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Natural polyphenols as anti-inflammatory agents

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1. ABSTRACT

Epidemiological studies have clearly shown that diets rich in plant foods protect human against degenerative diseases. Plant foods contain fiber, vitamins and polyphenols which can contribute to the health effects. Polyphenols, plant secondary metabolites, represents a wide variety of compounds, with different chemical structures and activities. These compounds possess several biological functions, such as antioxidant, anti-inflammation, and anticancer activities, able to counteract endogenous and exogenous biological stimuli. Recently, there is also increasing evidence for many potential benefits through polyphenolic-mediated regulation of cellular processes such as inflammation. Polyphenols, in fact, may act as inflammation modulatory agents by various mechanism, including down-regulation of NFkB, release of inflammatory mediators, chromatin remodeling, or the various enzymes involved, by inhibition of the activity of those enzymes, or by increasing the cells ability to scavenge ROS. So the polyphenols may be perceived as future pharmacological agents and may be used as antioxidant and anti-inflammatory enforcements to combat oxidative challenges.However, future studies are required to understand the effect of polyphenols on the pathology of different inflammatory disease states.

2. INTRODUCTION

2.1.1 Types of polyphenols

Polyphenols are phytochemicals found in food substances produced by plants. Phenolic compounds, secondary plant products commonly found in plant-derived foodstuffs, constitute a group of substances abundant in the plant kingdom, where more than 8000 are known, with different chemical structures and activities. Polyphenols contain one or more aromatic rings, bearing one or more hydroxyl moieties. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins (1). Examples of polyphenolic families include phenolic acids, stilbenes, coumarins, tannins, and flavonoids. Phenolic acids include derivatives of benzoic acid (flavonoids, stilbenes) and derivatives of cinnamic acid (coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, lignans). The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish, and onions. Furthermore, hydroxybenzoic acids are components of complex structures such as hydrolyzable tannins (gallotannins in mangoes and ellagitannins in red fruit, e.g. strawberries, raspberries, and blackberries) (2). The hydroxycinnamic acids are more common than hydroxybenzoic acids and

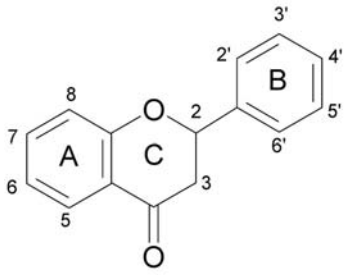
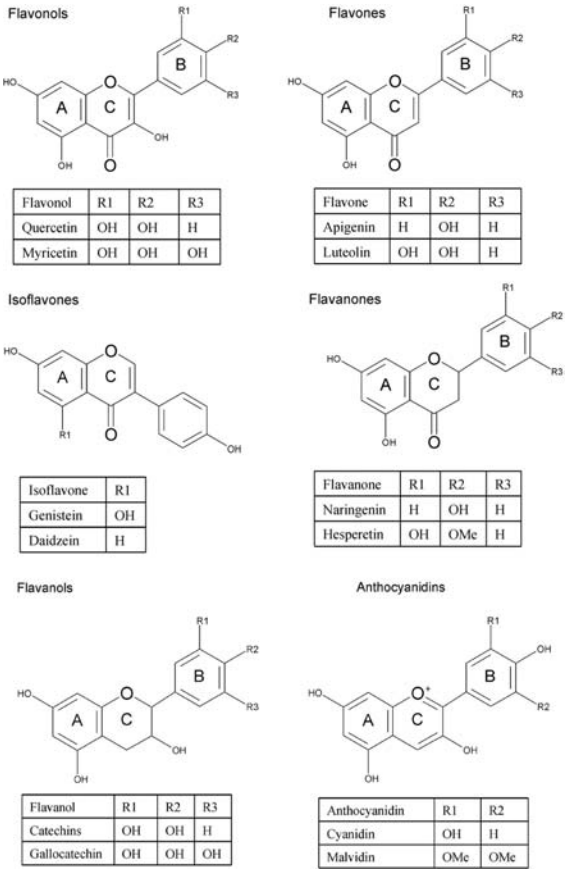


Figure 1. Basic chemical structure.



Flavonol	R1	R2	R3
Quercetin	OH	OH	H
Myricetin	OH	OH	OH

Flavone	R1	R2	R3
Apigenin	H	OH	H
Luteolin	OH	OH	H

Isoflavone	R1
Genistein	OH
Daidzein	H

Flavanone	R1	R2	R3
Naringenin	H	OH	H
Hesperetin	OH	OMe	H

Flavanol	R1	R2	R3
Catechins	OH	OH	H
Gallocatechin	OH	OH	OH

Anthocyanidin	R1	R2
Cyanidin	OH	H
Malvidin	OMe	OMe

Figure 2. Chemical structure of polyphenols.

consist chiefly of *p*-coumaric, caffeic, ferulic, and sinapic acids. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilization, or fermentation (2). The flavonoids, which share a common structure consisting of 2 aromatic rings (A and B) bound together by 3 carbon atoms forming an oxygenated heterocycle (ring C) in (Figure 1", may themselves be divided into 6 subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins) (Figure 2). They are constituents of fruits, vegetables, nuts and plant derived beverages such as tea, wine, and traditional Eastern medicines (3). Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol. They are generally present at relatively low concentrations of ~15–30 mg/kg fresh wt. The richest sources are onions (up to 1.2 g/kg fresh wt), curly kale, leeks, broccoli, and blueberries. Flavones consist chiefly of glycosides of luteolin and apigenin. The only important edible sources of flavones identified to date are parsley and celery. Cereals such as millet and wheat contain *C*-glycosides of flavones (4,5).

In human foods, flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present in high concentrations only in citrus fruit. he main aglycones are naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons. Flavanones are generally glycosylated by a disaccharide at position 7: either a neohesperidose, which imparts a bitter taste (e.g. to naringin in grapefruit), or a rutinose, which is flavorless (Figure 3). Isoflavones are flavonoids with structural similarities to estrogens. Although they are not steroids, they have hydroxyl groups in positions 7 and 4' in a configuration analogous to that of the hydroxyls in the estradiol molecule. This gives them pseudohormonal properties on them, including the ability to bind to estrogen receptors, and they are consequently classified as phytoestrogens. Isoflavones (genistein, daidzein) are found almost exclusively in leguminous plants. Flavanols exist in both monomer (catechins) and the polymer forms (proanthocyanidins). Catechins are found in many types of fruit. Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found in seeds of certain leguminous plants, in grapes, and more importantly in tea. Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers, and polymers of catechins that are bound together by links between C4 and C8 (or C6). Information on their mean degree of polymerization in foods is scarce: in cider apples, the mean degree of polymerization ranges from 4 to 11 (6). Anthocyanins are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit, to which they impart a pink, red, blue, or purple color (7). They exist in different chemical forms, both colored and uncolored, according to pH (Figure 4). Although they are highly unstable in the aglycone form (anthocyanidins), while they are in plant, they are resistant to light, pH, and oxidation conditions that are likely to degrade them. Degradation is prevented by glycosylation, generally with a glucose at position 3, and esterification with various organic acids (citric and malic acids) and phenolic acids. In the human diet, anthocyanins are found in red wine, varieties of cereals, and certain leafy and root vegetables (aubergines, cabbage, beans, onions, radishes), but they are most abundant in fruit. Cyanidin is the most common anthocyanidin in foods. Dietary polyphenols are derived from plants and are consumed in the forms of fruits, vegetables, spices, and herbs. Dietary intake of polyphenols fluctuates widely between cultures, ethnic groups, and even within a narrow geological location. Large percentages of dietary polyphenols are consumed in the form of flavonoids, although cultural and dietary habit will dictate which forms of polyphenols are taken up. Polyphenols, in particular flavonoids, also known

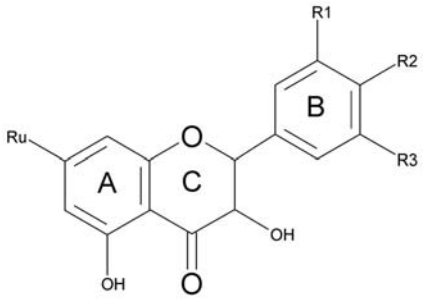


Figure 3. Chemical structure of glycosides. Naringine: R1=H; R2=OH; 3=H; Ru= disaccharide.

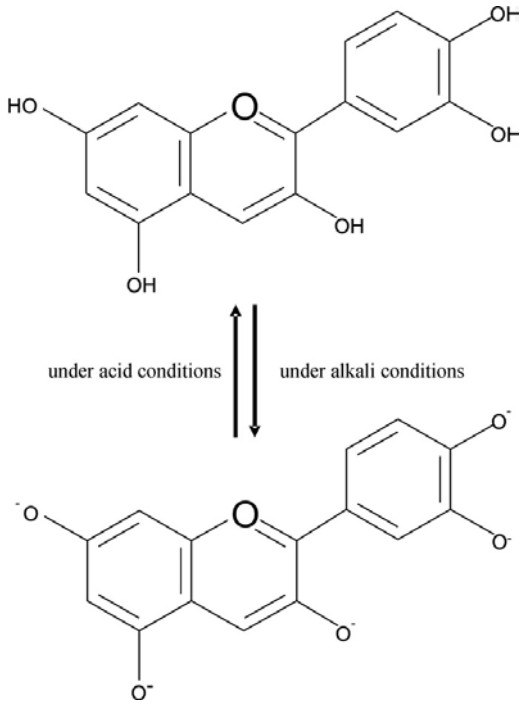


Figure 4. The anthocyanidins in flower, colours it red in acid soil and blue in alkali soil.

as "nature's tender drugs", possess various biological/pharmacological activities including anticancer, antimicrobial, antiviral, antiinflammatory, immunomodulatory, and antithrombotic activities (8).

3. BIOSYNTHESIS, BIOAVAILABILITY AND METABOLISM OF POLYPHENOLS

3.1. Functions and chemistry of polyphenols

Plants synthetize an enormous variety of metabolities classified as primary metabolities, produced for metabolic processes within the plant, as well as secondary metabolities stimulating ecological interactions between plants and their environment (9). It is already well established that polyphenols have a significant impact on various aspects of plant biology. They exhibit a wide range of functions in physiology, biochemistry, and ecology, for example in UV-protection, chelation of toxic heavy metals, anti-oxidant protection from free radicals generated during the photosynthetic process, flower coloration, interspecies interaction, and plant defense. Plants contain an enzyme, polyphenol oxidase, which polymerizes all available polyphenols, in the presence of oxygen at the site of an injury to the plant. Other highly remarkable properties of certain polyphenols are their nutritional values and medicinal benefits to humans, represented among others by antioxidant or putative anticancer activities.

Interestingly, these antioxidant/anti-inflammatory compounds may be responsible for the multitude of beneficial effects that have been reported for fruits and vegetables on an array of health-related bioactivities. Polyphenols, particularly flavonoids, derive their 15-carbon skeletons from two basic metabolites, malonyl-CoA and *p*-coumaroyl-CoA. The crucial biosynthetic reaction is the condensation of three molecules of malonyl-CoA with one molecule of *p*-coumaroyl-CoA to a chalcone intermediate. Flavonoids are characterized by phenylbenzopyran chemical structure (10). The general chemical structures include a C15 (C6-C3-C6) skeleton joined to a chroman ring (benzopyran moiety) that in turn bears an aromatic ring at C-2, C-3 or C-4 (Figure 1). The heterocyclic benzopyran ring is known as the "C" ring, the fused aromatic ring as the "A" ring, and the phenyl constituent as the "B" ring. The A ring can be of two types: a phloroglucinol type that is *meta*-trihydroxylated or a resorcinol type that is *meta*-dihydroxylated (11-13). The B ring can be monohydroxylated, ortho-dihydroxylated or vicinal-trihydroxylated. The center heterocycle most commonly exists in one of three forms: pyran, pyrilium, or gamma-pirone (11). The position of the chroman-aromatic linkage determines the benzopyran class: 2-phenylbenzopyrans comprise the flavonoids, 3-phenylbenzopyrans the isoflavonoids and 4-phenylbenzopyrans the neoflavonoids. All three groups share a chalcone precursor that exists in an open chain isomeric form. Flavonoids with a five member heterocycle ring are referred to as aurones. Other flavonoid modifications include methxylations, hydroxylgroup, O-glycosilation, presence of phenyl or alkyl groups covalently linked to flavonoid moieties and additional rings condensed to the flavonoid core (10). Based on C-ring saturation and oxidation status, the 2-phenylbenzopyrans are further divided into eight groups: flavan, flavanone, flavone, flavonol dihydroflavonol, flavan-3-ol, flavan-4-ol and flavan-3,4-diol. Most polyphenols exist in plants in glycosidic forms. Glycoside conjugates of polyphenols (*O*- and *C*-glycosides) are the major naturally occurring forms in leguminous plants, including soy (*Glycine max merrill*) and kudzu (*Pueraria lobata*) (14-16). Several isoflavone *C*-glycosides have recently been identified in *Pueraria lobata* root cell cultures by tandem mass spectrometry (17). The sugars of *C*-glycosyl isoflavones are linked to the isoflavonoid by a carbon-carbon bond, which is resistant to acid hydrolysis; in contrast, the sugars of isoflavone *O*-glycosides are *O*-linked at phenolic hydroxyl groups, forming a carbon-oxygen-carbon bond, and are acid-labile. Examples of mono-*C* glycosyl isoflavones are daidzein 8-

Table 1. Major effects of polyphenols

Effects	Mechanisms
Antioxidant	ROS ⇓ Antioxidant enzymes ⇑ Lipid peroxidation production ⇓ Non enzymic antioxidants ⇑
Antiviral	HIV replication ⇓ ROS ⇓
Anti-cancer	Apoptosis ⇑ DNA binding of NFkB DNA binding of tumor initiation ⇓
Anti-inflammation	Histamine Release ⇓ Lipoxygenase ⇓ NO⇓ iNOS ⇓, COX 2 ⇓, NFkB ⇓ Activation of PPARgamma

C-glucoside, apigenin 8-*C*-glucoside, and apigenin 6-*C*-glucoside.

3.2. Bioavailability and metabolism of polyphenols

The foods containg the various polyphenols have been reviewed elsewhere. One of the main objectives of bioavailability studies is to determine, among the hundreds of dietary polyphenols, which are better absorbed and which lead to the formation of active metabolites. Many researchers have investigated the kinetics and extent of polyphenol absorption by measuring plasma concentrations and/or urinary excretion among adults after the ingestion of a single dose of polyphenol, provided as pure compound, plant extract, or whole food/beverage. Polyphenols are apparently absorbed in the upper gastrointestinal tract by a number of mechanisms which have not been fully characterized. Some glycosides, mainly glucosides, but not rutinosides, appear to interact with the active sugar transporter (SGLT1), lactase phloridzin hydrolase or cytosolic beta-glucosidase (18). Most of the polyphenol *O*-glucosides undergo intestinal hydrolysis to release the respective aglycones by intestinal glucosidases/hydrolases. Because aglycones and their metabolites are more hydrophobic, they are more efficiently transported across the wall of the gastrointestinal tract than their respective glucosides. They are converted both in the gut wall and the liver, as well as at peripheral tissue sites, into phase I and phase II metabolites. In many animals, there are also extensive bacterial metabolites. Indeed, except for humans, the principal form of the soy isoflavone daidzein in the blood and urine of animals used in chemoprevention experiments is (*S*)-equol, a bacterial metabolite (19). There is a complex interplay between the chemical structure of glycosides and their rate of intestinal transport. Preliminary studies on puerarin metabolism and pharmacokinetics have indicated that puerarin is rapidly absorbed intact, reaching a maximum concentration and then declining within 1 h after oral administration (20, 21). Unlike bioflavonoid *O*-glycosides, unconjugated puerarin is a major component in the blood and urine after its oral administration, indicating that phase II metabolism is not the major metabolic pathway for puerarin excretion (22). The more hydrophobic aglycones appear to undergo passive diffusion. Only 5–10% of polyphenols are absorbed in this manner, the remainder passing into the colon (23). Colonic micro-flora appears able to break down flavonoids and procyanidins into simpler hydroxycinnamic, hydrocinnamic (phenylpropionic), phenylacetic and benzoic acid derivatives (24). For quercetin glycosides in humans it has been shown that maximum absorption occurs 0.5–0.7 h after ingestion of quercetin 4'-glucoside and 6–9 h after ingestion of the same quantity of rutin (quercetin-3-rutinoside). The bioavailability of rutin is only 15–20% that of quercetin 4'-glucoside. Similarly, absorption of quercetin is more rapid and efficient after ingestion of onions, which are rich in glucosides, than after ingestion of apples containing both glucosides and various other glycosides (2). Metabolites of polyphenols may follow two pathways of excretion; i.e., via the biliary or the urinary route. It is reported that large, extensively conjugated metabolites are more likely to be eliminated in the bile, whereas small conjugates such as monosulfates are preferentially excreted in urine (2). Research on polyphenol bioavailability must allow us to correlation of polyphenols intakes with potential health effects: many epidemiological studies have indicated that consumption of some foodstuffs and drinks with high phenolic content is associated with the prevention of some diseases. These compounds may possess various biological/pharmacological activities including anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities (2, 8, 25-26) (Table 1).

4. POLYPHENOLS AND HEALTH

4.1. Antioxidant capacity of phenolic compounds

Excess production of free radicals and reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$), is thought to cause damage in cells. This damage may be involved in the etiologies of diverse human diseases, such as atherosclerosis, ischemic injury, inflammation, cancer, ageing and neurodegenerative diseases (Parkinson and Alzheimer) (27-30). Cellular mechanisms involved in the production of oxidative stress include inflammatory response, free radical leakage from mitochondria, auto-oxidation of catecholamines, xanthine oxidase activation, pro-oxidant activities of toxic compounds, such as CCl_4 and exposure to ionizing radiation. ROS may cause cellular and subcellular damage by peroxidation of membrane lipids, denaturing cellular proteins and breaking DNA strands, disrupting cellular functions (30). It is reported that ROS have been implicated in initiating inflammatory responses through the activation of transcription factors, such as nuclear factor (NF)-kB and activator protein (AP)-1, and other signal transduction pathways, such as mitogen-activated protein (MAP) kinases and phosphoinositide-3-kinase (PI-3K), leading to enhanced gene expression of pro-inflammatory mediators (31-33). Recently, it has been shown that oxidative stress and the redox status of the cells can also regulate nuclear histone modifications, such as acetylation, methylation and phosphorylation, leading to chromatin remodeling and recruitment of basal transcription factors and RNA polymerase II, with consequent induction of pro-inflammatory mediators (34, 35). Aerobic cells are endowed with extensive antioxidant defense mechanisms including both low molecular weight scavengers (such us alfa-tocopherol, cysteine, beta–carotene, reduced

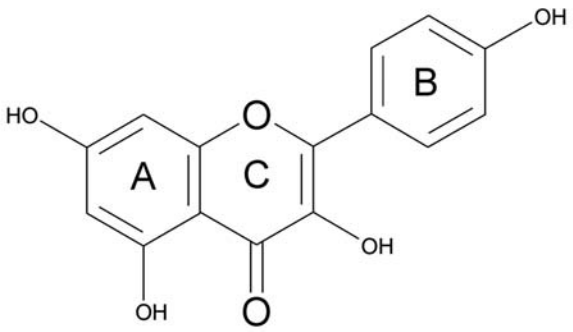


Figure 5. Flavonoids (C6-C3-C6), basic structure.

glutathione, ascorbic acid) and enzymatic systems, such us superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Red) and glucose-6-phosphate dehydrogenase (G6PD), which counteract the damaging effects of toxic oxygen species (36). However, when the balance between these species and antioxidants is altered, a state of oxidative stress results, possibly leading to permanent cellular damage. There is evidence that exogenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in vegetables, fruits, and medicinal herbs, which are candidates for the prevention of oxidative damage caused by free radical species (37-38).

It is reported that polyphenols can function in a similar manner to other antioxidant compounds by inactivating harmful free radicals, and by chelating metal ions, thereby reducing their oxidative potential (39). Since the antioxidant mechanism of polyphenols is still controversial, the antioxidant activity of phenolic phytochemicals has been widely investigated in recent years (40-42). Recent studies have shown that phenolic compounds may prevent lipid peroxidation and formation of atherosclerotic plaques (43-44); in fact the juice of blood orange containing large amounts of anthocyanins, such as cyanidin (Cy) and cyanidin-3-beta glucoside (C3G) contributes to the inhibitory effect of oxidation by low-density lipoproteins (LDL). In addition, it has been reported that polyphenols inhibit the activities of an array of enzymes, including lipoxygenase, cyclooxygenase, monooxygenase, xanthine oxidase, mitochondrial succinic oxidase and NADH-oxidase, phospholipase A2 and protein kinases (39). These biological effects are believed to come from the antioxidant properties of the related polyphenols, including their protection against iron-induced free radical reactions (39). Moreover, they have been shown to be highly protective against H_2O_2-induced damage in human keratinocytes, fibroblasts and in NG 108-15 cells (a mouse neuroblastoma-rat glioma hybrid cell line) (45). In agreement with other authors, our previous research demonstrated that polyphenols possess a significant protective effect on DNA cleavage induced by UV/photolysis of H_2O_2. These compounds, in fact, suppressed the formation of linear DNA (linDNA) caused by exposure of plasmid DNA to $^{\cdot}OH$ and induced a partial recovery of supercoiled DNA (scDNA) (41, 42). In our study, in evaluating the superoxide scavenging capacity of this natural product we used a method which excludes the Fenton-type reaction and xanthine/xanthine oxidase system (7). The free radical-scavenging activity of these compounds was also tested by their ability to bleach the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH). This assay provided information on the reactivity of test compounds with a stable free radical. Also in this assay polyphenols showed a DPPH quenching capacity in a dose-dependent manner. In addition, we evaluated the effects of polyphenols on xanthine oxidase enzyme, a physiological source of superoxide anions in eucaryotic cells. This study, therefore, also considered a possible inhibitory action of these natural compounds on the primary function of the enzyme as reported by Takahama (46). Polyphenols, in fact, determined a dose-dependent inhibition of xanthine oxidase activity. These data suggested that, in addition to their direct $O_2^{\bullet-}$ scavenging action, the inhibitory action of these compounds on XO activity might, at least in part, contribute to its antioxidant action. These results suggest that polyphenols are effective free radical scavengers and metal chelators, and the antioxidant property of these molecules is due to the presence of 1) the catechol structure of ring B; 2) the 2,3 double bond in conjugation with 4-oxo function; 3) the basis of the availability of –OH and the system of conjugated double bonds present in these molecules (Figure 5). To antioxidant properties of these compounds could be beneficial in diseases where oxidative stress is involved, even if many effects of polyphenols such as anti-inflammatory, anti-tumor, anti-atherogenic abilities cannot be explained solely on the basis of their antioxidant properties. Investigations on the mechanism of action of these molecules have thrown light on the fact that polyphenols may not merely exert their effects as free radical scavengers, but may also modulate cellular signaling processes during inflammation, or may themselves serve as signaleng agents.

5. INFLAMMATION AND POLYPHENOLS

5.1. Inflammation pathways

Inflammation is a complex, high sequential series of events that is provoked by a variety of stimuli including pathogens, noxious mechanical and chemical agents, and autoimmune responses. The subsequent cascade of events is characterized by signs and symptoms such as redness, swelling, heat, and pain. Inflammation is the primary process through which the body repairs tissue damage and defends itself against stimuli, but it also contributes to the pathophysiology of many chronic diseases. Low-grade inflammation is also involved in the etiology of cardiovascular disease, in inflammatory diseases such as arthritis, and in allergies such as asthma.The affected tissues release inflammatory mediators (cytokines) including the proinflammatory cytokines tumor necrosis factor-alfa (TNF) and interleukin-1 (IL-1). In a complex signaling cascade, these mediators up-regulate and modulate other inflammatory cytokines and immunoglobulins from activated leukocytes, which in turn have actions to up-regulate cellular adhesion molecules in inflamed tissue (47). Phagocytosis of bacteria by leucocyte

neutrophils leads to the neutrophil burst in which various ROS are generated in order to neutralize the invading organisms. There is also concomitant up-regulation of other enzyme systems which contribute to the protective and repair processes, including phospholipase A2, cycloxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), inducible nitric oxide synthase (iNOS) and the central regulator of the inflammatory process, nuclear factor-kappa B (NF-kB) (47-48). NF-kB is activated by numerous extracellular stimuli, including cytokines such as TNF-alfa and IL-1beta, viruses and environmental particulates (PM10s) and oxidative stress (47). NF-kB is ubiquitously expressed within cells, and not only controls induction of inflammatory genes in its own right, but also enhances the activity of other cell- and signal-specific transcription factors (49-51). The NF-kB/Rel complex is a family of redox-sensitive transcription factors composed of several key regulatory molecules controlling the expression of many inflammatory and protective/stress response genes. NFkB exists as a heterodimeric complex usually of p50 and p65/RelA subunits. In unstimulated cells, NF-kB is found in the cytoplasm as an inactive non-DNA binding form, associated with an inhibitor protein called inhibitor of kB (IkB), which masks the nuclear translocation signal, thus preventing NF-kB from entering the nucleus. he expression of several genes such as COX-2, matrix metalloproteinase-9 (MMP-9), iNOS, TNF, interleukin-8 (IL-8), eotaxin, cell surface adhesion molecules, and antiapoptotic proteins are regulated by NFkB (52). The cyclo-oxygenases are responsible for prostaglandin synthesis. Although cycloxygenase-1 (COX-1) is constitutive and required for normal "housekeeping" functions, COX-2 is inducible and barely detectable under normal physiologic conditions. It is rapidly but transiently induced as an early response to proinflammatory mediators and mitogenic stimuli, including cytokines, endotoxins, growth factors, oncogenes, and phorbol esters.

COX-1 is responsible for protection of mucosal surfaces, maintenance of renal function, and platelet activity and stability. COX-2 synthesizes series-2 prostaglandins (PGE2, PGF2alfa), which contribute to inflammation, swelling, and pain. Among the functions of PGE2 are promotion of IL-10, an immunosuppressive cytokine, and suppression of IL-12 (53). Another enzyme that plays a pivotal role in mediating inflammation is iNOS, activated by NFkB and acting in synergy with COX-2 to promote the inflammatory reaction. Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. In particular, some polyphenols were found to inhibit chronic inflammation in several experimental animals models. Polyphenols may act as antinflammation modulatory agents by various mechanisms, including down-regulation of NF-kB, or the various enzymes involved (including those that generate reactive oxygen species), by inhibition of the activity of those enzymes, or by increasing the cell ability to scavenge reactive oxygen species (ROS) directly or via glutathione peroxidase activity. olyphenols inhibit pro-inflammatory genes expression via inhibition of IkB kinases. In addition the polyphenols can blockade NFKB through MAPKs pathway modulation and/or the stimulation HO-1 expression by promoting dissociation of the Nrf2-Keap1 complex. Nrf2 is a member of the family of transcription factors. Nrf2 is expressed in a wide range of tissues, many of which are sites of expression for phase 2 detoxification genes. The antioxidant response elements (ARE) are regulatory sequences found on promoters of several phase 2 detoxification genes that are inducible by xenobiotics and antioxidants. Alteration of the Nrf2-keap1 interaction enables to Nrf2 to translocate into the nucleus, bind to the ARE and initiate the transcription of genes coding for detoxifying enzymes and cytoprotective proteins. This response is also favorite by polyphenolic compounds. In fact polyphenols may react with thiol groups of Keap1 promoting the dissociation of the Nrf2-Keap1 complex, leading to increased Nrf2 binding to HO-1. (Figure 6).

5.2. Effects of polyphenols

Recent *in vitro* studies have further potential of polyphenols to modulate various parts of the inflammatory process. It would therefore be worthwhile to continue to evaluate the anti-inflammatory activity of flavonoids, not only in order to establish the anti-inflammatory mechanisms, but also to develop a new class of anti-inflammatory agents. Several cellular action mechanisms have been proposed to explain *in vivo* anti-inflammatory activity of flavonoids. Polyphenols could regulate cellular activities of the inflammation-related cells: mast cells, macrophages, lymphocytes, and neutrophils. For instance, some flavonoids inhibit histamine release from mast cells and others inhibit T-cell proliferation. In addition, certain flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A2 (PLA2), COX, lipoxygenase (LOX) and nitric oxide synthase (NOS), the nitric oxide (NO) producing enzyme. An inhibition of these enzymes by flavonoids reduces the production of AA, prostaglandins (PG), leukotrienes (LT), and NO, crucial mediators of inflammation. Thus, the inhibition of these enzymes exerted by flavonoids is definitely one of the important cellular mechanisms of anti-inflammation. Furthermore, many lines of evidence support the idea that certain flavonoids are modulators of gene expression, especially of proinflammatory gene expression, thus leading to the attenuation of the inflammatory response. It is not known to what extent these proinflammatory gene expressions contribute to the inflammatory response, but it is evident that flavonoids show anti-inflammatory activity, at least in part, by the their suppression. The inhibitory activity of several flavonoid derivatives against AA metabolizing enzymes was investigated (33). Thereafter, numerous studies have evidenced the inhibitory effect of flavonoids on these enzymes. AA (a precursor of eicosanoids) is mainly released from membrane lipids in cells. The enzyme responsible for this release is PLA2, although some portion is attributed to the combined action of phospholipase C and diacyl-glycerol lipase. They are many isoforms of this enzyme, divided into in three principal categories, secretory PLA2 (sPLA2), cytosolic PLA2 (cPLA2), and calcium independent PLA2 (iPLA2). These PLA2s are distributed in a wide variety of tissues and cells. The first flavonoid inhibitor of PLA2 found was quercetin, which inhibited

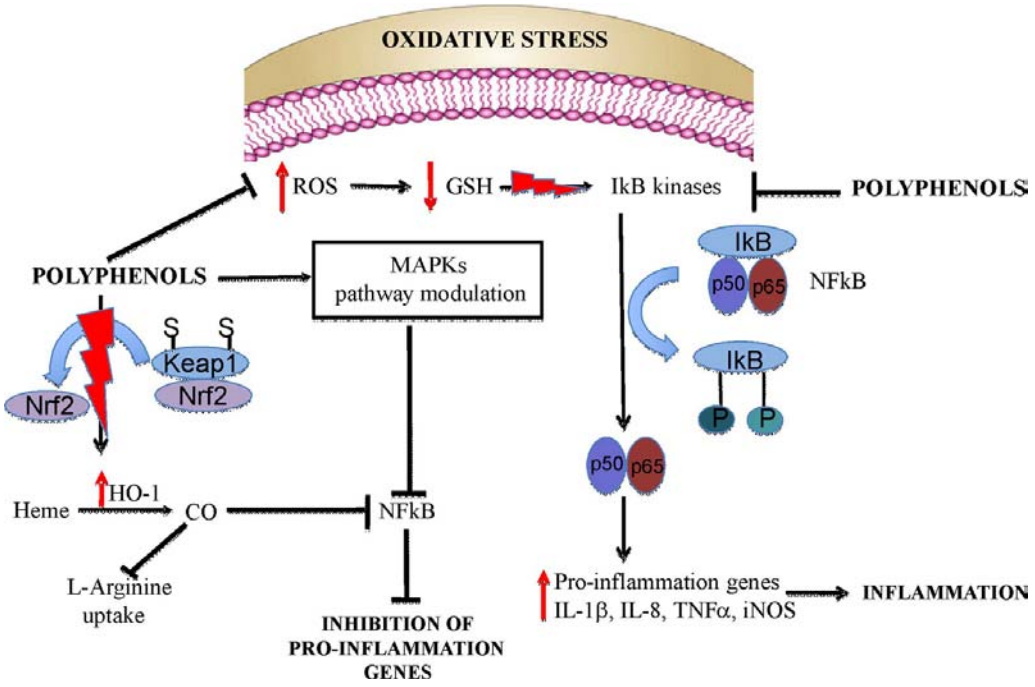


Figure 6. Possible mechanism of anti-inflammatory activity of polyphenols. Polyphenols may act as antinflammation modulatory agents by various mechanisms, including down-regulation of NF-kB, or by increasing the cell ability to scavenge reactive oxygen species (ROS) directly or via glutathione peroxidase activity. Polyphenols inhibit pro-inflammatory genes expression via inhibition of IkB kinases. In addition the polyphenols can blockade NFKB through MAPKs pathway modulation and/or the stimulation HO-1 expression by promoting dissociation of the Nrf2-Keap1 complex. Polyphenols may react with thiol groups of Keap1 promoting the dissociation of the Nrf2-Keap1 complex, leading to increased Nrf2 binding to HO-1.

PLA2 from human neutrophils (54). While flavanones, including flavanone, hesperetin, and naringenin, showed less inhibition, flavonols such as kaempferol, quercetin, and myricetin were found to considerably inhibit snake venom PLA2, indicating the importance of the C-ring-2,3-double bond. Since COX are involved in inflammation, inhibitors of these enzymes are being continuously developed to obtain safer anti-inflammatory drugs. Flavonoids and flavonoid-containing foods have been investigated as selective COX-2 inhibitors. Flavonoids with an ortho-dihydroxy (catechol moiety) in rings A or B are stronger inhibitors of COX-2 than those with a free 3-OH group. The presence of a C2–C3 double bond appears to be a major determinant of COX activity. In fact, when their structural activity relationships were compared, several flavone derivatives such as flavone and apigenin were found to be COX inhibitors, while some flavonol derivatives such as quercetin and myricetin were preferential LOX inhibitors. In particular, reduction of C-2,3-double bond and glycosylation reduced the inhibitory activity. Some chalcones having a 3,4-dihydroxycinnamoyl moiety were reported to inhibit COX and 12-LOX from mouse epidermis, being more active on LOX (55). While some flavonoid glycosides, including rutin and hypolaetin-8-glucoside, enhanced COX activity from sheep seminal vesicle, certain flavonoids such as flavone, kaempferol, and quercetin were repeatedly found to be COX inhibitors from rat peritoneal macrophages. In addition, some flavones/flavonols such as flavone, apigenin, luteolin, galangin and kaempferol are also potent COX-1 inhibitors (56). It is known that interference of signal transduction pathways or modulation of the inflammatory pathway through transcription factors are potential mechanisms of COX-2 transcription. Additionally, flavonoids can suppress COX-2 transcriptional activity by inhibition of phosphorylation signal transduction pathways. Structurally, the number of hydroxyl groups on the B ring may be related to the molecular conformation that influences the interactions between the flavonoids and enzymes such as tyrosine kinase and protein kinase C, which are involved in COX-2 transcriptional activity. In fact it has been reported that flavonoids with 3', 4', and 5' hydroxyl groups in the B ring such as myricetin and epigallocatechin do not to suppress the transcriptional activity of COX-2 in human adenocarcinoma cell line DLD-1 (56).

It has been found that some flavonoids are COX-1/COX-2 inhibitors, and *in vivo* anti-inflammatory activity may be contributed by these inhibitory properties to reduce prostanoid production. Another potential mechanism by which flavonoids mediate their inhibition of COX-2 gene expression is by alteration of the NF-kB pathway. Lee *et al.* reported that silibinin can suppress the expression of inflammatory genes such as CD80 and MHCclass I, inhibit the lipopolysaccharide (LPS)-induced activation of mitogen-activated protein (MAP) kinases (important in vascular gene regulation) and the nuclear translocation of the NF-kB p65 subunit in murine dendritic cells (57). Luteolin and other flavonoids interfere with LPS signaling pathways, reducing activation of several MAP kinase

family members, and inhibit inflammatory mediator expression (58). Similar effects were seen with an *A. polygama* extract in macrophages. This extract also reduced carrageenan-induced rat paw oedema, a model of inflammation (59). With regard to allergic inflammation, apple procyanidins can exert an anti-allergenic effect by inhibition of histamine release from mast cells, via inhibition of the interaction between IgE and its high-affinity receptor, FcepsilonRI (60). Asthma is an obstructive airway inflammation mediated by allergic activation of the immune system. A clinical correlation has been shown between apple consumption and healthy lung function in humans (61). Interestingly, anthocyannis and blackcurrant and boysenberry polyphenol extracts [our unpublished observations] have also been found to modulate allergy-mediated pathways in a mouse model of asthma and in lung epithelial cells, respectively (62). Tangeretin and nobiletin, polymethylated flavonoids from citrus peel, inhibit IL-1beta-induced COX-2 expression in human lung carcinoma cells. In a recent study, Birrel *et al.* (63) have demonstrated that *in vivo*, resveratrol, a phytoalexin stilbenic, can inhibit inflammatory cytokine expression in response to lipopolysaccharide (LPS) challenge in rat lungs.Furthermore, in both monocytic U937 cells and alveolar epithelial A549 cells, resveratrol inhibits NF-kB and activator protein-1 (AP-1) activation (64, 65). Resveratrol had no effect on the binding of NF-kB proteins to DNA, but it did block the tumor necrotic factor (TNF)-induced translocation of p65 subunit of NF-kB and reporter gene transcription. Similarly, the activation of c-Jun N-terminal kinases (JNK) and its upstream kinase mitogen-activated protein (MEK) are inhibited by resveratrol, which may explain the mechanism of suppression of AP-1 by resveratrol. In addition it has been reported that resveratrol is able to block COX-2 and iNOS expression by inhibiting NF-kB activation; this effect could be because the genes of COX-2 and iNOS are regulated by NF-kB. Other authors have reported that *cis* resveratrol can significantly modulate a wide variety of pro-inflammatory pathways by inhibiting the activation of NF-kB (65). In fact, *cis* resveratrol in peritoneal macrophages stimulated with LPS and gamma interferon (IFN-gamma) significantly attenuated the expression of NF-kB family of genes, adhesion molecules and acute-phase proteins (65). In addition, *cis*-resveratrol also inhibited transcription of Scya2 (chemokine monocyte chemotactic peptide-1 (MCP-1)), the chemokine RANTES (regulated on activation, normal T cell expressed and secreted), proinflammatory cytokines that attract monocyte–granulocyte cells such as colony-stimulating factor 1 (M-CSF), colony-stimulating factor 2 (GM-CSF) and colony-stimulating factor 3 (G-CSF), the transforming growth factor beta (TGF-beta) and the extracellular ligand interleukin 1 (IL-1a).However, the effect of resveratrol on the immune system does not seem to be as mechanistically simple as nonspecific inhibition of inflammation; resveratrol seems to enhance the immune response of mice treated with the arylating substance dinitrofluorobenzene and prevents immunosuppression by ethanol (66). Resveratrol also appears to protect mice from infection with herpes simplex viruses (67). The exact mechanisms by which resveratrol differentially inhibits and enhances the immune system have not been clearly elucidated.In rodent models of inflammatory colitis, intragastric resveratrol given acutely before and after colonic injury has been shown to reverse weight loss, increase stool consistency, improve mucosal appearance, improve histopathology, decrease inflammatory infiltrate, and decrease mucosal levels of interleukin (IL)-1beta, COX-2, and prostaglandin (PG) D_2. Some flavonoids can also inhibit TNF-induced adhesion molecule expression in human aortic endothelial cells, the first stage of atherosclerosis (68). Quercetin inhibits cytokine and iNOS expression through inhibition of the NF-kB pathway without modification of c-Jun N-terminal kinase activity in macrophages (69-71). It is reported that catechins, in particular epigallocatechin gallate (EGCG), markedly inhibited IL-1beta-mediated IL-1beta receptor-associated kinase (IRAK) degradation and the signaling events downstream from IRAK degradation: IKK activation, IkBalfa degradation, and NF-kB activation (72). The functional consequence of this inhibition was evidenced by inhibition of IL-8 gene expression. Catechins, monomers of flavonols with similar composition such as catechin, epicatechin, epigallocatechin, epicatechin gallate and EGCG, have also been shown to down-regulate CD11b expression on CD8+ T cells and thereby inhibit infiltration of these cells into sites of inflammation (73). Green tea polyphenols are also able to stimulate MAPK pathways in HepG2 cells (74) and can increase mRNA levels of immediate-early genes such as c-jun and c-fos. However, Chen *et al.* (75) have shown that not all polyphenols in green tea extracts have similar activity and their effects appear to be structurally related to the 3-gallate group. The degree of activation of MAPK by the five tea polyphenols was related to the structure, dose and time.

It is known that inducible iNOS is another enzyme that plays a pivotal role in mediating inflammation. NO is one of the cellular mediators of physiological and pathological processes (56, 75-77) such as inflammation. Compounds reducing NO production by iNOS without affecting endothelial (eNOS) or neuronal NOS (nNOS) may be desirable as anti-inflammatory agents. While a small amount of NO synthesized by eNOS and nNOS is essential for maintaining normal body function (homeostasis), a significantly increased amount of NO synthesized by iNOS participates in provoking inflammatory processes and acts synergistically with other inflammatory mediators (77). Therefore, inhibition of iNOS activity or down-regulation of iNOS expression may be beneficial in reducing the inflammatory response. It is reported that some flavonols, isoflavones and, particularly, flavones considerably inhibited NO production. On the other hand, flavonoid glycosides such as vitexin regardless of the chemical structures of aglycones did not significantly inhibit NO production up to 100 microM. In general, flavones showed stronger inhibition of NO production than flavonols. These results strongly suggest that the C-2,3-double bond is crucial for inhibiting NO production, and hydroxyl substitutions on A- and B-rings influence the inhibitory activity. A-ring 5-/7- and B-ring 3-/4-hydroxylation(s) gave favorable results while C-3 hydroxylation (flavonol) did not. It was also demonstrated that the active flavonoids did not significantly inhibit iNOS

activity, but instead strongly suppressed iNOS expression. In particular, flavone and several other amino-substituted flavones were reported to inhibit NO production (78). Several flavonoid derivatives including apigenin, quercetin, and morin also inhibited NO production from LPS/ interferon (IFN)-gamma-activated C6-astrocytes (79), the cellular mechanism was not however elucidated. Clearly the antioxidant and scavenger activities of polyphenols play an important role in the anti-inflammatory actions. It is known, in fact, that oxidants may play an important role in the modulation of histone deacetylase (HDAC) and inflammatory cytokine gene transcription (80-81). Furthermore, it has been shown that both oxidants and TNF-alfa caused an increase in histone acetylation (HAT activity) leading to IL-8 expression in monocytes and alveolar epithelial cells *in vitro* (82). It is known that glucocorticoid suppression of inflammatory genes requires the recruitment of histone deacetylase-2 (HDAC2) into the proinflammatory transcriptome complex by the glucocorticoid receptor (83). This results in deacetylation of histones and a decrease in inflammatory gene transcription. A reduced level of HDAC2 was associated with increased proinflammatory response (84). The restoration of a ROS-induced HAT/HDAC imbalance could therefore have a significant impact on inflammation; Rahaman *et al.*, in fact, showed inhibited pro-inflammatory gene expression through the activation of histone deacetylases (31). However, other possible mechanisms of polyphenol-mediated inhibition of the inflammatory response should not be overlooked, e.g. quenching or reversing post-translational protein modifications induced by oxidants and damaging reactive aldehydes. This might be achieved through the induction of enzymes such as tyrosine denitrase, carbonyl reductase or aldo–keto reductase. It is interesting to speculate that these dietary polyphenols and flavonols may not only act as antioxidant/anti-inflammatory agents, but it is also possible that they would increase the efficacy of glucocorticosteroids.

Another actor implicated in the anti-inflammatory response is the peroxisome proliferator activated (PPAR)gamma transcription factor. PPARs bind to specific response elements as heterodimers with the retinoid X factor and activate transcription in response to a variety of different exogenous or endogenous ligands such as arachidonic acid metabolites and some drugs (31, 85).Three PPAR isoforms differ in their tissue distribution and ligand specificity (86). PPARalfa is predominantly expressed in tissues exhibiting high catabolic rate of fatty acids (heart, liver, and kidney), whereas PPARgamma expression is ubiquitous, and its physiological role is unclear. PPARgamma is expressed predominantly in adipose tissue, the adrenal gland, spleen, large colon and the immune system (87-88). Several lines of evidence indicate that PPARgamma plays an important role in regulating adipocyte differentiation and glucose homeostasis (89). Both PPARalfa and PPARgamma have also been shown to have an anti-inflammatory action activated by arachidonic acid metabolites. PPARalfa bind to, and are activated by, leukotriene B4 (90), at levels induced at the transcriptional level by anti-inflammatory glucocorticoids (91). PPARgamma are activated by the prostaglandin D2 metabolite 15-deoxy-$\Delta^{12;14}$ prostaglandin J2 and synthetic anti-diabetic thiazolidinedione drugs, and negatively regulate the expression of pro-inflammatory genes, and suppress tumor cell growth (92-94). Liang *et al.* reported that several flavonoids can bind to PPARgamma *in vitro*, acting as possible PPARgamma ligands thereby blocking production of inflammatory cytokines in human monocytes. In particular these compounds might act as allosteric effectors, able to bind to and activate PPARgamma (85). Structurally these flavonoids possess lipophilic backbone characteristics similar to those of other PPARgamma ligands but lack their common acid moiety. The flavanones and flavan-3-ol were inefficient at activating PPARgamma, indicating that the C2–C3 double bond of the C ring is essential for activation (95). Flavones, flavonols, and isoflavones were able to activate PPARgamma, but this activation was dependent on the number and position of the hydroxyl groups. Important residues were the hydroxyl groups at position 5 and 7 on the A ring and the 4'-position on the B ring such as in kaempferol, apigenin, and chrysin. The presence of the 3'-hydroxyl group on the B ring, such as in luteolin and quercetin, resulted in a decrease in PPARgamma activation (95). Although these compounds are excellent candidates to explain the health benefits of diets rich in fruits and vegetables, there is still not enough information on food composition data, bioavailability, interaction with other food components and their biological effects to fully define the mechanisms involved.

6. SUMMARY

Different studies suggest that diets rich in polyphenols may have health beneficial effects. In fact these compounds, acting as antioxidants and free radical scavengers, may play a significant role in inflammation and in chronic inflammatory diseases.

Several cellular action mechanisms are proposed to explain their anti-inflammatory activity. In addition to anti-oxidative activity, they inhibit eicosanoid generating enzymes; and certain flavonoids, mainly flavone derivatives, modulate the expression of proinflammatory molecules, at least partly, via inhibition of transcription factor activation. Polyphenols seem to be important metabolic modulators by virtue of their ability to moderate and influence several cellular processes such as signaling, proliferation, apoptosis, redox balance, differentiation, *etc*. In view of their anti-inflammatory and antioxidant abilities and their capacity to modulate important inflammatory and anti-inflammatory signaling pathways, polyphenols, promise a novel approach to attenuation and possible reversal of the inflammation cascade. These compounds may thus represent new therapeutics agents in different inflammatory diseases.

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Abbreviations: AA: arachidonic acid; AP-1: activator protein; CAT: catalase; CO: carbone monoxide; COX: cyclooxygenase; COX-1: cycloxygenase-1; COX-2: cycloxygenase-2; cPLA2: cytosolic; Cy cyanidin; C3G: cyanidin -3- glucoside; DPPH: 1,1-diphenyl-2-picryl-hydrazyl radical; EGCG: epigallocatechin gallate; eNOS: endothelial nitric oxide synthase; G-CSF: colony-stimulating factor 3; G6PD: glucose-6-phosphate dehydrogenase; GM-CSF: colony-stimulating factor 2; GSH-Px: glutathione peroxidase; GSH-Red: gluthatione reductase; H_2O_2: hydrogen peroxide; HAT: histone acetylation activity; HDAC: histone deacetylase; HDAC2: histone deacetylase-2; HO-1: heme oxygenase 1; IFN-gamma: gamma interferon; IL-1: interleukin-1; IL-8: interleukin-8; iNOS: inducible nitric oxide synthase; iPLA2: calcium independent; Keap1: Kelch-like ECH-associated protein 1; LDL: low density lipoproteins; linDNA: linear DNA; LOX: lipoxygenase; 5-LOX: 5-lipoxygenase; LPS: lipopolysaccharide; LT: leukotrienes; M-CSF: colony-stimulating factor 1; MAP: mitogen-activated protein; MMP-9: metalloproteinase-9; NFkB: nuclear factor-kB; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; NOS: nitric oxide synthase; Nrf2: nuclear related factors 2; O_2^{-}: superoxide anion; $^{\cdot}OH$: hydroxyl radicals; $^1O_2$, singlet oxygen; PGF2alfa: prostaglandins; PI-3K: phosphoinositide-3-kinase; PLA2: phospholipase A2; PPAR: peroxisome proliferator activated; ROS: reactive oxygen species; scDNA: supercoiled DNA; SOD: superoxide dismutase; sPLA2: secretory PLA2; TGF-beta: transforming growth factor beta; TNF: tumor necrosis factor-alfa; UV: ultraviolet.

Key Words: Polyphenols, Flavonoids, Inflammation, Oxidative Stress, Antioxidant, NFkB, COX1, COX2, Review

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