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Purified high-dose anthocyanoside oligomer administration improves nocturnal vision and clinical symptoms in myopia subjects

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The aim of the present study was to determine the effect of purified high-dose anthocyanoside oligomer administration on nocturnal visual function and clinical symptoms in low-to-moderate myopia subjects. The study was a randomized, double-blind, placebo-controlled trial and involved sixty subjects with asthenopia and refractive errors between −1·00 and −8·00 diopters in both eyes. Thirty subjects were administered a purified high-dose anthocyanoside oligomer (100 mg tablet comprising 85 % anthocyanoside oligomer), and thirty were given a placebo in tablet form twice daily for 4 weeks. Prior to the treatment, the placebo and anthocyanoside groups were similar in terms of age and contrast sensitivity. Before and after treatment, subjects completed a questionnaire to determine their clinical symptoms and were also assessed for nocturnal visual function using contrast sensitivity testing. Questionnaire data analysis showed that, following treatment, twenty-two (73·3 %) anthocyanoside subjects showed improved symptoms, whereas only one placebo subject showed an improvement (Fisher's exact test, $P<0·0001$). Contrast sensitivity levels according to each cycle per degree significantly improved in the anthocyanoside group and remained stable in the placebo group. The mean contrast sensitivity change in the anthocyanoside group was 2·41 (SD) 1·91, compared with −0·66 (SD) 2·66 dB for the placebo group (unpaired Student's *t* test, $P<0·0001$). At all cycle per degree levels, contrast sensitivity changes in the anthocyanoside group were better than in the placebo group (unpaired Student's *t* test, $P<0·05$). The present data show that the administration of anthocyanoside oligomer appears to improve subjective symptoms and objective contrast sensitivity in myopia subjects with asthenopia.

Anthocyanoside oligomer: Mesopic contrast sensitivity: Asthenopia: Myopia

Bilberry (*Vaccinium myrtillus*) is a small deciduous shrublet that grows in wooded areas of central and northern Europe, northern Asia and North America. Bilberry fruit has been used in traditional European medicine for nearly a thousand years (Morazzoni & Bombardelli, 1996), as reported by twelfth-century German herbalist Hildegarde von Bingen (1098–1179 AD) and later by sixteenth-century herbalist Hieronymus Bock. During World War II, British Royal Air Force pilots reported that their night visual acuity improved after consuming bilberries. Previous studies have reported on the effects of bilberry fruit preparations on visual acuity in dim light (Jayle & Aubert, 1964; Cunio, 1993; Barrette, 1999), pigmentary retinitis when taken with β-carotene (Fiorini *et al.* 1965), night vision in normal subjects (Jayle *et al.* 1965), patients with diabetic retinopathy when taken in combination with β-carotene (Sevin & Cuendet, 1966), patients with significant visual problems in bright light (Zavarise, 1968), patients with macular degeneration, diabetic retinopathy, retinal inflammation and retinitis pigmentosa (Neumann, 1971), and patients with progressive myopia (Politzer, 1977). Later studies investigated the effect of bilberries on microcirculatory function in patients with various retinopathies (Scharrer & Ober, 1981), myopia, glaucoma and retinitis pigmentosa (Caselli, 1985). Additional studies also investigated the effect of bilberries on cataract formation in patients with senile cortical cataracts when taken in combination with vitamin E (Bravetti, 1989).

The most active components of bilberry fruit are the anthocyanoside oligomers, which occur as small anthocyanidin glycoside polymers, particularly in the form of dimers, trimers, tetramers and pentamers. Anthocyanidin oligomers are hydro- and lipo-soluble, giving high bioavailability, and are not known to accumulate in the body. Most commercially available bilberry preparations contain only 5–30 % anthocyanoside oligomer, with the remaining compounds comprising inactive non-anthocyanoside impurities such as free sugars, tannin and organic acids. Using specific fermentation processes (Fig. 1), anthocyanoside oligomers can be concentrated to levels higher than 85 %.

Myopia causes decreased contrast sensitivity and may be a cause of the vague eye discomfort arising from use of the eyes, known as asthenopia. A large adult population study showed that myopia that was fully corrected with spectacles was associated with lower mean contrast sensitivity in the higher spatial frequencies compared with the emmetropic condition (Ogden, 1994). Moreover, visual acuity and contrast sensitivity under reduced light conditions, namely mesopic visual function, was lower in myopia subjects who were corrected by spectacle and photorefractive keratectomy (Owsley & Sloane, 1987).

In the present study, we performed a randomized, double-blind, placebo-controlled trial in low-to-moderate myopia subjects to determine the effect of a nutrient supplement containing

Abbreviations: ACP, Visual Capacity Analyzer; CPD, cycles per degree; MSC, morphoscopic contrast sensitivity.

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anthocyanoside oligomers on nocturnal visual function and clinical symptoms of asthenopia.

Materials and methods

The study involved screening more than 200 potential subjects aged between 18 and 65 years who visited the Young Dong Severance Hospital during the period April to August 2000. The recruitment protocol was approved by the Severance Ethics Research Committee. All subjects received a full explanation of the procedures, and informed consent was obtained prior to the commencement of the study in accordance with the World Medical Association Declaration of Helsinki (48th General Assembly, Somerset West, Republic of South Africa, October 1996).

Inclusion criteria for the study were as follows: a refractive error between −1·00 and −8·00 diopters in both eyes, decreased night vision, and asthenopia classified as severe based upon results of a structured questionnaire. Nocturnal visual function was measured using the morphoscopic contrast sensitivity (MCS) programme under mesopic conditions with a Visual Capacity Analyzer (ACV; L2 Informatique, Paris, France). The lowest contrast level at a given target size was measured under mesopic conditions, which provides information on nocturnal visual performance. Subjects with poor nocturnal vision usually show an abnormal curve of contrast sensitivity at the middle- and high-frequency levels (between 6·0 and 30·0 cycles per degree (CPD)) under mesopic conditions (between approximately −2 and 0 log cd/m^2). Only subjects showing such an abnormality were included in the study. The exclusion criteria for the study were: a medical history of hypersensitivity to anthocyanoside or products containing anthocyanoside, age over 70 years, pregnancy or expecting a pregnancy, organic eye disease and/or previous eye surgery, a medical history of a disease that could affect vision (e.g. diabetes) or the current use of medicines known to affect the eyes.

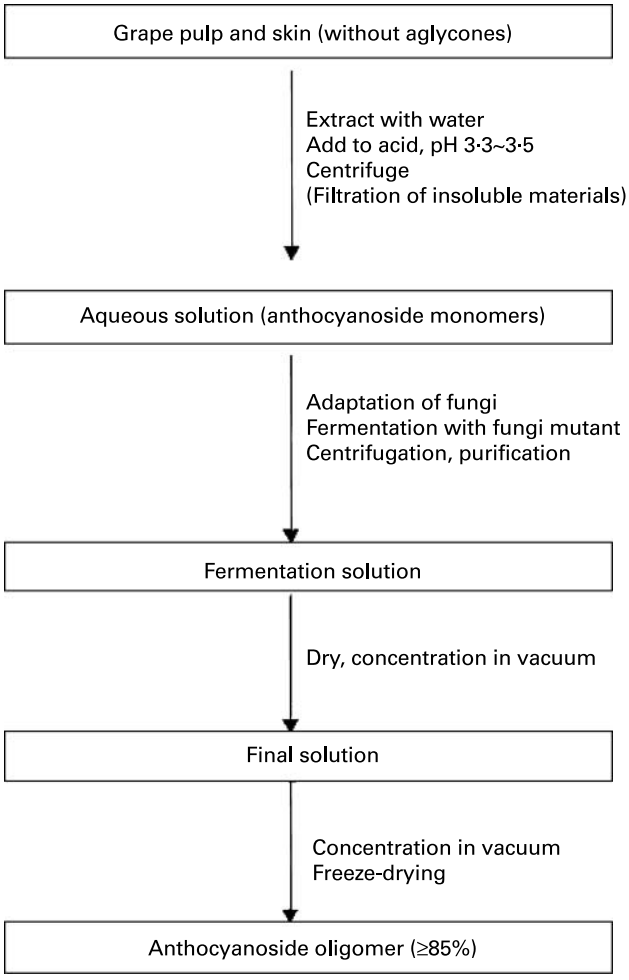

Fig. 1. Summary of the Eyezone fermentation and manufacturing process. Reproduced with permission from Hanmi Pharmaceuticals

Sixty people with poor nocturnal vision and symptoms of asthenopia were enrolled after giving signed informed consent. Each subject was randomly allocated into one of two groups using a series of serially numbered, opaque envelopes prepared from a list of computer-generated pseudorandom numbers of variable block size. The envelopes were prepared by an administrative clerk who was not otherwise involved in the study and were kept in a locked cabinet in the clinic. Patients took either purified high-dose anthocyanoside oligomers (*n* 30) or a placebo (*n* 30). The anthocyanoside oligomers were given in the form of the clinical investigation product Eyezone (Hanmi Pharmaceuticals, Seoul, Korea), which is produced by the specific fermentation process mentioned above (Fig. 1). Patients took a 100 mg Eyezone tablet (which consists of 85 % anthocyanoside oligomers), or a placebo, twice daily for a period of 4 weeks. The placebo tablet contained only inactive ingredients (lactose, magnesium stearate and colouring).

Subjects were assessed before and after treatment to determine the effect of anthocyanoside oligomer treatment. These investigations were performed by an investigator unaware of the treatment taken by the subjects. The effects on the clinical symptoms of asthenopia were determined using a questionnaire. Subjects answered seven questions regarding the severity/frequency of their eye symptoms using the following scale: 1 = no symptoms, 2 = 1 or 2 symptoms/week, 3 = about 3 or 4 symptoms/week, and 4 = symptoms every day (Table 1). Scores for each question were summated. A subject was deemed to have 'improved' if the symptom score after treatment was more than seven points below the score prior to treatment. The effect of treatment on nocturnal visual function was measured using the MCS programme, as described earlier. MCS was measured using the ACV, and the improvement in contrast threshold level according to each CPD was calculated by subtracting the initial values from the final values.

Table 1. Questionnaire used to determine the severity/frequency of eye symptoms

Questions
1. Frequent eye strain or pain at normal life?
2. Eye strain or pain at reading?
3. Dryness of eye or tearing at reading?
4. Eye strain at sudden bright lights?
5. Decreased vision in dark places?
6. Blurring of vision?
7. General weariness when reading?

Data analysis was performed using Statistical Analysis Systems statistical software package version 6·12 (SAS Institute, Cary, NC, USA). A probability value of $P<0·05$ was considered statistically significant. MCS measurements before and after treatment were compared using paired Student's *t* tests for both treatment groups. Age, refractive error and mesopic contrast sensitivity measured with the ACV were compared between the placebo and the anthocyanoside groups using an unpaired Student's *t* test. Symptom scores were compared between the two groups using Fisher's exact test.

Results

Sixty people qualified for the study; thirty were treated with placebo and thirty with anthocyanoside. The two treatment groups showed no statistically significant differences in terms of age or gender ($P>0·05$; Table 2). However, subjects in the placebo group were more near-sighted ($P=0·053$) and included a greater number of women ($P=0·071$). Following 4 weeks of treatment, results from the symptom questionnaire showed that the anthocyanoside group had a greater improvement than the placebo group (Fisher's exact test, $P<0·0001$; Table 3). However, not all the subjects in the anthocyanoside group were classified as 'improved'. The anthocyanoside group showed improved contrast sensitivity at each CPD level, whereas no such improvement was detected in the placebo group (Table 4). The mean MCS changes in the anthocyanoside group were superior to those of the placebo group at all CPD levels (unpaired Student's *t*-test, $p<0·05$; Fig. 2). No subjects complained of specific side-effects related to anthocyanoside use.

Table 2. Age, sex and refractive errors in placebo and anthocyanoside groups

	Placebo	SD	Anthocyanoside	SD	*P* value
Age (year)	36·0	12·6	41·1	13·1	0·133
Sex (M:F)	11:19		18:12		0·071
Refractive error (diopter)	−4·04	1·76	−3·39	1·83	0·053

Table 3. Effect of placebo and anthocyanoside treatment on eye symptoms

	Placebo	Anthocyanoside
Not improved	29	8
Improved	1	22

$P<0·0001$ (χ^2 test).

Discussion

The present data show that treatment with anthocyanoside oligomers appears to improve subjective symptoms and objective contrast sensitivity in myopia subjects with asthenopia.

Previous publications reporting on the effect of anthocyanoside oligomers on nocturnal vision have resulted in controversy. Early publications reported an improvement in nocturnal visual functions in normal individuals after taking anthocyanosides (Jayle & Aubert, 1964; Fiorini *et al.* 1965; Jayle *et al.* 1965; Belleoud *et al.* 1967; Ponte & Lauricella, 1969; Rouher *et al.* 1972; Sole *et al.* 1984). However, later reports demonstrated that anthocyanoside oligomers given in single or multiple oral doses had no effect on night vision (Levy & Glovinsky, 1998; Zadok *et al.* 1999; Muth *et al.* 2000; Mayser & Wilhelm, 2001). These conflicting conclusions may be caused by differences in the type of subject, the methods of evaluating night vision and the concentration and dose of anthocyanoside oligomers. For example, whereas some studies using normal subjects showed no effect of anthocyanoside oligomers (Levy & Glovinsky, 1998; Zadok *et al.* 1999; Muth *et al.* 2000; Mayser & Wilhelm, 2001), the oligomers were found to be effective in subjects with myopia and night blindness (Sole *et al.* 1984).

The design of the present study differs from that of most previous studies in terms of dose, duration and type of subjects. We used a purified anthocyanoside oligomer preparation in the form of Eyezone, which comprises more than 85 % anthocyanoside oligomers. Previous studies used typical commercially available bilberry preparations, which comprise only 5–30 % anthocyanoside oligomers. Subjects in the present study were treated for 4 weeks, which is longer than treatments reported in other studies. Therefore, our treatment regime of 100 mg Eyezone twice daily for 4 weeks resulted in a higher dose of anthocyanoside oligomers over a longer period of time compared with other

Table 4. Contrast sensitivity before and after administration in placebo and anthocyanoside group

	Placebo					Anthocyanoside				
Cycles per degree	Before (dB)	SD	After (dB)	SD	*P* value	Before (dB)	SD	After (dB)	SD	*P* value
0·6	30·77	4·09	31·26	3·11	0·356	31·44	3·81	33·45	2·73	0·000
1	29·82	4·29	29·54	3·72	0·626	30·68	4·20	32·54	2·66	0·000
1·5	29·66	4·41	29·05	4·00	0·251	30·29	4·45	31·97	3·35	0·001
3	26·98	4·22	26·03	4·89	0·108	27·92	30·67	30·67	4·11	0·000
6	21·80	7·44	19·40	6·82	0·001	22·75	6·41	25·04	7·05	0·000
7·5	18·94	7·76	17·21	7·73	0·007	20·63	8·11	22·77	7·89	0·000
9·5	15·87	8·06	14·45	7·48	0·072	16·72	8·56	19·61	8·19	0·000
12	10·41	7·43	10·02	6·63	0·454	11·46	8·06	14·03	9·05	0·000
15	6·89	6·09	6·32	5·57	0·362	8·01	7·07	10·9	8·79	0·000
19	0·84	2·67	0·67	1·79	0·474	1·51	3·88	5·31	6·67	0·001
24	0·67	1·75	0·74	1·96	0·816	2·00	3·25	4·32	5·70	0·003
30	0·46	1·60	0·57	1·86	0·643	1·12	2·43	2·84	4·44	0·000

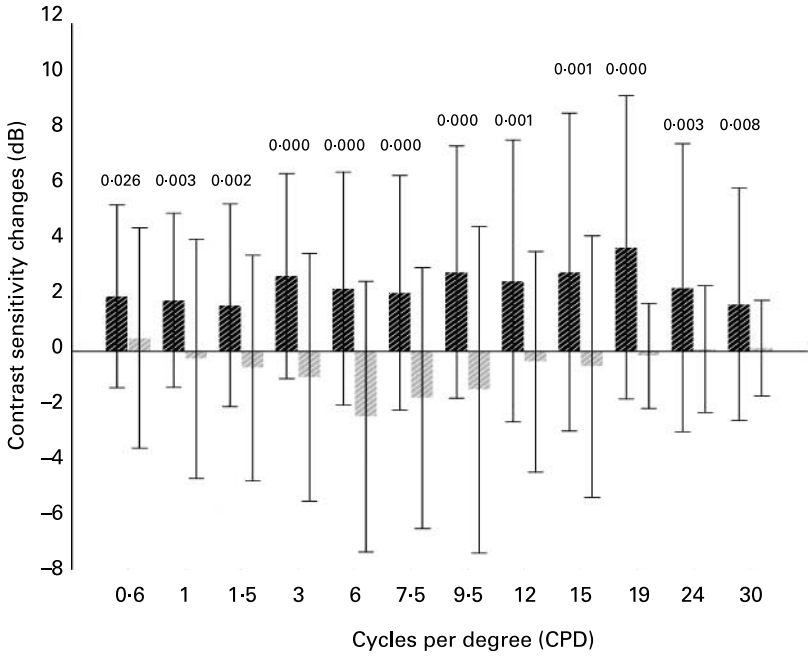

Fig. 2. Mean mesopic contrast sensitivity changes with SD after placebo (▨) or anthocyanoside (▨) treatment. The mean morphoscopic changes in the anthocyanoside group were superior to those of the placebo group at all cycle per degree levels (unpaired Student's *t* test, $P<0{\cdot}05$). The *P* value at each spatial frequency is displayed at the top of each bar.

studies that showed no effect of anthocyanoside oligomers on vision. The doses and times in those studies were 12–36 mg given once (Levy & Glovinsky, 1998), 12–24 mg given twice daily for 4 d (Zadok *et al.* 1999), 120 mg (25 % bilberry extract) daily for 21 d (Muth *et al.* 2000) and 160 mg (25 % bilberry extract) daily for 28 d (Mayser & Wilhelm, 2001). Another major difference from previous studies was that the present study enrolled myopic subjects with symptoms of asthenopia and decreased mesopic contrast sensitivity, whereas all other studies, other than Sole *et al.* (1984), used subjects with normal vision.

It is known that contrast sensitivity is lower in elderly persons (mesopic contrast sensitivity gradually deteriorating from 51 years of age; Puell *et al.* 2004), females and high myopes (Oen *et al.* 1994; Liou & Chiu, 2001). In addition, the bioavailability of lipid-soluble nutrients is different between male and female. Although statistical analysis indicated that these differences were not significant, baseline characteristics showed some numerical differences between groups, especially in terms of sex. In addition, the two treatment groups showed a significant difference in terms of refractive error ($P=0{\cdot}053$). The fact that subjects in the placebo group were slightly more near-sighted and included a greater number of females, and hence theoretically had greater depressed contrast sensitivity, might have biased the results in favour of the treatment group. In the present study, however, contrast sensitivity at each CPD level for the two groups before treatment showed no significant difference at any spatial frequency level. Moreover, because anthocyanoside oligomer is water soluble, it is not likely to be redistributed differently, based upon androgen-sensitive lipoprotein segregation in the liver as well as adipose sequestration. Therefore, the results obtained in the present study were unlikely to be the result of differences in group baseline characteristics.

Although results from the symptom questionnaire showed that the anthocyanoside group had a greater improvement than the placebo group, eight subjects out of thirty in the anthocyanoside group were classified as non-responders because the improvement in their symptom score was less than six points. However, they still showed some improvement in the symptom score. The symptom score improvement of non-responders was between two and five points. There were five male non-responders. The non-response rate was similar in males (28 %) and females (25 %).

We chose a new method for evaluating contrast sensitivity, namely the ACV. The ACV uses computer-based technology to measure MCS, which may have enhanced the sensitivity of detecting MCS changes. The reproducibility of the ACV method has been previously established (Lee *et al.* 2003). Whereas Snellen visual acuity measurement is an important clinical tool for the evaluation of visual function, recent studies have demonstrated that contrast sensitivity is useful for determining difficulties in performing everyday visual tasks. This test has therefore been widely promoted as an important adjunct to or even a replacement for visual acuity testing (Owsley & Sloane, 1987; Ogden, 1994). Contrast sensitivity is also suggested as an important factor in predicting mobility performance under mesopic conditions in people with poor vision (Kuyk *et al.* 1998; Kuyk & Elliott, 1999). A person who showed abnormalities in mesopic contrast sensitivity tests despite normal photopic visual acuity might show a poor performance in low illuminated conditions, especially night-driving (Lachenmayr *et al.* 1998).

Anthocyanosides are suggested to act by stabilising collagen, preventing capillary fragility and improving the microcirculation. They are also thought to have antioxidant activity (Timberlake & Henry, 1988; Pizzorno & Murray, 1993). Although they are reported to improve night vision, such an effect appears to be influenced by the type of subjects, the method of evaluation and

the dose and duration of treatment. Our results show that, for subjects with MCS abnormalities, treatment with purified high-dose anthocyanoside oligomers appears to improve subjective symptoms and objective MCS results. The present study indicates the need for further investigation into the optimal dose and duration of anthocyanoside oligomer treatment, the duration of an effect following cessation of the treatment, and the application of such treatment to individuals with eye diseases that could reduce night vision, such as retinitis pigmentosa, diabetic retinopathy and glaucoma.

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