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Review

Potential of the bioflavonoids in the prevention/treatment of ocular disorders

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Abstract

Objectives Flavonoids are a common group of plant polyphenols that give colour and flavour to fruits and vegetables. In recent years, flavonoids have gained importance in the pharmaceutical field through their beneficial effects on human health and are widely available as nutritional supplements. Several pharmacological actions of the bioflavonoids may be useful in the prevention or treatment of ocular diseases responsible for vision loss such as diabetic retinopathy, macular degeneration and cataract. This review aims to summarize the potential therapeutic applications of various bioflavonoids in different ocular diseases and also discusses delivery of these agents to the ocular tissues.

Key findings It is apparent that the flavonoids are capable of acting on various mechanisms or aetiological factors responsible for the development of different sight threatening ocular diseases. From a drug delivery perspective, ocular bioavailability depends on the physicochemical and biopharmaceutical characteristics of the selected flavonoids and very importantly the route of administration.

Summary The potential therapeutic applications of various bioflavonoids in ocular diseases is reviewed and the delivery of these agents to the ocular tissues is discussed. Whereas oral administration of bioflavonoids may demonstrate some pharmacological activity in the outer sections of the posterior ocular segment, protection of the retinal ganglionic cells *in vivo* may be limited by this delivery route. Systemic or local administration of these agents may yield much higher and effective concentrations of the parent bioflavonoids in the ocular tissues and at much lower doses.

Keywords anti-angiogenic; anti-inflammatory; antioxidant; flavonoids; ocular drug delivery

Introduction

The eye, the organ of sight, has a very unique structural and biochemical organization. Age and certain disease conditions, however, can affect the function of this vital organ. A report from the World Health Organization (WHO) in 2002 estimated that approximately 161 million people were suffering from visual impairment worldwide, out of which 37 million face blindness and 124 million suffer from low vision. Age seems to be a causative factor in blindness as 82% of the population with blindness are aged above 50 years.[1] In the USA, in 2002, among civilian non-institutionalized adults, 19.1 million people were suffering from visual impairment including 0.3% with blindness.[2] Although blindness is necessarily associated with ageing, cataract is still the leading cause of blindness followed by refractive error, glaucoma, age-related macular degeneration, trachoma, childhood blindness and diabetic retinopathy (Figure 1).[1,3]

Over the last century, flavonoids or bioflavonoids have been identified as the most common group of plant polyphenols that give colour and flavour to fruits and vegetables. They are secondary metabolites in plants and their biosynthesis takes place via the shikimate and arogenate pathways.[4] In plants, flavonoids occur as the glycosides (with attached sugar) or occasionally as the aglycones. Initially they were considered as vitamins and the term 'vitamin P' was coined to describe them; however, subsequently this term was discontinued. Flavonoids play an important role in plant physiology, including pigmentation, flavour, growth and reproduction. Furthermore, these molecules also provide resistance against pathogens.[5]

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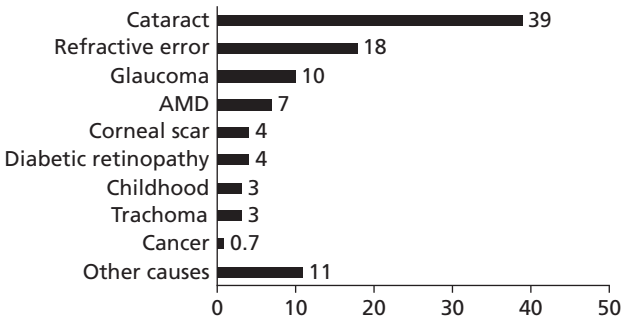

Figure 1 Proportion (percentage) of cases of blindness by major ocular diseases. Courtesy of World Health Organization.[1] AMD, age-related macular degeneration.

The common structural feature of the flavonoids is the flavone nucleus (2-phenyl chromone or 2-phenyl benzopyrone), characterized by a C_6-C_3-C_6 carbon skeleton with the C_6 component being aromatic in nature (Figure 2). This basic skeleton may contain numerous substituent groups: (1) hydroxyl groups, generally present at the 4′, 5 and 7 positions; (2) sugars, generally linked with the hydroxyl group positioned at 7; and (3) methyl and isopentyl units. Hydroxyl groups and sugars impart hydrophilicity, while methyl groups and isopentyl units impart lipophilicity to the flavonoids. Until now, more than 8000 polyphenolic compounds have been identified and these flavonoids can be classified into different subclasses, which include flavones, flavonols, flavanones, flavanols, anthocyanins and isoflavones (Figure 2, Table 1).[6]

Flavonoids have gained prominence in the pharmaceutical arena by virtue of their therapeutically beneficial properties. Bioflavonoids possess antioxidant, anti-angiogenic or anti-inflammatory activity and are also capable of reducing fluid retention and strengthening capillary walls. Interestingly, the aetiology of most ocular diseases involves free-radical-mediated oxidative damage, hypoxia, decreased blood supply to ocular tissues and, in certain conditions, angiogenesis, increased vascular permeability and leakage of vascular contents.[7,8] Thus, select bioflavonoids may be effective in the prevention or treatment of ocular diseases (e.g. diabetic retinopathy, macular degeneration and cataract) that lead to vision loss if left untreated.

This review summarizes different factors associated with the initiation and progression of some of these conditions, the potential therapeutic role of the flavonoids and ocular drug delivery aspects.

Ocular diseases/disorders

The following section briefly describes the pathophysiology of various ocular diseases and pathways that can be targeted by the flavonoids.

Cataract

Multiple mechanisms have been proposed with respect to the development of a cataractous lens such as non-enzymatic glycation, oxidative stress and the polyol pathway.[7,9,10] There is a body of evidence which suggests that H_2O_2 and hydroxyl radicals, the most reactive and damaging free radicals, contribute to cataract formation. When compared with normal eyes, significantly higher amounts of these free radicals were found in the cataractous lens and in the aqueous humour. Additionally, glutathione reductase activity was found to be inversely proportional to the severity of cataract formation.[7] In the case of the polyol pathway, when hyperglycaemia occurs, glucose is converted to sorbitol by aldose reductase. The sorbitol thus produced does not cross cell membranes easily and accumulates in the cells, causing a disturbance in homoeostasis. Intralenticular accumulation of

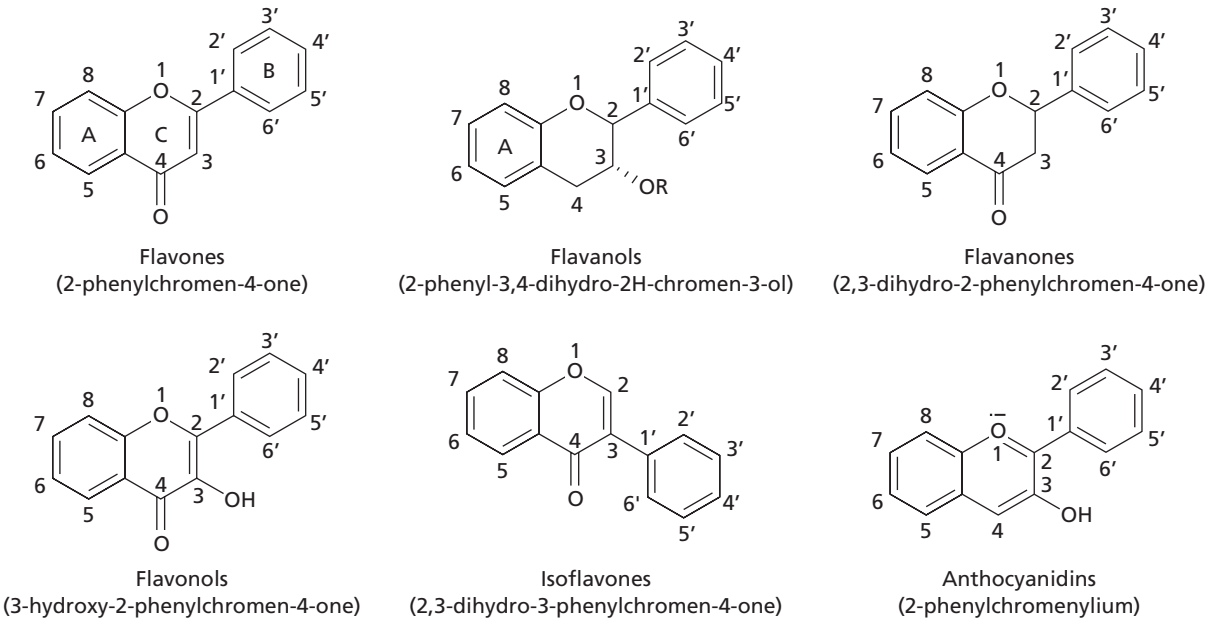

Figure 2 Basic chemical structures of different flavonoid classes.

Table 1 Major flavonoid classes: examples under each category with their substituent groups

Flavonoid class	Example	Substituent groups						
		3	5	7	3′	4′	5′	Others
Flavones	Apigenin	H	OH	OH	H	OH	H	
	Luteolin	H	OH	OH	OH	OH	H	
	Chrysin	H	OH	OH	H	H	H	
	Baicalein	H	OH	OH	H	H	H	6 = OH
	Nobiletin	H	OCH_3	OCH_3	OCH_3	OCH_3	H	6, 8 = OCH_3
	Wogonin	H	OH	OH	H	H	H	8 = OCH_3
Flavanols	Epicatechin	H	OH	OH	OH	OH	H	R = H
	ECG	H	OH	OH	OH	OH	H	R = Gallate
	EGCG	H	OH	OH	OH	OH	OH	R = Gallate
Flavanones	Naringenin	H	OH	OH	H	OH	H	
	Naringin	H	OH	O-Rha	H	OH	H	
	Taxifolin	OH	OH	OH	OH	OH	H	
	Eriodictyol	H	OH	OH	OH	OH	H	
	Diosmin	–	OH	O-Rha	OH	OCH_3	H	
	Hesperetin	H	OH	OH	OH	OCH_3	H	
	Hesperidin	H	OH	O-Rha	OH	OCH_3	H	
	Linarin	–	OH	O-Rha	H	OCH_3	H	
	Isorhoifolin	–	OH	O-Rha	H	OH	H	
Flavonols	Kaempferol	OH	OH	OH	H	OH	H	
	Galangin	OH	OH	OH	H	H	H	
	Morin	OH	OH	OH	H	OH	H	
	Myricetin	OH	OH	OH	OH	OH	OH	2′ = OH
	Quercetin	OH	OH	OH	OH	OH	H	
	Fisetin	OH	H	OH	OH	OH	H	
	Quercetrin	O-Rha	OH	OH	OH	OH	H	
Isoflavones	Daidzein	H	H	OH	H	OH	H	
	Genistein	H	OH	OH	H	OH	H	
	Puerarin	H	H	OH	H	OH	H	8 = O-Glucosyl
Anthocyanidins	Cyanidin	OH	OH	OH	OH	OH	H	
	Delphinidin	OH	OH	OH	OH	OH	OH	
	Malvidin	OH	OH	OH	OCH_3	OH	OCH_3	
	Petunidin	OH	OH	OH	OH	OH	OCH_3	

ECG, epicatechin-3-gallate; ECGC, epigallocatechin-3-gallate; Rha, rhamnoglucose.

polyols has long been suggested to be a major factor in acute models of sugar cataract.[10]

Diabetic retinopathy

Diabetes is considered to be the primary causative factor in the development of diabetic retinopathy. The disease can be broadly categorized into three stages: background diabetic retinopathy, pre-proliferative diabetic retinopathy and proliferative diabetic retinopathy. In the first stage of diabetic retinopathy, hyperglycaemia initiates thickening of capillary basement membrane and causes death of pericytes that support the vessel wall. Following this, microaneurysms and vascular leakage take place leading to blockage of retinal capillaries and induction of local hypoxia. Subsequently, endothelial cells die resulting in closure of capillaries and increased areas of non-perfusion. Pre-proliferative diabetic retinopathy is identifiable by the areas of increased retinal hypoxia and multiple haemorrhages because of loss of vascular patency. Increased areas of non-perfusion stimulates the generation of angiogenic factors leading to the formation of new blood vessels, a characteristic feature of proliferative diabetic retinopathy. Subsequently retinal detachment may take place, causing vision loss or blindness. Hyperglycaemia and hypoxia are the two principal factors in the initiation and progression of diabetic retinopathy. Production of a variety of local agents in the ocular tissues, such as vascular endothelial growth factor (VEGF), prostaglandins, cyclooxygenase-2 (COX-2) and nitric oxide (NO), is indicated in the process, all of which contribute to vascular permeability and angiogenesis.[11,12]

Age-related macular degeneration

Age-related macular degeneration (AMD) is another leading cause of vision impairment and blindness, especially in western countries.[13] The well-known risk factor for AMD is age. Retinal tissues most affected in this disease are the photoreceptors and the retinal pigmented epithelium (RPE). There are two types of AMD: an atrophic form, which is associated with pigmentary changes in the macula without haemorrhage or scar formation, and disciform macular degeneration, which is characterized by exudative mound formation and sub and intraretinal haemorrhage. However, leakage of plasma from small blood vessels in the macula following breakdown of the blood–retinal barrier can lead to macular oedema and can endanger vision. Besides age,

macular pigmentary change, hypertension, smoking and obesity are other risk factors. Importantly, in AMD, like in diabetic macular oedema, free radicals and reactive oxygen intermediates (ROI) are implicated in the initiation and progression of the disease.[7,8,14]

Glaucoma

Glaucoma is a complex disease that can damage the optic nerve of the eye, leading to vision loss and blindness. It is often called the 'silent blinder', since many people are incognizant about the presence of the disease. It is prevalent in almost all age groups; however, it most affects the elderly. There are different types of glaucoma – the most common ones are primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG), normal tension glaucoma and the less common types are congenital glaucoma and secondary open-angle glaucoma. Conventionally, POAG has been characterized as a disease of elevated intra-ocular pressure. However, recent scientific evidence suggests that both vascular and biochemical factors are also involved. It is now defined as group of ocular diseases that may cause changes in the optic nerve head, visual field, or both.[15,16]

Recent evidence also suggests that reactive oxygen species (ROS) play a significant role in the pathogenesis of POAG. Oxidative damage to the epithelial tissue regulating aqueous humour outflow (i.e. the trabecular meshwork) is significantly higher in glaucoma patients than in normotensive subjects. Moreover, oxidative damage to the retinal cells and neuronal cells of the optic nerve can also lead to POAG.[17,18]

In glaucoma patients, the average blood flow to various ocular tissues, such as iris, choroid, retina and optic nerve, is reduced. This reduction in blood flow is more noticeable in normal tension glaucoma than in high-tension glaucoma. Decreased ocular blood flow can lead to glaucomatous optic neuropathy (GON).[17,19]

Dry eye syndrome

The international dry eye workshop in 2007 described dry eye as a 'multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. This syndrome is most prevalent among the elderly and is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface'.[20] It has been classified into aqueous tear deficient dry eye and evaporative dry eye and there are additional subclasses under each category.

Dry eye is generally associated with inflammation of the surface of the eye, the lachrymal gland or the conjunctiva and any disease that changes the composition of tears. In dry eye syndrome, whatever may be the initial cause, chronic dryness of the surface of the eye leads to neurogenic inflammation, subsequent to activation of T cells and release of inflammatory cytokines into the lachrymal glands, tear fluid and conjunctiva. In later stages, these inflammatory mediators may even cause gradual dysfunction and destruction of the lachrymal glands and impairment of conjunctival epithelium. Thus, lachrymal glands are deprived of normal trophic stimulation required for regular maintenance. Once the disease is initiated, inflammation becomes the key mechanism of ocular surface injury. Recent evidence also suggests a role for oxidative stress in the primary initiating events that lead to the corneal, conjunctival and lachrymal gland injury. Thus, oxidative stress with associated inflammatory process can trigger this disease state.[21]

Pharmacological actions of flavonoids

The following section briefly describes various pharmacological activities of the bioflavonoids, which can be useful in the prevention or treatment of different ocular diseases.

Antioxidant activity

A number of flavonoids have been documented as potential antioxidants and include quercetin, apigenin, hesperidin, hesperetin, luteolin, epigallocatechin gallate, epicatechin gallate, rutin, cyanidin, naringenin, myricetin, chrysin, eriodictyol and kaempferol.[22–24] These compounds exhibit their antioxidant activity through different mechanisms.

By scavenging the free radicals directly

Because of their low redox potential (i.e. high reactivity of the hydroxyl groups) flavonoids are able to reduce the highly oxidizing free radicals (e.g. superoxide, peroxyl, alkoxyl and hydroxyl), resulting in more stable, less reactive radicals.[22,23]

By inhibiting nitric oxide production

Nitric oxide (NO) is produced by several types of cells, including endothelial cells and macrophages. The constitutive production of NO is necessary to maintain the dilation of blood vessels. However, inducible nitric oxide synthase (iNOS) is responsible for the production of higher concentrations of NO during oxidative damage. NO reacts with free radicals and generates the highly reactive and damaging

peroxynitrite. Flavonoids, through their free radical scavenging properties, can prevent the generation of peroxynitrite.[23] Moreover, the flavonoids appear to be capable of inhibiting iNOS directly and thus decrease production of NO.[25–28]

By inhibiting certain enzymes

Flavonoids have been reported to inhibit the enzymes responsible for the production of superoxide anions such as xanthine oxidase and protein kinase C. Some flavonoids are also capable of inhibiting cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all involved in ROS generation.[22]

By chelating trace elements

Trace elements (e.g. free iron and copper) are potential enhancers of ROS generation and also play an important role in oxygen metabolism. Certain flavonoids are capable of chelating these trace elements and thereby prevent the generation of ROS.[22]

Effect on ocular blood flow

A decrease in ocular blood flow can lead to diseases such as glaucoma, diabetic retinopathy and macular degeneration.[29] Literature suggests a significant effect of bioflavonoids on ocular blood flow.[30–32] In 1996, Liu *et al*. reported that

hesperetin was able to increase the blood flow in the iris, ciliary body and choroid.[32] Further investigations using different flavonoids revealed that this activity is dependent on the number of hydroxyl groups present in the flavones and flavanones and on dihydrogenation of the flavone molecules (Figure 2).[30,31,33] Topical administration of the flavonoids in rabbits suggested that flavonoids with two hydroxyl groups (e.g. hesperidin and naringin) had very little, or no, effect on blood flow, while those compounds with three hydroxyl groups (e.g. hesperetin and naringenin) exhibited highest activity with respect to increasing ocular blood flow. With flavanones containing four hydroxyl groups (e.g. rutin) a mixed effect was observed (i.e. blood flow increased at some time points and decreased at other time points). Interestingly, compounds with five hydroxyl groups, e.g. quercetin, had a negative effect on ocular blood flow. A significant improvement in ocular blood flow was also attained when the flavones were dehydrogenated to the flavanones. Additionally, compounds that increased blood flow also caused a marked increase in retinal function recovery following ischaemic insult.[31,34]

Ginkgo biloba extract (GBE), which contains approximately 25% flavone glycosides, was also found to increase blood flow in the ophthalmic artery, in healthy humans, when administered orally at a dose of 40 mg three times a day for two days.[35] No effect was observed on arterial blood pressure, heart rate or intra-ocular pressure. However, Wimpissinger *et al*. recently reported that a single administration of 240 mg GBE did not produce significant changes in the ocular and systemic haemodynamic parameters compared with placebo-treated group.[36]

In another study, administration of the food supplement Mirtogenol (Mirtoselect and Picnogenol), which contains anthocyanosides, to 38 asymptomatic subjects with intraocular hypertension resulted in a significant improvement in ocular blood flow (central retinal, ophthalmic and posterior ciliary arteries) and reduced the intra-ocular pressure.[37]

Effect on oxidative damage to the retinal cells

The ability of the flavonoids to protect the retinal ganglion cells (RGC) from oxidative stress-induced death, *in vitro*, was investigated by Maher and Hanneken.[38] Oxidative stress was induced by three different methods: glutathione depletion, t-butyl peroxide (*t*-BOOH) treatment and H_2O_2 treatment. Some of the flavones (baicalein and luteolin), flavonols (3,6-dihydroxyflavone, 3,7-dihydroxyflavone, galangin, fisetin and quercetin) and flavanones (eriodictyol) were found to be effective in preventing retinal cell death induced by the above three methods of oxidative stress. A few others were effective against either two or one of the oxidative stress induction methods. However, some flavonoids (myricetin and epigallocatechin gallate (EGCG)) were completely ineffective. The differences in activity were attributed to the ability of the flavonoids to induce the biosynthesis of glutathione and to prevent the accumulation of ROS.

In another in-vitro study by the same authors, ischaemia was induced in the rat retinal ganglion cell line, RGC-5, using iodoacetic acid (IAA), and the protective effect of different flavonoids was investigated.[39] Several of the neuroprotective flavonoids (3,6 dihydroxyflavone, 3,7 dihydroxyflavone, galangin, baicalein, luteolin, fisetin, quercetin and eriodictyol) were effective in preventing ischaemia-induced cell death. Interestingly, the classical antioxidants (vitamin E, vitamin C, trolox and resveratrol) demonstrated weak protection against IAA-induced toxicity. Another clinically significant observation made by the authors of this study was that the flavonoids were effective even when they were administered subsequent to the ischaemic insult to the RGC. The neuroprotective function of the flavonoids, in this study, was attributed to several mechanisms, such as prevention of ROS accumulation, inhibition of calcium influx and induction of the expression and activity of phase-II detoxification proteins.

Hanneken *et al*. evaluated the ability of specific dietary and synthetic flavonoids to protect ARPE-19 and human RPE cells from oxidative stress-induced death *in vitro*.[40] Oxidative stress was induced by treatment with t-BOOH or H_2O_2. It was found that some of the flavonoids exhibited good efficacy, high potency and low toxicity in RPE cells. Dietary flavonoids with good efficacy include fisetin, luteolin, quercetin, eriodictyol, baicalein, galangin and EGCG and synthetic flavonoids include 3,6-dihydroxy flavonol, and 3,7-dihydroxy flavonol. Structure–activity studies revealed that minor differences in the structures made a dramatic difference to the efficacy. For example, luteolin is very effective while apigenin is not and the only difference in their structure is a single hydroxyl group in the B ring (Figure 2). Additionally, it was observed that the effective flavonoids were hydrophobic in nature and mostly belonged to the flavone and flavonol class. Importantly, some of the flavonoids tested (quercetin, fisetin, luteolin and eriodictyol) were observed to be effective even after the RPE cells were exposed to oxidative stress, but before cell death occurred. The authors suggested that the flavonoids were probably acting through the inhibition of ROS accumulation and through induction of transcription factor Nrf2 (nuclear erythroid 2 p45-related factor 2), and its downstream phase-2 gene, heme-oxygenase 1, in human RPE cells.[40] Similar mechanisms in the protection of the ARPE-19 cells by eriodictyol were suggested by Johnson *et al*.[41]

Protection of retinal cells against oxidative stress and ischaemia/reperfusion *in vivo* were reported for baicalein[42] and EGCG.[43,44] Also, pretreatment with GBE in drinking water was able to protect the retinal ganglion cells in a rat model of chronic glaucoma.[45] High levels of NO mediate glutamate-induced neurotoxicity following interaction with oxygen radicals. The flavonoid content of GBE was reported to strongly inhibit NO free radicals. Thus GBE's flavonoid content can be effectively used for the treatment of glaucoma.

The retinal neuroprotective effect of various flavonoids studied to date, along with their effective and lethal concentrations, is summarized in Table 2.

Effect on angiogenesis and vascular leakage

Angiogenesis is the process of formation of new blood vessels and is characterized by early degradation of the extracellular matrix followed by migration and proliferation of the endothelial cells and, finally, maturation of the new

Table 2 The protective effect of various flavonoids on retinal cell lines against oxidative stress-induced cell death in terms of effective (EC50) and lethal (LD50) concentrations

Flavonoid class	Common name	ARPE-19[40]			RPE159[40]			RGC-5[39]		RGC-5[38]	
		EC50 (μM)		LD50 (μM)	EC50 (μM)		LD50 (μM)	EC50 (μM)		EC_{50} (μM)	
		t-BOOH	H_2O_2		t-BOOH	H_2O_2		IAA	Post-IAA	Glutamate plus BSO	t-BOOH
Flavone	Apigenin	No	No	<50	–	–				15	No
	Baicalein	14	No	84	8	21	>>100	3.5	8	3	10
	Luteolin	14	9	104	2	3	>50	3.5	7.5	2	7
Flavonol	Galangin	32	31	112	26	61	70	7.5	30	10	50
	Fisetin	15	11	101	3	5	>50	8	17	15	10
	Kaempferol	No	No	~50	~50	No	~50			1	No
	Quercetin	18	19	230	6	11	>50	14	24	17	18
	Myricetin	No	No	>50	>50	No	>>50			No	–
Isoflavone	Genistein	No	No	>>50	No	No	>50			100	No
Flavanone	Naringenin	No	No	>>50	No	No	>>50			No	–
	Eriodictyol	6	17	153	7	11	>100	20	34	5	25
	Hesperetin									50	–
Flavanol	Catechin	No	No	>50	No	No	>>50			No	–
	Epicatechin	No	No	>50	No	No	>>50			50	No
	Epicatechin-3-gallate	No	No	>50	22	30	>100			No	–
Anthocyanidin	Cyanidin	No	No	>100	No	No	>50			No	

No, little or no efficacy up to 50 μM; t-BOOH: *tert*-butyl hydroperoxide; H_2O_2, hydrogen peroxide; IAA, idoacetic acid; BSO, buthionine sulphoximine.

blood vessels. Several factors are associated with the pathophysiology of angiogenesis (e.g. matrix metalloproteases (MMP-2 and MMP-9) and pro-angiogenic factors expressed in response to local injury, ischaemia or inflammation, such as hypoxia-inducible factor-1α (HIF-1α), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), tumour necrosis factor-α (TNF-α) and interleukin (IL)-1, 6 and 8 and others.[46,47] The newly formed blood vessels are leaky in nature and this hyperpermeability causes interstitial oedema, which leads to physical compression of the capillaries resulting in a no-reflow phenomenon. Retinal vascular hyperpermeability is observed in the later stages of diabetic retinopathy and age-related macular degeneration.[8] Thus, treatment with agents capable of decreasing capillary hyperpermeability and inhibiting angiogenesis is highly desired. Some flavonoids have been reported to possess these properties.

The micronized purified flavonoid fraction (Daflon 500 mg) (MPFF) and its individual flavonoid components (diosmin, hesperidin, linarin and isorhoifolin) were evaluated for their anti-leakage effect in a hamster cheek-pouch model, where hyperpermeability was induced by ischaemia–reperfusion. The activity displayed by hesperidin, linarin and isorhoifolin was similar to, or greater than, that of diosmin, the major component (90%) of MPFF. MPFF activity was greater than that of any single flavonoid, indicating synergetic activity.[48] Furthermore, recently, it has been reported that hesperidin (at concentrations of 10 and 100 μM) is capable of inhibiting the expression of hypoxia-inducible factor-1α (HIF-1α) and inflammatory cytokine production in the human mast cell line (HMC-1) in addition to inhibition of TNF-α. HIF-1α is an important mediator of the inflammatory response and one of the major transcriptional activators of vascular endothelial growth factor (VEGF) gene expression, playing a critical role in the process of angiogenesis.[49]

Zou and Chiou demonstrated the ability of apigenin to inhibit the process of angiogenesis *in vitro* and *in vivo*.[50] Apigenin inhibited the proliferation of human umbilical vein endothelial cells (HUVECs) and also choroidal endothelial cells (CEC), *in vitro*, through the degradation of HIF-1α protein and inhibition of VEGF expression. Moreover, at doses of 15 and 30 mg/kg (intraperitoneal administration), apigenin exhibited a similar anti-angiogenic activity in a laser-induced rat model of choroidal neovascularization (CNV). In an in-vitro study, homoisoflavanone was also found to inhibit CNV. It was demonstrated that this flavonoid inhibits expression of fibroblast growth factor (FGF-2), responsible for the blood vessel growth in CNV, induced tube formation and cell invasion of HUVECs.[51]

Quercetin, abundantly found in red wine, grapes and other fruits, also inhibited retinal and choroidal angiogenesis, in the rhesus choroid-retina endothelial cell line, RF/6A. Quercetin prevented endothelial cell proliferation, migration and tube formation in a dose-dependent manner.[52] Quercetin's anti-angiogenic activity was thought to be mediated through the inhibition of MMP-2 activation.[53] Other reports also substantiate the anti-angiogenic activity of quercetin.[54,55] However, it has also been reported that one of the metabolites of quercetin has an opposite effect. Quercetin and quercetin-3′-glucuronide were found to inhibit the VEGF receptor-2 but quercetin-3′-sulfate stimulated the VEGF receptor-2.[56]

EGCG, a catechin component of green tea, is another potent inhibitor of angiogenesis. EGCG was reported to inhibit angiogenesis by inhibiting HIF-1α protein expression[57] and, in turn, VEGF expression.[57,58] Jung *et al*. observed that treatment with EGCG (intraperitoneal

administration) in nude mice decreased tumour growth, microvessel density and tumour cell proliferation. However, the authors reported that other tea catechins such as (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epicatechin (EC) were ineffective *in vitro* against Erk1/2 (extracellularly regulated kinase-1 and -2; important mediators in the up-regulation of VEGF expression) activation, whereas EGCG inhibited Erk1/2 activation in a dose-dependent manner.[59] Several other flavonoids, such as delphinidin,[60] silibinin,[60] kaempferol,[60] fisetin,[61,62] luteolin[62,63] and chrysin,[64,65] are also reported to be anti-angiogenic.

Recently, the effect of the number of hydroxyl groups on the B-ring (Figure 2) of the flavonoid nucleus on anti-angiogenic properties has been investigated. Quercetin, kaempferol, galagin and myrecitin were studied with regard to angiogenesis and cell adhesion inhibition in HUVECs. However, a correlation between the number of hydroxyl groups and VEGF inhibitory potential could not be established.[60]

Anti-inflammatory activity

Some flavonoids have been reported to inhibit several mediators that are activated in certain inflammatory conditions, such as NO, prostanoids, leukotrienes, cytokines and adhesion molecules.[66] NO is produced from L-arginine by three NOS enzymes: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). iNOS is responsible for the production of larger amounts of NO for longer durations. Prostanoids and luekotrienes are also involved in inflammation. Prostaglandins and thromboxane A2 are produced by the cyclooxygenases (COX-1, COX-2 and COX-3). Generally, COX-1 is expressed in most tissues (acting in response to hormones and other stimuli) whereas COX-2 is highly expressed in inflammatory cells. Leukotrienes are generated by lipooxygenases (LOX) and 5- and 12-LOXs are associated with the inflammatory processes. Moreover, different cytokines are involved in inflammation and can be pro-inflammatory (IL-1β, IL-2, IL-6, interferon-γ or TNF-α) or anti-inflammatory (IL-10, TGF-β). Adhesion molecules also play a role in inflammation; blood vessel endothelial cells characteristically respond to pro-inflammatory stimuli and recruit leukocytes by selectively expressing adhesion molecules on the surface, such as vascular cell adhesion molecules (VCAM-1), intercellular adhesion mole- cules (ICAM-1) and endothelial cell selectin (E-selectin).[66] Flavonoids can act on multiple pathways in the inflammation process (Table 3).

Baicalein has been reported to exert anti-inflammatory effects.[67] In a study by Yang *et al*., treatment with baicalein reduced the inflammatory process in a diabetic retinopathy rat model.[68] This was evident from a decreased secretion of inflammatory (IL-18 and IL-1β) or cytotoxic factors (TNF-α), or both. In another study Nakamura *et al*. studied the anti-inflammatory effects of baicalin, baicalein and wogonin on ARPE-19 cell line.[69] It was found that baicalin did not suppress IL-1β-induced IL-6 and IL-8 production, but baicalein and wogonin significantly suppressed IL-6 and IL-8 production. In addition, nuclear factor-kappaB binding activity was not suppressed by baicalin and baicalein, but was suppressed by wogonin.

Naringin and naringenin were able to suppress uveitis in rats induced by foot-pad injection of lipopolysaccharide.[70] To see the effect of these two compounds, they were administered intravenously (0.4, 4 and 40 μg/kg) at three different time points, simultaneously, 30 min before and 30 min after injection of the lipopolysaccharide. At the end of 24 h, aqueous humour was collected and prostaglandin E2 and nitric oxide were estimated. It was found that both compounds decreased the levels of prostaglandin E2 and nitric oxide, compared with a control group, in a dose-dependent manner.

Aldose reductase inhibitory activity

In diabetic patients, hyperglycaemia is also observed in the aqueous humour. Sugars can passively diffuse into the lens where aldose reductase converts glucose to sorbitol or galactose to galactilol. These polyols, thus generated at high levels, cannot diffuse out of the lens passively and either accumulate, or are converted to fructose. Therefore an osmotic gradient is generated, inducing diffusion of water into the lens.

Table 3 General mechanisms by which various flavonoids exert their anti-inflammatory activity

S. No.	Target pathway	Flavonoid
1	Inhibition of iNOS expression	Quercetin[27]
		Quercetin gallate[26]
		Baicalin, baicalein[25]
		Hesperidin[28]
2	Inhibition of COX-2 expression	Hesperidin[28,85]
		Quercetin, kaempferol, genistein, resveratrol[86]
3	Inhibition of COX-2 and iNOS expression	Hesperidin[28]
		Quercetin, galangin, apigenin, naringenin[87]
		Quercetin[88]
4	Inhibition of VCAM-1	Hesperidin[89]
5	Inhibition of VCAM-1, ICAM-1, E-selectin	Quercetin and kaempferol[88]
6	Inhibition of TNF-α, IL-1β, IL-6 and IL-8	Fisetin, quercetin, rutin[90]
7	TNF-α and IL-6	Myricetin[90]

iNOS, inducible nitric oxide synthase; COX, cyclooxygenase; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; E-selectin, endothelial cell selectin; TNF-α, tumour necrosis factor-α; IL, interleukin.

The resultant swelling and electrolyte imbalance leads to cataract formation.[10,71]

Several flavonoids are reported to inhibit the enzyme aldose reductase. Quercitrin is the most promising aldose reductase inhibitor and is used as a positive control in many studies. Additionally, a number of other flavonoids, including luteolin, luteolin-7-b-glucoside, nepetin and its 7-glucoside, nepitrin, kaempferol, kaempferol 3-O-glucuronic acid, eriodictyol 7-O-glucuronide and hesperidin, were also found to be effective.[10,71]

A comprehensive tabulation of various published reports investigating the potential benefits of different bioflavonoids, particularly those dealing with ocular manifestations, is presented in Table 4.

Ocular drug delivery

From the above discussion it is apparent that flavonoids hold immense potential in the prevention or treatment of several sight-threatening eye diseases or disorders. However, drug delivery to the ocular tissues is a challenging task.[72] Drug delivery to the target site can be attempted through topical, periocular, intravitreal, systemic or oral routes of administration depending on the physicochemical properties of the molecule.

Topical application is the most favoured for ocular conditions and involves application of solution, suspension or ointment formulations into the cul-de-sac of the eye. The topical route is mainly used to deliver drugs to the anterior segment of the eye. However, several factors, such as formulation (aqueous solubility and stability) and perme-ability/delivery (precorneal drainage, corneal ultrastructure and drainage through the conjunctival vasculature or nasolachrymal duct) issues, limit bioavailability of the administered drug by this route.[72,73] Periocular administration is the more effective, minimally invasive, route of drug administration for the posterior segment of the eye and includes subconjunctival, subtenon, retrobulbar, peribulbar and posterior juxtascleral routes. The physical barriers associated with this route are the sclera, choroid-Bruch's membrane and RPE. Systemic or oral administration is another option for delivering therapeutic agents to the ocular tissues. However, this route is challenged by several physiological barriers (blood–ocular barrier (BOB), blood–retinal barrier (BRB)), and involves unnecessary systemic exposure to the drug. Intravitreal administration, which delivers the drug directly into the vitreous humour, is very effective but is invasive in nature.

Physicochemical properties of drug molecules play a very important role in determining ocular bioavailability following topical application. In general there exists a parabolic relationship between the oil–water partition coefficient and corneal bioavailability.[72] Maximum corneal permeability was observed for compounds with log octanol–water partition coefficient (logP) in the range of 2–3, for a series of steroids tested.[74] Thus, for efficient ocular tissue permeation, compounds should be neither too lipophilic nor too hydrophilic.[72] Among the different flavonoids tested, aglycones are more lipophilic than their corresponding conjugates. LogP was found to be highly variable within the flavonoid subclass[75] and mostly range between −1.11 and 3.22. While considering drug delivery aspects, solubility issues are additional parameters encountered. Solubility of the flavonoids depends to a large extent on the form in which they are available; compounds with one or more sugar moieties are more polar while the aglycones are less polar, with the highly alkylated flavonoids being lipid soluble.[76] Besides solubility and logP, the three-dimensional configuration, isomeric structure, number of ring substituents, interaction with membrane influx and efflux transporters, molecular weight and hydrogen bond donors/acceptors of the molecule are other important determinants of ocular tissue diffusion of the flavonoids.

To date, only a few studies have investigated delivery of flavonoids to the eye. The following section briefly discusses some of these reports.

A pH-responsive in-situ gelling system for puerarin was developed by Wu *et al*. to improve the precorneal residence time and thus bioavailability of the drug.[77] Two polymers, carbopol 980NF and HPMC E4M, were used to develop the formulation. Based on gelling capability, pH, transparency, viscosity and in-vitro release profiles, a formulation containing 0.1% (w/v) carbopol and 0.4% (w/v) HPMC E4M was identified as an optimized formulation. Bioavailability from this formulation, following topical application, was evaluated *in vivo* in rabbits. Puerarin eye drops containing 4% PVP was used as a control. The pH-triggered in-situ gelling formulation yielded a 2.17-fold greater $AUC_{0\text{-}24h}$ than the aqueous solution. The authors also evaluated the in-vitro permeability of puerarin across the rabbit cornea and it was found that an aqueous peurarin solution containing 5% hydroxypropyl beta-cyclodextrin (HP-b-CD) exhibited a 2.5-fold higher permeability than 4% PVP solution.

Based on the same concept of increasing precorneal residence time of the drug, another thermosensitive and mucoadhesive in-situ gelling system for puerarin was developed by Qi *et al*.[78] Using a two-factor, five-level central composite design, a formulation containing 21% (w/v) poloxamer P407 and 5% (w/v) poloxamer P188 (F1) was considered to be ideal. The formulation exhibited a gelation temperature of 34.8°C on dilution with artificial tear fluid. To improve the mucoadhesive properties of the above formulation, 0.1% (w/v) (F2) and 0.2% (w/v) (F3) carbopol 1342P NF was incorporated. These formulations were evaluated *in vivo* in rabbits, in addition to other control formulations like aqueous solution of the drug in HP-b-CD (F4) and in 0.2% carbopol 1342 NF (F5). The concentration of puerarin in tear fluids and intra-ocular pressure-lowering effect of the drug were evaluated. The AUC of the drug in tears was 4.43 and 5.26 times higher for F2 and F3, respectively, compared with F4. All the formulations were observed to decrease the intra-ocular pressure (a maximum drop of about 4–5 mmHg); however, the effect of F2 and F3 lasted for 24 h compared with 8 h with F4 and 8–24 h for F1 and F5.

Zhang *et al*. studied the pharmacokinetics of topically applied baicalein in rabbits.[79] Two formulations of 1% (w/v) baicalein were tested; drug suspension and solution in 10% HP-β-CD. Drug concentrations in aqueous humour and cornea were determined at 5, 10, 20, 30, 45, 60, 90 and 120 min post dosing. Baicalein-HP-b-CD demonstrated a 2.1-fold higher bioavailability than the suspension formulation.

Table 4 Comprehensive review of various studies carried out to date investigating the potential use of different bioflavonoids in the prevention or treatment of ocular diseases or disorders

Flavonoid	Type of study	Type of model used/route of administration/dose	Inference
Anthocyanins	Ocular bioavailability[80]	Rabbits and rats; oral, i.v. and i.p. administration	Blackcurrant anthocyanins were absorbed and distributed in ocular tissues in intact forms following i.v. and i.p. administration
Anthocyanosides	Effect on night vision	Young normal volunteers; Single oral administrations of 12, 24 and 36 mg[71] and multiple oral administrations of 12 and 24 mg[72]	Single (12–36 mg) and multiple oral administrations (12 and 24 mg) of anthocyanosides twice a day had insignificant effect on night vision
Anthocyanins (delphinidin, cyanidin, petunidin and malvidin)	Antioxidant effect[91]	Human retinal pigment epithelia (ARPE-19) cell line	Anthocyanins can serve as antioxidants by suppressing photooxidative processes initiated in RPE cells by the lipofuscin fluorophore, A2E
Baicalein	Pharmacokinetic and bioavailability study[79]	Topical administration (1% w/v) in rabbits	Baicalein:HP-β-CD solution exhibited superior bioavailability in the aqueous humor compared with plain baicalein suspension
	Effect on inflammation[68]	Oral administration (150 mg/kg/day) in a diabetes induced rat model	Baicalein treatment was able to inhibit inflammatory processes, characterized by microglial activation and Müller cell dysfunction, and inhibited vascular abnormality and neuron loss in diabetic retinas
Baicalin	Preventive effect against ischaemic and oxidative insult to the retinal cells[42]	Intraperitoneal (12.5 mg/kg) administration just before and after ischaemic insult to the retina in rats	Baicalin statistically inhibited most of the effects induced by ischaemia/reperfusion (IR); however, the increase in caspase-3 and caspase-8 mRNAs caused by IR was unaffected
		Retinal ganglion cell-5 (RGC-5) line (0.1–10 μM)	Baicalin significantly attenuated the negative insult of light, hydrogen peroxide and serum withdrawal on RGC-5 cells in a dose dependent manner. In lipid peroxidation studies, baicalin was found to be equivalent to EGCG in terms of antioxidant activity
Baicalin, baicalein and wogonin	Anti-inflammatory activity[69]	ARPE-19 cell line	Baicalin did not suppress IL-1, induced IL-6 and IL-8 production, but baicalein, and wogonin, significantly suppressed IL-6 and IL-8 production. Baicalin and baicalein did not suppress NF-kappaB binding activity, which was suppressed by wogonin
Catechin	Protective effect against glutamate induced retinal toxicity[92]	Porcine retinal homogenates (8 nM concn)	Catechin suppressed the damage of retinal lipoproteins
(–)-Epigallocatechin gallate	Protective effect on UVA-induced damage[74]	ARPE-19 cell line	EGCG inhibited UVA-induced H_2O_2 production, MAPK activation, and expression of COX-2
Epigallocatechin gallate (EGCG)	Protective effect on UV irradiated human lens epithelial cells[93]	Cultured human lens epithelial cells	EGCG increased the cell count and cell viability after UV irradiation of cultured human lens epithelial cells
	Attenuating effect on light induced photoreceptor damage[94]	Oral administration in rats, 400 mg/kg/day	EGCG suppressed negative effects of light induced insult to the retina
	Protective effect against oxidative stress induced apoptosis[77]	Human lens epithelial cells (HLEB-3)	EGCG protected against cell death caused by H_2O_2 in HLEB-3 cells
	Protective effect on retinal ganglion cells following IR[95]	Intraperitonial injection in female Wistar rats before the IR	EGCG pretreatment decreased retinal ganglion cell death from IR by approximately 10% probably by attenuating neuronal nitric oxide synthase expression and activity
	Protective effect on retinal pigment epithelial cells[96]	ARPE-19 cell line	EGCG increased the cell count and cell activity after UV irradiation, suggesting a protective role
	Protective effect on retina[34]	Rats and RGC-5 cell line	EGCG provided protection to retinal neurons from oxidative stress and ischaemia/reperfusion

(Continued)

Table 4 *(Continued)*

Flavonoid	Type of study	Type of model used/route of administration/dose	Inference
	Protection against oxidation-induced retinal degeneration[97]	Wistar rats, 5 μl of a combination of sodium nitroprusside and EGCG (100 and 15 μM in vitreous chamber, respectively) was injected intravitreally	A single bolus injection attenuated the SNP-induced oxidative photoreceptor apoptosis
	IOP lowering effect[98]	Intravenous administration of flavonoids in normotensive rabbits (1 mg dose showed effect)	Phenolic antioxidants containing a pyrogallol B-ring system and nonaromatic C-ring (epigallocatechin, epigallocatechin gallate and myricetin) were found to be effective
Daflon 500 mg (diosmin and hesperidin)	Protective action in ischaemia[80]	Gerbil with IR injury. 200, 100 and 50 mg/kg, for 6 days before left carotid occlusion	Daflon 500 mg significantly reversed the increase of stroke index only at 100 mg/kg and significantly decreased levels of hydroxyl free radicals at all 3 doses with maximum effectiveness for the dose of 100 mg/kg
Deguelin	Protective effect in retinopathy[99,100]	Mouse model of retinopathy, (0.1 μM/1 μl i.v.)	Deguelin was found to be a potent inhibitor of choroidal neovascularization (CNV) and may be useful in the treatment of other vasoproliferative retinopathies
Eriodictyol	Long-term protective effect on RPE cells[41]	ARPE-19 cell line	Eriodictyol was able to induce Nrf2 and phase-2 proteins, heme-oxygenase (HO)-1 and quinone oxidoreductase (NQO)-1. These proteins play a significant role in protecting RPE against oxidative stress. Eriodictyol induced long-term protection was significantly greater than its short-term protection
Eriodictyol, luteolin, quercetin and taxifolin	Antioxidant activity[101]	Cultured retinal cells	Antioxidant activity was found to be in the order of eriodictyol > quercetin > luteolin > taxifolin
Ginkgo biloba extract (EGb 761)	Protective effect on retinal injury[102]	Ischaemic injury induced in cat retina, 100 mg/kg i.v	Free radical scavenger EGb 761 efficiently protected the retina from ischaemic injury
Fisetin	Antioxidant effect on lens epithelial cells[85]	SRA01/04 cell line	Fisetin exhibited anti-catarogenic activity by activating NF-kappaB and MAPK in UV-induced oxidative stress
Fisetin, genistein, luteolin	Effect on corneal neovascularization[62]	Topical application of microemulsion containing flavonoid, 0.5 and 1 ng/ml	Fisetin exhibited the strongest effect followed by genistein and luteolin
Genistein	Aldose reductase inhibitory activity[103]	Human epithelial cell line (HLE-B3)	Genistein inhibited aldose reductase activity in a dose-dependent manner. Genistein also exhibited antioxidant activity in HEL-B3 cells
	Effect on cataract[104]	Animal model of dietary galactose-induced cataracts in adult male rats (15 mg/kg)	Genistein was not able to completely prevent cataract formation, but did delay progression
	Protective effect on retinal neovascularization[105]	Retinal pigment epithelia-19 cell line (50, 100, 200 μM)	Pretreatment with genistein reduced the expression of IL-8, indicated in the development of retinal neovascularization, in a dose-dependent manner
		ARPE-19 cell line (10, 20, 50, 100 and 200 μM)[106]	Pretreatment with genistein reduced the expression of basic fibroblast growth factor, indicated in the development of retinal neovascularization, in a dose-dependent manner
		ARPE-19 cell line (50, 100 and 200 μM)[107,108]	After pretreatment with genistein, hypoxia-evoked HIF-1 and VEGF expression was inhibited. Activity was concentration dependent
	Effect on retinal vascular permeability[109]	Diabetic rats, 150 and 300 mg/kg with food, ad libitum	Chronic oral administration of genistein significantly reduced retinal vascular leakage in an animal model of diabetic retinopathy
	Effect on proliferative vitreoretinopathy[110]	Rat retinal pigment epithelial cell line, RPE-J	Genistein inhibited RPE cell growth and induced apoptosis. 10 mM inhibited cell proliferation, 50 μM caused growth inhibition and subsequent apoptotic death
		Model of IR injury in the rat retina. Intraperitonial administration[111]	Genistein (3.4 mg) inhibited the increase in tyrosine phosphorylation and protected the eyes from the induced ischaemic retinal degeneration. 0.034 mg and 0.34 mg did not show a significant effect

Hesperidin	Transocular permeation[84]	Isolated rabbit ocular tissues	Hesperidin is capable of permeating across ocular tissues like cornea, sclera and choroid-RPE
	Ocular blood flow[32]	Rabbits, topical application (50 μl of 1% w/v solution) Homoisoflavone	Hesperetin increased ocular blood flow in all eye tissues except retina
Homoisoflavanone	Inhibitory effect on CNV[51,112]	Mouse model of laser-photocoagulation-induced CNV, intravenous administration HUVECs	Homoisoflavanone significantly reduced CNV and capillary leakage. Homoisoflavanone effectively inhibited tube formation and cell migration of HUVECs, *in vitro*
Mirtogenol	Effect on ocular blood flow and intra-ocular hypertension[28]	Asymptomatic human subjects with intraocular hypertension, oral administration for six months	Treatment with mirtogenol is useful for lowering the risk of developing symptomatic glaucoma by controlling IOP and improving ocular blood flow
Myricetin, quercetin, kaempferol	Protective effect against retinal cells[113]	Bovine retinal cell line	Myricetin, quercetin and kaempferol exhibited approximately 100% protection against A2E (a major fluorophore of lipofuscin) induced toxicity but quercetin was ineffective and kaempferol was poorly active against blue light induced toxicity
Naringin and naringenin	Uveitis[70]	Rats, i.v. administration	Both exhibited anti-inflammatory activity by suppressing PGE2 and NO expression. Particularly 40 μg/kg dose (i.v.) demonstrated the activity
Puerarin	Protective effect against diabetic retinopathy[114]	Rats	Puerarin exerts significant protective effects in rats, by regulating the expression of angiogenesis factors (VEGF and HIF-1alpha)
	Retinal blood flow[115]	Rats, topical application	Puerarin and all its derivatives, except ET (puerarin disubstituted with $-CH_2CH_2OH$), showed marked increase of choroidal blood flow at various times
Quercetin	Protective action against oxidative stress[116]	Cultured human RPE cells	Quercetin was able to protect RPE cells from oxidative damage and cellular senescence *in vitro* in a dose-dependent manner
	Therapeutic benefit in cataract[117]	Cultured human lens epithelial (HLE) cell line	Quercetin inhibited both a UV- and H_2O_2-induced decrease of collagen type-I, which has a significant role in cataract formation, via the inhibition of JNK/c-Jun activity
	Therapeutic benefit in cataract[118]	HLE cell line	Quercetin, at a low concentration (0.1 μM), protected HLECs and reversed the toxic effects of DMSO (1% v/v). However, at higher concentrations, quercetin was toxic to HLECs with an LD50 of 90.85 μM
	Therapeutic benefit in cataract[119]	Rat lens	Quercetin and 3′-*O*-methyl quercetin both (10 μM) inhibited H_2O_2-induced (500 μM) sodium and calcium influx and lens opacification
		Rat lens organ cultured model[120]	Quercetin was active when incubated in the culture medium together with hydrogen peroxide, or when the lens was pre-treated with quercetin prior to oxidative insult, whereas (+)epicatechin and chlorogenic acid were much less effective
Flavonoids	Ocular blood flow[30,31,33]	Rabbit topical administration	Structure–activity relationship with respect to activity of various flavonoids to increase the ocular blood flow was elucidated (refer the text for details)
	Retinal function recovery[33,34]	Rat	Structure–activity relationship of various flavonoids on retinal function following ischaemic insult was elucidated (refer the text for details)
	Aldose reductase inhibitory activity[121]		Relationship between the structure and aldose reductase inhibitory activity was studied (see text for details)

i.v., intravenous; i.p., intraperitoneal; IL, interleukin; NF-kappaB, nuclear factor-kappaB; MAPK, mitogen activated protein kinase; HUVECs, human umbilical vein endothelial cells; IOP, intra-ocular pressure.

The distribution of blackcurrant anthocyanins (BAC) in the ocular tissues following oral (100 mg/kg, rats), intraperitoneal (108 mg/kg, rats) and intravenous (20 mg/kg, rabbits) administration was investigated by Matsumoto *et al*. to evaluate the barrier characteristics of the BOB and BRB.[80] Concentration of all four anthocyanins (delphidin-3-rutinoside, delphidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside) were determined in different ocular tissues and summed together. Following oral administration in rats, intact BACs were detected in the plasma and in the eye; however, the concentrations in the eye were very low. Interestingly, when these BACs were administered orally in rabbits (authors did not mention the dose), detectable levels were not observed even in plasma, suggesting poor absorption of anthocyanins in rabbits. Comparing the plasma AUCs of BACs in rats following oral (2.56 μg h/ml) and intraperitoneal (12.3 μg h/ml) administration, it can be speculated that oral bioavailability of BACs is low even in rats. Following intraperitoneal administration, detectable BAC levels were observed in all the ocular tissues tested and levels were in the following order 1 h after drug administration: sclera with choroid > cornea > ciliary body with iris > retina > aqueous humour > vitreous > lens. However, at the 24 h time point, BAC levels were not detectable in any of the tissues, indicating that anthocyanins were rapidly eliminated from the eye. Following intravenous administration in rabbits, the AUC in various ocular tissues were in the following order: choroid > sclera > ciliary body > cornea > aqueous
humour > iris > retina > vitreous > lens. The elimination half-life of the drug was in the range 1.4–1.8 h, explaining the absence of drug levels in the ocular tissues at 24 h after intraperitoneal administration. Overall, the results, taken together, suggest that BACs exhibit low oral bioavail- ability but are capable of permeating across the BOB and BRB to reach the inner ocular tissues following intravenous or intraperitoneal administration. Other reports also support the observation that following oral administration, the bioavailability is limited and mainly the respective phase-II metabolites (e.g. sulfate, glucuronide conjugates) of the flavonoids are principally observed in the systemic circulation.[81–83]

In a recent study, we investigated in-vitro permeability of hesperidin across various rabbit isolated ocular tissues.[84] Hesperidin appeared to be fairly permeable across the cornea, sclera, and sclera with choroid-RPE. Expectedly, its permeability across the sclera was much higher, almost 10-fold, compared with the cornea and sclera with choroid-RPE.

Conclusions

In summary, it is apparent that the flavonoids are capable of acting on various mechanisms or aetiological factors responsible for the development of different sight-threatening ocular diseases. Oral bioavailability of the flavonoids, however, is limited by poor intrinsic transmembrane diffusion characteristics, poor solubility and intestinal and hepatic metabolism. The activity of the flavonoid metabolites has not been properly evaluated as yet. From a drug delivery perspective, ocular bioavailability depends on the physicochemical and biopharmaceutical characteristics of the selected flavonoids and very importantly the route of administration. When administered by the oral route, diffusion of the hydrophilic metabolites (the parent compounds undergo rapid hepatic metabolism) from the plasma into the neural retina will be severely restricted by the inner and outer blood–retinal barriers. Thus, whereas oral administration may demonstrate some pharmacological activity in the outer sections of the posterior ocular segment, protection of the retinal ganglionic cells *in vivo* may be limited by this delivery route. Systemic or local administration of these agents may yield much higher and effective concentrations of the parent bioflavonoids in the ocular tissues and at much lower doses.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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