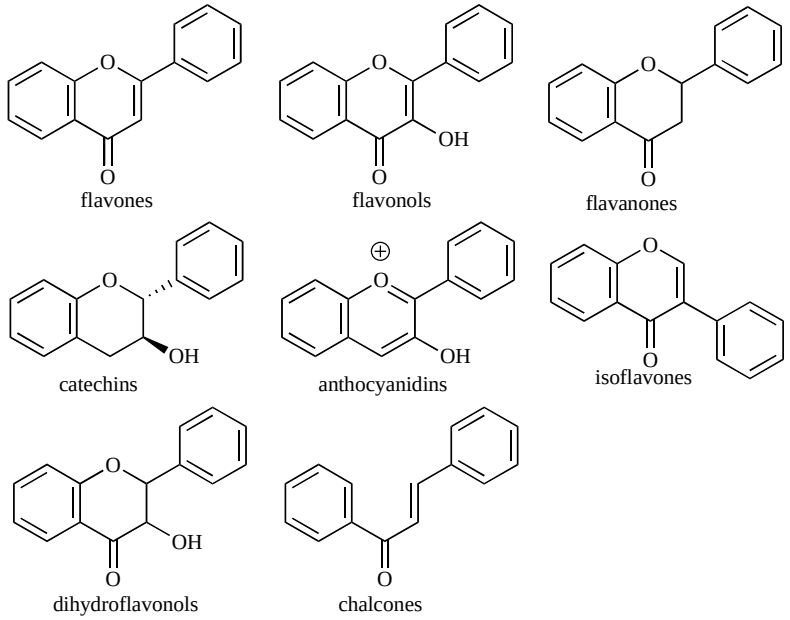


Fig. (2). The major subclasses of flavonoids.

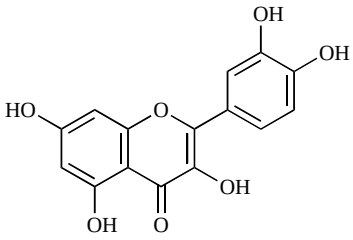
methoxychalcones that do not cause cellular damage. Their work also reveals that the Michael reaction acceptor functionality (i.e., the *α-β* unsaturated carbonyl group) of methoxychalcones is essential for heme oxygenase induction, which can be attenuated by thiols as well as inhibitors of the phosphatidylinositol-3 kinase pathway. Furthermore, an electron-withdrawing substituent in position 3 becomes crucial for HO-1 induction because of its influence on electron delocalization on the Michael acceptor functionality.

It was reported that quercetin (Fig. **3**) could upregulate HO-1 expression by activating nuclear factor erythroid 2 related factor (Nrf2) to bind with the antioxidant response element (ARE) in the *ho-1* gene promoter region [32-33]. However, the protective effect of quercetin in hepatocytes has some difference. In hepatocytes, Yao *et al.* demonstrated that HO-1 is induced by quercetin *via* p38, especially *via* ERK (extracellular signalregulated kinase)/Nrf2 transduction pathway. The ERK pathway is mainly responsible for quercetin-derived HO-1 induction while p38 is mainly responsible for ethanol-stimulated HO-1 induction [34]. Their study may provide a promising prophylactic and therapeutic strategy for alcoholic liver damage and other alcohol associated tissue damages through the supplementation of flavonoids/quercetin-rich food. Some differences in respect of the HO-1 expression are found in Microglial cell. Microglial cells are thought to be functionally equivalent to peripheral macrophages in the central nervous system (CNS). Activated microglia is thought to be involved in neuronal inflammation. Chen *et al.* [35] found quercetin can induce increases in HO-1 mRNA and protein expression in microglial cells. Data from antisense approach further suggest the involvement of HO-1 expression by quercetin in its downregulation action on iNOS and NO. The involvement of signal pathways in quercetin-induced HO-1 gene expression was associated with tyrosine kinase and mitogen-activated protein kinases activation. The results of their experiments highlight the therapeutic potential of quercetin as a novel anti-inflammatory and anti-neurodegenerative drug.

Wan *et al.* reported that HO-1 protein expression and activity in mouse liver were induced dose-dependently by baicalin (Fig. **3**) and pretreatment with baicalin dose-dependently reduced lipopolysaccharide/D-galactosamine-induced (LPS/D-GalN-induced) hepatic TNF-α (tumor necrosis factor-alpha) expression, serum TNF-α release and diminished TNF-α-induced the increase of myeloperoxidase activity [36]. In other animal models, decrease of TNF-α production by baicalin were also observed [37-39]. Their finding also suggested that the transcriptional inhibition of TNF-α by baicalin was probably due to blocking of NF-κB signaling pathway. These results suggest that baicalin can effectively prevent LPS/D-GalN-induced liver injury by inhibition of NF-κB activity to reduce TNF-α production and the underlying mechanism may be related to up-regulation of HO-1 protein and activity.

INHIBITOR OF 5-LIPOXYGENASE

Inflammation and leukocyte recruitment are considered to play key roles in atherogenesis [40]. Leukotriene B_4 (LTB_4), an eicosanoid, is one of the factors that mediate inflammatory processes in the vascular wall. There is evidence that LTB_4 are also involved in inflammatory diseases such as rheumatoid arthritis [41] and atherosclerosis [42]. 5-Lipoxygenase (LOX) activating protein (FLAP) acts as a docking protein on the nuclear membrane and together with leukotriene A_4 hydrolase and phospholipase A_2 completes the complex that is required for LTB_4 synthesis [43]. In flavonoids, flavonols such as quercetin and epicatechin (Fig. **3**) are pontent inhibitors of human 5- LOX. Nevertheless, other flavonoids such as sakuranetin (Fig. **3**), a flavanone, were also reported to directly inhibit this enzyme [44]. Loke *et al.* give evidences to argue that inhibition of 5-lipoxygenase by flavonoids is distinct from the antioxidant properties of their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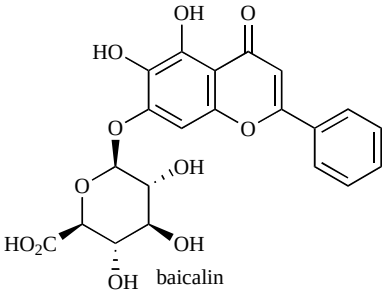
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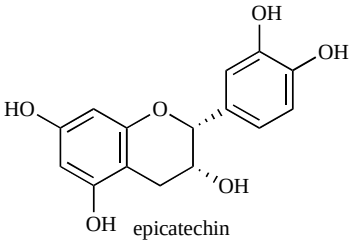
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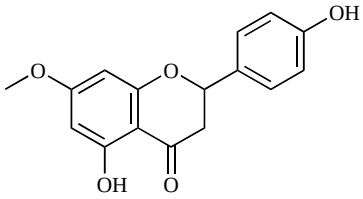
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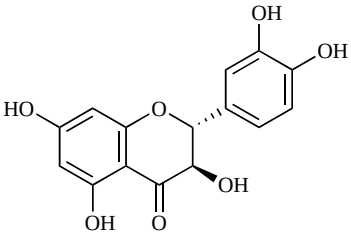
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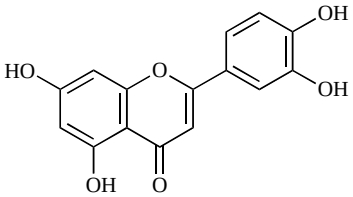
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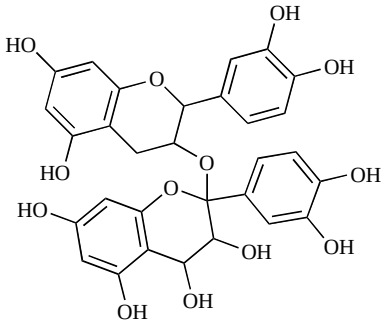
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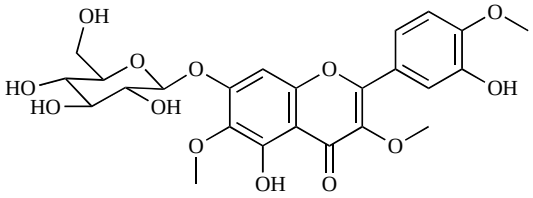
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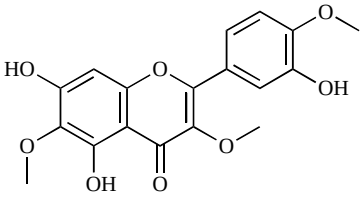
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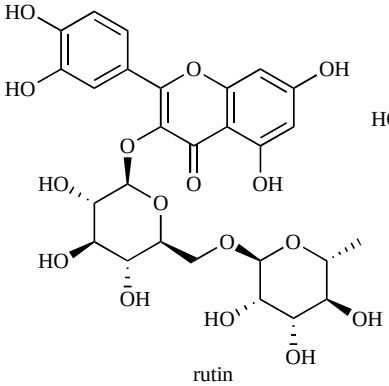
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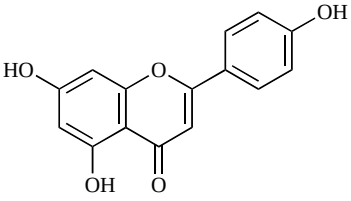
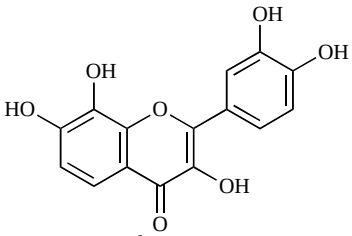

apigenin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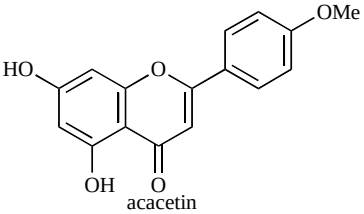
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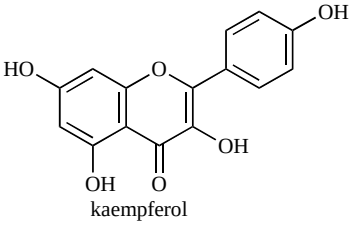
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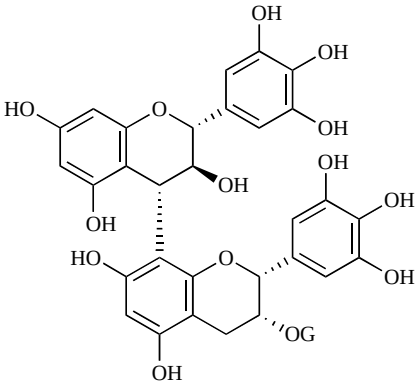
prodelphinidin B-4 3'-O-gallate; G: gallate

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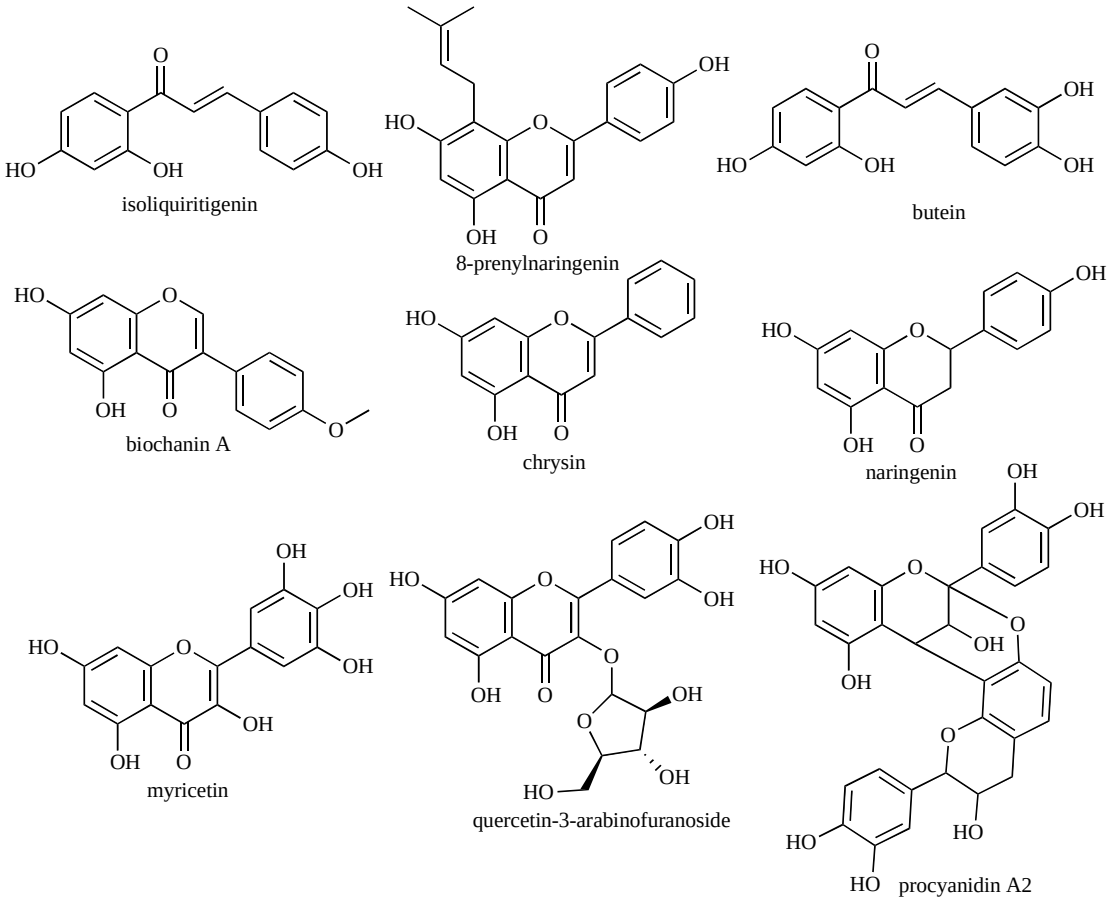

Fig. (3). The structures of some representative flavonoids.

studied compounds [45]. Their results comparing antioxidant activity with leukotriene inhibitory activity clearly demonstrate this distinction. For example, the 2,3-double bond is critical for leukotriene inhibitory activity but has little effect on antioxidant activity as observed with taxifolin (Fig. **3**). The C3-hydroxyl group is not critical for leukotriene inhibitory activity but its absence significantly reduces antioxidant properties as seen with luteolin (Fig. **3**). This observation is consistent with the work of Hernández *et al.*, in which sakuranetin was demonstrated to be a potent inhibitor of 5-lipoxygenase, but it clearly lacks the structural features necessary for antioxidative activity [44].

Recently, the protective effect of baicalin against ischemic brain injury has been reported. Ge *et al.* confirmed that baicalin protects rat primary cortical neurons against oxygen-glucose deprivation-induced ischemic-like injury, and showed that this protective effect relates to the inhibition of 5-LOX activation. Their results further indicate that baicalin inhibits 5-LOX activation *via* attenuating *N*-methyl-d-aspartic acid receptor-mediated responses, but not to merely inhibit 5-LOX enzymatic activity [46]. This finding provides valuable information for developing the flavonoid neuroprotective agents (with baicalin as a prototype) for ischemic neuronal injuries.

INHIBITORS OF ENDOTHELIAL NADPH OXIDASE

The NADPH (nicotinamide adenine dinucleotide phosphate) oxidase pathway is the predominant source of the $O_2^{\cdot-}$ release in angiotensin II stimulated human umbilical vein endothelial cells (HUVEC). $O_2^{\cdot-}$ could eliminate $^{\cdot}NO$ through diffusion-controlled formation of peroxynitrite, which either rapidly isomerizes to inert nitrate or causes oxidation and nitration of biomolecules [47]. Therefore, the $^{\cdot}NO/O_2^{\cdot-}$ balance is a key regulator of endothelial function, and an imbalance owing to elevated $O_2^{\cdot-}$ levels is implicated in many forms of cardiovascular disease. Various data indicate that production of reactive oxygen species (ROS) will induce an autoamplification loop, leading to an increased expression of NADPH oxidase [48], while antioxidant molecule inhibitors of the enzyme will reduce its expression. Inhibition of NADPH oxidase activity consequently leads to lower $O_2^{\cdot-}$ generation and protects HUVEC from signs of oxidative stress.

Some catechol-type flavonoids were repoted to have protection properties against ROS in endothelial cells due to their $O_2^{\cdot-}$-scavenging activity. Steffen *et al.* [49] provide evidence that a large variety of flavonoids and their metabolites are capable of protecting vascular endothelial cells against $O_2^{\cdot-}$ mainly through inhibition of NADPH oxidase and

in part possibly combined with $O_2^{\cdot-}$-scavenging. Especially, the monomethylated flavonoids such as 3'- and 4'-monomethyl ethers of (-)-epicatechin and 3'-monomethylated quercetin are the efficient inhibitors of NADPH oxidase. NADPH oxidase-inhibitory flavonoids may affect the assembly of the multi-protein complex [50-52]. For apocynin, impairment of the translocation of the oxidant-sensitive cytosolic component p47phox to the membrane has been proposed, but further efforts are needed to unravel the precise mode of action of this and other NADPH oxidase-inhibitory phenolics [53-54].

Study *in vivo* disclosed that chronic treatment with (red wine polyphenols) RWPs reduces hypertension and endothelial dysfunction in the model of deoxycorticosterone acetate-salt hypertension. The protective effect is most likely due to the ability of RWPs to prevent vascular oxidative stress by inhibiting NADPH oxidase activity and/or by reducing endothelin-1 release in hypertensive rats [54].

Al-Awwadi *et al.* explored the effect of a purified red grape skin polyphenolic extract enriched in anthocyanins and a grape seed extract rich in procyanidin (Fig. **3**) and galloylated procyanidins (PRO) on insulin resistance and the cardiovascular changes associated with it [55]. Their results show that both extracts lowered the production of ROS and the expression of NADPH oxidase. However, the different types of polyphenols do not have the same biological activity *in vivo*. Anthocyanins was able to correct high blood pressure and cardiac hypertrophy, while PRO normalized insulin sensitivity.

INDUCERS OF THE EPRE MEDIATED GENE EXPRESSION

Phase 2 enzymes are important detoxifying enzymes in cells that are suggested to play a role in the prevention against cancer [56]. The induction mechanism of detoxifying enzymes has been extensively studied, and activation of gene expression through electrophile-responsive element (EpRE), which was initially called the antioxidant-responsive element (ARE) [57-58], has been described for various flavonoids such as quercetin [59-60]. Among these compounds, flavones are found to be the most potent inducers of EpRE-mediated gene expression [61]. This may attribute to the the C2–C3 double bond in the C-ring and the resulted planar molecular structure [62-65]. Therefore, a stereospecific molecular interaction may be important for EpRE-mediated gene activation. The work of Lee-Hilz shows that flavonoids bearing a hydroxyl group at the 3-position are the best inducers of EpRE-mediated gene induction, and furthermore the degree of hydroxylation of the flavonoids do not seem to be important for the EpRE-mediated gene transcription [66]. The release of Nrf2 from the Keap1-Nrf2 (Kelch-like erythroid-cell-derived protein with CNC homology-associating protein 1-Nrf2) complex is a crucial step in the EpRE-mediated gene induction of detoxifying enzymes. One of the mechanisms for Nrf2 release from Keap1 suggests a direct oxidative modification of Keap1 by inducers of EpRE-mediated gene transcription. Flavonoids have been described to display pro-oxidant activity after donating electrons by antioxidant action, enzymatic oxidation, or autoxidation [67]. So the pro-oxidant action of flavonoids rather than their antioxidant activity is responsible for their inducing activity of an EpRE-mediated response and for their anticancer properties.

It has been proposed that induction of EpRE-mediated gene expression by so-called bifunctional inducers actually occurs in two steps, the first one involving induction of enzymes through XRE elements in the gene regulatory region, which would result in the generation of the actual inducer of EpRE-controlled gene transcription in the second step [68]. This order of consecutive molecular events suggests a direct relation between AhR and EpRE and places the EpRE pathway downstream of XRE-mediated gene expression in the case of a bifunctional inducer [69]. However, Lee-Hilz [70] demonstrated that flavonoids, although they are able to induce both XRE- and EpRE-controlled gene expression, and therefore are classified as bifunctional inducers, preferentially induce EpRE-mediated gene transcription in mouse hepatoma cells at physiologically relevant concentrations. In other words, EpRE-mediated gene expression induced by flavonoids is not a downstream reaction of XRE-mediated gene expression.

Boerboom *et al.* developed two stably transfected luciferase reporter cell lines, EpRE(hNQO1)-LUX and EpRE(mGST-Ya)-LUX which based on EpRE sequences from the human NADPH:quinine oxidoreductase (hNQO1) and the mouse glutathione-S-transferase Ya (mGST-Ya) gene respectively, as a validated tool for mechanistic studies of EpRE-mediated gene transcription [65]. Their observations suggested that there are some differences in the mechanism of EpRE-mediated transcription activation between quercetin and the standard inducer tert-butylhydroquinone. But extensive and detailed experimental analysis using selective inhibition of each pathway of Nrf2 activation by biochemical or genetic methods is needed to achieve comprehensive mechanistic understanding of EpRE-mediated gene transcription activation.

MODULATORS OF IFN-γ EXPRESSION

T cells are key players in human immunity including cellular and humoral immunity. Interferon gamma (IFN-γ) is a key cytokine produced by activated T cells. IFN-γ modulates a variety of immune responses including pathogen clearance, tumor eradication, T cell activation and inflammatory responses [71]. Thus, high-level production of IFN-γ is typically associated with effective host defense against intracellular pathogens and cancer [72-73]. For the first time, Chang *et al*. manifested that flavonoids, centaurein and centaureidin (Fig. **3**), were able to modulate IFN-γ transcription [74]. The effective dose of centaurein used to stimulate the IFN-γ production is relative high (50–100 μg/ml). In contrast, centaureidin, an aglycone of centaurein, at 2 μg/ml has similar effect as centaurein at 100 μg/ml on IFN-γ stimulation. On the other hand, centaurein and centaureidin in some cases induced IFN-γ transcription as well as apoptosis in T cells. This phenomenon was also observed in the PHA (phytohemagglutinin, a T cell stimulant) case and is known as activation-induced cell death [75]. How both flavonoids can cause T cell activation-induced death needs to be further examined. To better understand the mechanism by which centaurein augments IFN-γ transcription, they tested whether centaurein

can modulate nuclear factors. Their results indicated that centaurein modulates IFN-γ expression possibly *via* both nuclear factor of activated T cells and NF-κB.

Choosing peripheral blood mononuclear cells (PBMC) as *in vitro* model, Cherng *et al.* evaluated the immunomodulatory activities of quercetin and rutin (quercetin-3-rutinoside) (Fig. **3**). They found that the immunosuppressive effects of the abovementioned compounds were at cytotoxic concentrations on mitogen-activated lymphocytes. On the other hand, at non-toxic doses both compounds directly enhancing of lymphocyte activation and/or secretion multipotent cytokine IFN-γ [76]. Furthermore, quercetin concomitantly increased lymphocyte activation and IFN-γ secretion. However, rutin stimulated the secretion of IFN-γ, and left proliferation of human PBMC no elevation. Lymphocyte activation was correlated to increase the number of lymphocyte cells including $CD8^+$ T cells and activated PBMC, while the enhancement of the secretion of IFN-γ might be due to the $CD8^+$ T cells.

Apigenin (Fig. **3**) was reported to induce the apoptosis of human hepatoblastoma derived cell line Hep G2 [77]. The results show that TNF-α and IFN-γ levels in apigenin-pretreated groups were significantly and dose dependently elevated as compared to the control values. The anti-tumor effect of TNF-α is often augmented by IFN-γ [78], and IFN-γ is a potent activator of caspases [79]. TNF-α and IFN-γ release and induction of caspases activity cause programmed cell death.

INHIBITORS OF INOS and COX-2 gene expression

Prostaglandins (PGs) are one of the potent modulators in both inflammation and immune responses. As compared with the surrounding normal tissues, elevated levels of PGs, especially PGE2, have also been found in many human cancers [80]. Cyclooxygenase (COX) is the rate-limiting enzyme in PG synthesis. Therefore, COX-2 is an important enzyme that mediates inflammatory processes and plays a role in the development of tumors [81]. iNOS, another enzyme associated with inflammatory processes, is usually not detectable in healthy tissues but is expressed after immunological challenge or injury. Enhanced expression of iNOS and its enzymatic activity have also been observed in many human tumor tissues [82]. Thus, aberrant or excessive expression of iNOS and COX-2 is implicated in inflammatory disorders and the pathogenesis of cancer. Melanoxetin (Fig. **3**), a flavonol isolated from *Acacia confuse,* was reported to not only show strong inhibitory activity on iNOS mRNA expression but also to exhibit significant inhibition of the COX-2 mRNA expression in LPS-stimulated macrophages [83].

Quercetin and kaempferol (Fig. **3**), only minor difference in structure, significantly inhibited mRNA level of iNOS, COX-2, and CRP (Reactive C-protein) in Chang Liver cells. Kaempferol produced a significant concentration-dependent decrease of iNOS, COX-2 and CRP protein level at all concentrations, but the percentage of inhibition induced by quercetin was reduced at high concentrations. The inhibitory action of quercetin and kaempferol in Chang liver cells might be due to downregulation of iNOS, COX-2 and CRP expression by impairment of intracellular signal pathways. Data from García-Mediavilla further confirm that quercetin and kaempferol suppress the expression of iNOS and COX-2 *via* mechanisms likely to involve blockade of NF-κB activation. Their data also indicate that the minor structural differences between both compounds determine differences in their inhibitory capacity [84]. Similar to quercetin and kaempferol, molecular data revealed that prodelphinidin B-4 3'-O-gallate (Fig. **3**), a proanthocyanidin, inhibited LPS-induced expression of COX-2 and iNOS in mouse macrophage cell line RAW 264 *via* the downregulation of TAK1(TGF-β-activated kinase)-NF-κB pathway [85]. In addition to the above mentioned flavonols [86] and proanthocyanidins, some other subsets of flavonoids such as flavones (acacetin, Fig. **3**) [87] and chalcones (isoliquiritigenin, Fig. **3**) [88] are also reported to show inhibition of iNOS and COX-2 expression in macrophage. For neuroprotective effects, Ha *et al.* evaluated the anti-inflammatory effect of various flavonoid compounds by using BV-2 microglia [89]. Their experiments show that apigenin inhibited the production of nitric oxide and PGE2 by suppressing the expression of iNOS and COX-2 protein, respectively. Moreover, apigenin suppressed p38 mitogen-activated protein kinase, c-Jun N-terminal kinase phosphorylation without affecting the activity of extracellular signal-regulated kinase. Their *in vivo* data indicated that apigenin was able to protect neuronal cells from injury in middle cerebral artery occlusion.

INHIBITORS OF AROMATASE

Aromatase (CYP 19) is a key enzyme in the biosynthesis of steroids, and is mainly responsible for converting androgens to estrogens. Androgens and estrogens do not only play an important role in reproductive processes, but also in vascular function, lipid and carbohydrate metabolism, as well as bone mineralization in both sexes [90]. On the other hand, exposure to estrogens in general has been related to initiation and promotion of hormone-dependent diseases such as breast cancer. Briefly, the aromatase expression is significantly more induced in cancerous breast tissue compared to healthy tissue. The common strategies to treat estrogen-dependent breast cancer are to block estrogen receptor binding or to inhibit estrogen production. Estrogen production can be reduced by aromatase inhibitors. A recent study from Monteiro *et al.* illustrated that 8-prenylnaringenin (Fig. **3**) is a potent aromatase inhibitor with an IC_{50} of 65–80 nM [91-92]. Using MCF-7 cells stably transfected with aromatase as a model system for aromatase inhibition, some naturally occoured chalcones were evaluated in Wang group. The results indicated that butein (Fig. **3**) was the strongest inhibitor of aromatase with an IC_{50} value below 5 μM [93]. Rice *et al.* provided evidences that some phytoestrogens such as apigenin and biochanin A (Fig. **3**) could reduce aromatase activity by down-regulation of its expression. Their studies also show the reduction in aromatase activity was significantly delayed in time compared with the reduction in $mRNA_{arom}$ [94].

Many other flavonoids such as chrysin, naringenin and apigenin (Fig. **3**) also significantly inhibit aromatase with IC_{50}s below 10 μM [95]. Therefore some of these compounds could have beneficial value in the prevention or treatment of hormone-dependent types of breast cancer. In general, the capacity to reduce aromatase activity strongly

correlates to hydroxylation of the A ring and to a lesser extent to hydroxylation of the B ring. Some flavonoids have aromatase inhibitory as well as estrogenic properties, which can be considered as opposing effects with respect to estrogenicity. Thus, the overall effect of both mechanisms on estrogen responsive tissue is of interest.

INHIBITORS OF GLUCOSYLTRANSFERASES

Gregoire *et al.* reported that flavonols could inhibit the activity of surface-adsorbed glucosyltransferases (GTFs) [96]. The GTFs secreted by *Streptococcus mutans* are found in whole human saliva and are also incorporated in the pellicle that is formed on the tooth surface in a highly active form [97]. The surface-adsorbed GTFs synthesize complex glucans from dietary sucrose *in situ*, which are critical for bacterial accumulation on the tooth surface and contribute to the bulk and structural integrity of the biofilms [98]. In general, the flavonols myricetin (aglycone) and quercetin-3-arabinofuranoside (O-glycoside form), and procyanidin A2 (A-type PAC dimer) (Fig. **3**) showed moderate biological activity on the *in vitr*o parameters tested in their study, whereas, the phenolic acids displayed negligible inhibitory effects. Furthermore, as for PACs, the degree of polymerization is associated with their biological activity. The dimer to be more effective than monomer in reducing the activity of purified GTF, and PACs from cacao bean husk and apple that are larger than trimers were more effective inhibitors than monomers and dimmers.

CONCLUSIONS

The evidences from published literatures demonstrated that some flavonoids show the same health benefits due to acting on the same targets. On the other hand, some flavonoids with diverse chemoprotections may be of their high affinity to many targets. In summary, evaluation of the molecular mechanisms of flavonoids may help to choose flavonoids or combinations of flavonoids for clinical development, and may be a promising area for the development of new flavonoid-based agents useful in the treatment of various inflammatory diseases.

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