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Therapeutic Potential of Flavonoids in the Treatment of Diabetic Retinopathy: An Updated Review

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ABSTRACT:

Diabetic retinopathy (DR), one of the most common complications of diabetes, is the leading cause of legal blindness among adults of working age in developed countries. After 20 years of diabetes, almost all patients suffering from type I diabetes mellitus and about 60% of type II diabetics have DR. Several studies have tried to identify drugs and therapies to treat DR though little attention has been given to flavonoids, one type of polyphenols, which can be found in high levels mainly in fruits and vegetables, but also in other foods such as grains, cocoa, green tea or even in red wine. Flavonoids have anti-inflammatory, antioxidant and antiviral effects. Since it is known that diabetes induces oxidative stress and inflammation in the retina leading to neuronal death in the early stages of the disease, the use of these compounds can prove to be beneficial in the prevention or treatment of DR. In this review, we summarize the molecular and cellular effects of flavonoids in the diabetic retinopathy.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both, leading to various serious damaging consequences such as heart attacks, blindness, kidney failure, leg amputation and stroke. Diabetes contributes to several visual impairments such as cataracts, glaucoma and, most importantly, diabetic retinopathy, one of the most common complications of diabetes [1]. Although there are significant differences between various regions of the globe, a recent study calculates the global prevalence of DR at 27% [2]. This means that of the 463 million diabetic adults as of 2019, 125 million have DR. Furthermore, the already disturbing number of diabetic adults is estimated to increase even further, reaching 700 million in the year 2045 [3] in which, assuming the prevalence of DR among diabetic adults is maintained, will originate approximately 189 million cases of DR worldwide. After 20 years of diabetes, nearly all patients with type 1 diabetes (T1D) and 60% of patients with type 2 diabetes (T2D) have diabetic retinopathy [4]. A recent case–control study showcased how DR influences people's quality of life and life satisfaction, using two groups of T2DM patients, one with 70 patients with DR and another group with 70 patients without DR. Patients with DR had significantly worse scores in all scales related to quality of life and life satisfaction compared to patients without DR [5]. Risk factors for DR can be classified in two main categories: non-modifiable and modifiable risk factors. Non-modifiable risk factors associated with DR include duration of diabetes, renal disease [6], puberty and pregnancy [7] as it is estimated that diabetes affects 17% of pregnancies worldwide [8]. Modifiable risk factors associated with DR include obesity, smoking, hyperglycemia, hypertension and dyslipidemia [9]. All these factors contribute to the development of diabetes and ultimately increase the risk of DR. Other eye complications of diabetes, such as cataracts, also might contribute to increase the risk of developing DR. Cataract surgery in diabetic patients who do not have DR increases the risk of developing non-proliferative DR (NPDR) when receiving the surgery [10].

2. Pathogenesis of Diabetic Retinopathy

Retinal stress in DR can be induced by many factors. These include inflammation, oxidative stress, epigenetic mechanisms and neurodegeneration [11].

Inflammation

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The pathogenesis of DR is complex but is mainly triggered by hyperglycaemia (≥150 mg/dl) and subsequent metabolic stress on the retinal cells. Prolonged elevation of blood glucose induces the production of advanced glycation end-products (AGEs). AGEs are principally formed through the Maillard reaction and deposition of AGEs has been shown to have detrimental effects on cells and tissues. The role of AGEs in the pathology of DR can be attributed to the crosslinking effects (with proteins, lipids and nucleic acids) and the binding of AGEs to receptors of AGE (RAGE) that in turn trigger multiple cell-signalling pathways [12]. AGEs and AGE–RAGE interactions are important contributors to pericyte drop-out, endothelial dysfunction and vascular inflammation, leading to the increase in transcription of vascular endothelial growth factor (VEGF), adhesion molecules and proinflammatory cytokines and chemokines such as tumour necrosis factor (TNF)α, interleukin (IL)-6 and IL-1β [13]. In addition, hyperglycaemia is known to be responsible for chronic low-grade inflammation that leads to upregulation of adhesion molecules such as intercellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, as well as the upregulation of proinflammatory cytokine VEGF, which controls angiogenesis, abnormal vascular permeability and inflammation. Consequently, retinal hypoxia and ischemia will proceed and cause loss of pericytes, endothelial dysfunction and retinal mitochondrial degeneration. Hypoxia-activated macrophages and microglia migrate to the hypoxic areas and stimulate the release of hypoxiainducible factor (HIF)-1, monocyte chemotactic protein (MCP)-1, TNFα, IL-6 and IL-8, and further increase the levels of VEGF. The inflammatory state of DR can also be aggravated by modifications in the retinal fatty-acid metabolism via the mitogen-activated protein (MAP) kinase pathway. Oxidised glycated low-density lipoprotein has been demonstrated to cause death of retinal pigment epithelium, Müller cells and pericytes seen in early DR [14].

Oxidative stress

Prolonged hyperglycaemia also results in the overproduction of reactive oxygen species (ROS) that lead to the disruption of normal cellular physiology. Increased ROS levels result in cellular damage via lipid peroxidation, DNA modification, protein misfolding and mitochondrial damage [15]. The increased ROS production can be attributed to increased glucose metabolism by the glycolysis and citric acid cycle pathways. Disturbances in these pathways results in increased production of reduced forms of nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FADH). The reduced forms, NADH and FADH2, are vital in the generation of ATP and superoxide radicals in the mitochondria [16]. This increase in superoxide levels has been demonstrated in *in vivo* and *ex vivo* models of DR. In addition, the activity of enzyme superoxide dismutase (SOD) which is responsible for the conversion of superoxide into oxygen and hydrogen peroxide has been shown to be downregulated in streptozotocin (STZ)-induced diabetic rats [17]. These models also demonstrated an upregulation of pro-oxidant and proapoptotic enzymes that exacerbate this oxidative stress. Moreover, increased oxidative stress has been linked to neurodegeneration in DR [18].

Neurodegeneration

Several studies have demonstrated that dysregulation of excitotoxic metabolites such as glutamate, homocysteine, endogenous peptides and neurotrophic factors is closely linked to the neurodegeneration seen in early DR [19]. Glutamate is the most important excitatory neurotransmitter in the brain and retina and numerous studies have shown that disparity in glutamate homeostasis is associated with neuronal damage and initiation of DR. Owing to the activation of the *N*-methyl D-aspartate (NMDA) receptors by glutamate, there is an influx of calcium and sodium within the cells leading to the generation of free radicals and death of retinal ganglion cells (RGCs) [20]. Müller cells are specialised glial cells in the inner retina and control the intra- and extra-cellular uptake of excess glutamate. They aid in lowering postsynaptic excitotoxicity seen in progressing DR. An imbalance between the influx of calcium, sodium and the amount of glutamate produced induces stress on the Müller cells; leading to their death. Another contributor to retinal neurodegeneration seen in DR is the elevated levels of homocysteine (Hcys). Hcys is a sulfur-containing amino acid that depends on vitamin B12 and folate for efficient degradation. Vitamin B12 deficiency and hyperhomocysteinemia have been linked to early DR [21]. Hcys acts as an agonist at the glutamate site of the NMDA receptors and induces RGC apoptosis, reduces rod responses and can affect glial cell viability. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and pigmentepithelium-

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derived growth factor (PEDF) play important parts in maintaining healthy interactions between neuronal, vascular and retinal cells, preventing ischemia and neuronal death. BDNF is secreted by glial cells and, in DR, absence of BDNF is associated with RGC loss. Other studies show neuroprotective effects of BDNF via the upregulation of glutamine synthetase in Müller cells [22]. In addition, overexpression of PEDF in Müller cells counteracts the effects of VEGF, reduces neovascularisation and aids in the survival of RGCs, axons and neurons. Thus, strategies that can increase the levels of neurotrophic factors could assist in managing the early stages of DR [23].

Epigenetics

In the past decade, epigenetic processes have been reported to mediate various pathological events that occur in DR. Global DNA hypermethylation patterns have been associated with increasing severity of DR and low levels of intermediates for DNA methylation (e.g., folic acid and vitamin B12) increase the risk of this ocular disease [24]. The methylation of histones can also play a part in the impaired redox pathways seen in DR. Glutathione (GSH) is an important intracellular antioxidant and its production is mediated by the activity of the glutamate-cysteine ligase catalytic subunit (Gclc) and the transcription factor nuclear factor erythroid 2 like 2 (Nrf2). Changes in histone methylation can alter the Nrf2-GclcGSH cascade [25]. Dimethylation of histone H3 at lysine 4 (H3K4me2) at the Gclc-antioxidant response element region (ARE) 4 is increased in retinal endothelial cells in a diabetic rat model, whereas H3K4me1 and H3K4me3 are decreased. RNA interference of histone demethylase LSD1 reverses the decreased binding of Nrf2 at Gclc–ARE4 and Gclc transcripts, thus restoring the normal activity of these key antioxidant enzymes [26]. In addition, retinal cells from hyperglycaemic diabetic rats that have been transfected with short-interfering RNA (siRNA) of the methyltransferase enzyme SetD7 promote an upregulation of Nrf2 and downregulate its cytoplasmic repressor Keap1 [27]. However, reversal of hyperglycaemia does not affect the methylation of *Nrf2* and *Keap1* genes. This indicates that epigenetic modifications might not reverse the expression of genes once the DR phenotype begins to manifest. Hypoxic conditions in the retina account for the increased expression of VEGF which promotes neovascularisation in DR. Pisani *et al.* (2018) revealed that the astrocyte marker glial water channel aquaporin (AQP) 4 can regulate the methylation status of the HIF-1 binding site. In the absence of AQP4, demethylation of the HIF-1 binding site becomes impaired, HIF-1 binding to the VEGF gene promoter is prevented and VEGF-induced retinal damage as a result of hypoxia is attenuated [28]. Hypomethylation of the IL-17 receptor C gene promoter resulting in the overexpression of its gene in choroidal blood vessels and retinal pigmented epithelial (RPE) cells was also found under hypoxic conditions. Thus, this gene can be a biomarker of choroidal neovascularisation and RPE cell degeneration. In 2015, a genome-wide analysis of DNA methylation in type 1 diabetes patients revealed an association between epigenetic changes with proliferative DR. A total of 349 differentially methylated phosphorylated cytosine-guanine (CpG) sequences within genes including TNF, chitinase-3-like protein 1, glycine receptor subunit alpha-1, B cell lymphoma (BCL)-6 corepressor-like protein 1 and glutathione peroxidase 1 were detected in patients with DR compared with normal healthy controls. These genes encode proteins that are involved in cell signalling, metabolism and redox reactions and most of these CpG sites experienced DNA hypomethylation. Another interesting observation was the significant positive correlation between natural-killer-cell-mediated cytotoxicity and these CpG sites; thus suggesting the influence of DNA methylation status on innate immunity. Sirtuin (Sirt)1 is a deacetylase enzyme that mediates multiple cellular functions including gene transcription and it is inhibited in the diabetic retina. Mishra *et al.* (2018) investigated Sirt1-overexpressing diabetic mice and these animals showed an alleviation of hypermethylated Sirt1 promoter DNA but no changes in the retinal vasculature or degree of apoptosis were observed compared with wild-type diabetic mice [29]. By contrast, Sirt1 expression mediated by protein arginine methyltransferase 1 conferred protection to human RPE cells against oxidative damage and apoptosis. A normal Sirt1 phenotype is also required to impede increases in p300, endothelin-1 and transforming growth factor (TGF)-β1 expression for the prevention of hyperglycaemic stress in the kidneys and retina of Sirt1-overexpressing mice. These conflicting findings suggest that there could be differences in the responses to epigenetic stimulations between *in vivo* and *in vitro* DR models. Another Sirt family member, Sirt6, has also been implicated in the regulation of cellular aging, metabolism and

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degeneration [30]. In the presence of high concentrations of glucose, retinal cells from normal wildtype rats demonstrated an increase in VEGF gene expression that was accompanied by reduced Sirt6 activity and increased H3K9 and H3K56 acetylation. When Sirt6 was silenced, levels of VEGF gene expression increased. The overexpression of the Sirt3 gene was also found to attenuate diabetic injury via deacetylation and activation of manganese SOD, the scavenging enzyme crucial for eliminating the build-up of ROS in the mitochondria of retina cells. Additionally, concurrent elevation of the levels of matrix metalloproteinase (MMP)-9, VEGF, HIF-1α and insulin growth factor-1 were also observed. Taken together, these findings indicate that normal expression of Sirt family members is required for the maintenance of healthy retinal vascular and neuronal homeostasis [31].

3. The Importance of Nutraceuticals

Despite numerous studies that have sought to identify possible drugs for the prevention and treatment of DR, little attention has been given to nutraceuticals. Nutraceuticals are natural functional foods that promote various health benefits, which include a wide variety of compounds such as vitamins, antioxidants, minerals, fatty acids and amino acids that can prevent or delay the progression of certain diseases. Various studies have shown that nutraceuticals promote multiple therapeutic benefits and provide protection against various diseases. In diabetes, the use of nutraceuticals contributes to improve insulin sensitivity, metabolism regulation and lower hyperglycemia [32]. Molecules such as flavonoids and carotenoids have been proven to have significant antioxidant and anti-inflammatory effects. In many animal models and humans studies, it has been shown that flavonoids, a large family of compounds that are extracted from plants, can prevent or attenuate complications associated with DR as they can modulate lipid and carbohydrate metabolism and insulin resistance, mitigate hyperglycemia and suppress oxidative stress and inflammatory processes [33]. Red fruits are among the most sought-after by consumers with health concerns. These fruits, such as raspberry (*Rubus idaeus*), blueberry (*Vaccinium corymbosum*), strawberry (*Fragaria ananassa*), blackberry (*Rubus ulmifolius*) and cranberry (*Vaccinium macrocarpon*), are rich in dietary fiber and organic acids, low in fat and calories and contain high amounts of antioxidant molecules, such as polyphenols, that prevent the fruit from oxidation against environmental factors, such as light, oxygen and microbiological contamination [34]. Polyphenols have various important biological properties such as anti-viral, anti-bacterial, anti-inflammatory, anti-cancer and, most importantly, antioxidant, which promote the capacity of radical scavenging that is based on the ability to turn free radicals into more stable ones, or by reducing the production of ROS caused by intracellular mechanisms in the mitochondria [35]. This capability is the main antioxidant defense system as intracellular antioxidants neutralize the damaging effect of free radicals preserving the cellular redox homeostasis. Due to these effects, polyphenols have also been explored to create cosmetics, creams, nutraceuticals and dietary supplements which also have proven benefits to human health.

4. Diabetic Retinopathy and the Benefits of Flavonoids

Polyphenols, chemically characterized as compounds with phenolic structural features, constitute one of the most numerous and widely distributed groups of natural products in the plant kingdom. This group of natural products is highly diverse and contains several sub-groups of phenolic compounds. Flavonoids, one polyphenol sub-group that accounts for about 60% of all polyphenols [36], can be found in fruits and vegetables with specific biological characteristics that include anti-inflammatory, antiviral and antioxidant effects. Flavonoids can be categorized in six classes according to their chemical structure, namely anthocyanins, flavanols, flavanones, flavones, flavonols and isoflavones [37] (Figure 1). Flavonoids can modulate carbohydrate and lipid metabolism, improve insulin resistance, attenuate hyperglycemia, improve β-cell function and improve the management of inflammatory processes, which could help to prevent the development of long-term chronic diabetic complications, such as diabetic retinopathy [38].

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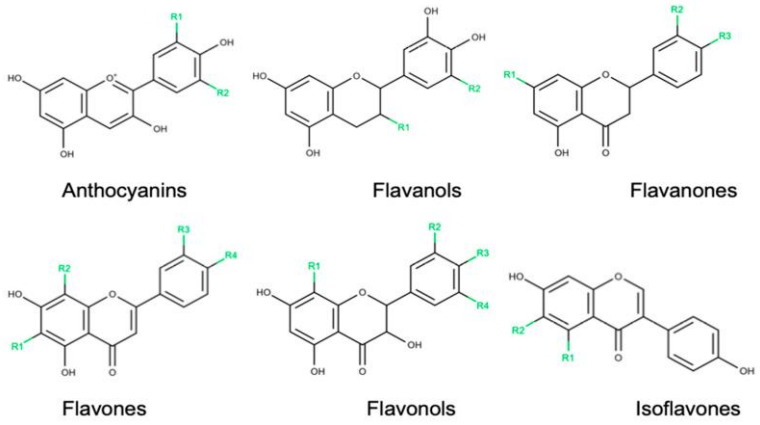

Fig.1. Chemical structures of flavonoid subclasses.

Anthocyanins

Anthocyanins are natural pigments that provide blue, red and purple colors in flowers, fruits and other plant structures. This flavonoid subclass is present in various fruits, being in high concentrations in berries and cherries [39]. Besides being used as colorants, anthocyanins have various beneficial effects on DR. A study performed in human retinal capillary endothelial cells (HRCECs) showed that blueberry anthocyanin extract (BAE) and its predominant constituents, malvidin (Mv), malvidin-3-glucoside (Mv-3-glc) and malvidin-3-galactoside (Mv-3-gal), prevented the high glucose-induced injury in HRCEs. It was observed that a reduction of the inflammatory and oxidative environment, caused by a decrease in pro-oxidant enzymes endothelial nitric oxide synthase (eNOS) and NADPH oxidase 4 (Nox4), increased antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), and inhibition of the intercellular adhesion molecule-1 (ICAM-1) and NF-κB pathway. Additionally, angiogenesis was compromised by a decrease in the VEGF level and inhibition of the protein kinase B (Akt) pathway [40]. The protective effects of BAE were also evaluated in vivo. The anthocyanins were administered orally in streptozotocin (STZ)-induced T1DM rats. As observed in vitro, ROS levels lowered, antioxidant enzymes glutathione (GSH) and glutathione peroxidase (GPx) increased, and VEGF levels diminished. Additionally, malondialdehyde (MDA) and IL-1β levels decreased. These results show that BAE has anti-inflammatory and antioxidant effects. Since BAE augmented nuclear factor erythroid 2-related factor-2 (Nrf2) and HO-1 mRNA levels and the nuclear location of Nrf2 and HO-1 protein levels, it was suggested that the oxidative stress and inflammation could be regulated by the Nrf2/HO-1 pathway [41]. Kim and colleagues showed that bilberries can prevent or delay the onset of DR by preventing BRB disruption. STZ-induced T1DM rats, treated with *Vaccinium myrtillus* extract (VME) that contains 15 different anthocyanins, exhibit less VEGF expression and more tight junction proteins (zonula occludens-1, occludin and claudin-5) in the retina. Furthermore, treated rats had less fluorescein leakage. These results show that BRB breakdown was inhibited by VME [42].

Flavanols

Flavanols, also referred to as flavan-3-ols or catechins, are present in high concentrations in cocoa, grapes, tea and red wine. Advanced glycation end products (AGEs) are direct contributors in the initiation and progression of diabetic retinopathy [43]. (-)—Epicatechin may improve retinal vascular cell injuries by reducing the AGEs burden in vitro and in vivo. Glycated human serum albumin isolated from diabetic patients incubated with (-)—epicatechin presented higher AGE breaking activity. This was also verified in normoglycemic rats injected with AGE-modified rat serum albumin. Additionally, the AGE burden lowered, as well as vascular apoptosis [44]. Skopinski and colleagues showed that plant-derived flavanols (epigallocatechin (EGC) and epigallocatechin gallate (EGCG)) inhibited the angiogenic effects of sera obtained from type 2 diabetic patients with non-proliferative retinopathy in Balb/c mice [45]. Green tea (*Camellia sinensis*) is characterized by its high flavonoid content (20–30% of the dry weight). Green tea exerts protective effects against glutamate toxicity in the diabetic retina. Silva and colleagues have shown that oral administration of green tea reduced some retinal complications of diabetes in two animal models. The increase in the expression of glial fibrillary acidic

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protein (GFAP), oxidative retinal markers and glutamine synthetase levels was prevented by green tea. In addition, the decrease in occludin, NMDAr1 subunit and GLAST-1 (GLutamate ASpartate Transporter 1) verified in diabetic animals was also reduced in green tea-treated animals. Diabetic spontaneously hypertensive rats (SHR) also exhibit BRB breakdown and impaired electroretinography recordings. Müller cells exposed to high-glucose medium produced higher levels of ROS and glutamine synthetase, but reduced levels of GSH, glutamate transporter and glutamate receptor [46]. Similarly, ARPE-19 cells exhibited increased ROS production accompanied by decreased expression of claudin-1 and glutamate transporter. Treatment with green tea fully restored all the above-mentioned alterations in diabetic animals as well as in retinal cells. Additionally, green tea also decreased superoxide production, acellular capillaries and pericyte ghosts in vivo [47]. Metalloproteinase-9 (MMP-9) promotes neovascularization and vascular permeability present in late DR. EGCG, green tea's most active compound, inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced MMP-9 and TNF-α mRNA and protein expression levels in human retinal pigment epithelial cells (HRPECs). EGCG exerted antiapoptotic effects by decreasing ROS levels and attenuating mRNA expression of MMP-9, VEGF and VEGF Receptor-2 in ARPE-19 cells. Furthermore, HRPECs exposed to VEGF and EGCG presented less proliferation, vascular permeability and tube formation. In vivo, EGCG reduced VEGF-induced vascular leakage and permeability [48]. STZ-induced T1DM rats treated with catechin presented diminished pro-inflammatory cytokines levels (IL-1β, IL-6 and TNF-α) as well as downregulated NF-κB. These results show that catechin exerts anti-inflammatory effects [49].

Flavanones

Flavanones are abundant in fruits and fruit juices of the *Citrus* genus such as oranges, bergamots, lemons and grapefruit [50]. Naringenin presents antioxidant, neuroprotective and anti-apoptotic effects in the diabetic retina. It ameliorated the oxidative stress by lowering GSH levels and thiobarbituric acid reactive substances (TBARs) in STZ-induced T1DM rats. Furthermore, brain-derived neurotrophic factor (BDNF), tropomyosin related kinase B (TrkB) and synaptophysin were augmented, preventing neurodegeneration. Naringenin also improved the level of apoptosis-regulatory enzymes by decreasing Bax and caspase-3 and increasing Bcl-2 levels [51]. Eriodictyol exerts antioxidant, anti-inflammatory and antiapoptotic effects via the Nrf2/HO-1 pathway. This flavanone attenuated retinal inflammation by diminishing TNF-α, ICAM-1, VEGF and eNOS levels in STZ-induced T1DM rats. By decreasing these factors, eriodictyol prevented BRB breakdown. Moreover, eriodictyol inhibited plasma lipid peroxidation, a feature induced by ROS [52]. In vitro, eriodictyol also ameliorated the oxidative stress by lowering ROS levels and augmenting SOD, GPx and CAT activity. Additionally, eriodictyol enhanced cell viability, heme-oxygenase-1 expression and Nrf2 nuclear translocation, an important regulator of oxidative stress. Hesperitin presents vasoprotective and antioxidant effects in the diabetic retina. PKC-β is an important mediator in the VEGF pathway, being involved in vascular tissues anomalies. This flavanone diminished PKC-β and VEGF expression in STZ-induced T1DM rats. Hesperitin also reduced vascular permeability, leakage and dilation of the vessels, and reduced vascular basement membrane (BM) thickness [53]. Additionally, hesperitin restored GSH levels, SOD and CAT activities. Pro-inflammatory cytokines levels (TNF-α and IL-1β) decreased, as well as caspase-3 and GFAP expression. Aquaporin-4 (AQP4) is highly expressed in Müller cells and astrocytes, being associated with neuronal and glial swelling when overexpressed. This overexpression observed in diabetic retinas was reduced by hesperitin [54]. Photoreceptors are responsible for converting light stimuli into electrical stimuli for visual processing. Accordingly, if there is degeneration of these sensory neurons, it will lead to visual loss. Light and transmission electron microscopic studies showed that hesperitin reduced photoreceptors cell death. It was also observed that edematous Müller cells' feet and BM thickness were diminished [55]. Hesperidin suppresses BRB injuries and prevents the reduction in retina thickness induced by diabetes in an STZ-induced T1DM rat model. Hyperglycemia is involved in the development of DR via increasing aldose reductase activity. Hesperidin reduced blood glucose levels, aldose reductase activity, ICAM-1, IL-1β, TNF-α, VEGF and AGEs. Additionally, MDA levels were significantly reduced, and SOD activity increased, improving the oxidative state [56]. This was also observed in vitro by the downregulation of ROS, MDA

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and protein carbonyl levels, and the increase in SOD, CAT, GPx and GSH levels. High glucose-exposed retinal ganglion cell 5 (RGC-5) and ARPE-19 cells treated with hesperidin had less cell loss and restored mitochondrial function, including an increase in the mitochondrial membrane potential and inhibition of cytochrome c release, preventing programmed cell death. Additionally, this flavanone inhibited cell apoptosis via downregulating caspase-9, caspase-3, caspase-2 and the Bax/Bcl-2 ratio. Lastly, the phosphorylation of c-Jun N-terminal kinase (JNK) and p38 MAPK was diminished, protecting retinal cells against ROS injury and cellular death [57].

Flavones

Flavones are extensively distributed in cereal grains, reported to also be in herbs. 12/15-lipoxygenase is linked to the development of microvascular dysfunction during DR. Othman and colleagues suggested that baicalein had anti-inflammatory, antioxidant and anti-hyperpermeability effects in diabetic mouse retinas, acting as a 12/15-lipoxygenase (12/15-LOX) inhibitor. Mice treated with baicalein had reduced ICAM1, VCAM-1, IL-18, TNF-α, IL-1β and IL-6 expression levels, reduced GFAP and VEGF expression in Müller cells and diminished vascular abnormality and ganglion cells loss in the retina [58]. The treatment with this flavone also prevented the increase and the activation of microglia, restored zonula occludens-1 (ZO-1) and protein tyrosine phosphatase pSHP1 levels. Protein tyrosine phosphatase decrease has a role in maintaining endothelial barrier function, potentially by inhibiting the VEGF/VEGF-R2 pathway. Additionally, baicalein reduced pVEGF-R2 levels and significantly decreased 12- and 15-hydroxyeicosatetreanoic acids, ROS generation and NADPH oxidase 2 (NOX2) expression in diabetic retina [59]. Baicalin reduced cell death and apoptosis, inhibited the release of IL-1β, IL-6 and IL-8 and diminished ROS levels, ameliorating the inflammatory and oxidative states of ARPE-19 and human retinal microvascular endothelial cells (HRMEC) exposed to high glucose. MicroRNA (miRNAs) constitute a class of small non-coding RNA that regulate the expression of genes at the post-transcriptional level by binding to target sites directly or promoting mRNA degradation [60]. miR-145 is one of various miRNAs that are involved in DR, and several works have shown that miR-145 levels are altered in diabetic retinas and in cellular models of DR. Treatment with baicalin increased the levels of miR-145 and consequently inhibited the NF-κB and p38MAPK pathways which are linked to a greater permeability of the blood vessels, one of the abnormalities observed in DR [61]. Mei and colleagues showed that scutellarin alleviates BRB breakdown in animal and cellular models of DR. This flavone diminished NF-κB and TNF-α expression in the BV-2 cell line exposed to high glucose. TNF-α-exposed HRECs and ARPE-19 cells treated with scutellarin augmented claudin-1 and claudin-19 expression, ROS formation and Nrf2 nuclear accumulation. These results were also observed in STZ-induced T1DM rats. Both in vivo and in vitro, this flavone reduced microglia cell activation and phosphorylation of ERK1/2 [62]. Scutellarin also decreased cell viability and VEGF levels via reducing hypoxia-inducible factor 1 (HIF-1α) mRNA and protein levels in HRECs exposed to high glucose and hypoxia-mimetic agent. Additionally, scutellarin diminished the activity of NADPH oxidase, the principal source of ROS [63].

Nepetin decreased production and expression of IL-6, IL-8 and MCP-1 in IL-1β-treated ARPE-19 cells. The nuclear translocation of NF-κB p65 subunit was lowered via suppressing the phosphorylation of inhibitor of nuclear factor kappa B (IκB) and IκB kinase (IKK). Moreover, nepetin decreased the phosphorylation of ERK1/2, JNK and p38 MAPK induced by IL-1β. These results show that nepetin ameliorated the inflammatory responses via suppressing the NF-κB and MAPKs pathways [64]. Silybin diminishes obliterated retinal capillaries, a hallmark of early morphological pathology in DR. Additionally, silybin significantly reduced retinal vascular leukocyte adhesion (leukostasis) and the ICAM-1 level in STZ and high-fat diet-induced T2DM rats [65]. Chrysin exerts protective effects in diabetes-associated visual cycle impairment. Chrysin ameliorated glucose-induced neovascularization by diminishing VEGF and IGF-1 levels and restoring pigment epithelium-derived factor (PEDF) levels in RPE cells. Chrysin-treated db/db mice restored the ONL thickness and augmented visual cycle-related enzymes levels [66]. Chrysin decreased AGE production and RAGE induction in glucose-treated RPE cells and increased PEDF, RPE65, LRAT and RDH5 in AGE-BSA-exposed RPE cells. In glucose-exposed RPE cells and mice, chrysin inhibited endoplasmic reticulum (ER) stress sensor proteins

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IRE1α and ATF6, preventing ER stress-mediated loss of visual cycle proteins via the AGE/RAGE pathway [67].

Diosmin, a flavone glycoside (30,5,7-trihydroxy-40-methoxyflavone-7-rhamnoglucoside) found in citrus fruits, is a venoactive and a vascular protector. It is the main component of Daflon, a dietary nutraceutical, and is used in the treatment of symptoms and signs related to venous insufficiency (heavy legs, pain, tiredness, edema) and in the symptomatic treatment of hemorrhoidal crisis [68]. The administration of diosmin in an animal model of ischemic/reperfusion showed protective effects in the BRB, avoiding the increase in its permeability, reducing the levels of VEGF and relieving edema. It was also observed that diosmin also exerted a protective effect in the neural component of the retina, preventing the reduction in the total thickness of the retina, avoiding the reduction in the number of ganglion cells and the changes in the a- and b-waves of the electroretinograms induced by the I/R. In this same model, it was also observed that the administration of diosmin reduced the levels of MDA and increased the activity of the antioxidant enzymes SOD, CAT and GSH-Px in the retina [69]. Similar results were observed in ARPE-19 cells exposed to high glucose. In this cell line, diosmin increased cell viability, through the decrease in apoptosis, and ameliorated the decrease in SOD and GSH-Px enzymatic activities, with a consequent reduction in the ROS levels [70].

Flavonols

Flavonols are usually present in a variety of vegetables, fruits, tea and wine. Under high-glucose conditions, HRECs presented elevated VEGF and placenta growth factor (PGF) mRNA and protein levels. Kaempferol incubation suppressed the increase in both angiogenic factors, therefore inhibiting angiogenesis. Moreover, kaempferol inhibited high glucose-induced expression of PI3K and the phosphorylation of specific kinases (Src, Akt1 and Erk1/2), suggesting that this flavonol exerts the anti-angiogenic effects by inhibition of the Src-Akt1-Erk1/2 signaling pathway [71]. α-Glucosidase and α-amylase are enzymes in the digestive system that hydrolyze dietary carbohydrates and produce absorbable glucose. The degradation of dietary starch leads to postprandial hyperglycemia in patients with diabetes. Kaempferol inhibits these key enzymes and, through this mechanism, can reduce and control postprandial blood glucose spike, which is an effective approach to alleviate and treat T2DM [72]. Quercetin has a protective action against injuries caused by diabetes in the retina. Quercetin attenuated high glucose-induced apoptosis and inflammation by lowering ROS levels, pro-inflammatory molecules, MCP-1 and IL-6. It has, recently, been suggested that miR-29b may be beneficial in the treatment of DR, as it has antiangiogenic effects on diabetic retinas through inhibition of retinal microvascular endothelial cells proliferation, migration and angiogenesis [73]. miR-29b expression was higher in ARPE-19-treated cells, being noticed that quercetin protective effects were lower when miR-29b was suppressed. Moreover, PTEN/Akt pathway activation was promoted and the NF-κB pathway was inhibited via a miR-29b-dependent way. In vivo, quercetin also inhibited NF-κB expression and caspase-3, presenting antiapoptotic effects. This flavonol downregulates proteins that play an important role in neovascularization, namely MMP-9 and VEGF. Quercetin augmented antioxidant enzymes (GSH, SOD and CAT) and diminished pro-inflammatory cytokines (TNF-α and IL-1β). Additionally, quercetin lowered GFAP and AQP4 expression [74].

5. Clinical studies

Very few studies have been conducted to understand how nutraceuticals can improve diabetes and DR. A clinical trial with 10,054 participants, of which 546 were addressed to measure the risk of developing diabetes, demonstrated that higher intake of quercetin and myricetin for one year decreases the risk of developing T2DM [75]. A meta-analysis of randomized controlled clinical trials evaluated the impact that green tea catechins, with or without caffeine, can have on glycemic control markers in 1584 subjects. Results showed that the administration of these substances could significantly reduce fasting blood glucose, although there were no verified significant differences in fasting blood insulin, glycated hemoglobin (HbA1c) and homeostatic model assessment of insulin resistance (HOMA-IR) [76]. A randomized controlled trial assessed the effects of an oral combination of flavonoids, *Centella asiatica* and *Melilotus* for the treatment of diabetic cystoid macular edema without macular thickness in 70 type 2 diabetic patients. Results demonstrated that retinal sensitivity was preserved in treated patients when compared to the untreated group, although no significant differences in visual acuity, stability fixation, mean central retinal

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thickness, HbA1c percentage, microalbuminuria and blood pressure were observed [77]. Another study, which evaluated the effects of purified anthocyanins in 160 patients for 12 weeks, exhibited that the supplementation of anthocyanins promoted an increase in serum adiponectin and a decrease in fasting glucose in newly diagnosed diabetics. Mahoney et al. conducted a study that used information of 381 diabetic people from the National Health and Nutrition Examination Survey (NHANES) between 2003 and 2006 to evaluate if a flavonoid-rich diet impacts DR and diabetes-related biomarkers. The results demonstrated that participants with a high intake of flavonoids diet lowered the risk of developing DR by 30%. Additionally, they also presented lower C-reactive protein, HbA1C and glucose levels [78]. A clinic-based case–control study revealed that people who regularly drink green tea for at least a year presented a 50% lower risk of developing DR than those who do not drink green tea. A multi-center field study that assessed Pycnogenolfi's effect on the progression of visual acuity in patients with T1DM and T2DM who had DR, in which 1169 people were treated with Pycnogenolfi for six months, demonstrated that Pycnogenolfi prevented the progression of visual loss. However, there were no significant improvements in patients' sight [79]. Another study demonstrated that people treated with antioxidant supplementation containing Pycnogenolfi for six months presented lower ROS levels and central macular thickness. Additionally, Steigerwalt et al. evaluated the effects of Pycnogenolfi in the early stages of DR. Results exhibited that people treated with Pycnogenolfi for two months presented visual and baseline improvement [80]. Although some of these clinical trials show positive effects in diabetic patients, difficulties arise in analyzing the clinical trial's results since they are variable and, in some cases, even controversial. Many of these studies focus on T2DM patient populations and some clinical trials deal with patients that are in different stages of the disease, making it more difficult to correlate with the accuracy of the results [81].

6. Conclusions

Flavonoids can provide an effective and safe alternative to conventional drugs and therapies that are used to prevent and treat DR, one of the major complications of diabetes. As shown by several studies performed in vivo, using animal models of diabetes, and in vitro, using different cell cultures, flavonoids can prevent the disruption of the BRB, decrease the release of proinflammatory mediators, improve the oxidative state and prevent the reduction in retina thickness by attenuating apoptosis and neurodegeneration. These may contribute to the beneficial effects of the consumption of flavonoids observed in clinical studies. Despite the small number, these studies have shown that consumption of flavonoids, in the diet or through supplements, exerts beneficial effects at several stages: preventing the onset of diabetes, the development of DR in diabetics and, in diabetics with DR, flavonoids prevent the worsening of DR. However, nutraceuticals should be used as supplements to a healthy and balanced diet and not as a magic bullet that in itself prevents all the complications of diabetes. In conclusion, the data presented in this review strongly suggest that dietary supplementation with flavonoids or with flavonoids-rich nutraceuticals may be an effective, economical and safe way to prevent or limit the progression of DR and the concomitant visual impairments, thus improving the quality of life of millions of diabetics.

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