**Ginkgo biloba** Extract and Bilberry Anthocyanins

Improve Visual Function in Patients with Normal Tension Glaucoma

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ABSTRACT  **Ginkgo biloba** extract (GBE) and anthocyanins are considered beneficial for various vascular diseases. This study was performed to evaluate the effect of GBE and anthocyanins on visual function in patients with normal tension glaucoma (NTG) based on the vascular theory of mechanisms of glaucomatous optic nerve damage. Retrospective analysis was carried out by a chart review of 332 subjects (209 men and 123 women) who were treated with anthocyanins (n = 132), GBE (n = 103), or no medication (control, n = 97). Humphrey Visual Field (HVF) test, logarithm of the minimal angle of resolution best-corrected visual acuity (logMAR BCVA), intraocular pressure, blood pressure, and fasting blood glucose were determined before and after treatment. Complete ocular and systemic examinations were performed. The mean follow-up duration was 23.82 ± 9.84 (range, 12–59) months; the mean anthocyanin treatment duration was 24.32 ± 10.43 (range, 6–53) months, and the mean GBE treatment duration was 23.81 ± 10.36 months (range, 6–59) months. After anthocyanin treatment, the mean BCVA for all eyes improved from 0.16 (± 0.34) to 0.11 (± 0.18) logMAR units (P = .008), and HVF mean deviation improved from −6.44 (± 7.05) to −5.34 (± 6.42) (P = .001). After GBE treatment, HVF mean deviation improved from −5.25 (± 6.13) to −4.31 (± 5.60) (P = .002). A generalized linear model demonstrated that the final BCVA was not affected by demographic differences among the groups. These results suggest that anthocyanins and GBE may be helpful in improving visual function in some individuals with NTG.

KEY WORDS: • anthocyanins • antioxidants • bilberry • gingko

INTRODUCTION

NORMAL TENSION GLAUCOMA (NTG) is a form of open-angle glaucoma in which damages to the optic nerve and visual field are present, despite normal intraocular pressure (IOP).1 The exact mechanisms of the anatomic and functional damage in NTG are still unknown, but two theories of how NTG occurs are the vascular theory (reduced blood flow to the optic nerve) and the mechanical theory (relatively high IOP).2 Some patients with NTG continue to have visual field damage despite medical therapy or surgical procedures. Therefore, the pathogenic role of other factors warrants consideration.3 Retinal ganglion cell loss is the main process of glaucomatous optic nerve and is triggered by various factors, including depletion of neurotrophin,4 glutamate neuroexcitotoxicity,5–9 ischemia,10 nitric oxide and the formation of reactive oxygen species,11 autoimmune processes,12 and inflammation.13

Based on these theories, **Ginkgo biloba** extract (GBE) is used for numerous retinal disorders and glaucoma. GBE is also used by patients with peripheral vascular disease and to treat cerebral insufficiency.14 Mechanisms of action of GBE have been described: (1) effects on blood circulation, such as vasoregulatory activity and rheological effects (decreased viscosity and antagonism of platelet-activating factor receptors); (2) metabolic changes, for example, effects on neuron metabolism such as increased tolerance to anoxia; (3) beneficial effects on neurotransmitter disturbances; and (4) prevention of damage to cell membranes caused by free radicals.14

In addition to GBE, anthocyanins from *Vaccinium myrtillus* or bilberry are increasingly being used in ophthalmology. In fact, *V. myrtillus* has a long history of medical use.15 The active components, flavonoid anthocyanosides (anthocyanins), have a particular affinity for the eye and vascular tissues.16 Several mechanisms of action of anthocyanins have been described: (1) strong antioxidant properties;17 (2) stabilization of collagen fibers and promotion of collagen biosynthesis;18,19 (3) decreased capillary permeability and fragility;20 (4) inhibition of platelet aggregation;21 (5) prevention of the release and synthesis of...
proinflammatory compounds such as histamine, prostaglandins, and leukotrienes; and (6) lower blood glucose levels.

Recently, favorable effects of anthocyanins and GBE have been reported, although the quality of most of these investigations is questionable. Based on the reported favorable effects of anthocyanins and GBE on blood circulation and their antioxidative effects, anthocyanins and GBE are used by clinicians to treat patients with NTG and diabetic retinopathy. Therefore, we aimed to evaluate the effects of anthocyanins and GBE on the general visual function of patients with NTG.

**MATERIALS AND METHODS**

We first performed a retrospective study with a chart review. Subjects with a 12-month or more follow-up were recruited from the outpatient glaucoma service of the Department of Ophthalmology, Kangbuk Samsung Hospital, Seoul, Korea, from January 2005 to December 2010. This study was performed in adherence with the Declaration of Helsinki and after approval from the Institutional Review Board and Ethics Committee of the Kangbuk Samsung Hospital in Seoul, Korea. We studied 332 consecutive patients (664 eyes), 209 men and 123 women (mean age, 52.90 years; range, 20–89 years), who had monocular/binocular NTG. Among 332 consecutive NTG patients, 132 patients were assigned to the anthocyanin treatment group, 103 to the GBE treatment group, and 97 to the control (nontreatment) group. The control patients consisted of subjects who had monocular/binocular NTG and were recruited during the same period at the same center.

All subjects underwent a full medical and ocular history and a detailed ocular examination, including visual acuity, IOP measurement using the Goldman applanation tonometer, the Humphrey visual field (HVF) test, red-free disc photo, stereo disc photo, optical coherence tomography (OCT) examination, gonioscopy, slit-lamp examination, and fundus examination. Systolic and diastolic blood pressure and fasting blood glucose were measured.

Included were patients with a diagnosis of NTG, defined as optic disc abnormalities consistent with glaucomatous optic neuropathy with or without visual field loss. Glaucomatous optic disc abnormality was defined as neuroretinal rim thinning, notching, excavation, or a retinal nerve fiber layer (RNFL) defect. Glaucomatous visual field loss was defined as a pattern standard deviation outside 95% normal limits or a glaucoma hemifield test result that was not within normal limits and abnormally high sensitivity, confirmed with two or more consecutive more visual field tests. Other inclusion criteria for both normal and glaucomatous subjects were (1) IOP of 21-mm Hg or less without topical hypotensive therapy (highest values recorded during diurnal measurements, made from 9 AM to 5 PM, every 2 h by the Goldmann applanation tonometer at first visit); (2) 20 years or older; (3) logarithm of the minimal angle of resolution best-corrected visual acuity (logMAR BCVA) 0.70 or better; (4) no history of amblyopia; (5) no history of ocular or neurologic disease or surgery that might cause test results or vision changes that confound recognition of a test result solely due to glaucoma; and (6) mental and physical capacity to perform the tests. Exclusion criteria were (1) active ocular disease; (2) use of other ocular medications or therapies that might have a substantial effect on IOP; (3) history of ocular surgery; or (4) use of other similar systemic medications (e.g., ergoloid mesylate derivative).

The anthocyanin product used in the study was *V. myricoides* extract (Kukje Pharma Ind. Co., Ltd., Gyeonggi-do, Korea). The anthocyanin product was provided in the form of capsule; each capsule contained anthocyanins 60.0 mg. The GBE product used in the study was *Ginkgo* leaf extract (SK Chemicals Co., Ltd., Gyeonggi-do, Korea), which is composed of ginkgo flavones glycosides and terpenelactons from the *Ginkgo* leaf. GBE was provided in the form a tablet containing 80.0 mg of the GBE. The subjects were instructed to take one capsule or tablet after breakfast and supper, two capsules or tablets daily. Patients were surveyed using a questionnaire to assess the duration of actual intakes of anthocyanins and GBE for the treatment duration analysis.

The HVF Analyzer, BCVA, IOP, blood pressure, fasting blood glucose, RNFL thickness, stereoscopic disc photography were performed at every visit, and complete ocular and systemic examinations were performed.

Visual field examinations of both eyes were performed by an experienced technician using the HVF Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) with the 30-2 SITA standard algorithm at each visit. For analysis, the average of the last two of the three visual field examinations within the first six months was compared with the visual field examinations of the last two visits. For unreliable tests, the subject was excluded from the analysis. After the first six months, a follow-up examination was performed within four to six months. In the HVF, the mean deviation is a measure of the average difference from the normal expected value for that age. Therefore, negative values denote decreased overall visual field sensitivity. The pattern standard deviation is the standard deviation of the mean difference between the threshold value at each test location and the expected normal value. It is a measure of the extent to which the threshold determinations at different locations in the visual field differ from each other. Therefore, positive high values mean that there is a localized loss, possibly indicating glaucoma.

The BCVA was checked at every visit, and all visual acuity values were converted to the logarithm of the minimal angle of resolution for statistical analysis. The logMAR scale converts the geometric sequence of a traditional chart to a linear scale. Positive values indicate vision loss, whereas negative values indicate normal or better visual acuity.

IOP and blood pressure and fasting blood glucose were recorded before and after treatment (a single measurement was taken at the same time of the day for all phases of the trial).

Complete ocular examinations and questionnaires that addressed ocular or systemic complications were performed at every visit.
**Statistical methods**

Baseline demographic and clinical parameters were compared among treatment groups using a one-way analysis of variance (ANOVA). Paired t-tests compared differences before and after medication with baseline values within treatment groups. In addition, a generalized linear model was used to assess the relationship between final visual acuity and multiple variables. All data were analyzed using the SPSS statistical software system version 18.0 (SPSS, Inc., Chicago, IL, USA). A P value of <.05 was considered statistically significant. In cases of bilateral NTG, only the right eye was considered.

**RESULTS**

Investigated were 332 patients (332 eyes) of which 209 were men and 123 were women. The mean age was 52.90 ± 13.74 years (range, 20–89 years). The mean follow-up duration was 23.82 ± 9.84 months (range, 12–59 months), and 78 patients (23.4%) had diabetes mellitus, and 103 (30.9%) had hypertension (Table 1).

Groups were 132 patients in the anthocyanin treatment group, 103 in the GBE group, and 97 in the control (non-treatment) group. Significant differences among the three groups were mean age (one-way ANOVA, \( P = .001 \)), dial-stolic/systolic blood pressure (one-way ANOVA, \( P = .006 \), \( P = .026 \), respectively), but not fasting blood glucose (one-way ANOVA, \( P = .156 \)), diabetes mellitus (one-way ANOVA, \( P = .814 \)), or hypertension (one-way ANOVA, \( P = .662 \)) prevalence rates (Table 2). The mean anthocyanin treatment duration (actual duration of intake from the patient survey) was 24.32 ± 10.43 (range, 6–53) months, and the mean GBE treatment duration was 23.81 ± 10.36 (range, 6–59) months. Significant differences were seen in mean age, diastolic/systolic blood pressure, diabetes mellitus, and hypertension prevalence rate between the anthocyanins and GBE treatment groups (independent sample t-test, \( P < .001, .011, .007, < .001, \) and .001, respectively), but not in the mean treatment duration and fasting blood glucose (independent sample t-test, \( P = .706, .225 \), respectively) (Table 2).

In the anthocyanin treatment group (132 eyes of 132 patients), the mean baseline BCVA of all eyes was 0.16 (±0.34) logMAR units. After anthocyanin treatment, the mean BCVA for all eyes improved to 0.11 (±0.18) logMAR units (paired sample t-test, \( P = .008 \)). The HVF mean deviation improved from \(- 6.44 (± 7.05)\) to \(- 5.34 (± 6.42)\) after anthocyanin treatment (paired sample t-test, \( P = .001 \)) (Table 3).

In the GBE treatment group (103 eyes of 103 patients), the mean baseline BCVA of all eyes was 0.06 (±0.13) logMAR units. After GBE treatment, the mean BCVA for all eyes was 0.07 (±0.15) logMAR units (paired sample t-test, \( P = .22 \)). The HVF mean deviation changed from \(- 5.25 (± 6.13)\) to \(- 4.31 (± 5.60)\) after GBE treatment (paired sample t-test, \( P = .002 \)) (Table 3).

The control group (97 eyes of 97 patients) showed a trend of reduction in the mean BCVA from 0.07 (± 0.11) logMAR units to 0.13 (± 0.23) logMAR units after follow-up (paired sample t-test, \( P = .009 \)). The HVF mean deviation changed from \(- 5.41 (± 4.64)\) to \(- 5.06 (± 6.32)\) after GBE treatment (paired sample t-test, \( P = .725 \)) (Table 3).

The mean BCVA logMAR unit change was significantly different among the anthocyanin treatment group (\(- 0.05 ± 0.21\)), the GBE treatment group (\(0.01 ± 0.06\), and the nontreatment group (\(0.05 ± 0.19\) (one-way ANOVA, \( P < .001 \)) (Table 4). However, differences in the HVF mean

### Table 1. Characteristics of All Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>332 (332 eyes)</td>
</tr>
<tr>
<td>Gender</td>
<td>209 male, 123 female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.90 ± 13.74 (20–89)</td>
</tr>
<tr>
<td>Mean follow-up (months)</td>
<td>23.82 ± 9.84 (12–59)</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>13.59 ± 3.03 (7–21)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>77.56 ± 7.97 (49–102)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121.95 ± 12.31 (90–163)</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>107.23 ± 22.95 (63–219)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>78 patients (23.4%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>103 patients (30.9%)</td>
</tr>
</tbody>
</table>

*Mean ± SD (range).

IOP, intraocular pressure.

### Table 2. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Anthocyanins (n = 132)</th>
<th>Gingko biloba extract (n = 103)</th>
<th>Control (n = 97)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.98 ± 10.52</td>
<td>46.98 ± 12.53</td>
<td>52.27 ± 16.04</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Mean treatment duration (months)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.32 ± 10.43</td>
<td>23.81 ± 10.36</td>
<td>-</td>
<td>-</td>
<td>.706</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.24 ± 6.85</td>
<td>75.50 ± 7.00</td>
<td>78.82 ± 7.89</td>
<td>.006</td>
<td>.011</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>124.08 ± 10.58</td>
<td>119.83 ± 13.37</td>
<td>121.30 ± 12.99</td>
<td>.026</td>
<td>.007</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>110.82 ± 26.64</td>
<td>103.94 ± 16.78</td>
<td>103.92 ± 20.80</td>
<td>.156</td>
<td>.225</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>80 patients (60.6%)</td>
<td>90 patients (87.4%)</td>
<td>84 patients (86.6%)</td>
<td>.814</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>76 patients (57.6%)</td>
<td>80 patients (77.7%)</td>
<td>73 patients (75.3%)</td>
<td>.662</td>
<td>.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>One-way ANOVA.

<sup>b</sup>Independent sample t-test between anthocyanins and Gingko biloba extract.

<sup>c</sup>Mean ± SD.

ANOVA, analysis of variance.
deviation changes among the anthocyanin (0.73 ± 2.99), the GBE (0.76 ± 2.57), and nontreated (0.24 ± 5.62) groups were not significant (one-way ANOVA, \( P = .55 \)) (Table 4). Differences in the mean BCVA logMAR unit change between the anthocyanin and GBE treatment groups were significant (independent sample \( t \)-test, \( P = .004 \)) (Table 4). However, differences in the HVF mean deviation change were not significant between the anthocyanins and GBE treatment groups (independent sample \( t \)-test, \( P = .926 \)) (Table 4).

To describe the linear association between the final BCVA and a set of exploratory variables, including the systemic treatment (anthocyanins/GBE), age, diabetes mellitus, hypertension, a generalized linear model was developed by a stepwise method. Our result demonstrated that the final BCVA was affected only by systemic treatment (anthocyanins \( P < .001 \), GBE \( P = .015 \)) and not affected by different demographics between the groups (age \( P = .402 \), diabetes mellitus \( P = .114 \) and hypertension \( P = .357 \)).

No ocular or systemic side effects were noted in any patient during the follow-up.

**DISCUSSION**

Our results suggest that systemic administration of anthocyanins and GBE improves visual function in some individuals with NTG.

The anthocyanin treatment group showed improved BCVA logMAR units and HVF mean deviation. In the GBE treatment group, BCVA logMAR units were not changed significantly, but the HVF mean deviation was improved. In the control group, the BCVA logMAR units deteriorated, and the HVF mean deviation was not changed after follow-up.

Although differences were significant for mean age, diastolic/systolic blood pressure, diabetes mellitus, and hypertension prevalence rates between the anthocyanins and GBE treatment groups, the final BCVA was affected only by systemic treatment and not by demographic factors of the groups in a generalized linear analysis.

From these results, our study suggested that anthocyanins and GBE might be effective for improving visual function in patients with NTG. We think that the mechanisms of anthocyanins and GBE action are effects on blood circulation and antioxidant properties.

Several studies examined the effects of anthocyanins and GBE on blood circulation. In animal studies, anthocyanins are beneficial for improving vascular tone and blood flow by redistribution of microvascular blood flow and interstitial fluid formation. Clinical trials in humans yielded similar results. A study of 47 patients with various venous diseases, giving 480 mg/day of bilberry extract, resulted in a reduced capillary flow as well as elimination of microstagnation and blood stasis of the foot. A review of uncontrolled trials from 1979 to 1985 on a total of 568 patients with venous insufficiency of the lower limbs showed that anthocyanins were effective in decreasing symptoms and improving both venous microcirculation and lymph drainage. The results of these studies indicate that the anthocyanins also can improve ocular blood circulation.

Reports on the effects of GBE on ocular circulation include a prospective study of 11 healthy volunteers treated in a random order with GBE 120 mg/day for 2 days. GBE significantly increased the end-diastolic velocity in the ophthalmic artery, without changes in arterial blood pressure, heart rate, or IOP. In another study, the same dose of GBE for four weeks increased the microcircular blood

### Table 3. Best-Corrected Visual Acuity, Visual Field Indices with Treatment

<table>
<thead>
<tr>
<th></th>
<th>Anthocyanins (n = 132)</th>
<th>Gingko biloba extract (n = 103)</th>
<th>Control (n = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BCVA, logMAR(^a)</td>
<td>0.16 ± 0.34</td>
<td>0.06 ± 0.13</td>
<td>0.07 ± 0.11</td>
</tr>
<tr>
<td>MD (dB)(^a)</td>
<td>0.73 ± 2.99</td>
<td>0.76 ± 2.57</td>
<td>0.24 ± 5.62</td>
</tr>
<tr>
<td>PSD (dB)(^a)</td>
<td>−0.10 ± 1.75</td>
<td>−0.13 ± 1.76</td>
<td>0.19 ± 4.13</td>
</tr>
</tbody>
</table>

\(^{a}\) Paired sample \( t \)-test.
\(^{b}\) Mean ± SD.

### Table 4. Changes in Best Corrected Visual Acuity (logMAR), Visual Field Indices Before and After Treatment

<table>
<thead>
<tr>
<th></th>
<th>Anthocyanins (n = 132)</th>
<th>Gingko biloba extract (n = 103)</th>
<th>Control (n = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BCVA, logMAR(^c)</td>
<td>−0.05 ± 0.21</td>
<td>0.01 ± 0.06</td>
<td>0.05 ± 0.19</td>
</tr>
<tr>
<td>MD (dB)(^c)</td>
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<tr>
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<td>−0.13 ± 1.76</td>
<td>0.19 ± 4.13</td>
</tr>
</tbody>
</table>

\(^{c}\) Paired sample \( t \)-test between anthocyanins and Gingko biloba extract.
\(^{a}\) One-way ANOVA.
\(^{b}\) Mean ± SD.
velocity, flow, and volume in healthy volunteers for four weeks. In another study, GBE administration (160 mg/day for 4 weeks) increased the mean blood flow, volume, and velocity in NTG patients.

Many studies show that anthocyanins and GBE are potent antioxidants. In animal studies, long-term daily intake of anthocyanins in the diet affects antioxidant enzyme activity and expression, and enhances oxidative markers in healthy rats. The antioxidative properties of GBE are from its direct radical-scavenging activity. GBE prevents oxidative damage to mitochondria, exhibits neuroprotective properties, and inhibits LDL oxidation.

Another possible explanation of the beneficial effects of anthocyanins and GBE in NTG patients is the effects of anthocyanins and GBE on improvements in the cognitive function. This effect has been demonstrated in patients with cerebral vascular insufficiency. Cerebral small-vessel ischemia is more common in NTG patients than in normal subjects, so a reasonable assumption is that anthocyanin or GBE administration improves BCVA and the mean deviation in VF indices via increased cerebral blood flow, thus improving the ocular blood flow, and improving retinal sensitivity, concentration, and alertness.

The exact explanation for this effect is not clear, but GBE and anthocyanins have anti-inflammatory properties, and several studies report that the chemopreventive activity of anthocyanins is related to effects on signal transduction and apoptosis.

Some studies have demonstrated effects of anthocyanins and GBE on visual function. Lee et al. reported that purified high-dose anthocyanoside oligomer administration improves nocturnal vision and clinical symptoms in myopia subjects. After administering a purified high-dose anthocyanoside oligomer (100 mg tablet, 85% anthocyanoside oligomer) twice daily for 4 weeks, subjective symptoms and objective contrast sensitivity improved in myopia subjects with asthenopia.

Quaranta et al. reported an effect of GBE on a pre-existing visual field damage in patients with NTG. After 40 mg of orally administered GBE, three times daily for 4 weeks, a significant improvement in visual field indices (the mean deviation and the corrected pattern standard deviation) was recorded without changes in the arterial blood pressure, heart rate, or IOP. These properties support the therapeutic value of anthocyanins and GBE in treating NTG.

This study mainly investigated objective rather than subjective values, and the treatment duration was six months or more, much longer than other studies. This article is the first study on the direct effect of anthocyanins on NTG patients. In rare cases, mild gastrointestinal complaints, headache, and allergic skin reactions have been reported when GBE was used. A review of studies comprising over 1000 patients taking anthocyanins reported mild side effects affecting the gastrointestinal, cutaneous, or nervous system, but in the present study, no ocular or systemic adverse events related to the use of anthocyanins or GBE were recorded. The results are in accordance with a long-term randomized clinical trial in which no significant difference in the incidence of adverse events was found when anthocyanin-treated patients were compared with a control group. The results of these studies suggest that anthocyanins and GBE were tolerable for most NTG patients.

However, our study had several limitations. First, because of its retrospective nature, we cannot exclude the possible effects of selection bias. However, we recruited consecutive patients to minimize selection bias. Prospective validation of our results might be necessary. Second, our study did not give the information on how long the effects of anthocyanin and GBE administration last and how long patients should take the systemic medication. Further study is required to investigate the duration of the effect and the optimal administration schedule for GBE and anthocyanin treatment for NTG patients.

In conclusion, this study suggests that anthocyanins and GBE could be effective for improving visual function in patients with NTG. These results can help clinicians manage patients with NTG.

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AUTHOR DISCLOSURE STATEMENT

None of the authors has financial or proprietary interest in any of the materials mentioned.

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