Natural flavonoids as potential multifunctional agents in prevention of diabetic cataract

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ABSTRACT
Cataract is one of the earliest secondary complications of diabetes mellitus. The lens is a closed system with limited capability to repair or regenerate itself. Current evidence supports the view that cataractogenesis is a multifactorial process. Mechanisms related to glucose toxicity, namely oxidative stress, processes of non-enzymatic glycation and enhanced polyol pathway significantly contribute to the development of eye lens opacity under conditions of diabetes. There is an urgent need for inexpensive, non-surgical approaches to the treatment of cataract. Recently, considerable attention has been devoted to the search for phytochemical therapeutics. Several pharmacological actions of natural flavonoids may operate in the prevention of cataract since flavonoids are capable of affecting multiple mechanisms or etiological factors responsible for the development of diabetic cataract. In the present paper, natural flavonoids are reviewed as potential agents that could reduce the risk of cataract formation via affecting multiple pathways pertinent to eye lens opacification. In addition, the bioavailability of flavonoids for the lens is considered.

KEY WORDS: diabetic cataract; natural flavonoids; oxidative stress; antioxidants; aldose reductase inhibitors; glycation inhibitors

Introduction
Diabetic patients are susceptible to the development of chronic health complications responsible for a significant increase in their morbidity and mortality. Cataract, eye lens opacification, is one of the earliest secondary complications of diabetes mellitus (Kyselova et al., 2004; Obrosova et al., 2010). Diabetic patients are about 60% more likely to develop these eye conditions. People with diabetes also tend to get cataract at a younger age with a faster progression (Bron et al., 1998).

The association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies (Bron et al., 1998; Polleisz and Schmidt-Erfurth, 2010; Obrosova et al., 2010). Since extracellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the most affected body parts in diabetes mellitus. The understanding of mechanisms by which glucose exerts its toxicity is of utmost importance for rational pharmacological interventions to treat diabetic cataract. The etiology of diabetic cataract is multifactorial; multiple hyperglycemia-dependent mechanisms leading to an array of subtle post-translational modifications in the lens structural proteins contribute to its development. The lens is a closed system with limited capability to repair or regenerate itself. The proteins of the lens are extremely long-lived and there is virtually no protein turnover, which provides great opportunities for post-translational modification to occur (Bron et al., 2000; Harding, 2002; Krishna Sharma & Santhoshkumar, 2009).

Oxidative stress and its sequelae are clearly involved in the etiology of senile cataract, while mechanisms related to glucose toxicity, namely oxidative stress, enhanced polyol pathway, and processes of non-enzymatic glycation, significantly contribute to the development of eye complications in diabetic patients (Baynes & Thorpe, 1999; Kyselova et al., 2004; Brownlee, 2005; DelCorso et al., 2008; Alexiou et al., 2009; Obrosova et al., 2010).

Under conditions of diabetes, the need of tight blood glucose control is a key prerequisite to reduce the incidence, progression, and severity of cataract. Yet periods of hyperglycemia in the daily regimen of a diabetic patient...
cannot be avoided, with all of the aforementioned deleterious consequences of glucose toxicity. Therefore additional adjunct therapy interfering with the pathological processes at molecular level, e.g., based on antioxidants, aldose reductase inhibitors and anti-glycation agents, is needed to attenuate the noxious effects of glucose.

Innovative strategies in treatment of diseases of multifactorial origin are oriented on a rational design of chemical entities able to affect simultaneously multiple key mechanisms involved. This approach increases the chance of successful therapeutic intervention, decreases the risk of side effects and is economical. An example of the "multi-target" strategy in treatment of diabetic complications are the bifunctional compounds combining the aldose reductase inhibitory activity with the antioxidant effect, including pyrido-pyrimidines (La Motta et al., 2007), pyridazines (Coudert et al., 1994), benzopyranes (Constantino et al., 1999) and carboxymethylated pyridoindoles (Stefek et al., 2008) or compounds combining the aldose reductase inhibitory activity with the ability to attenuate nonenzymatic glycation (Demopoulos et al., 2005). Considering the detoxification role of aldose reductase against toxic carbonyl products, significantly enhanced in diabetic tissues (Baynes, 1991; Thorpe & Baynes, 1996; Thornalley, 2005; Turk, 2010), the concurrent antioxidant action of a multifunctional drug can counterbalance its inhibition. In addition, antioxidant activity may suppress processes of advanced glycation (glycoxidation) at the level of free radical intermediates (Baynes, 1991; Thorpe & Baynes, 1996; Brownlee, 2000; Giacco & Brownlee, 2010).

Recently, considerable attention has been devoted to the search for phytochemical therapeutics. There is epidemiologic evidence that a sufficient intake of fruit and vegetables can lower the risk of cataract in humans (Taylor, 1993; 1999). A variety of constituents, like vitamins, minerals, fiber, and numerous phytochemicals, including flavonoids may contribute to the protective effect of fruits and vegetables. Indeed, several pharmacological actions of flavonoids may operate in the prevention of both age-related and diabetic cataract since flavonoids are capable of affecting multiple mechanisms or etiological factors responsible for the development of sight threatening ocular diseases (Bhimanagouda, 2009; Majumdar et al., 2010; Kalt et al. 2010).

At the pre-clinical level of animal models, flavonoids (Figure 1) have been shown to be protective against eye lens opacification. In the present paper, flavonoids are reviewed as potential agents that could reduce the risk of cataract formation via affecting multiple key pathways pertinent to eye lens opacification, including oxidative stress, non-enzymatic glycation and polyol pathway. In addition, the bioavailability of flavonoids to the lens is considered.

Anticataract action of flavonoids

Models in vitro

Under in vitro conditions, using rat lens organ culture endowed with hydrogen peroxide, low micromolar levels of flavonol quercetin inhibited oxidation-induced sodium and calcium influx and loss of lens transparency (Sanderson et al., 1999). As shown later by Cornish et al. (2002), quercetin was rapidly lost from the media and readily entered the lens where it was methylated to 3'-O-methyl quercetin. Both quercetin and its metabolite were active in inhibiting oxidative damage in the lens.

The glucoside of isorhamnetin (methylated quercetin), isolated as a bioactive flavonoid from the leaves of Cochlospermum religiosum (Gayathri et al., 2010) and flavonoid fraction isolated from fresh leaves of Vitex negundo (Rooban et al., 2011) protected enucleated rat eye lenses against selenite-induced cataract in an in vitro culture model.

The flavonoid venoruton, a mixture of mono-, di-, tri- and tetrahydroxymethylrutosides, significantly reduced the degree of opacification and the leakage of lactate dehydrogenase in rat lens organ culture simulating diabetic conditions (Kilic et al., 1997).

Animal models in vivo

As early as in 1977, Varma et al. (1977) studied the effect of quercetin rhamnoside (quercitrin) on the development of cataract in the rodent Octodon degus made diabetic by a single intraperitoneal dose of streptozotocin. The control diabetic animals not receiving quercitrin developed nuclear opacity by about the tenth day after the onset of hyperglycemia. In contrast, the diabetic animals treated with quercitrin did not develop cataracts even 25 days after the onset of diabetes, although they had a blood glucose concentration similar to that of the control diabetic group. In a similar study performed by Lu et al. (2008) in streptozotocin diabetic rats, high-isoflavone soy protein markedly decreased the death rate and incidence of cataracts in the diabetic animals. At the same time, reduced serum glucose and methylglyoxal were recorded in the treated rats. Nakano et al. (2008) reported lower incidence of cataract in streptozotocin diabetic rats treated with
flavangenol, a complex mixture of bioflavonoids with oligomeric proanthocyanidin as main constituents.

Topical administration of quercetin to the orbital pouch of the galactosemic neonatal rat diminished cataractogenesis in the corresponding lens. Comparison with the contralateral lens indicated that quercetin reduced intracellular edema, prevented extracellular fluid accumulation and maintained cellular interdigitation of the superficial anterior cortical fiber. In addition to preserving fiber integrity, topical application of quercetin maintained lens growth as evidenced by radius and dry weight measurements (Beyer-Meyers & Farnsworth, 1979). Analogically, in the same galactosemic rat model, Mohan et al. (1988) recorded anticataract action of quercetin and structurally related myricetin after topical administration. In galactosemic rats, oral treatment with quercetin resulted in a significant correction of eye lens electrolyte disturbances and normalization of lens protein levels (Ramana et al., 2007). The results imply that inclusion of quercetin contributes to lens transparency through the maintenance of characteristic osmotic ion equilibrium and protein levels of the lens. The isoflavone genistein delayed the progression of cataracts induced in rats by dietary galactose (Huang et al., 2007).

The rat selenite cataract model (Shearer et al., 1997; Ghupta et al., 2009; Kyselova, 2010) was extensively used to study the anticataract action of flavonoids. The results of Orhan et al. (1999) showed that ethanol extract of propolis, rich in flavonoids (Scheller et al., 1990), and quercetin prevented cataract formation to the extent of 70 and 40%, respectively. Standardized extract of Ginkgo biloba (Egb761) did not affect cataract formation. The flavonoid fraction from Emilia sonchifolia was reported (Lija et al., 2006) to decrease the rate of maturation of selenite cataract more efficiently than quercetin. Activities of superoxide dismutase, catalase and reduced glutathione were found to be increased in the group treated with Emilia sonchifolia, while thiobarbituric acid reacting substances were decreased compared with the selenite induced group.

Rutin (quercetin rutinoside) was reported (Isai et al., 2009) to prevent selenite-induced cataractogenesis in rat pups. At the end of a 30-day study period, all the rat pups that had received only selenite were found to have developed a dense nuclear opacity in the lens of each eye, whereas only 33.3% of pups that had received selenite and been treated with an intraperitoneal dose (175 mg/kg of body weight) of rutin hydrate were found to have mild lenticular opacification in each eye. The other 66.7% of pups in that group had clear lenses in both eyes, as in normal pups. The mean activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione transferase, and glutathione reductase were found to be significantly lower in the lenses of cataract-untreated rat pups than in normal control rat lenses. However, in lenses treated with rutin hydrate, the mean activities of antioxidant enzymes were significantly higher than the values in rat pups with untreated cataracts.

Onion is a flavonoid-rich foodstuff and the major flavonoids contained have been identified as quercetin, quercetin-4’-glucoside and quercetin-3,4’-diglucoside (Fossen et al., 1998; Miean and Mohamed, 2001). In the study of Javadzadeh et al. (2009), the instillation of fresh juice of crude onion into the rat eyes was found to prevent selenite-induced cataract formation by 75%. This effect was associated with higher mean total antioxidant level as well as higher mean activities of superoxide dismutase and glutathione peroxidase in the lenses of rats receiving fresh juice of crude onion and subcutaneous injection of sodium-selenite, compared with those rats which received only sodium-selenite injection. The onion juice, as a flavonoid-rich source, was postulated to provide an additional support to the antioxidant agents, leading to the elevation of total antioxidant levels and superoxide dismutase and glutathione peroxidase activities in the rat lens, in spite of exposure to sodium-selenite.

Flavonoid fractions isolated from natural sources including green and black tea (Thiagarajan et al., 2001; Gupta et al., 2002), Ginkgo biloba (Thiagarajan, 2002), grape seeds (Durukan et al., 2006), Emilina sonchifolia (Lija et al., 2006), Vetex negundo (Rooban et al. 2009) and broccoli (Vibin et al., 2010) were shown to have anticataract activity in selenite-induced experimental cataract in rats. In addition, Ginkgo biloba extracts were found to protect rats against radiation-induced cataract (Ertekin et al., 2004).

Among others, damage to the lens epithelium is considered a possible cause of cataract formation (Hightower, 1995). Catechin was found to inhibit apoptotic cell death in the lens epithelium of rats after cataract induction with N-methyl-N-nitosourea (Leed et al., 2010). Grape seed extract rich in flavonoids reduced hydrogen-peroxide-induced apoptosis of human lens epithelial cells and depressed H2O2-induced activation and translocation of NF-xB (Jia et al., 2011). Similarly, the flavonoid fisetin was found to protect human lens epithelial cells from UVB-induced oxidative stress by inhibiting the generation of reactive oxygen species and modulating the activation of NF-xB and MAPK pathways (Yao et al., 2008).

**Flavonoids as multifunctional agents**

As reviewed below, flavonoids efficiently affect the multiple key molecular mechanisms involved in the etiology of both age-related and diabetic cataract, namely oxidative stress, non-enzymatic glycation and polyol pathway. Structural requirements of flavonoids for efficient inhibition of the above mentioned processes are summarized. Flavonoids may interfere also with lens calpain proteases and lens epithelial cell signaling, which is however outside the scope of this review.

**Antioxidant action of flavonoids**

The antioxidant action of flavonoids, the best described biological activity of this group of natural polyphenolic substances, is covered by a number of excellent reviews (Bors et al., 1990; Cao et al., 1997; Pietta, 2000; Rice-Evans, 2001; Nijveldt et al., 2001; Bors & Michel, 2002;
The general structural requirements for effective radical scavenging and/or the antioxidant potential of flavonoids are known as Bors’ criteria (Bors & Michel, 2002; Amič et al., 2007), namely (i) the presence of a catechol group in ring B, capable of readily donating hydroxyl (electron) to stabilize a radical species, (ii) the presence of 2,3-unsaturation in conjugation with a 4-oxo-function in the C-ring, which is responsible for electron delocalization, and (iii) the presence of a 3-hydroxyl group in the heterocyclic ring which increases the radical-scavenging activity. The catechol moiety may also contribute to an ability to chelate transition metal ions, such as copper and iron.

Flavonoids were found also to scavenge efficiently the model free radicals of 2,2-diphenyl-1-picrylhydrazyl and α,α′-bisphenylene-β-phenyllalyl (Butković et al., 2004). Flavonoid chelating activity for transition metal ions has been well documented (Nijveld et al. 2001; Pietta, 2000; Williams et al., 2004). Flavonoids inhibit xanthine oxidase, the enzyme responsible for superoxide anion production (Hamaski et al., 1994). Interestingly, isorhamnetin (3-methylquercetin) was found to inhibit xanthine oxidase, even more efficiently than the aglycone form of quercetin (Nagao et al., 1999).

As reviewed below, the high antioxidant efficacy of flavonoids is accompanied by their ability to inhibit aldose reductase and non-enzymatic glycation – activities of high relevance to the development of diabetic cataract.

### Aldose reductase inhibition by flavonoids

The accumulation of polyol sorbitol within the lens is a primary contributing factor to the formation of diabetic cataract (Yabe-Nishimura, 1998; Del Corso et al., 2008; Alexiou et al., 2009; Obrosova, 2010), a mechanism different from senile cataract. In diabetes, glucose is in a high concentration in the aqueous humor and can diffuse passively into the lens. The enzyme aldose reductase within the lens converts glucose to sorbitol. This polyol cannot diffuse passively out of the lens and accumulates or is converted to fructose.

Aldose reductase inhibitors represent a potential therapeutic strategy for preventing the onset or progression of diabetic cataract (Costantino et al., 2000; Miyamoto, 2002; Suzen et al., 2003; Alexiou et al., 2009; Obrosova, 2010). Pharmacophoric requirements for aldose reductase inhibitors are determined by the structural features of the inhibitor binding site of aldose reductase, which was shown to be formed by a large hydrophobic pocket (El-Kabbani et al., 2004). This pocket is mainly composed of two regions: a hydrophilic anionic binding site which accommodates acidic functionalities and a region of hydrophobic residues that binds the hydrophobic aromatic ring system of the inhibitors. Inhibitor binding is therefore a consequence of polar and non-polar interactions between the inhibitor and the complementary residues that line the enzyme binding pocket. It has been proposed that the specificity for the inhibitor was mainly due to inhibitor-enzyme interactions at the non-polar domain (El-Kabbani & Podjarny, 2007).

To date, two main classes of active aldose reductase inhibitors have been reported and classified on the basis of the ionizable group which allows them to anchor to the catalytic site: carboxylic acids (substitution derivatives of acetic acid) and spirohydantoins, with epalrestat and sorbinil being the most representative members of each respective family (Costantino et al., 2000; Miyamoto, 2002; Suzen et al., 2003; Alexiou et al., 2009). They are generally referred to as carboxylate-type and hydantoin-type inhibitors. A third class of aldose reductase inhibitors represents flavonoids. Since the mid-1970s, a number of studies have been reported on the inhibition of aldose reductase by flavonoids (Varma et al., 1975; Varma and Kinoshita, 1976; Okuda et al., 1982; Nakai et al., 1985; Lim et al., 2001; Jung et al., 2002; Matsuda et al., 2002; Kawanishi et al., 2003; Lee et al., 2010). Structural features required for a firm anchoring to the catalytic site of the aldose reductase enzyme, were summarized by Matsuda et al. (2002) as follows: (i) the presence of a 7-hydroxyl group and catechol moiety at the B ring guarantees the strong activity; (ii) the 5-hydroxyl moiety does not affect the activity; (iii) the 3-hydroxyl and 7-O-glucosyl moieties reduce the activity; (iv) the 2–3 double bond enhances the activity; (v) the flavonols having the catechol moiety (the 3′,4′-dihydroxy moiety) at the B ring exhibit stronger activity than those with the pyrogallol moiety (the 3′,4′,5′-trihydroxyl moiety).

Inhibitory action of active flavonoid components isolated from natural products against rat lens or human recombinant aldose reductase were reported, often in comparison with quercetin used as a positive control (Matsuda et al., 2002; Suryanarayana et al., 2004; Wirasathien et al., 2007; Chethan et al., 2004; Carbone et al., 2009; Jung et al., 2008; Suzen et al., 2009; Lee et al., 2010).

Aldose reductase inhibitors of the flavonoid class, in contrast to those of the carboxylate type whose acidic nature results in poor biological availability, possess a higher pKₐ value, which is a prerequisite for their better pharmacokinetics.

In the light of the above mentioned biological activities of natural flavonoids, they serve as an example of bifunctional agents for the “multi-target” approach to the treatment of diabetic cataract by combining the aldose reductase inhibitory activity with its antioxidant action. In addition, starting from the flavonol quercetin as a lead
structure, a series of 4H-1-benzopyran-4-one derivatives was designed and developed as semi-synthetic agents, with dual antioxidant/ aldose reductase inhibition activity (Costantino et al., 1999).

Advanced glycation inhibition by flavonoids

The process of non-enzymatic glycation is well known to be one of the key mechanisms leading to diabetic cataract (Shamsi et al., 2000; Brownlee, 2001, 2005; Stitt, 2005; Monnier et al. 2005; Nagaraj et al., 2010). In compliance with the glycation theory of aging (Monnier & Cerami, 1981), accumulation of advanced glycation end products in the aging lens, yet to a lesser extent in comparison with the diabetic eye, may contribute to age-related lens opacity. In seeking potential anti-cataract drugs, clinically useful anti-glycation agents are a reasonable option. As reviewed below, a number of naturally occurring flavonoids were reported to exhibit inhibitory effects on advanced glycation end products formation.

Four flavonoids of the methanol extract of Thymus vulgaris, quercetin, eriodictyol, 5,6,4’-trihydroxy-7,8,3’-trimethoxyflavone, and cisilinole suppressed the levels of advanced glycation end products and fructosamines of bovine serum albumin under in vitro conditions (Morimitsu et al., 1995). By using the bovine serum albumin glycation model, Beaulieu et al. (2010) demonstrated that the flavonoid components of the Vaccinium vitis-idaea berry extract were potent anti-glycation agents, while Wu et al.(2005) recorded significant inhibitory activity of the natural flavonoids luteolin, quercetin, and rutin. Rutin and metabolites of rutin were found to inhibit glycation product formation, including both fluorescent and nonfluorescent AGEs, induced by glucose in collagen 1 in vitro (Cervantes-Laureau, 2006). Flavonoids markedly reduced pentosidine formation in collagen from bovine Achilles tendon incubated with glucose in vitro in the following decreasing order of their specific inhibitory activity: myricetin ≥ quercetin > rutin > (+)-catechin > kaempferol (Urios et al., 2007). Rutin and its metabolites 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxystilbene were found to inhibit histone H1 glycation by the powerful glycat ing agent ADP-ribose, as effectively as did aminoguanidine (Pashikanti et al., 2010). Rutin and G-rutin, a water soluble glucose derivative of rutin, suppressed glycation of muscle and kidney proteins exposed to glucose in vitro (Nagasawa et al., 2003a). Under in vivo conditions, G-rutin was found to inhibit glycation reactions in muscle, kidney and plasma proteins of streptozotocin-induced diabetic rats (Nagasawa et al., 2003b).

In their study based on 62 flavonoids, Matsuda et al. (2003) formulated structural requirements of flavonoids for inhibition of protein non-enzymatic glycation: (i) as the hydroxyl groups at the 3′-, 4′-, 5′-, and 7-positions increased in number, the inhibitory activities became stronger; (ii) the activities of flavones were stronger than those of corresponding flavonols, flavanones, and isoflavones; (iii) methylation or glucosylation of the 4′-hydroxyl group of flavones, flavonols, and flavanones reduced their activity; (iv) methylation or glucosylation of the 3-hydroxyl group of flavonols tended to increase activity; (v) glucosylation of the 7-hydroxyl group of flavones and isoflavones reduced their activity. Yet these principles should be further corroborated.

In seeking more efficient multifunctional flavonoids combining antioxidant activity with both aldose reductase and advanced glycation inhibitory action, for potential pharmacological prevention of diabetic cataract and other long-term diabetic complications, all three sets of the aforementioned criteria should be applied in screening available databases of flavonoid structures.

Bioavailability of flavonoids

Biological activity of flavonoids is often assessed by using in vitro models; in almost all such studies, cells are treated with aglycones and data are reported at concentrations that elicited a response. However, plasma and tissues are not exposed in vivo to flavonoids in these forms. The forms reaching the blood and tissues are, in general, neither aglycones nor the same as the dietary source glycosides. In blood, flavonoids are present as conjugates of glucuronate or sulfate, with or without methylation of the catechol functional group. As a consequence, the flavonoid conjugates are likely to possess different biological properties and distribution patterns within tissues and cells than have flavonoid aglycones. Although deconjugation can potentially occur in vivo to produce aglycone, it occurs only at certain sites. Thus, the extent to which in vitro effects produced by the aglycones can be extrapolated to the in vivo situation, in particular in humans, is poorly understood (Kroon et al., 2004).

Flavonoids occur in plants mainly in the form of O-glycoside conjugates linked to sugars like glucose, galactose, arabinose or rhamnose (Bravo, 1998; Arts et al., 2004). The bioavailability is primarily determined by the type of the sugar moiety (Arts et al., 2004; Crozier et al., 2010). In the case of flavonoid-O-β-D-glucosides, the aglycone can be enzymatically released in the small intestine by the brush border lactase phlorizin hydrolase (Day et al., 1998) or by cytosolic β-glucosidase activity (Ioku et al., 1998). The enzyme of lactase phlorizin hydrolase exhibits broad substrate specificity for flavonoid-mono- and disaccharides and the released aglycone may then enter the small intestine epithelial cells by passive diffusion (Day et al., 2000). Alternatively, cytosolic β-glucosidase functions within the epithelial cells after the glucosides entered via the sodium-dependent glucose transporter 1 (Gee et al., 2000). On the other hand, e.g. quercetin-3-rutinoside (rutin) is not a substrate of lactase phlorizin hydrolase. The quercetin aglycone is released hydrolytically by bacterial α-rhamnosidases and β-glucosidases in the lower gastrointestinal tract. Thus absorption of quercetin from rutin is delayed, and quercetin bioavailability is much smaller in comparison with that of quercetin-glucosides. This example stresses the role of sugar type for bioavailability of natural flavonoid glycosides (Hollman et al.,
be expected to halve the number of cataract extractions (Brian & Taylor, 2001). This calls for a search of alternative pharmacological measures to treat this disorder.

In diabetic patients, tight control of hyperglycemia is the first prerequisite to attenuate the risk of cataract. In addition, adjunct therapy is needed to help preserve vision in diabetic patients, aimed at correcting biochemical and metabolic abnormalities in the hyperglycemic milieu of the diabetic individual.

The current data on natural polyphenols in relation to cataract, along with epidemiological knowledge on diet and lens opacity, demonstrate that flavonoids may play a role in cataract prevention. Yet future clinical trials are needed to assess the benefits of flavonoids in lowering the risk of both age-related and diabetic eye complications.

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