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CHAPTER

41

c0041 # Natural Products and Retinal Ganglion Cells: Protective Roles of Edible Wild Vegetables Against Oxidative Stress in Retinal Ganglion Cells

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s0010 ## INTRODUCTION

p0010 Glaucoma is a chronic disease that causes progressive damage to the optic nerve and to retinal ganglion cells (RGCs), resulting in visual field damage from the side to the center vision.[1] This condition is expected to affect up to 80 million individuals worldwide by 2020, with at least 6–8 million individuals becoming blind in both eyes.[2,3]

p0015 Several risk factors have been suggested to play a role in the pathogenesis of glaucoma, including intraocular pressure (IOP) and mechanical damage to RGC axons,[4] ischemic insults to RGCs axons in the optic nerve head,[5,6] and genetic risks.[7]

p0020 Glaucoma has been classified into specific types, including primary open-angle glaucoma and closed-angle glaucoma. These types are specified by the contact between the iris and the trabecular meshwork, which in turn obstruct the aqueous humor from the eye. These types cause increased IOP in the eye, which is the most common risk factor for glaucoma.[4] Increased IOP (normal range 10–20 mm Hg) causes damage to the optic nerve head through the mechanical compression of RGC axons, and causes axonal transport blockade, thereby altering the appropriate nutritional requirements for the survival of RGCs.[8]

p0025 Thus, the current therapy for glaucoma is focused on the reduction of IOP.[9] Topical medications that reduce IOP have been used for treatment, including cholinergic agonists, cholinesterase inhibitors, carbonic anhydrase inhibitors, adrenergic agonists, β-blockers, and prostaglandin analogues.[3] Some epidemiologic studies have shown that reducing IOP is effective in slowing the progression of glaucoma in approximately 90% of cases.[4]

p0030 Although reduction of IOP is effective in most cases, not all patients with glaucoma are protected from disease progression with such a treatment.[10,11] This is because many glaucoma patients have normal IOP, but exhibit a decreased blood supply to or a weakness in the optic nerve; moreover, not all ocular hypertensive patients develop glaucoma.[11]

p0035 According to epidemiologic studies conducted in Korea, the prevalence of glaucoma among the population of rural Sangju in South Korea was 3.4%. Notably, open-angle glaucoma with low IOP was found to be the most common form, accounting for 94.4% of the total number of cases.[12] Greater knowledge of the underlying pathogenesis of glaucoma has resulted in new therapeutic approaches, and the prevention of RGC degeneration rather than IOP reduction is the goal of current treatment strategies.[13–15]

p0040 Several putative compounds with neuroprotective effects exist for the potential treatment of glaucoma, including memantine and brimonidine, which is currently in application for clinical trials.[3] Memantine failed its randomized phase III clinical trials in patients with glaucoma as it did not show significant efficacy that was similar to prior studies.[16] This lack of reported efficacy may be due to the various modes of action for neuroprotection in glaucoma, and the difficulty in detecting effects with present methodologies.[17]

Handbook of Nutrition, Diet, and the Eye
http://dx.doi.org/10.1016/B978-0-12-401717-7.00041-1

10041-PREEDY-9780124017177

p0045 Natural products that possess multiple properties, including antioxidant capacity, may provide neuroprotection in glaucoma and may potentially be used to prevent or treat such neurodegenerative diseases.[18] In this chapter, the neuroprotective role of natural products, particularly edible wild vegetables, in RGCs is investigated. One of these putative wild vegetables, *Gymnaster koraiensis*, was shown to have antiapoptotic and antioxidant properties in transformed RGCs, and protective effects on RGCs in an *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity model and a partial optic nerve crush (PONC) model *in vivo*. The potential value of natural products and the possible application of edible wild vegetables as neuroprotective agents for glaucoma are summarized and discussed.

NEUROPROTECTION OF RETINAL GANGLION CELLS

s0015

p0050 RGC axons are the output of the eye and integrate information from the outer retina to the brain where they synapse at the thalamus, the hypothalamus, and the superior colliculus.[19] Neuroprotection in glaucoma is defined by the preservation and functional maintenance of damaged RGCs through interference with injury and death pathways.[20]

p0055 The reasons why and the processes involved in how RGCs actually die in glaucoma are still being investigated, although many studies have been carried out to identify the relevant mechanisms of action. The death of RGCs in glaucoma was shown to occur by apoptosis in various experimental models.[21–24]

p0060 Advances in the area of RGC neuroprotection can be achieved using knowledge derived from laboratory studies to predict clinical applications. Several approaches for the neuroprotection of RGCs have been proposed, including stopping or preventing apoptosis, preventing tumor necrosis factor activation, stabilizing Ca^{2+} homeostasis, blocking glutamate excitotoxicity, normalizing mitochondrial dysfunction, inhibiting nitric oxide production, modulating the expression of heat shock proteins, supplying neurotrophins, and improving blood flow to the optic nerve.[14,25–27]

p0065 There is a significant amount of evidence implicating the role of oxidative stress in glaucoma.[28] The mechanisms that have been proposed in RGCs, including excitotoxicity, IOP, and ischemia, can induce oxidative stress by various insults, resulting in RGC death.[29]

p0070 Reduced antioxidative capacity has been shown in the aqueous humor of glaucoma cases, and reduced glutathione (GSH) and increased malondialdehyde (MDA) plasma levels have also been found in the plasma of glaucoma patients.[30,31] In a clinical correlation study in patients with primary open-angle glaucoma, IOP elevation was shown to cause visual field damage by DNA oxidative damage in the human trabecular meshwork.[32] This finding may explain why oxidative stress is an important factor in the pathogenesis of glaucoma.

p0075 Therefore, it would be desirable for neuroprotective substances for RGCs to also possess antioxidant properties. Natural products, with their antioxidative properties, have long been shown to have beneficial effects in humans and can be tolerated when taken regularly. Thus, natural products may be beneficial in the treatment of a chronic disease such as glaucoma.[33]

USE OF NATURAL PRODUCTS TO PROTECT RETINAL GANGLION CELLS

s0020

p0080 There have been numerous reports of natural products, mostly from plants, with protective effects on RGCs, as summarized in Table 41.1. One of the natural products in clinical trials is *Ginkgo biloba*. *Ginkgo biloba* is a well-known dietary supplement containing many different flavonoids, terpenoids, and other polyphenols that have proven strong antioxidative properties.[73] Two well-known pharmacologic applications of *G. biloba* are in the treatment of blood disorders and the enhancement of memory.[74] Laboratories studies have shown that *G. biloba* enhances microcirculation by improving endothelium-dependent vasodilation,[75] which contributes to *G. biloba* acting as a putative substance for the treatment of glaucoma.

p0085 Notably, *G. biloba* extracts can increase ocular blood flow velocity and have various activities, including neuroprotection in RGCs, as shown by clinical studies in patients with normal tension glaucoma (Table 41.1).[54–61] [AU1]

p0090 Green tea is an ancient tea from *Camellia sinensis*, and is widely used worldwide. Green tea contains high amounts of polyphenols, including (+)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate (EGCG).[76] Green tea and its main constituents have various beneficial actions on conditions such as inflammation and thrombosis, as well as age-related neurodegeneration, by increasing learning and memory with a particular focus on the hippocampal formation.[77,78]

p0095 EGCG, the main constituent of green tea, has been shown to have beneficial effects on the neuroprotection of RGCs. Orally administered EGCG has been shown to attenuate retinal neuronal death in rats, and inhibit apoptosis caused by various insults, such as ischemia, oxidative stress, and light in transformed RGCs (RGC-5 cells) (Table 41.1).[44–48] EGCG tablets (Epinerve, Sifi, Italy) have been prescribed by ophthalmologists to treat glaucoma in Italy. [AU2]

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t0010 **TABLE 41.1** Evidence Suggesting Protective Effects of Natural Substances in Retinal Ganglion Cells

Natural Product (Raw Materials)	Biologically Active Compounds	Methods	References
Thuja orientalis	Isoquercitrin	Oxidative stress-induced RGC-5 cell death	Jung *et al.* (2010)[33]
Carotenoid	Zeaxanthin, astaxanthin, lutein	Oxidative stress-induced RGC-5 cell death	Li and Lo (2010), Nakajima *et al.* (2008, 2009)[34–36]
Olive oil	Squalene	Alcohol damage in the chick embryo retina	Aguilera *et al.* (2005)[37]
Scutellaria baicalensis	Baicalin	Ischemia–reperfusion injury in retina, oxidative stress-induced RGC-5 cell death	Jung *et al.* (2008)[38]
Garlic	*S*-Allyl-L-cysteine (SAC)	Ischemia–reperfusion injury in retina, hypoxia-induced RGC-5 cell death	Chen *et al.* (2012)[39]
Phyllostachys nigra	Luteolin 6-*C*-(6″-*O*-trans-caffeoylglucoside)	Oxidative stress-induced RGC-5 cell death	Lee *et al.* (2010)[40]
Lycium barbarum	Polysaccharide	Ocular hypertension in rat, neuroprotection in primary cultured RGCs	Chan *et al.* (2007), Chiu *et al.* (2009), Yang *et al.* (2011)[41–43]
Green tea	Epigallocatechin gallate (EGCG), epicatechin (EC)	Rotenone-induced RGC-5 cell death, light-induced cell death in RGC-5 cell, ischemia–reperfusion injury in retina	Kamalden *et al.* (2012), Osborne *et al.* (2010), Zhang and Osborne (2006), Zhang *et al.* (2007, 2008)[44–48]
Brazilian green propolis	Dicaffeoylquinic acid	Oxygen–glucose deprivation-induced RGC-5 cell death, NMDA in rat	Inokuchi *et al.* (2006), Nakajima *et al.* (2007, 2009)[49–51]
Morus alba	–	Diabetes and hypercholesterolemia in mother rat	El-Sayyad *et al.* (2011)[52]
Rhus verniciflua	Fustin, fisetin, sulfuretin, butein	Oxidative stress-induced RGC-5 cell death	Choi *et al.* (2012)[53]
Ginkgo biloba	–	Clinical study for normal tension glaucoma, chronic glaucoma model by IOP elevation in cautery of three episcleral vessels in rats, optic nerve crush in rat	Chung *et al.* (1999), Hirooka *et al.* (2004), Ma *et al.* (2009, 2010), Park *et al.* (2011), Quaranta *et al.* (2003), Shim *et al.* (2012), Wang *et al.* (2011)[54–61]
Zuogui pill	–	Optic nerve crush in rat	Wang *et al.* (2011)[62]
Annatto (*Bixa orellana*)	Bixin	Endoplasmic reticulum stress-induced retinal damage, tunicamycin-induced cell death in rat	Tsuruma *et al.* (2012)[63]
Bilberry (*Vaccinium myrtillus*)	Anthocyanins	Oxidative stress-induced RGC-5 cell death, NMDA-induced cell death in rat	Matsunaga *et al.* (2009)[64]
Crocus sativus, *Gardenia jasminoides*	Crocetin	Light-induced cell retinal damage in rat	Yamauchi *et al.* (2011)[65]
Curcuma longa	Curcumin	Staurosporine-induced retinal damage in mice	Burugula *et al.* (2011)[66]
	Diosmin	Ischemia–reperfusion injury in retina	Tong *et al.* (2012)[67]
Eisenia bicyclis	Phlorotannins	Oxidative stress-induced RGC-5 cell death, NMDA-induced cell death in rat	Kim *et al.* (2012)[68]
Grape	Resveratrol	Endoplasmic reticulum stress-induced retinal damage in RGC-5 cells, ischemia–reperfusion injury in mice	Li *et al.* (2012), Yang *et al.* (2012)[69,70]
–	Flavonoids	Oxidative stress-induced RGC-5 cell death	Maher and Hanneken (2005)[71]
Aegle marmelos fruit	–	Water loading and steroid-induced models	Agarwal *et al.* (2009)[72]

RGC-5: transformed retinal ganglion cells; NMDA: *N*-methyl-D-aspartate; IOP: intraocular pressure.

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p0100 The bioavailability of green tea is poor and small molecules, including epicatechin and epigallocatechin, have higher bioavailability than large molecules, such as EGCG.[79] Therefore, increasing the bioavailability of EGCG as the active compound in green tea is very important for the development of pharmaceuticals or dietary supplements.

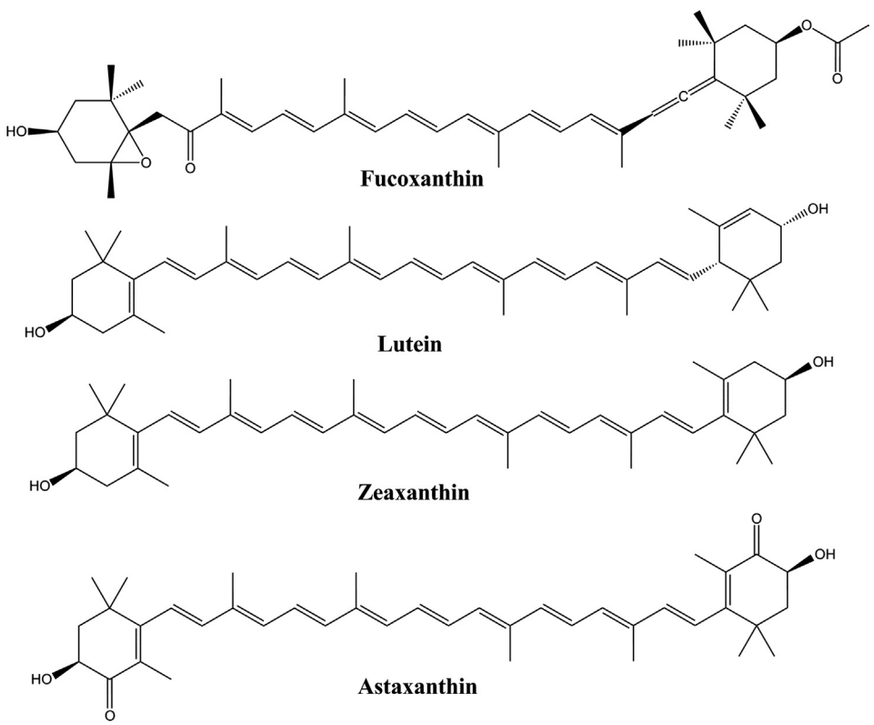
p0105 Carotenoids are tetraterpenoids and can be classified into two specific classes: xanthophylls and carotenes. The chemical structures of carotenoids are shown in Figure 41.1. These molecules can absorb blue light, which can protect the eye, and particularly the macula lutea, from damaging blue and near-ultraviolet (UV) light. In this regard, oxygen-containing carotenoids (xanthophylls) such as lutein and zeaxanthin have been shown to play an important role in the prevention of age-related macular degeneration.[80] Given that humans are not able to synthesize xanthophylls, these must be consumed from natural products, including certain fruits, vegetables, and eggs.[81] Several studies have shown that lutein and zeaxanthin have beneficial antioxidative properties for neuroprotection against oxidative stress-induced RGC damage [AU3] (Table 41.1).[34–36]

The well-known natural antioxidants resveratrol and anthocyanins, which are found in grapes and berries, respectively, have been shown to have protective effects on endoplasmic reticulum stress-induced retinal damage (Table 41.1).[64,69,70] Propolis has also been suggested as a putative natural substance for RGC protection, with effects shown both *in vitro* and *in vivo* (Table 41.1).[49–51] [AU4]

p0110

p0115 Previous studies have shown that several natural products and their isolated compounds have protective effects on oxidative stress-induced RGC death. Flavones, such as isoquercitrin isolated from *Thuja orientalis*, were found to be effective at blunting the negative influence of oxidative insults to RGCs (Table 41.1).[33]

p0120 There are many reports on the biologic effects of *Scutellaria baicalensis*. Its active compound, baicalin, was shown to have neuroprotective effects on ischemia–reperfusion in the hippocampi of gerbils via antioxidative and antiapoptotic pathways.[82] Moreover, baicalin was found to attenuate inflammatory reactions and cerebral ischemia injury in rats, involving toll-like receptor 2 and 4 (TLR2/4) and the downstream nuclear factor-κB (NF-κB).[83] The neuroprotective effect of baicalin was also demonstrated by its inhibition



f0010 **FIGURE 41.1** Chemical structure of carotenoids.

of NMDA receptor-mediated 5-lipoxygenase activation in rat cortical neurons.[84] Studies have shown that baicalin is a powerful neuroprotectant in RGCs. Baicalin attenuated ischemia–reperfusion injury in rats and oxidative stress-induced transformed RGC (RGC-5) death.[38]

p0125 Black bamboo, *Phyllostachys nigra*, and *Rhus verniciflua* have also been suggested as possible natural products for the treatment of glaucoma (Table 41.1).[40,53]

p0130 It has been reported that the hydroxyl groups of flavonoids at the catechol positions are effective in preventing microsomal lipid peroxidation and enable increased antioxidative properties.[85] Many flavonoids have catechol moieties and are potentially able to scavenge free radicals, including peroxynitrite.[86] This ability may be due to their strong reactivity with free radicals, and their efficient metal chelation and electron delocalization.[87,88]

p0135 The neuroprotective effects of isoquercitrin isolated from *T. orientalis*, baicalin isolated from *S. baicalensis*, and luteolin (6-C-6″-*O*-*trans*-caffeoylglucoside) isolated from *P. nigra* appear to be primarily related to the antioxidant characteristics of these substances. These compounds each have a catechol group, which may underlie the protective effects of the above compounds on RGCs.

p0140 In a recent study investigating the effects of seaweed on RGCs, the ethanol extract of *Eisenia bicyclis* was found to protect RGCs from NMDA-induced excitotoxicity in rat retinas and oxidative stress-induced RGC death. These protective effects on RGCs are due to the antioxidative properties of the extract, with phlorotannins being the likely active compounds (Table 41.1).[68] The beneficial effects of *E. bicyclis* may also be due in part to fucoxanthin (Fig. 41.1), a derivative of carotenoids that was isolated from *E. bicyclis* by centrifugal partition chromatography.[89]

p0145 Other suggested natural products that have been demonstrated to be effective in various experimental models are shown in Table 41.1.

s0025 USE OF WILD VEGETABLES TO PROTECT RETINAL GANGLION CELLS

p0150 Wild vegetables grown in Korea are consumed as healthy food, and several species have also been used as fermented food, particularly Kimchi. It is known that wild vegetables contains higher levels of minerals and vitamins than cultivated vegetables.[90] Moreover, it has also been reported that wild vegetables have higher carotene and calcium concentrations than locally cultivated species.[91] This may be because wild vegetables are under the constant stress of harsh conditions, including competition with other plants and with insects.

p0155 In recent years, the multiple roles of wild vegetables as both food and medicinal sources have been widely documented. Despite several publications on the importance of edible wild vegetables used in Korea, data have not been collected systematically and detailed biologic studies have not been conducted.

p0160 Research has investigated whether edible wild vegetables grown in Pyeongchang, Korea, exhibit any biologic activities (Daegwanryeong project), including neuroprotective effects in transformed RGCs (RGC-5). In particular, studies have focused on the family of Compositae; the list of vegetables belonging to this family is shown in Table 41.2. In total, 50 wild vegetables from the Compositae family consumed in Korea were tested for protective effects on oxidative stress-induced RGCs. Among the vegetables investigated, *Gymnaster koraiensis* N. showed the highest protective effect, and additional studies were carried out to further clarify this effect.

s0030 Protective Effect of *Gymnaster koraiensis* on Oxidative Stress-Induced Retinal Ganglion Cells

p0165 *Gymnaster koraiensis* N. (Compositae) (Fig. 41.2) is a wild vegetable that grows in Korea and has been used as a source of food in this country for many years. The reported biologic activities of *G. koraiensis* include inhibitory activities on AKR1B10 to prevent cancer,[92] on osteoclast formation by suppressing NFATc1 and DC-STAMP expression,[93] on aldose reductase and advanced glycation end-products,[94] and on nuclear factor of activated T cells (NFAT) transcription factor,[95] as well as hepatoprotective effects.[96]

p0170 The chemical constituents of *G. koraiensis* have been reported to include polyacetylenes, benzofurans, terpenes, squalenes, and several flavonoids [14,94,97–99] (Fig. 41.3), as well as dicaffeoylquinic acids. Brazilian green propolis also contains high amounts of dicaffeoylquinic acid, which has been shown to have significant neuroprotective effects on oxidative stress-induced retinal damage.[49] Therefore, dicaffeoylquinic acid may be the chemical constituent underlying the neuroprotective effects of *G. koraiensis*.

p0175 Many glaucoma risk factors lead to the overproduction of reactive oxygen species (ROS), which may play a major role in the demise of RGCs, as discussed in the 'Introduction' section.[100] It is clear that oxidative stress distorts intercellular homeostasis and that reducing oxidative stress in target tissues is a strategy worthy of consideration for the treatment of glaucoma. *Gymnaster koraiensis* has antioxidative properties that were shown to significantly reduce the production of ROS caused by different sources of oxidative stress, such as hydrogen peroxide (H_2O_2), $O_2^{\cdot-}$, or ·OH.[99] These findings indicate that *G. koraiensis* is effective in reducing ROS in RGCs.

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t0015 **TABLE 41.2** List of Compositae

No.	Code	Scientific Name	Parts
1	D-002	*Saussurea pulchella* (Fisch.) Fisch.	Whole plant
2	D-011	*Gymnaster koraiensis* (Nakai) Kitam.	Leaves, stem, and flower
3	D-014	*Cirsium nipponicum* (Maxim.) Makino	Leaves and stem
4	D-015	*Aster glehni* var. *hondoensis* kitamura	Leaves
5	D-016	*Solidago virgaurea subsp. gigantea* (Nakai) kitam	Leaves and stem
6	D-021	*Atractylodes ovata* (Thunb.) DC.	Leaves and stem
7	D-026	*Achillea alpina* (Ledeb)	Leaves and stem
8	D-027	*Lactuca indica* L. var. *laciniata* (O.Kuntze) Hara	Leaves, stem, and flower
9	D-037	*Lactuca indica* L. var. *laciniata* Hara	Leaves and stem
10	D-039	*Adenocaulon himalaicum* Edgew.	Whole plant
11	D-041	*Youngia sonchifolia* Maxim.	Leaves, stem, and flower
12	D-043	*Youngia denticulata* Kitamura	Whole plant
13	D-044	*Taraxacum officinale* Weber	Whole plant
14	D-050	*Artemisia dubia* Wall.	Leaves
15	D-058	*Ligularia fischeri* (Ledeb.) Turcz.	Leaves and stem
16	D-060	*Ligularia fischeri* var. *spiciformis* Nakai	Leaves and stem
17	D-061	*Ligularia fischeri* (Ledeb.) Turcz.	Leaves and stem
18	D-062	*Cirsium setidens* (Dunn) Nakai	Leaves and stem
19	D-066	*Lactuca indica* L. var. *laciniata* (O.Kuntze) Hara	Leaves
20	D-067	*Ligularia fischeri* (Ledeb.) Turcz.	Leaves, stem, and flower
21	D-068	*Aster scaber* Thunberg	Leaves, stem, and flower
22	D-076	*Taraxacum coreanum* Nakai	Leaves
23	D-080	*Carpesium abrotanoides* L.	Whole plant
24	D-082	*Artemisia gmelini* Weber ex Stechm	Leaves, stem, and flower
25	D-083	*Aster tataricus* L.f.	Whole plant
26	D-084	*Sonchus brachyotus* DC.	Whole plant
27	D-090	*Cirsium japonicum* var. *maackii Cirsium japonicum* var. *ussuriensis* (Kitam)	
28	D-094	*Synurus deltoides* (Aiton) Nakai.	
29	D-099	*Cirsium pendulum* Fisch. Ex DC.	
30	D-102	*Syneilesis palmata* (Thunb.) Maxim	
31	D-103	*Crepidiastrum chelidoniifolium* (Makino)	
32	D-110	*Erigeron canadensis* L.	
33	D-111	*Erigeron canadensis* L.	
34	D-112	*Erigeron annuus* L.	
35	D-122	*Solidago virgaaurea* var. *asiatica Nakai*	Leaves and shoot
36	D-135	*Breea segeta* Kitam.	Leaves and shoot
37	D-136	*Carpesium macrocephalum* Fr.	Leaves

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TABLE 41.2 List of Compositae—cont'd

No.	Code	Scientific Name	Parts
38	D-142	*Parasenecio auriculata* var. *kamtschatica* (Maxim.) H.Koyama	Leaves and shoot
39	D-147	*Saussurea macrolepis* (Nakai) Kitam.	Leaves and shoot
40	D-148	*Saussurea seoulensis* Nakai	Leaves and shoot
41	D-153	*Synurus excelsus* (Makino) Kitam.	Leaves
42	D-156	*Lactuca triangulata* Maxim.	Leaves and stem
43	D-164	*Carpesium cernuum* L.	Leaves and stem
44	D-167	*Parasenecio auriculata* var. *kamtschatica* (Maxim.) H.Koyama	Leaves and stem
45	D-168	*Syneilesis palmata* (Thunb.) Maxim.	Leaves and stem
46	D-169	*Ixeris repens* (L.) A.Gray	Leaves, stem, and root
47	D-175	*Xanthium strumarium* L.	Stem and fruit
48	D-176	*Picris hieracioides* var. *koreana* Kitam.	Leaves, stem, and flower
49	D-187	*Helianthus tuberosus* L.	Leaves and stem
50	D-189	*Sigesbeckia glabrescens* (Makino) Makino	Leaves, stem, and flower

Fifty wild vegetables of Compositae were collected from 2007 to 2010 in the vicinity of Gangneung, Korea, and the voucher specimens were deposited at the Herbarium of KIST Gangneung, Korea.

f0015

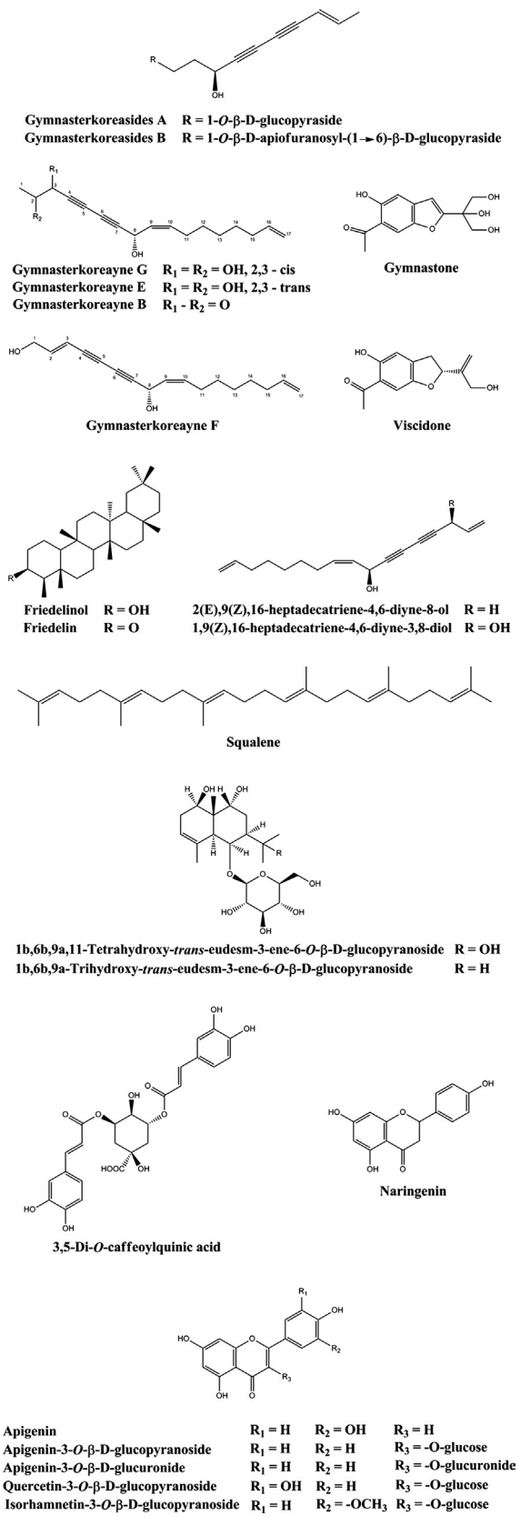
FIGURE 41.2 *Gymnaster koraiensis*. The *G. koraiensis* was collected in 2009 from the vicinity of Gangneung, Korea, and the voucher specimen (voucher no. D-011) was deposited at the Herbarium of KIST Gangneung, Korea.

p0180 Studies by Wei *et al.* showed that nuclear factor erythroid 2-related factor 2 (Nrf2) is cytoprotective against neuronal and capillary degeneration in a model of retinal ischemia–reperfusion injury. The authors suggested that Nrf2 could be a new therapeutic target for retinal diseases.[101]

p0185 The author's previous studies investigated the cytoprotective effects of isolated compounds from *G. koraiensis* on oxidative stress-induced cytotoxicity in HepG2 cells.[96] Gymnasterkoreayne B, a polyacetylene derivative isolated from *G. koraiensis*, was shown to induce detoxification enzymes through the nuclear translocation of Nrf2, which is known to regulate antioxidant responsive element (ARE)-driven phase II detoxification genes.[102] Thus, these data suggest that the neuroprotective effects of *G. koraiensis* in RGCs may be due to both direct and indirect antioxidant mechanisms.

p0190 Online high-performance liquid chromatography (HPLC)–2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), an online rapid screening method, was used to identify the antioxidative compound in *G. koraiensis* (Fig. 41.4). The detection of antioxidative compounds from complex extracts is possible by the online coupling of HPLC separation. The HPLC separation eluate is mixed with a stabilized solution of $ABTS^+$ radicals from different HPLC pumps, and the mixed solution is detected with a UV/Vis detector monitoring absorbance at 734 nm. The $ABTS^+$ radicals scavenging detection chromatogram is detected as a negative peak on the absorbance profile. The more rapidly the absorbance decreases, the more potent the antioxidant activity of the compound in terms of hydrogen-donating ability.[103]

p0195 Figure 41.4 shows the online HPLC–$ABTS^+$ analysis of an extract of *G. koraiensis*. From the combined UV (positive signals) and ABTS+ quenching (negative signals) chromatograms, one major compound was identified as showing the highest free radical scavenging activity. The major compound was isolated from the ethyl acetate fraction of *G. koraiensis* (EAGK) using Diaion HP-20 and preparative HPLC, and the chemical structure was elucidated by spectral analysis as 3,5-di-*O*-caffeoylquinic acid (3,5-DCQA).[99] Both the EAGK and the isolated 3,5-DCQA



f0020 **FIGURE 41.3** Chemical composition of *Gymnaster koraiensis*. Several compound derivatives have been isolated from *G. koraiensis*, including polyacetylenes, terpenes, squalenes, caffeoylquinic acids, and flavonoids.

were found to protect RGC-5 cells against H_2O_2-induced oxidative stress in a concentration-dependent manner (Fig. 41.5a–c).[99]

p0200 H_2O_2-induced cell death in RGC-5 cells involves changes in the expression of various apoptotic proteins: cleaved poly (ADP-ribose) polymerase (PARP), apoptosis-inducing factor (AIF), and cleaved caspase-3 (Fig. 41.5d). EAGK and 3,5-DCQA were shown to attenuate the upregulation of cleaved PARP and cleaved caspase-3 proteins caused by exposing cultures to H_2O_2.[99] Moreover, H_2O_2 is known to cause AIF nuclear translocation. However, EAGK and 3,5-DCQA were found to significantly reduce the upregulation of AIF proteins in a concentration-dependent manner.[99]

p0205 Glutamate neurotoxicity caused by excessive excitatory neurotransmitter glutamate has been shown to cause glaucoma.[104] Excessive glutamate binds to the NMDA receptor and to other receptor subtypes, and is thought to activate an intracellular Ca^{2+} influx that increases ROS production and causes neurotoxicity.[105] Therefore, blocking glutamate neurotoxicity by inhibiting the NMDA receptor and/or decreasing glutamate release is a target strategy for neuroprotection in glaucoma.

p0210 Exposure to NMDA causes thinning of retinal thickness in the inner plexiform layer (IPL) of the retina and the apoptosis of RGCs, as shown by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) in the ganglion cell layer (GCL) (Fig. 41.6). EAGK and 3,5-DCQA were found to have protective effects on the thinning of the IPL and an increased number of TUNEL-positive cells in NMDA-induced rat retinas.[99] The neuroprotective effects of EAGK and 3,5-DCQA may be due not only to their direct antioxidative-mediated anti-apoptotic effects, but also to their role in modulating the downstream signaling pathways involved in NMDA-induced excitotoxicity.

p0215 PONC is commonly used in the study of glaucoma and has been shown to cause chronic glaucoma that has been well characterized by optic nerve transections. PONC can be carried out by clamping the optic nerve for several minutes after surgically exposing the optic nerve.[106] PONC is known to induce the generation of ROS, causing the slow, chronic, and synchronous death of RGCs.[107]

p0220 To determine whether the EAGK has a neuroprotective effect in the retina, the PONC model with retrograde labeling using Fluoro-Gold into the superior colliculus was used (Fig. 41.7). Fluorescence in the Fluoro-Gold-labeled RGCs was observed 1 week after PONC. As shown in Figure 41.7, the average density of RGCs was significantly decreased by PONC; however, pretreatment with EAGK significantly decreased the loss of RGCs.

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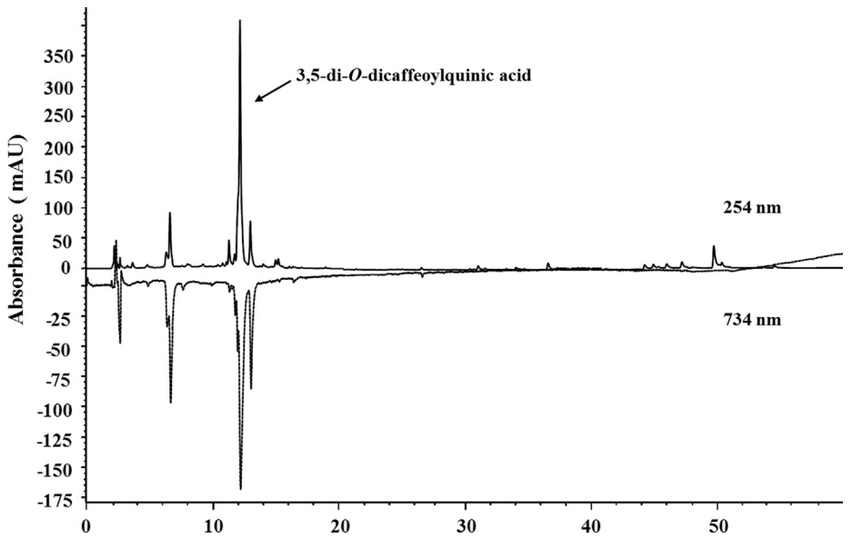

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FIGURE 41.4 Online high-performance liquid chromatography (HPLC) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) analysis of the ethyl acetate fraction of *Gymnaster koraiensis*. The column conditions were as follows: an Eclipse SB-C_{18} Rapid Resolution column (150 mm × 4.6 mm inner diameter, 3.5 μm, Agilent) was used; the column temperature was maintained at 25°C; and the mobile phase consisted of 0.1% trifluoroacetic acid (TFA; solvent A) and acetonitrile (solvent B) with a flow rate of 0.7 mL/minute. The gradient program conditions were as follows: 0–5 minutes, 10% to 20% B in A; 5–15 minutes, 20% B; 15–30 minutes, 20% to 35% B; 30–35 minutes, 35% B; 35–43 minutes, 35% to 100% B; 43–45 minutes, 100% to 10% B; 45–55 minutes, equilibration at 10% B; diode array detector at 254 nm (positive trace) prior to reaction with $ABTS^+$ radicals and the analysis of antioxidant potential at 734 nm (negative trace); 5 mg/mL sample concentration; and 10 μL of injection volume. *Source: Unpublished data*.

s0035

CONCLUSIONS

p0225 The neuroprotective effects were investigated of edible wild vegetables collected from Pyeongchang, Korea, in transformed RGCs. Among them, *G. koraiensis* and 3,5-DCQA were found to possess potent neuroprotective effects both *in vitro* and *in vivo*. These effects may be due to their antiapoptotic properties by preventing the activation of caspase-3, PARP, and AIF (Fig. 41.8). The neuroprotective effects of *G. koraiensis* and 3,5-DCQA may also result from their antioxidant properties and involve the replenishment of the intracellular reduced GSH (rGSH):oxidized GSH (GSSG) ratio and the detoxification of H_2O_2 by stimulating antioxidant enzymes such as catalase and Gpx-1 (Fig. 41.8).[99]

p0230 It has been reported that excessive NMDA induces neurotoxicity in RGCs. A clinical study has shown that the level of glutamate in the vitreous body of glaucoma patients is two-fold higher than that observed in a control population of patients with only cataract.[108]

p0235 The NMDA receptor has a relatively high permeability to Ca^{2+} ions, which increase the production of ROS. Moreover, calcium overload changes mitochondrial membrane potential and induces apoptosis in the retina.[109,110] Intravitreal injection of NMDA caused a decrease in the levels of antioxidant proteins, such as Cu/Zn superoxide dismutase (SOD-1), catalase, and glutathione peroxidase-1.[99] However, the decreased level of antioxidant proteins was found to be attenuated by EAGK and 3,5-DCQA,[99] which may be explained by their ability to block the NMDA receptor or by indirectly reducing ROS production caused by the excessive activation of NMDA (Fig. 41.8).

p0240 *Gymnaster koraiensis* has long been used as a source of food and medicine in East Asia. Therefore, a major advantage of its use is that it is known to be well tolerated, even when considerable amounts are consumed. Edible wild vegetables, including *G. koraiensis*, may have a potential role in the prevention of glaucoma by inhibiting the overproduction of ROS. Most active compounds in natural products are phenolic compounds because of their antioxidant properties. 3,5-DCQA, the active compound in *G. koraiensis*, is also a phenolic compound and may serve as a biologically active compound template for standardization in the development of natural product medicines.

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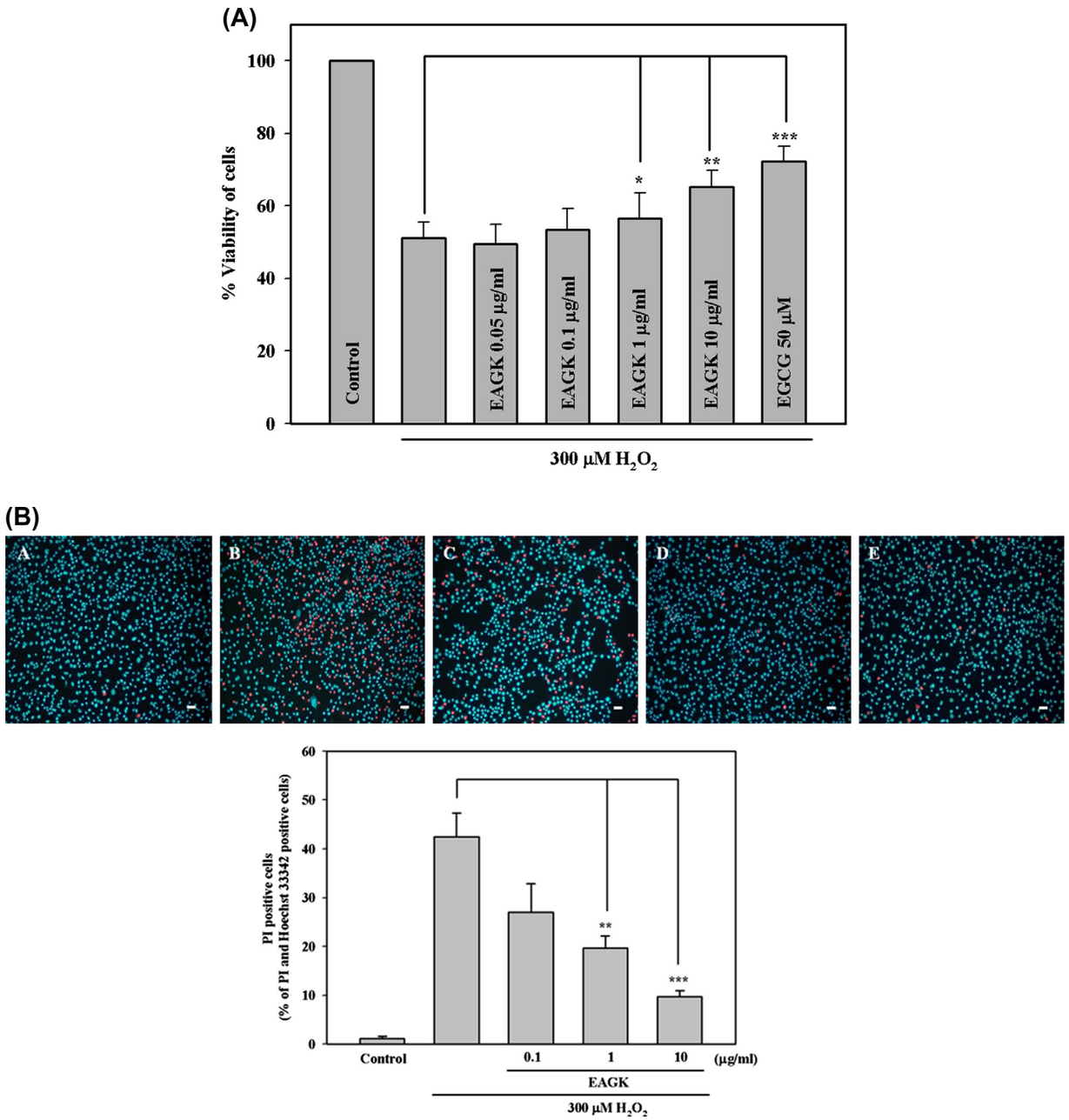

FIGURE 41.5 (A) Cell viability assay of the transformed retinal ganglion cells (RGC-5) exposed to 300 µM hydrogen peroxide (H_2O_2). The viability of the RGC-5 cells that were exposed to H_2O_2 was concentration dependent, with 300 µM H_2O_2 causing death in approximately 50% of the cells. The ethyl acetate fraction of *Gymnaster koraiensis* (EAGK) at 1 µg/mL and 10 µg/mL (as well as 50 µM epigallocatechin 3-gallate (EGCG)) significantly attenuated H_2O_2-induced cell death. Experimental values are expressed as the percentage of viable cells, with error bars indicating ±SEM from four independent experiments. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ indicate statistically significant differences compared with the cells exposed to H_2O_2 alone. (B) Microscopic analysis of RGC-5 cells using propidium iodide (PI) and Hoechst 33342 double staining. The RGC-5 cells were incubated with EAGK before exposure to 300 µM H_2O_2 for 24 hours, and then examined using fluorescence microscopy after staining with Hoechst 33342 and PI. Early apoptotic and necrotic cells were stained with PI (red), whereas apoptotic cells, having condensed and fragmented nuclei, were stained with Hoechst 33342 (blue). Control cells (A), and cells treated with 300 µM H_2O_2 (B), 300 µM H_2O_2 plus EAGK (0.1 µg/mL) (C), 300 µM H_2O_2 plus EAGK (1 µg/mL) (D), and 300 µM H_2O_2 plus EAGK (10 µg/mL) (E) clearly showed that EAGK blunted the negative effect of 300 µM H_2O_2, resulting in fewer red-stained cells. PI-positive cells were counted using a cell counter under a fluorescence microscope at 100× magnification, and four representative images were used to estimate the percent of PI-positive cells out of the total cell numbers (a minimum of 200 cells/well were counted), as shown in (F). The scale bar represents 50 µm. Experimental values are expressed as PI-positive cells, with error bars indicating mean ± SEM from three independent experiments (**$P<0.01$, ***$P<0.001$).

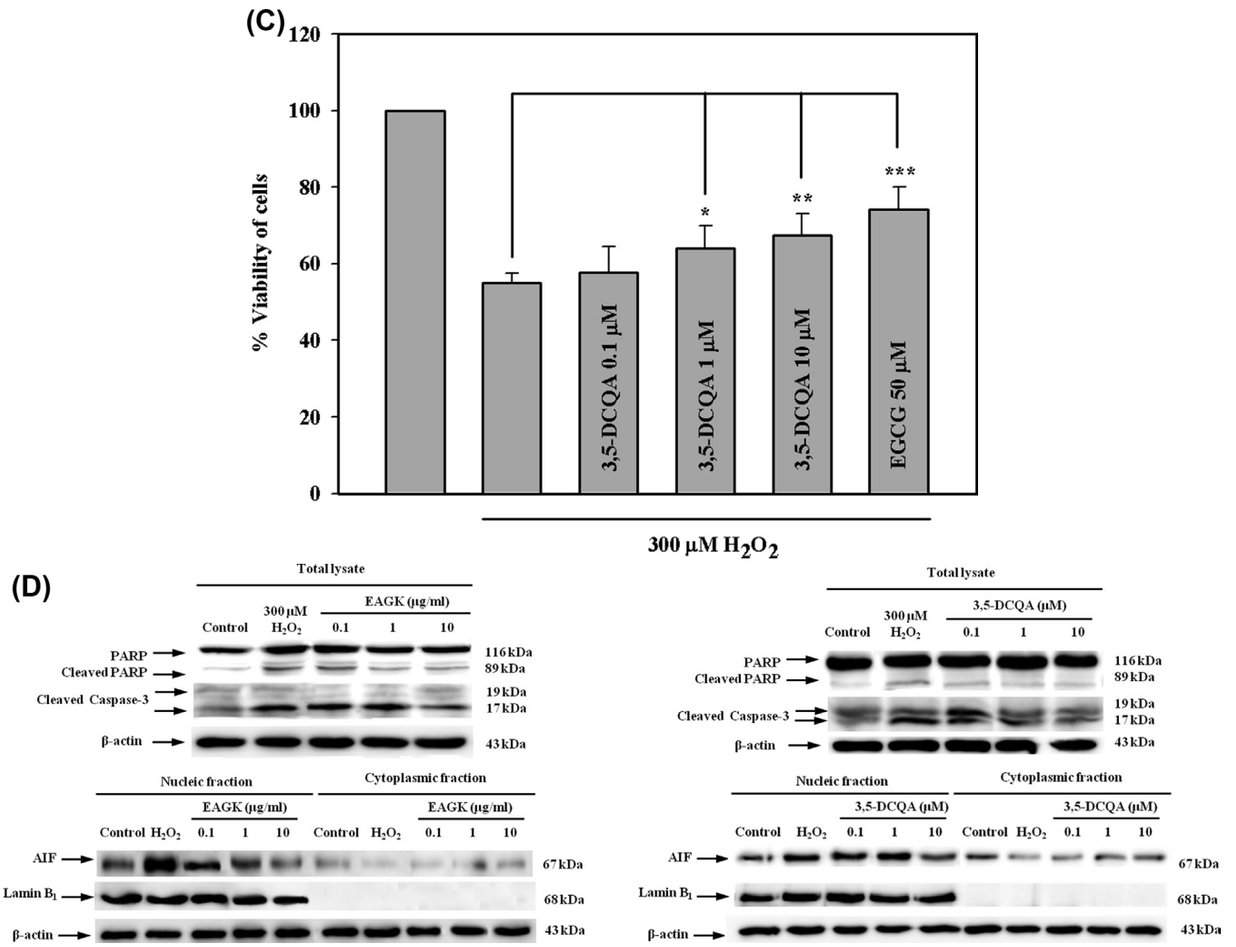

FIGURE 41.5 Cont'd (C) Effect of 3,5-di-*O*-caffeoylquinic acid (3,5-DCQA) isolated from EAGK on the viability of RGC-5 cells exposed to 300 μM H_2O_2 for 24 hours in culture, as measured by the MTT assay. Clearly, 1 μM and 10 μM 3,5-DCQA (as well as 50 μM EGCG) dose-dependently attenuated H_2O_2-induced cell death. Experimental values are expressed as a percentage of viable cells, with error bars indicating ± SEM from four independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate statistically significant differences in the cells exposed to H_2O_2. (d) Evaluation of the levels of various apoptotic proteins (poly (ADP-ribose) polymerase (PARP), apoptosis-inducing factor (AIF), and cleaved caspase-3) in RGC-5 cells exposed to 300 μM H_2O_2, with or without either EAGK or 3,5-DCQA treatment. Representative Western blot shows the expression levels of the apoptotic proteins in the total lysates, nucleic fractions, and cytoplasmic fractions isolated from the RGC-5 cells exposed to 300 μM H_2O_2, with or without either EAGK or 3,5-DCQA treatment. The Western blot densitometric analysis of apoptotic protein levels in the RGC-5 cells is shown. Protein level values are expressed as mean ± SEM from three independent experiments. *Source: Data from Kim* et al. *(2011).*[99] *Reproduced with permission from Elsevier.*

s0040

TAKE-HOME MESSAGES

p0245 • Natural products may be putative substances for the prevention and treatment of glaucoma by enabling retinal ganglion cell (RGC) neuroprotection.

u0015 • Numerous reports have been published demonstrating the protective effects of natural products on RGCs.

u0020 • *Gymnaster koraiensis* prevents RGC death caused by oxidative stress.

• *Gymnaster koraiensis* has potent direct and/or indirect antioxidant activities. u0025

• *Gymnaster koraiensis* protects against retinal degeneration caused by *N*-methyl-D-aspartate and optic nerve crush *in vivo*. u0030

• 3,5-Di-*O*-caffeoylquinic acid is the active compound in *G. koraiensis*. u0035

• Edible wild vegetables may have a potential role in the prevention of glaucoma through inhibition of the overproduction of reactive oxygen species. u0040

f0035

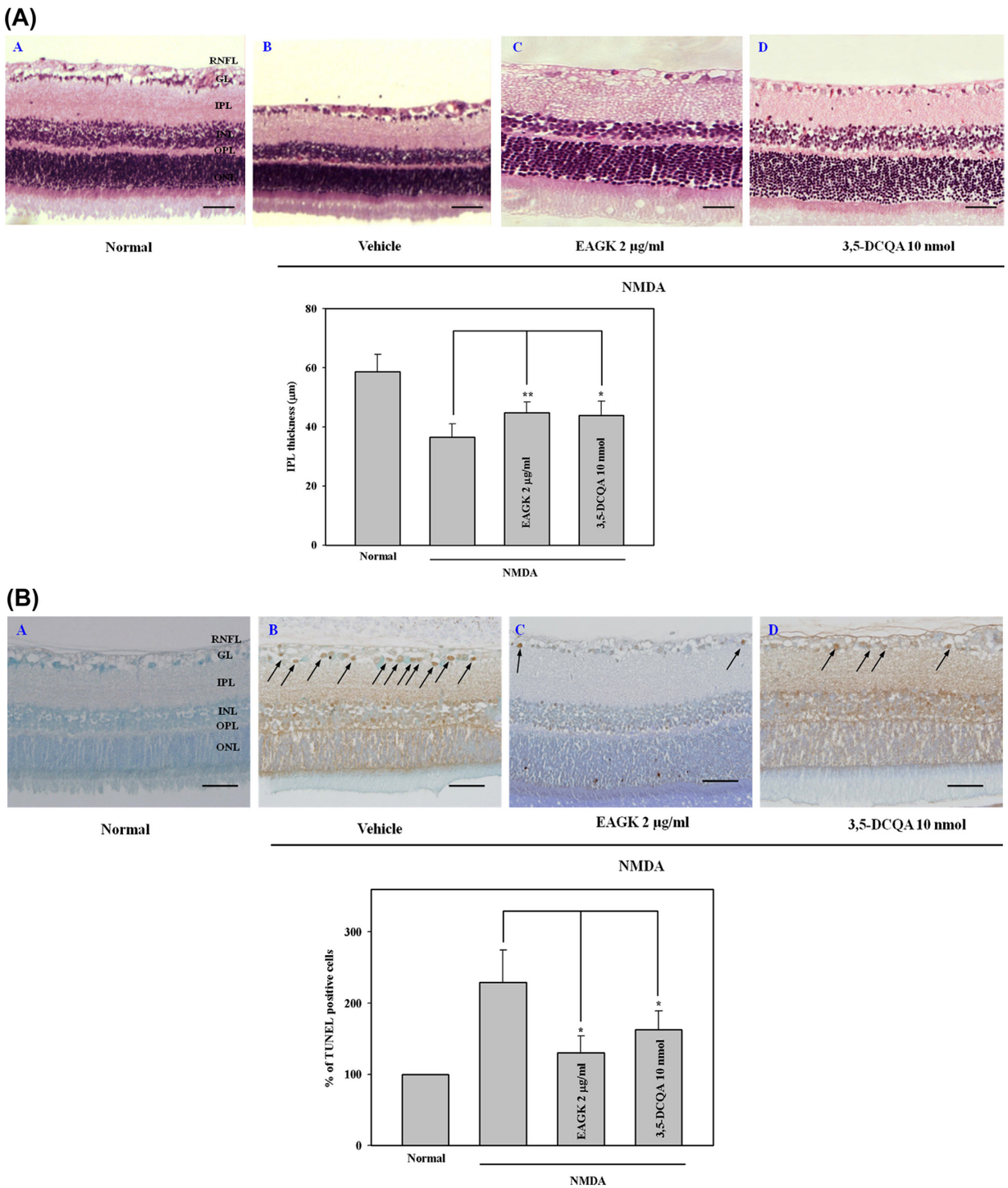

FIGURE 41.6 (A) Representative photomicrographs showing the histologic appearance of the retina induced by an intravitreous injection of *N*-methyl-D-aspartate (NMDA; hematoxylin and eosin staining, 400×). Non-treated (A), NMDA-treated (B), NMDA (5 nmol) plus the ethyl acetate fraction of *Gymnaster koraiensis* (EAGK, 2 μg/mL)-treated (C), and NMDA (5 nmol) plus 3,5-di-*O*-caffeoylquinic acid (3,5-DCQA; 10 nmol)-treated (D) retinal cross-sections after 7 days with or without NMDA are shown. The scale bar represents 50 μm. (F) shows the thickness of the inner plexiform layer (IPL). The results shown are the mean values with error bars indicating mean ± SEM from six independent experiments (*$P < 0.05$, **$P < 0.01$). (B) Antiapoptotic effect of EAGK or 3,5-DCQA demonstrated by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay (400×). Retinal damage was induced with an intravitreous injection of NMDA. Non-treated (A), NMDA-treated (B), NMDA (5 nmol) plus EAGK (2 μg/mL)-treated (C), and NMDA (5 nmol) plus 3,5-DCQA (10 nmol)-treated (D) retinal cross-sections after 24 hours with or without NMDA are shown. The arrows indicate TUNEL-positive cells (brown stain). The number of TUNEL-positive cells increased after NMDA injection, but NMDA (5 nmol) plus EAGK at 2 μg/mL and 3,5-DCQA at 10 nmol decreased the NMDA-induced retinal damage. The scale bar represents 50 μm. (F) shows the TUNEL-positive cells. The results are the mean values with error bars indicating mean ± SEM from six independent experiments (*$P < 0.05$). *Source: Data from Kim* et al. *(2011).*[99] *Reproduced with permission from Elsevier.*

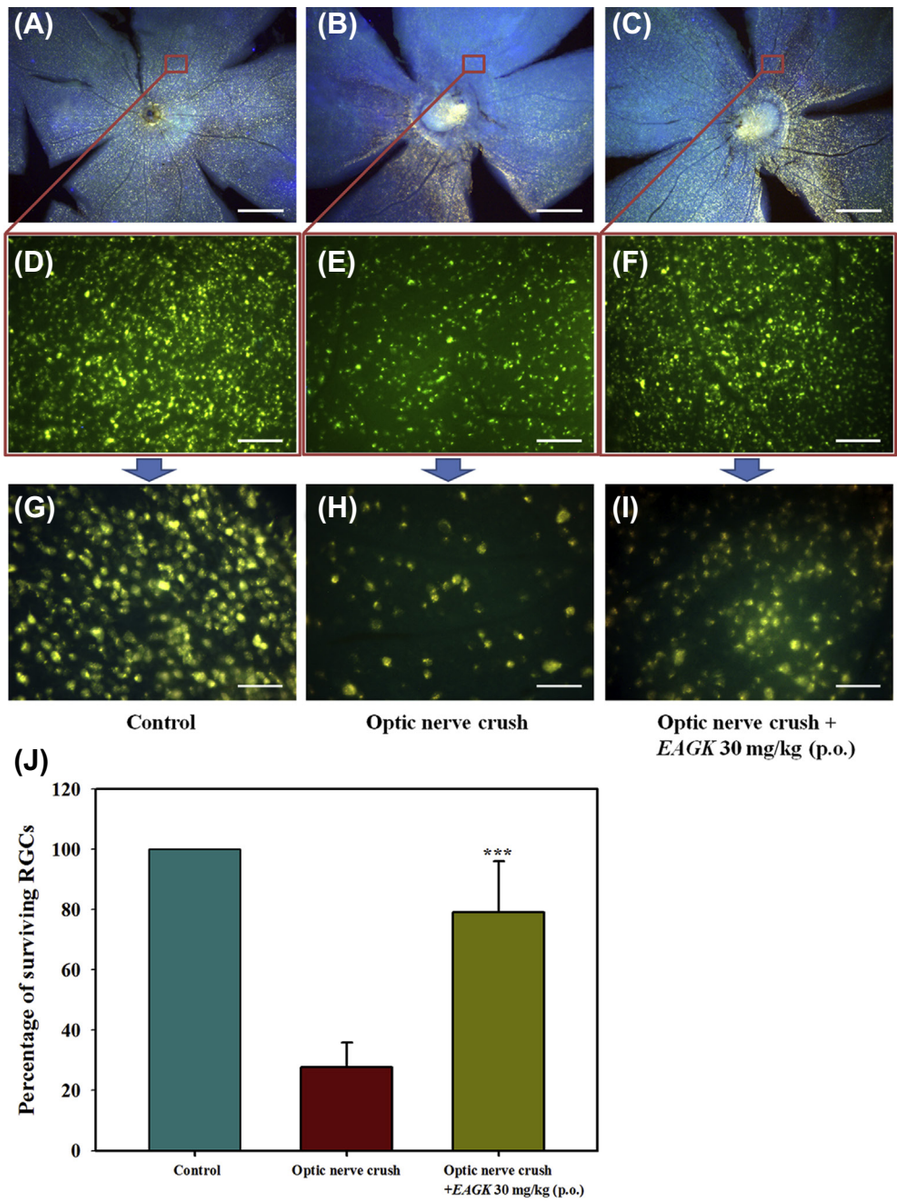

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FIGURE 41.7 Fluoro-Gold-labeled retinal ganglion cells (RGCs) in the mouse 1 week after partial optic nerve crush (PONC). Retrograde-labeled RGCs of mice with uninjured and injured optic nerves are shown. RGCs were labeled by injecting 3% Fluoro-Gold into the superior colliculi of the brain. The figure shows representative micrographs of normal retina (A) and damaged retina 12 days after PONC with (C) or without the ethyl acetate fraction of *Gymnaster koraiensis* (EAGK, B). The scale bars in (A), (B), and (C) represent 500 μm. Low-magnification (200×) images of labeled RGCs designated by the dark red boxes in (A), (B), and (C) are shown in (D), (E), and (F). The scale bars in (D), (E), and (F) represent 100 μm. High-magnification (400×) images of (A), (B), and (C) are shown in (G), (H), and (I). The scale bars in (G), (H), and (I) represent 50 μm. The results showed a significant increase in RGC survival after EAGK treatment compared with vehicle treatment. (J) Experimental values are expressed as the percentage of surviving RGCs with error bars indicating mean ± SEM from three independent experiments (***$P < 0.001$). *Source: Unpublished data.*

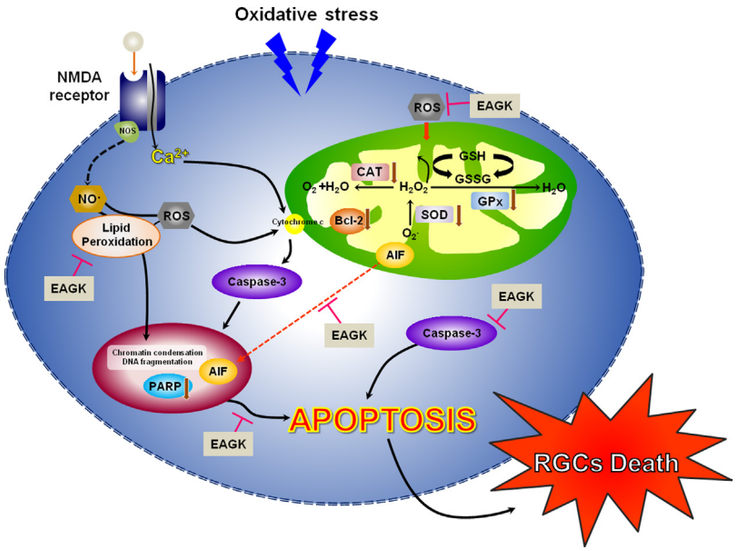

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FIGURE 41.8 Proposed mechanisms of action underlying the protective effects of *Gymnaster koraiensis* on oxidative stress-induced retinal damage. *Gymnaster koraiensis* can attenuate oxidative-stress induced retinal ganglion cells by inhibiting apoptotic proteins such as caspase-3, poly (ADP-ribose) polymerase (PARP) and apoptosis-inducing factor (AIF), and by inhibiting radical species and lipid peroxidation. Bcl-2: B-cell lymphoma 2; CAT: catalase; EAGK: ethyl acetate fraction of *Gymnaster koraiensis*; GPx: glutathione peroxidase; GSH: glutathione; GSSG: oxidized glutathione disulfide; NMDA: *N*-methyl-D-aspartate; NO: nitric oxide; NOS: nitric oxide synthase; RGCs: retinal ganglion cells; ROS: reactive oxygen species; SOD: superoxide dismutase.

s0045

References

1. McKinnon SJ. Glaucoma, apoptosis, and neuroprotection. *Curr Opin Ophthalmol* 1997;**8**:28–37.
2. Quigley HA. Glaucoma. *Lancet* 2011;**377**:1367–77.
3. Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. *Nat Rev Drug Discov* 2012;**11**:541–59.
4. AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol* 2000;**130**:429–40.
5. Morgan JE. Optic nerve head structure in glaucoma: astrocytes as mediators of axonal damage. *Eye (Lond)* 2000;**14**(Pt 3B):437–44.
6. Flammer J, Orgul S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res* 2002;**21**:359–93.
7. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol* 1998;**116**:1640–5.
8. Anderson DR, Hendrickson A. Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. *Invest Ophthalmol Vis Sci* 1974;**13**:771–83.
9. Rahman KM, Sugie S, Watanabe T, Tanaka T, Mori H. Chemopreventive effects of melatonin on diethylnitrosamine and phenobarbital-induced hepatocarcinogenesis in male F344 rats. *Nutr Cancer* 2003;**47**:148–55.
10. Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Arch Ophthalmol* 2003;**121**:48–56.
11. Tielsch JM, Katz J, Singh K, Quigley HA, Gottsch JD, Javitt J. Sommer AA population-based evaluation of glaucoma screening: the Baltimore Eye Survey. *Am J Epidemiol* 1991;**134**:1102–10.
12. Kim JH, Kang SY, Kim NR, Lee ES, Hong S, Seong GJ, et al. Prevalence and characteristics of glaucoma among Korean adults. *Korean J Ophthalmol* 2011;**25**:110–5.
13. Levin LA, Peeples P. History of neuroprotection and rationale as a therapy for glaucoma. *Am J Manag Care* 2008;**14**:S11–4.
14. Dat NT, Van Kiem P, Cai XF, Shen Q, Bae K, Kim YH. Gymnastone, a new benzofuran derivative from *Gymnaster koraiensis*. *Arch Pharm Res* 2004;**27**:1106–8.
15. Levin LA. Retinal ganglion cells and neuroprotection for glaucoma. *Surv Ophthalmol* 2003;**48**(Suppl. 1):S21–4.
16. Danesh-Meyer HV. Neuroprotection in glaucoma: recent and future directions. *Curr Opin Ophthalmol* 2011;**22**:78–86.
17. Osborne NN. Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned. *Acta Ophthalmol* 2009;**87**:450–4.
18. Mozaffarieh M, Grieshaber MC, Orgul S, Flammer J. The potential value of natural antioxidative treatment in glaucoma. *Surv Ophthalmol* 2008;**53**:479–505.
19. Rodieck RW. Maintained activity of cat retinal ganglion cells. *J Neurophysiol* 1967;**30**:1043–71.
20. Danesh-Meyer HV, Levin LA. Neuroprotection: extrapolating from neurologic diseases to the eye. *Am J Ophthalmol* 2009;**148**:186–91. e182.
21. Berkelaar M, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. *J Neurosci* 1994;**14**:4368–74.
22. Garcia-Valenzuela E, Gorczyca W, Darzynkiewicz Z, Sharma SC. Apoptosis in adult retinal ganglion cells after axotomy. *J Neurobiol* 1994;**25**:431–8.
23. Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci* 1995;**36**:774–86.
24. Kerrigan LA, Zack DJ, Quigley HA, Smith SD, Pease ME. TUNEL-positive ganglion cells in human primary open-angle glaucoma. *Arch Ophthalmol* 1997;**115**:1031–5.
25. Marcic TS, Belyea DA, Katz B. Neuroprotection in glaucoma: a model for neuroprotection in optic neuropathies. *Curr Opin Ophthalmol* 2003;**14**:353–6.
26. Cheung W, Guo L, Cordeiro MF. Neuroprotection in glaucoma: drug-based approaches. *Optom Vis Sci* 2008;**85**:406–16.
27. Baltmr A, Duggan J, Nizari S, Salt TE, Cordeiro MF. Neuroprotection in glaucoma – is there a future role? *Exp Eye Res* 2010;**91**:554–66.

28. Levkovitch-Verbin H, Martin KR, Quigley HA, Baumrind LA, Pease ME, Valenta D. Measurement of amino acid levels in the vitreous humor of rats after chronic intraocular pressure elevation or optic nerve transection. *J Glaucoma* 2002;**11**:396–405.
29. Kumar DM, Agarwal N. Oxidative stress in glaucoma: a burden of evidence. *J Glaucoma* 2007;**16**:334–43.
30. Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. Oxidative stress markers in aqueous humor of glaucoma patients. *Am J Ophthalmol* 2004;**137**:62–9.
31. Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL. Systemic reduction in glutathione levels occurs in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2005;**46**:877–83.
32. Sacca SC, Pascotto A, Camicione P, Capris P, Izzotti A. Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. *Arch Ophthalmol* 2005;**123**:458–63.
33. Jung SH, Kim BJ, Lee EH, Osborne NN. Isoquercitrin is the most effective antioxidant in the plant *Thuja orientalis* and able to counteract oxidative-induced damage to a transformed cell line (RGC-5 cells). *Neurochem Int* 2010;**57**:713–21.
34. Li SY, Lo AC. Lutein protects RGC-5 cells against hypoxia and oxidative stress. *Int J Mol Sci* 2010;**11**:2109–17.
35. Nakajima Y, Inokuchi Y, Shimazawa M, Otsubo K, Ishibashi T, Hara H. Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress *in vitro* and in mice *in vivo*. *J Pharm Pharmacol* 2008;**60**:1365–74.
36. Nakajima Y, Shimazawa M, Otsubo K, Ishibashi T, Hara H. Zeaxanthin, a retinal carotenoid, protects retinal cells against oxidative stress. *Curr Eye Res* 2009;**34**:311–8.
37. Aguilera Y, Dorado ME, Prada FA, Martínez JJ, Quesada A, Ruiz-Gutiérrez V. The protective role of squalene in alcohol damage in the chick embryo retina. *Exp Eye Res* 2005;**80**:535–43.
38. Jung SH, Kang KD, Ji D, Fawcett RJ, Safa R, Kamalden TA, Osborne NN. The flavonoid baicalin counteracts ischemic and oxidative insults to retinal cells and lipid peroxidation to brain membranes. *Neurochem Int* 2008;**53**:325–37.
39. Chen YQ, Pan WH, Liu JH, Chen MM, Liu CM, Yeh MY, et al. The effects and underlying mechanisms of *S*-allyl-*l*-cysteine treatment of the retina after ischemia/reperfusion. *J Ocul Pharmacol Ther* 2012;**28**:110–7.
40. Lee HJ, Kim KA, Kang KD, Lee EH, Kim CY, Um BH, Jung SH. The compound isolated from the leaves of *Phyllostachys nigra* protects oxidative stress-induced retinal ganglion cells death. *Food Chem Toxicol* 2010;**48**:1721–7.
41. Chan HC, Chang RC, Koon-Ching Ip A, Chiu K, Yuen WH, Zee SY, So KF. Neuroprotective effects of *Lycium barbarum* Lynn on protecting retinal ganglion cells in an ocular hypertension model of glaucoma. *Exp Neurol* 2007;**203**:269–73.
42. Chiu K, Chan HC, Yeung SC, Yuen WH, Zee SY, Chang RC, So KF. Modulation of microglia by wolfberry on the survival of retinal ganglion cells in a rat ocular hypertension model. *J Ocul Biol* 2009;**2**:47–56.
43. Yang M, Gao N, Zhao Y, Liu L-X, Lu X- J. Protective effect of *Lycium barbarum* polysaccharide on retinal ganglion cells *in vitro*. *Int J Ophthalmol* 2011;**4**:377–9.
44. Kamalden TA, Ji D, Osborne NN. Rotenone-induced death of RGC-5 cells is caspase independent, involves the JNK and p38 pathways and is attenuated by specific green tea flavonoids. *Neurochem Res* 2012;**37**:1091–101.
45. Osborne NN, Ji D, Abdul Majid AS, Fawcett RJ, Sparatore A, Del Soldato P. ACS67, a hydrogen sulfide-releasing derivative of latanoprost acid, attenuates retinal ischemia and oxidative stress to RGC-5 cells in culture. *Invest Ophthalmol Vis Sci* 2010;**51**:284–94.
46. Zhang B, Osborne NN. Oxidative-induced retinal degeneration is attenuated by epigallocatechin gallate. *Brain Res* 2006;**1124**:176–7.
47. Zhang B, Rusciano D, Osborne NN. Orally administered epigallocatechin gallate attenuates retinal neuronal death *in vivo* and light-induced apoptosis *in vitro*. *Brain Res* 2008;**1198**:141–52.
48. Zhang B, Safa R, Rusciano D, Osborne NN. Epigallocatechin gallate, an active ingredient from green tea, attenuates damaging influences to the retina caused by ischemia/reperfusion. *Brain Res* 2007;**1159**:40–53.
49. Inokuchi Y, Shimazawa M, Nakajima Y, Suemori S, Mishima S, Hara H. Brazilian green propolis protects against retinal damage *in vitro* and *in vivo*. *Evid Based Complement Alternat Med* 2006;**3**:71–7.
50. Nakajima Y, Shimazawa M, Mishima S, Hara H. Water extract of propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. *Life Sci* 2007;**80**:370–7.
51. Nakajima Y, Shimazawa M, Mishima S, Hara H. Neuroprotective effects of Brazilian green propolis and its main constituents against oxygen–glucose deprivation stress, with a gene-expression analysis. *Phytother Res* 2009;**23**:1431–8.
52. El-Sayyad HI, El-Sherbiny MA, Sobh MA, Abou-El-Naga AM, Ibrahim MA, Mousa SA. Protective effects of *Morus alba* leaves extract on ocular functions of pups from diabetic and hypercholesterolemic mother rats. *Int J Biol Sci* 2011;**7**:715–28.
53. Choi SJ, Lee MY, Jo H, Lim SS, Jung SH. Preparative isolation and purification of neuroprotective compounds from *Rhus verniciflua* by high speed counter-current chromatography. *Biol Pharm Bull* 2012;**35**:559–67.
54. Chung HS, Harris A, Kristinsson JK, Ciulla TA, Kagemann C, Ritch R. *Ginkgo biloba* extract increases ocular blood flow velocity. *J Ocul Pharmacol Ther* 1999;**15**:233–40.
55. Hirooka K, Tokuda M, Miyamoto O, Itano T, Baba T, Shiraga F. The *Ginkgo biloba* extract (EGb 761) provides a neuroprotective effect on retinal ganglion cells in a rat model of chronic glaucoma. *Curr Eye Res* 2004;**28**:153–7.
56. Ma K, Xu L, Zhan H, Zhang S, Pu M, Jonas JB. Dosage dependence of the effect of *Ginkgo biloba* on the rat retinal ganglion cell survival after optic nerve crush. *Eye* 2009;**23**:1598–604.
57. Ma K, Xu L, Zhang H, Zhang S, Pu M, Jonas JB. The effect of *Ginkgo biloba* on the rat retinal ganglion cell survival in the optic nerve crush model. *Acta Ophthalmol* 2010;**88**:553–7.
58. Park JW, Kwon HJ, Chung WS, Kim CY, Seong GJ. Short-term effects of *Ginkgo biloba* extract on peripapillary retinal blood flow in normal tension glaucoma. *Korean J Ophthalmol* 2011;**25**:323–8.
59. Quaranta L, Bettelli S, Uva MG, Semeraro F, Turano R, Gandolfo E. Effect of *Ginkgo biloba* extract on preexisting visual field damage in normal tension glaucoma. *Ophthalmology* 2003;**110**:359–62. discussion 362–4.
60. Shim SH, Kim JM, Choi CY, Kim CY, Park KH. *Ginkgo biloba* extract and bilberry anthocyanins improve visual function in patients with normal tension glaucoma. *J Med Food* 2012;**15**:818–23.
61. Wang YS, Xu L, Ma K, Wang JJ. The protective effects of *Ginkgo biloba* extract on cultured human retinal ganglion cells. *Chin J Ophthalmol* 2011;**47**:824–8.
62. Wang YQ, Li XF, Zhou X, Liu XQ, Wang WP. Protective effects of Chinese herbal medicine Zuogui Pill on retina ganglion cells after optical nerve clipping injury in rats. *J Chin Integr Med* 2011;**9**:991–7.
63. Tsuruma K, Shimazaki H, Nakashima K, Yamauchi M, Sugitani S, Shimazawa M, et al. Annatto prevents retinal degeneration induced by endoplasmic reticulum stress *in vitro* and *in vivo*. *Mol Nutr Food Res* 2012;**56**:713–24.

10041-PREEDY-9780124017177

64. Matsunaga N, Imai S, Inokuchi Y, Shimazawa M, Yokota S, Araki Y, Hara H. Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage *in vitro* and *in vivo*. *Mol Nutr Food Res* 2009;**53**:869–77.
65. Yamauchi M, Tsuruma K, Imai S, Nakanishi T, Umigai N, Shimazawa M, Hara H. Crocetin prevents retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity. *Eur J Pharmacol* 2011;**650**:110–9.
66. Burugula B, Ganesh BS, Chintala SK. Curcumin attenuates staurosporine-mediated death of retinal ganglion cells. *Invest Ophthalmol Vis Sci* 2011;**52**:4263–73.
67. Tong N, Zhang Z, Gong Y, Yin L, Wu X. Diosmin protects rat retina from ischemia/reperfusion injury. *J Ocul Pharmacol Ther* 2012;**28**:459–66.
68. Kim KA, Kim SM, Kang SW, Jeon SI, Um BH, Jung SH. Edible seaweed, *Eisenia bicyclis*, protects retinal ganglion cells death caused by oxidative stress. *Mar Biotechnol* 2012;**14**:383–95.
69. Li C, Wang L, Huang K, Zheng L. Endoplasmic reticulum stress in retinal vascular degeneration: protective role of resveratrol. *Invest Ophthalmol Vis Sci* 2012;**53**:3241–9.
70. Yang H, Lee BK, Kook KH, Jung YS, Ahn J. Protective effect of grape seed extract against oxidative stress-induced cell death in a staurosporine-differentiated retinal ganglion cell line. *Curr Eye Res* 2012;**37**:339–44.
71. Maher P, Hanneken A. Flavonoids protect retinal ganglion cells from oxidative stress-induced death. *Invest Ophthalmol Vis Sci* 2005;**46**:4796–803.
72. Agarwal R, Gupta SK, Srivastava S, Saxena R, Agrawal SS. Intraocular pressure-lowering activity of topical application of *Aegle marmelos* fruit extract in experimental animal models. *Ophthalmic Res* 2009;**42**:112–6.
73. Ou HC, Lee WJ, Lee IT, Chiu TH, Tsai KL, Lin CY, Sheu WH. *Ginkgo biloba* extract attenuates oxLDL-induced oxidative functional damages in endothelial cells. *J Appl Physiol* 2009;**106**:1674–85.
74. Mahadevan S, Park Y. Multifaceted therapeutic benefits of *Ginkgo biloba* L.: chemistry, efficacy, safety, and uses. *J Food Sci* 2008;**73**:R14–9.
75. Wu Y, Li S, Cui W, Zu X, Du J, Wang F. *Ginkgo biloba* extract improves coronary blood flow in healthy elderly adults: role of endothelium-dependent vasodilation. *Phytomedicine* 2008;**15**:164–9.
76. Kanwar J, Taskeen M, Mohammad I, Huo C, Chan TH, Dou QP. Recent advances on tea polyphenols. *Front Biosci (Elite Ed)* 2012;**4**:111–31.
77. Andrade JP, Assuncao M. Protective effects of chronic green tea consumption on age-related neurodegeneration. *Curr Pharm Des* 2012;**18**:4–14.
78. Tedeschi E, Suzuki H, Menegazzi M. Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition. *Ann N Y Acad Sci* 2002;**973**:435–7.
79. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev* 2002;**11**:1025–32.
80. Kijlstra A, Tian Y, Kelly ER, Berendschot TT. Lutein: more than just a filter for blue light. *Prog Retin Eye Res* 2012;**31**:303–15.
81. Calvo MM. Lutein: a valuable ingredient of fruit and vegetables. *Crit Rev Food Sci Nutr* 2005;**45**:671–96.
82. Cao Y, Mao X, Sun C, Zheng P, Gao J, Wang X, et al. Baicalin attenuates global cerebral ischemia/reperfusion injury in gerbils via anti-oxidative and anti-apoptotic pathways. *Brain Res Bull* 2011;**85**:396–402.
83. Tu XK, Yang WZ, Shi SS, Chen Y, Wang CH, Chen CM, Chen Z. Baicalin inhibits TLR2/4 signaling pathway in rat brain following permanent cerebral ischemia. *Inflammation* 2011;**34**:463–70.
84. Ge QF, Hu X, Ma ZQ, Liu JR, Zhang WP, Chen Z, Wei EQ. Baicalin attenuates oxygen-glucose deprivation-induced injury via inhibiting NMDA receptor-mediated 5-lipoxygenase activation in rat cortical neurons. *Pharmacol Res* 2007;**55**:148–57.
85. Heijnen CG, Haenen GR, Vekemans JA, Bast A. Peroxynitrite scavenging of flavonoids: structure activity relationship. *Environ Toxicol Pharmacol* 2001;**10**:199–206.
86. Heijnen CG, Haenen GR, van Acker FA, van der Vijgh WJ, Bast A. Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups. *Toxicol In Vitro* 2001;**15**:3–6.
87. Wang L, Tu YC, Lian TW, Hung JT, Yen JH, Wu MJ. Distinctive antioxidant and antiinflammatory effects of flavonols. *J Agric Food Chem* 2006;**54**:9798–804.
88. Yang B, Kotani A, Arai K, Kusu F. Estimation of the antioxidant activities of flavonoids from their oxidation potentials. *Anal Sci* 2001;**17**:599–604.
89. Kim SM, Shang YF, Um BH. A preparative method for isolation of fucoxanthin from *Eisenia bicyclis* by centrifugal partition chromatography. *Phytochem Anal* 2011;**22**:322–9.
90. Rajyalakshmi P, Geervani P. Nutritive value of the foods cultivated and consumed by the tribals of south India. *Plant Foods Hum Nutr* 1994;**46**:53–61.
91. Ogle BM, Johansson M, Tuyet HT, Johannesson L. Evaluation of the significance of dietary folate from wild vegetables in Vietnam. *Asia Pac J Clin Nutr* 2001;**10**:216–21.
92. Lee JY, Song D, Lee EH, Jung SH, Nho CW, Cha KH, Pan C. Inhibitory effects of 3,5-*O*-dicaffeoyl-epi-quinic acid from *Gymnaster koraiensis* on AKR1B10. *J Korean Soc Appl Biol Chem* 2009;**52**:731–4.
93. Kim HJ, Hong J, Jung JW, Kim TH, Kim JA, Kim YH, Kim SY. Gymnasterkoreayne F inhibits osteoclast formation by suppressing NFATc1 and DC-STAMP expression. *Int Immunopharmacol* 2010;**10**:1440–7.
94. Lee J, Lee YM, Lee BW, Kim JH, Kim JS. Chemical constituents from the aerial parts of *Aster koraiensis* with protein glycation and aldose reductase inhibitory activities. *J Nat Prod* 2012;**75**:267–70.
95. Dat NT, Cai XF, Shen Q, Lee IS, Lee EJ, Park YK, et al. Gymnasterkoreayne G, a new inhibitory polyacetylene against NFAT transcription factor from *Gymnaster koraiensis*. *Chem Pharm Bull (Tokyo)* 2005;**53**:1194–6.
96. Lee SB, Kang K, Oidovsambuu S, Jho EH, Yun JH, Yoo JH, et al. A polyacetylene from *Gymnaster koraiensis* exerts hepatoprotective effects *in vivo* and *in vitro*. *Food Chem Toxicol* 2010;**48**:3035–41.
97. Jung HJ, Min BS, Park JY, Kim YH, Lee HK, Bae KH. Gymnasterkoreaynes A-F, cytotoxic polyacetylenes from *Gymnaster koraiensis*. *J Nat Prod* 2002;**65**:897–901.
98. Park J, Min B, Jung H, Kim Y, Lee H, Bae K. Polyacetylene glycosides from *Gymnaster koraiensis*. *Chem Pharm Bull (Tokyo)* 2002;**50**:685–7.
99. Kim KA, Kang KD, Lee EH, Nho CW, Jung SH. Edible wild vegetable, *Gymnaster koraiensis* protects retinal ganglion cells against oxidative stress. *Food Chem Toxicol* 2011;**49**:2131–43.
100. Aslan M, Cort A, Yucel I. Oxidative and nitrative stress markers in glaucoma. *Free Radic Biol Med* 2008;**45**:367–76.
101. Wei Y, Gong J, Yoshida T, Eberhart CG, Xu Z, Kombairaju P, et al. Nrf2 has a protective role against neuronal and capillary degeneration in retinal ischemia–reperfusion injury. *Free Radic Biol Med* 2011;**51**:216–24.
102. Harvey CJ, Thimmulappa RK, Singh A, Blake DJ, Ling G, Wakabayashi N, et al. Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radic Biol Med* 2009;**46**:443–53.
103. Wu JH, Huang CY, Tung YT, Chang ST. Online RP-HPLC-DPPH screening method for detection of radical-scavenging phytochemicals from flowers of *Acacia confusa*. *J Agric Food Chem* 2008;**56**:328–32.

10041-PREEDY-9780124017177

104. Osborne NN, Ugarte M, Chao M, Chidlow G, Bae JH, Wood JP, Nash MS. Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Surv Ophthalmol* 1999;**43**(Suppl. 1):S102–28.
105. Yu SP, Yeh C, Strasser U, Tian M, Choi DW. NMDA receptor-mediated K^+ efflux and neuronal apoptosis. *Science* 1999;**284**:336–9.
106. Leung CK, Lindsey JD, Crowston JG, Lijia C, Chiang S, Weinreb RN. Longitudinal profile of retinal ganglion cell damage after optic nerve crush with blue-light confocal scanning laser ophthalmoscopy. *Invest Ophthalmol Vis Sci* 2008;**49**:4898–902.
107. Steinsapir KD, Goldberg RA. Traumatic optic neuropathy. *Surv Ophthalmol* 1994;**38**:487–518.
108. Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol* 1996;**114**:299–305.
109. Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. *J Neurosci* 1996;**16**:6125–33.
110. Kwong JM, Lam TT. *N*-Methyl-D-aspartate (NMDA) induced apoptosis in adult rabbit retinas. *Exp Eye Res* 2000;**71**:437–44.

10041-PREEDY-9780124017177

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Non-Print Items

Abstract
Progressive degeneration and death of retinal ganglion cells (RGCs) is a major risk factor for glaucoma. Increased intraocular pressure (IOP) is also known as a major risk factor and an underlying mechanism in the pathogenesis of glaucoma. Therefore, reduction of IOP is a primary target in glaucoma treatment. However, alternative approaches are needed because reduction of IOP is not always effective in patients with glaucoma (e.g., normal-tension glaucoma). One possible way to prevent or treat glaucoma is to protect RGCs by inhibiting the apoptotic cascade. Oxidative stress also plays an important role in the pathogenesis of glaucoma, and natural products such as natural antioxidants may be putative substances for preventing and treating glaucoma via neuroprotection of RGCs. This chapter summarizes the available data on natural products with potential neuroprotective effects for RGCs, and discusses the use of edible wild vegetables, which may have a role in the prevention of glaucoma by inhibiting the overproduction of reactive oxygen species.

Keywords: apoptosis; edible wild vegetable; glaucoma; intraocular pressure; natural product; neuroprotection; retinal ganglion cell.