Antioxidant Capacities of Flavones and Benefits in Oxidative-Stress Related Diseases

Marcelo D. Catarino[1], Jorge M. Alves-Silva[1], Olívia R. Pereira[1,2] and Susana M. Cardoso[1,3*]

[1]*CERNAS, School of Agriculture, Polytechnic Institute of Coimbra, Bencanta, Coimbra, Portugal;*

[2]*DTDT, School of Health Sciences, Polytechnic Institute of Bragança, Bragança, Portugal;*

[3]*QOPNA, Department of Chemistry, University of Aveiro, Portugal*

Address reprint request to Susana M. Cardoso, QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Telephone: +351 234 802940; Fax: +351 273 239 802979. E-mail: susanacardoso@ua.pt

Abstract:

Flavonoids, a group of secondary metabolites widely distributed in the plant kingdom, have been acknowledged for their interesting medicinal properties. Among them, natural flavones, as well as some of their synthetic derivatives, have been shown to exhibit several biological activities, including antioxidant, anti-inflammatory, antitumor, anti-allergic, neuroprotective, cardioprotective and antimicrobial. The antioxidant properties of flavones allow them to demonstrate potential application as preventive and attenuating agents in oxidative stress, i.e., a biological condition that is closely associated to aging process and to several diseases. Some flavones interfere in distinct oxidative-stress related events by directly reducing the levels of intracellular free radicals (hydroxyl, superoxide and nitric oxide) and/or of reactive species (e.g. hydrogen peroxide, peroxynitrite and hypochlorous acid) thus preventing their amplification and the consequent damage of other biomolecules such as lipids, proteins and DNA. Flavones can also hinder the activity of central free radical-producing enzymes, such as xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) or inducible nitric oxide synthase (iNOS) and can even modulate the intracellular levels of pro-oxidant and/or antioxidant enzymes. The evaluation of flavones antioxidant ability has been extensively determined in chemical or biological *in vitro* models, but *in vivo* therapy with individual flavones or with flavones-enriched extracts has also been reported. The present manuscript revises relevant studies focusing the preventive effects of flavones on stress-related diseases, namely the neurological and cardiovascular diseases, and diabetes and its associated complications.

Keywords: Antioxidant activity; coronary heart diseases, diabetes, flavones, neurodegenerative disorders, oxidative stress-related diseases, structure–activity relationship;

Running title: Protection of flavones on oxidative-stress related diseases

1. INTRODUCTION

1.1. General Structure and Function

Flavones are one of the main classes of flavonoids i.e., a group compounds characterized by a C15 basic skeleton composed of a benzene ring (A-ring) fused to a heterocyclic pyran ring (C-ring), having a phenyl substitution most often at the 2-position (B-ring). In particular, flavones are characterized by the presence of a double bond between the 2- and 3- positions in the heterocyclic C-ring and the lacking of oxygenation at the 3-position of the same ring (Fig. **1**).

Typical variations in the basic structure of the flavones include OH- and OMe-substitution, mainly in the A- and B-rings. Other groups such as *C*-methyl, methylenedioxy, *C*- and *O*-prenyl, pyran, furan and aromatic have also been described [1]. Moreover, natural flavones occur as aglycone or alternatively, as hexosides or acylated glucosides [2].

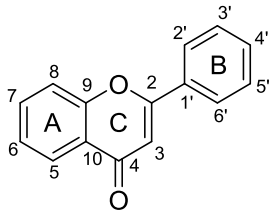


Fig. (1). General structure of flavones.

The natural flavones are secondary metabolites from vascular plants and, likewise other flavonoids, they are key players in plant development and growth. Some flavones are also involved in plant survival due their ability to act as ultraviolet filters, as well as to protect the plants from microbial, insect and even from mammalian herbivor attack [2-3]. Althoug flavones are classified as colourless compounds, they can act as co-pigments of anthocyanins, providing attractive colours to plant pollinators [2-3].

Main natural flavones comprise chrysin, balcalein, scutellarein, nobiletin, luteolin, apigenin, tangeritin and 6-hydroxyflavone. From those, luteolin and apigenin are widespread in grains, leafy vegetables, and herbs and are considered to be the most representative ones in food sources [1, 4]. High concentrations of luteolin are particularly found in celery seeds (aproximatly 800 mg/100 g) while moderate amounts are found in thyme, sage, oregano, olives, peppermint, green peppers, chilli pepper green, parsley, lemon, red lettuce and sweet pepper red. Apigenin is mainly found in parsley and celery seeds [1, 5].

The daily intake of flavones is widely variable amongst populations, depending on their specific dietary food habits. It has been estimated that the mean intake of apigenin and luteolin by Chinese population is aproximatly 1.1 and 3.8 mg/day [6], respectively, while the total intake of these two flavones by Australian and Spanish populations accounts up to 0.05 and 3.6 mg/day [7], [8]. Correlations between the intake of flavones and their *in vivo* effects are still under debate, as their bioavailability is not completely elucidated. Indeed, although it is presently accepted that ingested flavones (likewise other simple phenolics) can be partially absorbed in the small intestine and suffer metabolization in the liver as

signalization for excretion from the body, further studies need to be carried out in order to fully understand the effective concentrations of flavones in the target organs (e.g elucidation of absorbable low-molecular-weight phenolic metabolites which are produced by gut microbial flora and possible accumulation in body tissues) [5, 9].

1.2. Oxidative Processes

Mitochondria is the primary site of generation of reactive oxygen species (ROS) in aerobic cells, since the univalente reduction of triplet-state molecular oxygen results in the production of superoxide anion ($O_2^{\bullet -}$) [10]. This species can also be produced by other celular enzymes such as xanthine oxidase and NADPH-oxidases (Fig. **2**). Despite the relatively low reactivity of $O_2^{\bullet -}$, this species can be converted, through enzymatic or nonenzymatic reactions, to highly reactive ROS (e.g. hydroxyl radical ($OH^{\bullet}$)) or reactive nitrogen species (RNS), namely peroxynitrite ($ONOO^-$) [11]. The former results from its conversion of $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2) and it's subsequent reduction, which occurs either in the absence or in the presence of reduced transition metals. In turn, $ONOO^-$ results from the reaction of $O_2^{\bullet -}$ with nitric oxide ($NO^{\bullet}$), a reactive species that is produced by nitric oxide synthases (NOS) when converting arginine into citruline. Elevated amounts of $NO^{\bullet}$ are particularly produced by inducible nitric oxide synthase (iNOS), a pro-oxidant enzyme which is highly expressed in inflammatory cells upon stimulation by exogenous or endogenous stimuli [12-15].

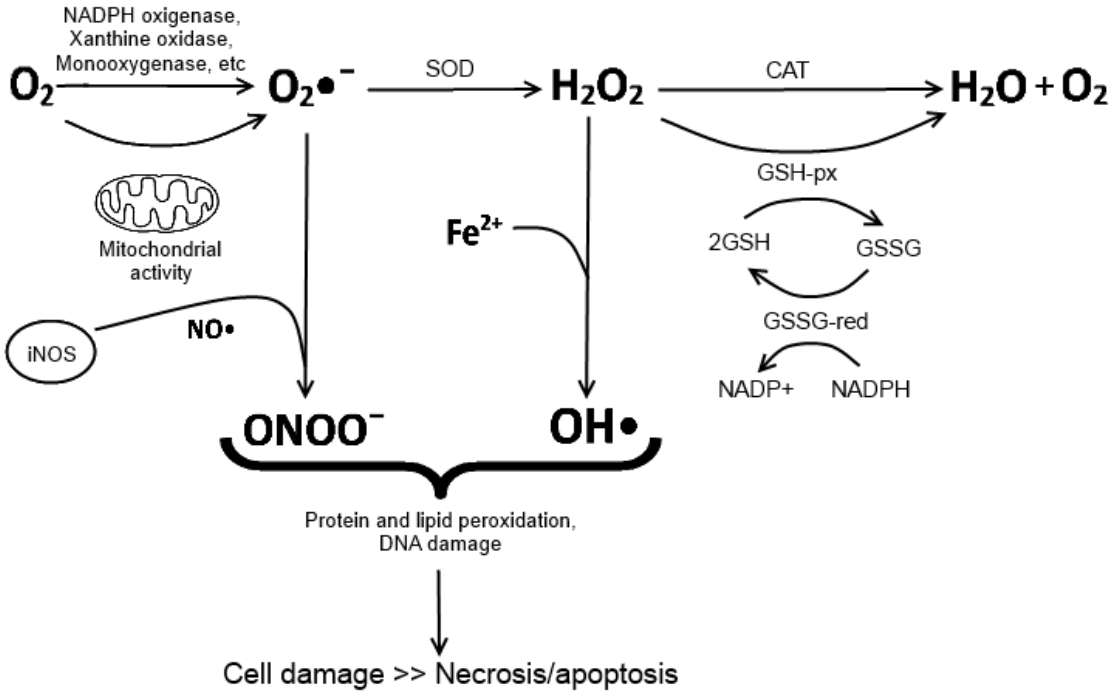


Fig. (2). Schematic representation of formation and enzymatic neutralization processes of the superoxide radicals and its derivatives. Superoxide anion ($O_2^{\bullet -}$) is converted into the $ONOO^-$ (a highly reactive species), in the presence of nitric oxide (NO•), thus causing serious cellular damages that might end in necrosis and/or apoptosis. In turn,

superoxide dismutase (SOD) is an antioxidant enzyme capable of converting $O_2\bullet^-$ into hydrogen peroxide (H_2O_2). The latter might follow two paths: 1- In presence of Fe^{2+}, the Fenton reaction occurs, converting H_2O_2 into hydroxyl radicals (OH•) which, similarly to $ONOO^-$, is highly reactive and will cause cellular damage/death; 2- Converted into water (H_2O) and oxygen (O_2) by the antioxidant enzymes catalase (CAT) or glutathione peroxidase (GSH-px). This latter enzyme uses the reduced form of glutathione (GSH) as an electron donor to convert the H_2O_2, producing gluthatione disulfide (GSSG), which in turn can be converted back again into GSH by glutathione reductase (GSSG-red) using NADPH as an electron donor.

Notably, cells have several mechanisms to maintain the redox homeostasis, i.e., the balance between ROS and RNS generation and their elimination [16]. The consumption or deactivation of said compounds occurs via the action of both enzymatic and non-enzymatic/simple antioxidants [11, 17]. For instance, superoxide radical is converted to oxygen and hydrogen peroxide by the enzyme superoxide dismutase (SOD) where the latter is transformed to water and oxygen by the enzyme catalase (CAT), while glutathione peroxidase (GSH-px) reduces lipid hydroperoxides to their corresponding alcohols and reduces free hydrogen peroxide to water (see Fig. **2**) [18]. The latter enzyme makes use of glutathione (GSH) as an electron donor, converting it into glutathione disulfide (GSSG), which in turn is regenerated by glutathione reductase (GSSG-red) into GSH again. Therefore, GSH is a pivotal endogenous molecule on cellular antioxidant defenses. It is also important to refer to the central role of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is known as the "master regulator" of the antioxidant response, since it is responsible for the modulation of the expression of hundreds of genes, including those that encode the antioxidant enzymes mentioned before. The activity of this transcription factor is triggered on oxidative stress conditions, causing its translocation to the nucleus where it will upregulate the expression of several genes of antioxidant and cytoprotective enzymes in order to restore the balance. In turn, vitamin A, C and E, as well as caffeine are examples of non-enzymatic antioxidants [19-20].

When the balance for production vs. elimination of ROS and RNS is disrupted, the cell enters into an oxidative stress state, which will trigger the activation of some signalling cascades. One of the most important cell responses is mediated by nuclear factor-kB (NF-kB), a transcription factor that plays a crucial role in inflammation, immunity, cell proliferation, apoptosis and other cellular cycles.

This transcription factor is normally maintained as inactive in the cytoplasm of non-stimulated cells by endogenous inhibitors, namely inhibitor of kB (I-kB). Under stress conditions, this transcription factor dissociates from its inhibitor and translocates to the nucleus, binding to DNA's promoter or enhancer regions, causing an increase in the expression of several genes that in turn will promote the transcription of several pro-inflammatory cytokines and enzymes, resulting in an overall increment of oxidative stress [21]. In a similar way, the activation of mitogen activated protein kinases (MAPKs) signalling cascade, also triggered by oxidative stress conditions, causes dimerization of c-Jun and c-Fos into activator protein 1 (AP-1) [21].

Hence, the overproduction of reactive species is settled in a vicious cycle way in oxidative stress conditions, since the high concentration of one reactive species stimulates further formation of ROS and RNS [17]. As an overall result, reactive species may cause damage in lipids, proteins, DNA and other macromolecules [16-17, 22], resulting in several pathological conditions [19].

2. ANTIOXIDANT PROPERTIES: STRUCTURE-FUNCTION RELATIONSHIPS

In recent decades, a wide range of biological activities have been described for flavones [23], with particular emphasis on their antioxidant and protective ability on oxidative stress-related conditions. These capacities render flavones a great application in several fields, including the food, cosmetic and pharmaceutical industries, as well as in medicine [4].

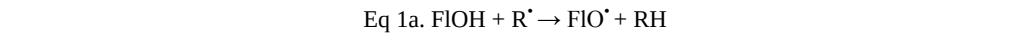
However, as referred before, the bioavailability of these compounds is still subject to debate, as this is influenced by many factors which are distinct in between different populations and even within the same population. Notwithstanding, it is presently accepted that once ingested, only a portion of low-molecular-weight polyphenols may be readily absorbed in the small intestine, while 90-95% accumulate in the large intestinal lumen. Recent literature data also suggest that these non-absorbable compounds can be subjected to the enzymatic activities of the gut microbial flora and transformed into a series of absorbable low-molecular-weight phenolic metabolites [24-26].

Nonetheless, it is believed that flavones have both direct and indirect antioxidant properties. The direct effects include their ability to scavenge free radicals (e.g. superoxide anion radicals, hydroxyl radicals), to quench ROS (e.g. singlet oxygen) and to chelate metal ions and inhibit lipid peroxidation. In turn, the indirect effects of flavones are related to the modulation of the activity of key enzymes and/or interaction with receptors [27]. The main structural-fuction relationships elucidated so far, regarding the antioxidant abilities of flavones, are summarized below.

2.1. Direct antioxidant effects

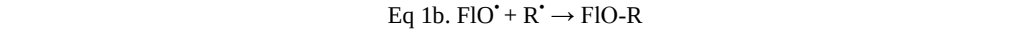
Similarly to other antioxidants, flavones counteract radicals mainly by two mechanisms, namely Hydrogen Atom Transfer (HAT) and by Single Electron Transfer (SET).

As a result of an HAT reaction, an hydrogen atom is transferred from the flavone (FlOH) to the radical. The reaction between a flavone and a free radical results in a flavone phenoxyl radical ($FlO^{\bullet}$) and a stable substance (RH) (Eq 1a). The flavone phenoxyl radical formed could then react with other radicals ((Eq 1b) $R^{\bullet}$ or (Eq 1c) $FlO^{\bullet}$) by radical-radical termination reactions, resulting in the formation of an unreactive compound i.e., (Eq 1b) FlO-R or (Eq 1c) FlO-OFl, respectively) [4, 28-30]. On the other hand, in a SET reaction, the flavone transfer one electron to reduce the radical, metals or carbonyls (Eq 2) [31].



Eq 1a. $FlOH + R^{\bullet} \rightarrow FlO^{\bullet} + RH$

Scavenging reaction



Eq 1b. $FlO^{\bullet} + R^{\bullet} \rightarrow FlO\text{-}R$

Radical-radical coupling reaction

Eq 1c. $FlO^{\bullet} + FlO^{\bullet} \rightarrow FlO\text{-}OFl$

Radical-radical coupling reaction

Eq 2. $FlOH + R^{\bullet} \rightarrow FlOH^{\bullet} + R^{-}$

Single-electron transfer reaction

Note that HAT and SET mechanisms may occur in parallel, the main mechanism being determined by the structural properties of the antioxidant, together with pH, solubility, partition coefficient and system solvent [31]. At present, researchers believe that HAT is the most relevant mechanism to human biology [32-33].

Generalistic methods for measuring radical scavenging capacity of antioxidants, in particular the chemical assays that use molecular probes e.g. trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging capacity, ferric ion reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and trapping antioxidant parameter (TRAP), have also been extensively applied to flavones [15, 34]. With the exception of the last two, the remaining are simple methods to measure the ability of an oxidant to undergo single electron transfer reactions [32]. On the other hand, TRAP and ORAC assays evaluate the capability of an antioxidant to inhibit peroxyl radical-induced oxidations, through H-atom donation [32].

The main structural features of flavones for conditioning their radical scavenging activity enclose (A) the 2,3-double bond in the C-ring in conjugation to 4-keto group in the C ring; (B) the ortho-dihydroxy (catechol) group in the B-ring and (C) the presence of an hydroxyl group at position 5 (Fig. **3**) [23, 35-36].

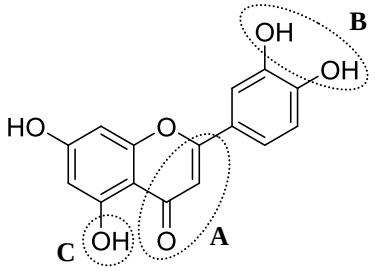
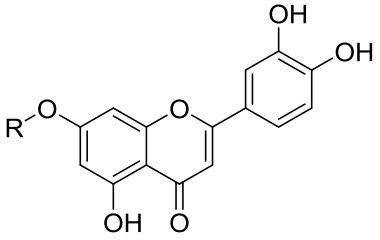


Fig. (3). Major structural requirements for radical scavenging activity of flavones.

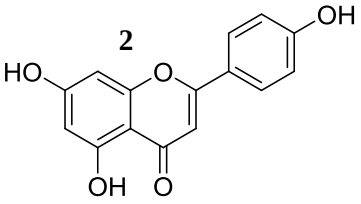
Notably, the 2, 3 double bond, in conjugation with the 4-keto group in the C-ring is responsible for the electronic delocalization starting from the B-ring [36], allowing the semiquinone radical to donate an electron and forming the stable-quinone structure, which is essential for SET mechanism. This capacity is improved by OH groups on the B-ring that decrease the O-H bond dissociation energy (BDE) and act as electron-donating groups [23].

The catechol moiety on the B-ring confers high stability on the radical species through H-bond formation and also participates in electron delocalization, by increasing the electron density at the hydroxyl group and lowering the oxygenhydrogen bond energy [3, 27, 36]. The catechol group has been associated to the promotion of scavenging activity against peroxyl, superoxide and peroxynitrite radicals [30, 37]. Leopoldini and colleagues [36] showed that flavonoids with this dihydroxy functionality are the most active in donating an H atom while Rice-Evans et al. [38] concluded that this functionality contributes at about 25% for the antioxidant activity of luteolin **(1)** comparing to that of apigenin **(2)** and chrysin **(3)** (Fig. **4**).



1 R= H
9 R= glucopyranoside

2



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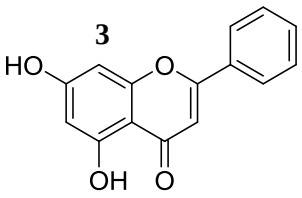

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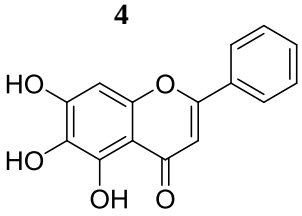

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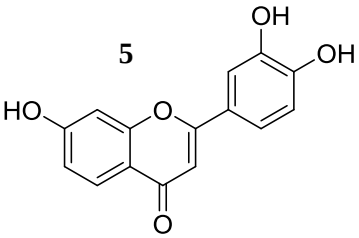

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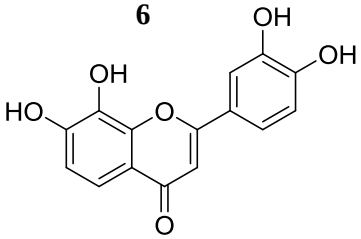

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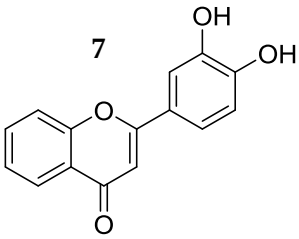

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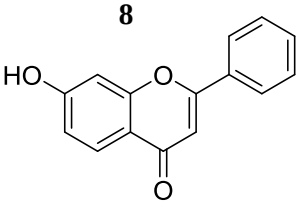
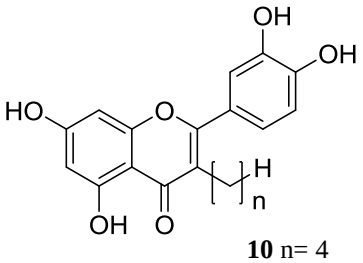
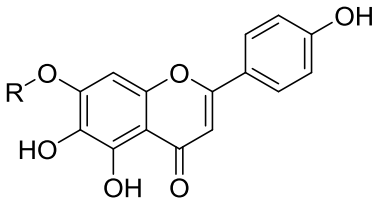
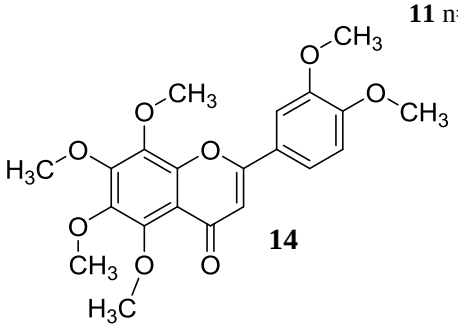




10 n= 4
11 n= 6



12 R= H
13 R= glucuronide



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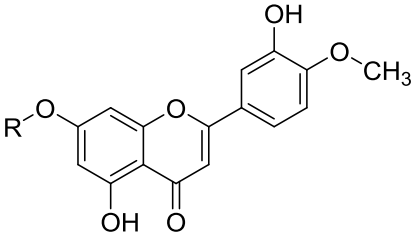
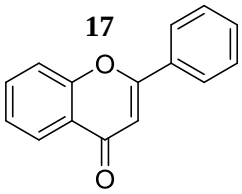


15 R= rutinoside
16 R= H

17



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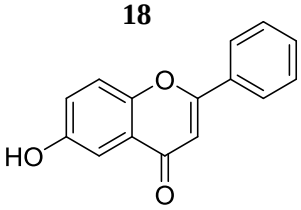

Fig. (4). Chemical structures of flavones. The reference numbers for the compound structures are used throughout the manuscript.

When present, the hydroxyl group at 5-position forms hydrogen bonds with the 4-keto group and in this condition the B-ring is slightly tilted with respect to the plane of A and C rings, thus facilitating the antioxidant action. The presence of additional OH group(s) on B-ring enhances its antioxidant action. Apigenin and luteolin are good candidates for the one-electron-transfer mechanism due to their planar conformation and the extended electronic delocalization between nearby rings [36].

Besides the previous mentioned factors, some additional properties can be marked as conditioning factors for the the scavenging properties of flavones. E.g. the synergistic interaction between flavones and other physiological antioxidants such as ascorbate or tocopherol is described as important in improving the radical scavenging capacity of flavones [23]. Baicalein **(4)** is an example of this phenomenon. Albeit this flavone has low antioxidant capacity, it has been shown to have a good anti-lipoxidation effect in 2,2′-azobis(2,4-dimethylvaleronitrile)-induced liposomal membranes, due to synergistic effects with beta-carotene [39].

Chelating of metal ions such as the chelating of catalytically active metal (e.g. Cu (I), Fe (II) and Fe (III)) is also a relevant mechanism for the antioxidant activities of flavones with important role in cellular protection. The reaction of a phenoxyl radical and metal ions produces a radical anion that is the most stable structure.

Remarkably, the 5-hydroxyl group associated with the 4-keto and catecholic hydroxyl groups are extremely important to this capacity. In flavones, the metal-complexing sites are thought to occur between the hydroxyl at 5-position and the 4-keto group, as well as in between the ortho-hydroxyls on the B-ring (Fig. **5**). Additionally, a study performed by Mira *et al* [40] indicated that the combined presence of 2,3-double bond (C-ring) and cathecol (B-ring) is an important feature for Fe^{3+} reducing activity while the cathecol group and the number of hydroxyl groups in A-ring plays a central role to Cu^{2+} reducing activity [40-41].

These reactions prevent the generation of oxidizing species (e.g. acting as initiators of lipid peroxidation or of the lipoxygenase reaction) and also highly reactive hydroxyl radicals that eventually could be formed by Fenton-type reactions [42].

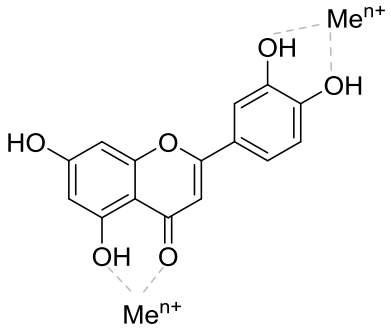

Fig. (5). Possible sites for chelating the transition metal ions on flavones (adapted from [23]).

2.2. Indirect antioxidant effects

Xanthine oxidase (XO) is the unique enzyme for which structure-fuction relations have been partially clarified for flavones. This is a molybdoflavoprotein that is involved in the metabolism of

purines by catalyzing the conversion of hypoxanthine to xanthine and that of xanthine to uric acid (Fig. **6**), with the release of superoxide anion radical or hydrogen peroxide [43-45].

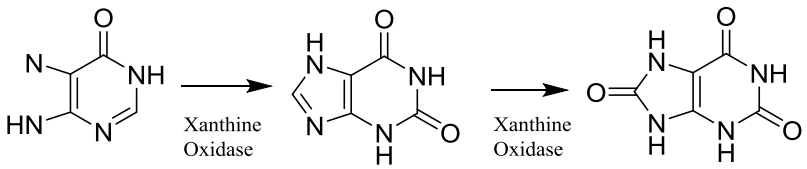

Fig. (6). Conversion of hypoxanthine to xanthine and of xanthine to uric acid, by xanthine oxidase.

In this regard, the inhibition of XO is very important because it prevents the production of excessive uric acid thus avoiding hyperuricemia, as well as the prevention of excessive levels of ROS [23]. Table **1** shows the IC_{50} values of distinct flavones for XO inhibiton.

Table 1. IC_{50} values of distinct flavones for XO inhibition.

Flavone	IC_{50} (µM)	Reference
Apigenin	1/0.70	**[46-47]**
Baicalein	2.79	**[47]**
Chrysin	2.5/ 0.84	**[46-47]**
Luteolin	0.75/0.55	**[46-47]**
7, 3,4'-trihydroxyflavone **(5)**	4	**[46]**
7, 8, 3', 4'-tetrahydroxyflavone **(6)**	10	**[46]**
3',4'-dihydroxyflavone **(7)**	40	**[46]**
7-hydroxyflavone **(8)**	40	**[46]**

Rastelli and co-workers [48] proposed a model for flavones-xanthine oxidase interaction, based on similarities between the flavones and the substrates or inhibitors of the enzyme. Relevant points comprised (A) the matches of the negative electrostatic potential of oxygen in C-7 of flavones skeleton with that of the carbonyl group at C-6 in xanthine, due to the extended delocalization of negative charges over the entire benzo-pyrone structure; (B) the lone-pair minima of O-4 approaching the negative potential of N-3 and N-9 of hypoxanthine and xanthine, respectively; (C) the superimposition of 2-phenyl rings of flavones with the phenyl group of the most potent purine inhibitors of the enzyme (as a consequence of the carbonyl superimposition, a group that is essential for activity), thus suggesting that 2-phenyl ring is responsible for hydrophobic interactions with the XO in the same location as the inhibitors; (D) the presence of a substituent at C-4' (in addition to an hydroxyl group at C-7) enhanced the flavone´s activity mainly because it is involved in dispersion interactions with XO. Notably, the presence of a hydroxyl group at C-7 is established as fundamental to the inhibitory effect of flavones on XO, mainly because this is responsible for the binding of flavone to the active site of the enzyme and it has a low pKa thus ensuring that there is enough dissociated form at physiological pH. Moreover, this group allows hydrophobic interactions between the flavone and XO [23, 48].

From experimental and theoretical results obtained more recently, several authors concluded that along with the mentioned factors, the substitution of hydroxyl groups at 5- and 7-positions, as well as the

substitution of a catechol or a 3',4',5'-pyrogallol functionality, are also structurally important factors contributing to the inhibition of XO by flavones [23, 47].

Notwithstanding, there are already some findings of the structure-activity relation between flavones and other enzymes such as iNOS. In the investigation of Kim *et al*. (1999), the authors have concluded that the most active flavonoids inhibiting the iNOS were those containing a C-2,3 double bond (such as in flavones) and 5,7-dihydroxyl groups in the A-ring. Furthermore, the substitution of hydroxyl groups at 4'- or 3',4'- in the B-ring (apigenin and luteolin, respectively) may contribute to the inhibitory effect on iNOS [49-50].

Moreover, evidence–points to these same structural features are related to the capacity of attenuating MAPKs signaling by interfering with c-Fos, and c-Jun gene expression expressions and AP-1 transcriptional activity, as well as interfering with IkB kinases (IKK)/NF-kB pathaway [51].

3. ROLES OF FLAVONES IN OXIDATIVE STRESS-RELATED DISEASES

Oxidative stress, i.e., the physiological condition arising from imbalance between the rates of production and release of free radicals, is closely associated to several diseases including cancer, diabetes, osteoporosis, neurodegenerative and cardiovascular diseases and many other aging-associated disorders [52].

In opposition, diet-derived antioxidants (including flavones) are regarded as potential protective agents in oxidative stress-related diseases. In fact, recent studies have demonstrated promising results regarding to the protective effects of flavonoids and/or flavones against stress-related diseases, both *in vitro* and *in vivo* models of diseases. Epidemiological studies and meta-analyses also suggest an inverse relationship between the consumption of flavonoid-rich diets and the development of distinct age-related diseases [53-55]. Still, despite these evidences, it should be remarked that the mechanisms underlying the protective effects of most flavonoids and/or flavones remain unclear and hence, there is a great demand on structure-activity studies on this area. Amongst the several oxidative-stress related disorders, the beneficial effects of flavones discussed below will be focused on the most relevant data reported on literature for flavones i.e., those correlated with neurodegenerative disorders, diabetes and its associated complications and with coronary heart diseases.

3.1. Neurodegenerative disorders

The brain is responsible for 20% of the total oxygen consumption due to its high metabolic requirements. Thus, this organ is characterized by high activity of the mitochondrial electron transport chain and high ROS production ratios [56]. The combination of those factors with weak tissue regeneration makes the brain one of the most susceptible organs to the oxidative stress [16]. In cerebral pathophysiologic conditions, oxidative damage occurs in proteins, lipids, DNA and takes place in modulation of apoptosis and necrosis [13]. Moreover excitotoxicity, mitochondrial dysfunction and intra or extracellular protein aggregation also contribute for the increment of oxidative stress and neuronal deregulation and death [57]. Hence, overall, oxidative stress is considered to be the major cause of the

neuronal loss occurring in chronic neurodegenerative diseases such as Alzheimer, Parkinson and Huntington [58], as well as in acute insults (ischemic and hemorrhagic stroke). In turn, reported data suggest that flavones can exert important protective roles in several models of neurological diseases (Table **2**).

Cerebral ischemia results from a transient or permanent reduction in cerebral blood flow that is restricted to the territory of a major brain artery, during which a series of phenomena such as excitotoxicity, oxidative stress, inflammation and apoptosis occur [59].

In their study, Zhao *et al*. [60] used the ischemic/ reperfusion (I/R) rat model to investigate the anti-ischemic potential of luteolin. The intraperitoneal injection of the encapsulated flavone upon I/R, for a period of 13 days, caused a noteworthy dose-dependent prevention of the induced injuries, due to the capacity of luteolin in reducing the increasedmitochondrial ROS levels as well as enhancing the activity of GSH and CAT.

Quiao and co-workers [61] additionally showed that luteolin is able to counteract diret and indirect oxidative stress events on I/R model. In more detail, the authors showed that this flavone could significantly stimulate the activity of the two antioxidant enzymes CAT and SOD-1 and overall decreased the oxidative stress marker malondialdehyde (MDA). The treatment also induced a decrease on the levels of the proapoptotic protein Bax and raised those of the anti-apoptotic protein Bcl-2. These results were reinforced by Zhang *et al*. [62], who reported that oral administration of luteolin (4 mg/kg) inhibited the neuronal death in a similar I/R model, suggesting that its neuroprotective action was not only due to its antioxidant properties but also to its capacity to induce nuclear factor erythroid-derived 2-like 2 (Nrf2) activity. In turn, this theory was recently supported by Xu et al. [63] who have demonstrated in traumatic brain injury cultured mice neurons that, besides restoring the levels of MDA and glutathione peroxidade (GSH-px), luteolin (at 10-50 μM) could enhance the Nrf2 translocation to the nucleus and subsequently caused the up-regulation of its downstream products, concomitantly lowering the intracellular ROS levels and increasing neuron survival.

Luteolin derivatives, either natural or synthetic, have also been suggested as potential agents in prevention and/or treatment of diverse neurological disorders. E.g. in a Parkinson disease model, cynaroside (luteolin-7-*O*-β-D-glucopyranoside) **(9)** has been shown to efficiently scavenge ROS-related products and to increment GSH levels, as well as to reduce the activities of the pro-aptotic caspase-3 and -8, thus protecting the cells from oxidative stress and promoting their viability [58]. The neuroprotective activity of this flavone on the same cellular model has been recently reaffirmed [64].

In turn, two synthetic 3-alkyl-luteolin derivatives bearing alkyl chains of 4 **(10)** and 6 **(11)** carbons (at 10-25 μM) were shown to rescue the intracellular ROS generation and caspase-3-like activity in striatal cells derived from Huntington disease knock-in mice, expressing mutant huntingtin [65].

Besides luteolin and/or luteolin derivatives, other flavones have already been tested in distinct models of neurological diseases. In hippocampal cells, the treatment with apigenin (at 5-60 μM) inhibited kainic acid-induced excitotoxicity (analogous of glutamate) in a dose-dependent manner, decreasing the intracellular ROS generation and increasing the GSH levels, hence demonstrating its neuroprotective potential [66]. Moreover, the treatment of cooper-stimulated APPsw cells (i.e., a model of Alzheimer disease manifested by an overexpression of amyloid precursor protein (APP) and a severe redox

imbalance) with apigenin (at 0.1-10 μM) resulted in a dose-dependent reduction of ROS levels and an enhancement of SOD and GSH-px activities. The authors also reported that the treatment with this flavone blocked the ROS-induced MAPK (mitogen-activated protein kinase) signaling pathways, preserved mitochondrial function and regulated apoptosis [67].

In addition, the oral administration of 10-20 mg/kg of apigenin to mice *in vivo* in a model of Alzheimer's disease caused the reduction of oxidized hydroethidine (a representant of superoxide anion levels on the cerebral cortex) in the brain when compared to those of untreated mice [68]. Recently, identical results were obtained by Zhao *et al*. [69], who additionally reported an enhanced SOD and GSH-px activities induced by apigenin, with respect to those observed in the control mice.

Scutellarein **(12)** and/or its derivatives, which are naturally found in *Scutellaria* plants, are also promising neuroprotective agents. In particular, Liu *et al*. [70] have shown that the treatment of H_2O_2-induced primary cultures of rat neuronal cells with scutellarin (scutellarein-7-glucuronide) **(13)** for 10-100 μM, caused a significant dose-dependent decrease on the MDA and NO• levels, also enhancing the cells viability with respect to controls. Further analysis lead the authors to conclude that the decrement of intracellular $NO^{\bullet}$ levels was resultant from the scutellarin´s capacity in inhibiting the neuronal NOS activity.

In turn, Hu *et al*. [71] reported that scutellarin caused up-regulation of eNOS and down-regulation of iNOS, as well as of vascular endothelium growth factor and of basic fibroblast growth factor (VEGF and bFGF, respectively), overall preventing the cerebral injury caused by I/R on Sprague-Dawley rats. In addition, further research revealed that the levels of SOD, CAT and GSH were significantly increased in ischemic brain tissues of scutellarin-treated rats, enhancing the endogenous antioxidant activity. Moreover, the addition of sculletarin to an *in vitro* neuron culture under an oxygen and glucose deprivation treatment, inhibited the levels of ROS generation and decreased the percentage of apoptotic cells [72].

Protective effects of scutellarin have also been suggested against Alzheimer´s disease since the treatment of Aβ-treated rat brains with this flavone induced the simultaneous increase of SOD´s activity and the decrease on MAO´s (monoamine oxidase) activity. The treatment also diminished the levels of inflammatory cytokines, hence overall lowering the oxidative stress and inflammation events, and resulting in an effective amelioration of the memory and learning abilities of the rats [73].

Despite the majority of experiments were performed with the glycosidic form of the flavone (i.e. scutellarin), it is important to highlight that the main the main in vivo metabolite of this flavone, i.e. scutellarein, has been demonstrated to exhibit stronger antioxidant capacities and to further protect PC12 cells against H2O2-induced cytotoxicity than its glycoside scutellarin [74]. Similar results were obtained for the neuroprotective effects of these two flavones on a cerebral I/R model, suggesting that scutellarein is preferential for therapeutical effects [75].

Another flavone, the O-methylated flavone nobiletin **(14)** isolated from citrus peels, has been shown to be able to counteract oxidative stress events in H2O2-induced PC12 cells [76]. The exposure of these cells to the flavone at 3-25 μM induced a dose-dependent increase on SOD and GSH activities, the decrease of MDA levels and lipid peroxidation, together with the regulation of mitochondrial membrane potential and the inhibition of caspase-3 activity [76]. Moreover, the treatment of senescence accelerated mice

(SAMP8) with this flavone (10 - 50 mg/kg) was also able to restore the glutathione derivatives GSH/GSSG ration, increasing the GSHpx and SOD activities and reducing the phosphorylation of tau protein in the hippocampus of the mouse brain, which lead to the restoration of learning and memory deficits, typical symptoms of Alzheimer's disease [77].

Overall, these results suggest that flavones (in particular those that are found in natural food sources) are potential candidates to be used in the intervention for neurodegenerative diseases, either in a preventive manner or as a possible therapy.

Table 2. Protective effect of flavones on neurodegenerative disorders.

Compound	Model	Test Conditions	Effects	Ref
Luteolin	I/R rat model	5 and 20 mg/kg/day for 13 days, intraperitoneal injection	↓ behavioural deficit scores; ↓ infarct volume; ↑ CAT levels; ↓ GSH levels; ↓ ROS production on hippocampus, frontal cortex and striatum	[60]
	I/R rat model	10 and 25 mg/kg	↓ neurological deficits score; ↓ infarct volume; ↓ Bax protein/mRNA levels; ↑ Bcl-2 and claudin-5 protein/mRNA levels; ↑ SOD-1/CAT; ↓ MDA levels	[61]
	SH-SYS cell	2-50 µM prior to treatment with 200, 500 or 800 µM H_2O_2	↑ Nrf2/HO-1 expression levels; ↓ H_2O_2-induced cell death; ↓ ROS production	[62]
	I/R rat model	4 mg/Kg , tail vein injection	↓infarct area; ↓caspase-3 cleavage	[62]
	TBI mice; mouse neurons	10, 30 and 50 mg/kg , intraperitoneal injection	↑ motor performance; ↓ apoptotic index; ↓ MDA levels; ↑ GPx expression; ↑ Nrf2 translocation to nucleus; ↑ Nrf2-AREs binding; ↑ Nrf2 downstream proteins; ↓ intracellular ROS production and TBI-induced cell damage	[63]
Luteolin-7-*O*-[58] β-D-glucopyranoside	PC12 cell	25-100 µM for 6h prior 6-OHDA (175 µM), H_2O_2 (87.5 µM) and 6-OHDA (175 µM) + CAT (87.5 U) (*p*-quinone) treatment	↓ *p*-quinone- and H_2O_2-induced cell death; ↓ ROS production; ↓ caspase-3 and -8 levels; ↓ OH radicals; ↑ GSH levels	[58]
	PC12 cell	100 µM for 6h prior 6-OHDA (175 µM) treatment	↓6-OHDA-induced neurotoxicity	[64]
3-alkyl-luteolin	ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines	10-25 µM	↓ intracellular ROS levels; ↓ caspase-3 activity	[65]
Apigenin	Hippocampal cells	5-60 µM 0.5-1h before KA (100 µM)	↓KA-induced neurotoxicity; ↓ROS production	[66]
	ICR mice	25-50 mg/kg followed by KA (40 mg/kg), intraperitoneal injection	↓ behaviour and electrical seizures induced by KA; ↓ GSH depletion on convulsive mice; ↓ KA-induced neuronal damage on hippocampal CA3 regions	
	APPsw cells	0.1-10 µM prior to a 24h 200 µM Cu incubation	↓ Cu-induced cell death; ↓ APP expression and $A\beta_{1-42}$ secretion; ↓ ROS generation; ↑ GSH levels; ↑ intracellular SOD and GPx levels; ↓ mitochondrial dysfunction; ↓ cyt c release; ↓ nuclear condensation; ↓ p38 MAPK-MK2-Hsp27 and SAPK/JNK-c-Jun pathways; ↓ caspase-3 and -9 activity	[67]
	APP/PS-1 mice	40 mg/kg/day for 5 days, oral administration	↓ spatial learning and memory impairment; ↓ Aβ burden by decreasing $A\beta_{1-40}$ and $A\beta_{1-42}$ insoluble forms; ↓ BACE-1 levels; ↓ OHEt signals; ↑ SOD and GSH levels; ↑ BDNF, p-ERK1/2 and CREB expression on cerebral cortex	[69]
	$A\beta_{25-35}$-induced amnesia mice models	10 and 20 mg/kg/day for 8 days, oral administration	Ameliorates spatial learning and memory deficits; protects microvessels integrity and attenuate neuronal loss; ↓ OHEt signals on cytosol and neurovascular interface; ↑occuldin, ZO-1 and claudin-5 levels; ↓ AChE activity; ↑ BDNF/ACh levels; ↑TrkB and pCREB expression on cerebral cortex	[68]
Scutellarein	Neuronal cells	10-100 µM prior to 2 mM H_2O_2 exposure	↓ NO release; ↓ cNOS activity; ↓ MDA levels; ↓ H_2O_2-induced cell death	[70]
	I/R rat model	25-75 mg/kg/day for 7 days, intragastric injection	↓ infarct area; ↓ neurological score; ↓ BBB permeability; ↓ NO_x production ; ↑ eNOS expression;↓ bFGF/VEGF/iNOS expression	[71]
	I/R rat model	20-60 mg/kg, intraperitoneal injection	↓ neurological scores; ↓ infarct area; ↑ SOD/CAT activity; ↑ GSH activity	[72]

	cortical neurons	25-100 μM on a OGD system	↓ LDH release; ↓ apoptotic cells; ↓ ROS generation	
	Rats with $A\beta_{25-35}$ aggregates	10 mg/day for 20 days, intragastric injection	Ameliorates learning and memory dysfunction associated with Aβ aggregates; ↑ SOD activity;↓ MAO activity; ↓ IL-1β/IL-6/ TNF-α; ↓ apoptotic neurons	[73]
	PC12 cell line	1-100 μM co incubated with 400 μM H_2O_2, pre incubated for 30 min and pre incubated for 3h before H_2O_2	↓ H_2O_2-induced cell death	[74]
	I/R rat model	25-100 mg/kg, intragastic injection	↑ neurological score; ↓ infarct area	[75]
Scutellarein	SAMP8 mice	10-50 mg/kg, intraperitoneal injection	↓ cell death; ↓ LDH leakage; ↓ MDA levels; ↑ GSH and SOD expression levels; ↑ mmp; ↓ ROS generation; ↓ caspase-3 activity	[76]
Nobiletin	SAMP8 mice	10-50 mg/kg, intraperitoneal injection	Reversed recognition memory and context-dependent fear memory impairment; ↑ Mn-SOD at 50 mg/kg in striatum and GPx in cerebral cortex, hippocampus and striatum; ↓ the GSH/GSSG ratio loss in cerebral cortex, hippocampus, striatum and cerebellum; ↓ protein carbonyl levels in cerebral cortex and hippocampus; ↓ tau protein hyperphosphorylation	[77]

6-OHDA – 6-hydroxydopamine; ACh – acetylcholine; AChE – acetylcholinesterase; Api – apigenin; APP – amyloid protein precursor; APPsw – swedish mutant APP; ARE – antioxidant response element; BACE-1 – β site APP-cleaving enzyme; BBB – blood brain barrier; BDNF – brain-derived neurotrophic factor; bFGF- basic fibroblast growth factor; CAT – catalase; cNOS – constitutive nitric oxide synthase; CREB – cAMP response element-binding protein; eNOS – endothelial nitric oxide synthase; ERK1/2 – extracellular signal-regulated kinase; GPx – glutathione peroxidase; GSH – reduced glutathione; Hsp27 – heat shock protein 27; I/R – ischemia/reperfusion; iNOS – inducible nitric oxide synthase; KA – kainic acid; LDH – lactate dehydrogenase; Lut – luteolin; MAO – monoamine oxidase; MAPK – mitogen activated protein kinase; MDA – malondialdehyde; MK2 – MAPKAP kinase 2; mmp – mitochondrial membrane potential; Nar – naringin; Nob – nobiletin; Nrf2 – nuclear factor erythroid 2-related factor 2; OHEt – oxidized hydroethidine; PC12 – rat pheochromocytoma cell line; PS-1 – presenilin-1; ROS – reactive oxygen species; SAMP-8 – senescence-accelerated mouse prone 8; Scut – scutelarin; SH-SYS – human derived neuroblastoma cells; SOD – superoxide dismutase; ST*Hdh*$^{7/7}$/$^{111/111}$ – striatal cells expressing normal huntingtin/mutant huntingtin; TBI – traumatic brain injury; TrkB – tropomyosin related kinase B; VEGF – vascular endothelial growth factor; ZO-1 – zona occuldens protein-1

3.2. Diabetes and associated complications

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes due to glucose oxidation, non-enzymatic glycosylation of proteins and the subsequent oxidative degradation of glycated proteins. The abnormal high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can result in the damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress promote the development of other diabetes-associated complications [78].

Table **3** resumes relevant reported data for the protective effects of flavones on diabetes and diabetes-associated diseases.

Pancreatic β-cells are known to be particularly sensitive to oxidative stress, a fact that may contribute to the impaired β-cell function that is characteristic of diabetes. The pre-treatment of H_2O_2-stimulated pancreatic βTC1 cells with chrysin, quercetin or catechin (all at 50 μM) has been found to significantly protect the cells against the generated oxidative stress. Interestingly, despite being the most hydrophobic of the three flavonoids and lacking the hydroxyl group on the B-ring (which increases antioxidant activity), chrysin was the compound that conferred better protection to the cells [79].

Reducing sugars (e.g. glucose and 2-deoxy-D-ribose) produce ROS through autoxidation and protein glycosylation, hence contributing for progressive β-cell failure. In this context, Suh and co-workers [80] have demonstrated that apigenin conferred protection on 2-deoxy-D-ribose-induced HIT-15 pancreatic cells through regulation of the mithocondrial membrane potential, as well as through decrement of intracellular ROS levels. A previous study have also demonstrated that apigenin and luteolin

could protect RINm5F rat insulinoma cells from interleukine (IL)-1β- and interferon (IFN)-γ-induced damage, since they inhibit $NO^{\bullet}$ production, mainly by reducing the iNOS mRNA and protein expression, apparently through the inhibition of nuclear factor-κB (NF-κB) activation [81].

More recently, some flavonoid components from extracts of Gelam honey, including luteolin and chrysin, were tested on high glucose-stimulated HIT-15 pancreatic cells. The pretreatment of cells with these flavones prior to culturing in a high glucose level medium resulted in a significant dosedependent decrease of the intracellular ROS generation, along with those of MDA and of glucose-induced lipid peroxidation, which lead to the general enhancement of the cells insulin contents and their viability [82].

As the metabolic disorder progresses, defects in glucose metabolizing machinery restrains the physiological system from correcting the imbalance in glucose levels, thus resulting in chronic hyperglycemia, which in turn is associated with long-term complications such as retinopathy, nephropathy, neuropathy, cardiomyopathy among other complications [83-84].

In a streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats, Srinivasan and Pari [84] tested the protective effect of diosmin **(15)** against consequent oxidative stress damage. After a period of 45 days of oral administration of diosmin (100 mg/kg/day), these rats had their plasma levels of glucose decreased and those of insulin increased. Furthermore, on these same diosmin treated rats, increased activity of the antioxidant enzymes SOD, CAT, GSH-px, glutathione-S-transferase (GST) and levels of non-enzymatic antioxidants vitamin C, vitamin E and GSH were observed, along with decreased levels of lipid peroxidation markers in kidney and liver tissues. Chrysin has also been suggested to display hepatoprotective properties, since it was able to reduce the levels of MDA and lipid peroxidation in liver of alloxan-induced diabetic mice [85].

In addition, luteolin has already been shown to display positive results in protection against nephropathy (diabetesassociated kidney disorders). This flavone was introduced (200mg/kg) in the diet of Sprague-Dawley rats, after 48h of STZ-diabetes induction. The gathered data confirmed that upon 8 weeks of treatment, the blood glucose levels of luteolin- treated rats was significantly reduced in comparison to that of controls. The authors also reported that levels of MDA on the kidneys of the luteolin-treated rats was signifycantly lowered, while the levels of SOD and the phosphorylation of Akt/PKB (serine/threonine-specific protein kinase) were significantly increased, evidencing the protective effects of luteolin against diabetic nephropathy [86].

Besides protection on kidneys disorders, luteolin has been suggested as a promising protective agent against diabetic-associated cardiomyopathy. Quian et al. [87] showed that the treatment of diabetic-Sprague–Dawley rats with luteolin revealed a marked attenuation of the endothelium-dependent relaxation impairment, as well as the strong reversion of the increased ROS levels and $OH^{\bullet-}$ formation, together with decreased $NO^{\bullet}$ levels and NOS and SOD activities. In addition, rats fed with this flavone (200 mg/kg) before the induction of diabetes-stimulus were demonstrated to have lower levels of MDA, lactate dehydrogenase (LDH) and LDL cholesterol, and increased levels of HDL cholesterol, SOD and Akt phosphorylation, with respect to the controls [88].

Luteolin also show positive results in diabetic-associated neuropathy. According to the work of Liu et al. [83], the administration of luteolin (50-100 mg/kg) for a period of 8 weeks to Sprague-Dawley rats upon

the STZinduction of diabetes, resulted in the decrement of cerebral MDA and lipid peroxidation levels, while the levels of GSH, SOD and CAT were substantially increased, resulting in effective counteraction of the neuronal damage and cognitive dysfunction. Besides luteolin, both chrysin and diosmetin **(16)** have also been suggested as protective agents in diabetic neuropathy. In fact, male Wistar rats treated with chrysin after diabetes induction have improved their cognitive deficits [89]. These effects were related not only to the reduction of the MDA levels and an increase of SOD, CAT and GSH levels, thus relieving the oxidative stress, but also to the suppression of the p65 subunit of NF-κB, IL-1β an IL-6 activities, which prevented the inflammation process. In turn, diosmin has shown its potential in preventing the progression of early diabetic neuropathy in rats. Type-2 diabetes was induced on Sprague-Dawley rats and this was followed by the oral administration of diosmin (50 and 100 mg/kg/day) for 4 weeks. After treatment with the flavone, the elevated blood sugar and lipid profiles were restored, together with those of the increased levels of MDA and $NO^{\bullet}$, and the decreased levels of SOD and GSH. Overall, this treatment with diosmin resulted in alleviation of thermal hyperalgesia, cold allodynia and walking function of the diabetic rats [90].

Taking all this data into account, it is pertinent to say that flavones have shown promising results that could make them potentially useful for the development of future therapies to treat and/or prevent diabetes and diabetes-associated complications.

Table 3. Protective effect of flavones on diabetes and diabetes-associated diseases.

Compound	Model	Test conditions	Effects	Ref
Apigenin	HIT-T15 cell line	0.01-10 µM apigenin for 30min prior to dRib 30 µM for 24h	↑ cell survivability; ↓ apoptosis, ROS generation and loss of mmp; ↓ NFkB and AP-1 expression	[80]
Apigenin, Luteolin	RINm5F	IL-1β- and IFN-γ-induced oxidative stress	↓cytotoxicity; ↓ NO production; ↓ iNOS mRNA/protein levels; Inhibits NFkB binding activity and IkBα degradation on cytosol; ↓ p50 and p65 content on nucleus; ↑ insulin secretion	[81]
Chrysin	HIT-T15 cell line	50 µM + Mb 30 µM for 24h prior to GO/metMb for 20h	↓ damage of H_2O_2/metMb-induced oxidative stress	[79]
	STZ-induced diabetic rats	30 and 100 mg/kg, intraperitoneal injection	↓ Glucose; Alleviates diabetes-associated cognitive deficits; ↓ MDA, p65 of NFkB, TNF-α, IL-1β and IL-6 content and caspase-3 activity; ↑ SOD, CAT and GSH levels	[89]
	Alloxan-induced diabetic mice	50 mg/kg, intraperitoneal injection	↓ MDA levels	[85]
Chrysin, Luteolin	HIT-T15 cell line	20-80 µM for 24h prior to a 24h incubation with 20 or 50 mM glucose	Protected cells from glucose-induced damage; ↓ ROS generation; ↓ MDA levels; ↓ F2 isoprostane content; ↑ insulin content	[82]
Diosmin	STZ-induced diabetic rats	100 mg/kg, intragastric injection	↑ Plasma insulin; ↓ plasma glucose; ↓ TBARS/hydroperoxides; ↑ SOD; ↑ CAT/GST; ↑ GPx; ↑ GR; ↑ Vit. C/Vit. E/GSH; ↓ GSSG; ↑ GSH/GSSG ratio	[84]
	STZ and high fat diet-induced diabetic rats	50 and 100 mg/kg, oral administration	↓ Glucose; ↓ TC/TG; ↑TP; ↓ thermal hyperalgesia and cold allodynia; ameliorates on walking function test; ↓ MDA levels; ↑ GSH/SOD levels; ↓ $NO^{\bullet}$ generation	[90]
Luteolin	STZ-induced diabetic rats	200 mg/kg, intragastric injection	↓ Glucose/BUN/Creatinine/TC/TG/LDL levels; ↑ HDL levels; ↓ 24h urea protein; ↓ TC/TG; ↓ SOD activity; ↓ MDA levels; ↑ HO-1 expression; ↑ Akt/Pkb phosphorylation	[86]
	HG-mediated impairment of endothelium	0.5-90 µM with 44 mM glucose	↑ Endothelium-dependent vasorelaxation; ↓ ROS; ↓ OH; ↑ SOD/cNOS; ↓ iNOS; ↑ $NO^{\bullet}$ levels	[87]
	STZ-induced diabetic rats	10 , 50 and 100 mg/kg/day for 8 days	↑ Endothelium-dependent vasorelaxation	
	STZ-induced diabetic rats	200 mg/kg, oral administration	↓ CK/LDH; ↓ TC/TG/LDL levels; ↑ HDL levels; ↓ MDA levels; ↑ SOD levels; ↑ HO-1 levels; ↑ Akt/Pkb levels; ↓ CTGF levels	[88]
	STZ-induced diabetic rats	50 and 100 mg/kg, oral administration	↓Glucose; ↓ diabetes-associated cognitive decline; ↓ ChE activity; ↓ MDA levels; ↑ GSH levels; ↑ SOD/CAT activity	[83]

AP-1 – activator protein-1; Api – apigenin; CAT – catalase; ChE – cholinesterase; Chr – chrysin; CK – creatine kinase; cNOS – constitutive nitric oxide synthase; DS – diosmin; dRiB – 2-deoxy-D-ribose; GO – glucose oxidase; GPx – glutathione peroxidase; GR – glutathione reductase; GSH – reduced glutathione; GST – glutathione-S-transferase; HDL – high density lipoprotein; HG – high glucose; HIT-T15 – insulin-secreting hamster β-cells; HO-1 – hemeoxygenase-1; IFN-γ – interferon-γ; IL-1β – interleukin-1β; IL-6 – interleukin-6; iNOS – inducible nitric oxide synthase; LDH – lactate dehydrogenase; LDL – low density lipoprotein; Lut – luteolin; Mb – myoblobin; MDA – malondialdehyde; metMb – metmyoglobin; mmp – mitochondrial membrane potential; NF-kB – nuclear factor-kappa B; ROS – reactive oxygen species; RINm5F – rat insulinoma cell line; SOD – superoxide dismutase; STZ – streptozotocin; TBARS – thiobarbituric acid reactive substances; TC – total cholesterol; TG – total triacylglycerol

3.3. Coronary heart diseases

Atherosclerosis (AS) i.e., the main cause of cardiovascular diseases (CVD), has also been closely associated to oxidative stress events. In fact, high levels of ROS are known to generate an increment of the oxidative stress in the vessel wall, as well as to promote the oxidation of the serum lowdensity lipoprotein (LDL) cholesterol, being the latter recognized as the major cause of AS and other cardiovascular diseases [91-92]. Elicitation of endothelial cells by the oxidized LDL (oxLDL) and other factors further stimulate the intracellular production of ROS, which in turn act as key second messengers, being responsible for initiating a series of intracellular signaling pathways [93]. In particular, the injured cells start expressing cellular adhesion molecules (CAMs) that promote the binding and recruitment of circulating leukocytes. These immune cells engulf oxLDLs and consequently form the foam cells that migrate to the intimal layer of the vessel where they further stimulate inflammatory mediators (including cytokines, chemokines and $NO^{\bullet}$), contributing to additional increment of the oxidative stress [94].

Several authors have reported protective effects of flavones against coronary heart diseases (Table **4**). Yi *et al.* [95] tested several flavonoids including flavone **(17)**, chrysin, apigenin, luteolin, 6-hydroxyflavone **(18)**, baicalein and 7-hydroxyflavone on oxLDL-induced human umbilical vein EA.hy926 cells, in order to assess their protective potential on AS. Among the tested flavones, the authors concluded that the treatment with apigenin and luteolin (at 40 μM) promoted $NO^{\bullet}$ release, suggesting a particular effect of the two flavones on the endothelial secretory function and endothelium-dependent vasorelaxation. Other positive effects of the apigenin and luteolin included the inhibition of MDA and ICAM-1 and cell viability amelioration. Further investigation performed in a similar cellular model corroborated that apigenin and luteolin (80 μM) could maintain the cell viability, as well as regulate intracellular ROS production [96]. The authors also observed that the two flavones had a notable inhibitory effect on the oxLDL-induced p38MAPK phosphorylation and NF-κB (p65) translocation to the nucleus, together with a deep reduction on the mRNA expression of several NF-κB-mediated genes, hence blocking the generation of more ROS.

When the inflammatory endothelial response is settled, TNF-α, a key cytokine in inflammation is released. This cytokine has a multifunctional role via the activation of numerous intracellular signaling pathways, including MAPK and transcription of NF-κB that in turn will stimulate the production of more cytokines (including itself) and increase ROS formation, resulting in a vicious cycle [94]. In order to evaluate luteolin capacity to counteract the effects of TNF- α, Xia *et al.* [97] tested the human umbilical endothelium cells' (HUVEC) response in presence/absence of the flavone. The treatment with luteolin (6.25-25 μM) was able to suppress the TNF- α -induced ROS generation, as well as the expression of the superoxide producing enzyme NADPH oxidase-4 and its subunit p22phox. The flavone also suppressed the expression of ICAM and VCAM, caspase-3 and -9, and enhanced Bcl-2, consequently ameliorating

the cells viability. Finally, the treatment with luteolin could inhibit transcriptional activity of NF-kB, and p38 and ERK 1/2 phosphorilation, overall attenuating oxidative stress and inflammatory processes.

One of the main complications of atherosclerosis is the acute myocardial infarction (AMI) [98-99]. This is characterized by the interruption of blood supply (ischemia) to a part of the heart. Ischemia and ensuing oxygen shortage induce myocardium the death of heart cells, thus, reperfusion therapy must be applied as soon as possible in order to attenuate the ischemic injury [100]. Luteolin has also demonstrated potential in the prevention of ischemic-associated oxidative stress. In Sprague-Dawley rats subjected to myocardial ischemia/reperfusion, luteolin significantly reduced myocardial infarct size, as well as MDA production in the injured tissue samples. Moreover, treatment with this flavone (10 μg/kg) decreased plasma LDH and NO· levels, and also down-regulated iNOS protein and mRNA expressions [101].

More recently, diosmin cardioprotective effects have been shown by Senthamizhselvan *et al.* [102], who observed significant decrease of LDH and creatine kinase (CK)-MB activities, along with increased levels of glutathione and antioxidant enzymes SOD, CAT and GSH-px activities on Langendorff-I/R rats. Moreover, lipid peroxidation and *in vitro* O2·- and OH· generation were reversed by diosmin.

Despite the few studies demonstrating the effects of flavones on the ischemic-associated oxidative stress, many others have been performed reporting the efficacy of several flavones (including apigenin, scutellarin, chrysin among others) in the protection of myocardial I/R injuries through interaction with other signaling pathways such as PI3K/Akt, MAPK and apoptotic cascade pathways, and NF-kB activation [103-107].

Table 4. Protective effect of flavones on coronary heart diseases.

Compound	Model	Test conditions	Effects	Ref
Apigenin, Luteolin	EA.hy926	40 μM for 2h prior to a 24h incubation with 100 μg/mL oxLDL	↑ Cell viability; ↓ MDA levels; ↑ NO· release; ↓ ICAM-1	[95]
Diosmin	I/R rat	50 and 100 mg/kg for 30 min prior to I/R, oral administration	↑ rate pressure product; ↓ LDH release; ↓ CK-MB expression; ↑ SOD/CAT/GPx activity; ↑ GSH levels; ↓ TBARS/LOOH levels; ↑ mitICDH/mitMDH activity; ↑ mit α-KGDH activity; ↑ mitSDH activity; ↑ ATP level; ↓ Bcl-2 downregulation	[102]
Luteolin, Apigenin	EA.hy926	40 μM for 2h prior to 24h incubation with 100 μg/mL oxLDL	Inhibited oxLDL-induced cytotoxicity; ↓ ROS generation; ↓ $O_2^{\cdot-}$ generation; ↓ p38MAPK phosphorylation; ↓ NF-kB translocation to nucleus; ↓ NF-kB-mediated transcriptional activity; ↓ NF-kB-mediated gene expression activity of ICAM-1, VCAM-1, E-selectin, MMP-1/-2/-9	[96]
Luteolin	HUVECS	6.25, 12.5, 25 μM for 12h prior to 24h with TNF-α 50 ng/mL	↓ LDH release; ↑ SOD activity; ↑ GSH activity; ↓ ROS generation; ↓ Nox-4 and p22phox mRNA/protein expression, caspase-3/-9, ICAM-1, VCAM-1 expression, nuclear p65 levels and p65, p38 and ERK1/2 phosphorylation; ↑ Bcl-2 expression and IkB-α cytosolic levels	[97]
	Myocardial I/R rat	0.01-10 μg/kg prior to ischemia 0.01-1 μg/kg prior to reperfusion, jugular vein injection	↓ Ischemia- and reperfusion-induced arrhythmias; ↓ LDH expression and NO_x release; ↓ myocardial infarct area; ↓ iNOS mRNA/protein expression; ↓ MDA levels	[101]
	I/R rat	40 μmol/L for 30 min before I/R,	Ameliorates I/R-induced impairment of hemodynamic parameters; ↓ infarct area; ↓ LDH release	[103]

		perfusion		
	Cardiomyocytes in simulated I/R	2, 4, 8, 16 µmol/L	↑ shortening amplitude; ↑ Bcl-2 expression; ↓ Bax expression; ↓ apoptotic cells; ↑ total Akt, PLB expression levels; ↑ p-Akt/p-PLB/SERCA2a expression	
	Cardiomyocytes in simulated I/R	0.5, 1.5, 2.5, 5.0 µg/mL	↓ necrotic cells; ↓ LDH release; ↑ shortening amplitude; ↓ apoptotic cells; ↓ caspase-3/Bax expression; ↑ Bcl-2 expression; ameliorated cardiac systolic/diastolic function and heart rate	[106]
	I/R on STZ-induced diabetic rats	10 µg/kg for 30 min prior to I/R, tail vein injection	↓ LDH release; ↓ Arrhythmic events; ↓ Infarct area; ↑ hemodynamic parameters on left ventricle; ↓ apoptotic cells; ↓ caspase-3; ↑ FGRF2, LIF, Bcl-2 expression and Akt and BAD phosphorylation; ↓ Bax expressin; ↓ MPO activity; ↓ IL-6/IL-1α/TNF-α levels	[104]
	I/R rat model	40 µmol/L	↓ hemodynamic parameters impairment; ↓ infarct area; LDH release; ↓ apoptotic cells	[107]
	Cardiomyocytes in simulated I/R	2, 4, 8, 16 µmol/L	↓ necrotic cells; ↓ LDH release; ↑ shortening amplitude; ↑ p-ERK1/2, Bcl-2, SERCA2a and p-PLB levels; ↓ p-JNK, Bax and p-PP1 levels	

6-OHFlav – 6-hydroxyflavone; 7-OHFlav – 7-hydroxyflavone; Akt – protein kinase B; Api – apigenin; BAD – Bcl2-associated death promoter; Baic – baicalein; CAT – catalase; Chr – chrysin; CK-MB – creatine kinase-MB; DS – Diosmin; EA.hy926 – human umbilical vein cell line; ERK1/2 – extracellular signal-regulated kinase; FGFR2 – fibroblast growth factor receptor 2; Flav – flavone; GPx – glutathione peroxidase; GSH – reduced glutathione; HUVECS - Human Umbilical Vein Endothelial Cells; I/R – ischemia/reperfusion; ICAM-1 – intracellular adhesion molecule-1; IL-6/-1α – interleukin-6/-1α; JNK – c-Jun N-terminal kinase; LDH – lactacte dehydrogenase; LIF – leukemia inhibitory factor; LOOH – peroxide; Lut – luteolin; MAPK – mitogen-activated protein kinase; MDA – malondialdehyde; mitICDG – mitochondrial isocitrate dehydrogenase; mitMDH – mitochondrial malate dehydrogenase; mitSDH – mitochondrial succinate dehydrogenase; mitαKGDH – mitochondrial α-ketoglutarate dehydrogenase; MMP-1/-2/-9 – matrix metalloproteinase-1/-2/-9; MPO – myeloperoxidase; NF-kb – nuclear factor-kappa B; Nox4 – NADPH oxidase-4: oxLDL – oxidized low density lipoprotein; P22phox - human neutrophil cytochrome b light chain, NAD(P)H oxidase essential component

4. CONCLUSION

Flavones are phenyl substituted chromones characterized by the presence of a double bond between 2 and 3 position in the heterocyclic C-ring and the lacking of oxygenation at the 3-position of the same ring. These compounds have been the focus of attention of much research, due to their potential health benefits. Particular emphasis has been given to their antioxidant capacities, which can occur through direct and/ or indirect ways.

Chronic and acute neurological insults, diabetes and atherosclerosis are pathological disorders closely associated with oxidative stress. Indeed, promising results regarding to the protective effects of some flavones have been demonstrated in *in vitro* and *in vivo* models of such diseases. However, further research needs to be done in order to better comprehend the mechanisms underlying these protective effects. Still, the introduction of flavonoids and/or flavones rich foods in our diet, can be the first step to prevent of the development oxidative stress diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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