Original Article

Effects of French maritime pine bark/bilberry fruit extracts on intraocular pressure for primary open-angle glaucoma

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We tested oral French maritime pine bark (40 mg)/bilberry fruit extract (90 mg) supplements for intraocular pressure-lowering effects in Japanese subjects with primary open-angle glaucoma. Eighteen subjects (29 eyes) were recruited with intraocular pressure of ≥15 mmHg who used one to three bottles of antiglaucoma medications. After a 2-week observation (period 1), subjects ingested a tablet/day of Sante® Glagenox for 4 weeks (period 2). The mean intraocular pressure (17.2 ± 2.3 mmHg) decreased significantly to 15.7 ± 1.9 mmHg (8.7% reduction) at week 4 ($p = 0.0046$). The mean morning intraocular pressure (14.1 ± 3.1 mmHg) self-measured using the iCare HOME tonometer during period 1 decreased significantly to 13.3 ± 2.9 mmHg (5.7% reduction) during period 2 ($p = 0.0291$). Blood redox parameters, diacron reactive oxygen metabolites, biologic antioxidant potential, and sulfhydryl tests were unchanged after 4-week supplementation. Intra-subject comparisons, compared to period 1, showed pooled, self-measured, period-2 intraocular pressures was significantly lower in nine subjects (50%), unchanged in six subjects (33%), and elevated in three subjects (17%), suggesting some non-responders. Four-week supplementation with French maritime pine bark/bilberry fruit extracts can further reduce intraocular pressure even in Japanese patients with controlled primary open-angle glaucoma. Further study should confirm the intraocular pressure-lowering effects and mechanisms of this supplement in glaucoma management. The study was registered in UMIN (ID: UMIN000033200).

***Key Words*:** **Pycnogenol®, procyanidin, Mirtoselect®, anthocyanin, diacron reactive oxygen metabolites (dROM) test, biological antioxidant potential (BAP) test, sulfhydryl (SH) test**

Glaucoma, which is characterized by progressive "glaucomatous" optic neuropathy and visual field loss, is a leading cause of irreversible blindness worldwide,[1] including Japan.[2] Retinal ganglion cell (RGC) death resulting from apoptosis and RGC axon loss leads to glaucomatous optic neuropathy, in which elevated intraocular pressure (IOP) is the primary risk factor.[3] In open-angle glaucoma (OAG), including primary OAG (POAG) and glaucoma secondary to pseudoexfoliation syndrome (EX), the IOP increases as the result of reduced aqueous humor outflow at the trabecular meshwork (TM).[4] This results from dysfunctional TM cells and consequent changes in the amount and quality of the extracellular matrix in the TM.[5] Clinical and experimental studies have reported that oxidative stress and/or inflammation associated with TM cell dysfunction and aqueous outflow resistance increase.[6–8]

We previously identified significantly lower antioxidant capacity level and higher hydroxylinoleates, oxidation products of linoleates, in blood samples from subjects with POAG and EX compared with controls.[9–11] Interestingly, the lower level of blood antioxidant capacity was associated with higher IOP values in patients with glaucoma and control subjects[12] and with worse visual field defects in OAG.[13,14] Previously, supplementation with flavonoids containing French maritime pine bark/bilberry fruit extracts improved eye health and microcirculation.[15] This evidence prompted us to evaluate the effect of pine tree French maritime pine bark/bilberry fruit extracts on the IOP in Japanese patients with glaucoma.

Subjects and Methods

Subjects. The study adhered to the tenets of the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The institutional review boards of Shimane University Hospital and Matsue Red Cross Hospital reviewed and approved the research. All subjects provided written informed consent. The study was registered in UMIN (ID: UMIN000033200) before data collection. Twenty-nine eyes of 18 Japanese subjects with POAG who fulfilled the inclusion and exclusion criteria were recruited at both hospitals. All subjects underwent ophthalmologic examinations including measurements of the best-corrected visual acuity (BCVA) and IOP by Goldmann applanation tonometry (IOP_{GAT}). POAG was diagnosed based on an open iridocorneal angle; the characteristic appearance of glaucomatous optic neuropathy, such as enlargement of the optic disc cup or focal thinning of the neuroretinal rim; corresponding visual field defects in at least one eye, and no evidence of secondary glaucoma bilaterally. The inclusion criteria included patients of both genders, age from 20 years to younger than 70 years; ability to understand the study and provide written informed consent; POAG in at least one eye; POAG with stable IOP control achieved using a minimum of one bottle and a maximum of three bottles of antiglaucoma medications; the absence of the need for a glaucoma and other ocular surgeries within 1 year after inclusion in the study; the desire to take antioxidative stress supplementation; a IOP_{GAT} with use of antiglaucoma medication(s) of 15 mmHg or higher in at least one eye; and the ability to measure the IOP using the self-tonometer (iCare Home rebound tonometer, M.E. Technica, Tokyo, Japan) (IOP_{RBT}).

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The exclusion criteria included a history of severe systemic diseases based on interviews with the subjects; sight-threatening ocular diseases other than glaucoma and visually insignificant cataract; liver, kidney, and heart disease that could have affected the blood examination in this study; use of antioxidant supplementations (e.g., extracts of pine bark and bilberry fruits; flavonoids such as catechin, anthocyanin, and procyanidin; flavonoids such as lutein, zeaxanthin, and astaxanthin; and vitamins C and E) within 6 months before study entry or a plan to use those supplements during the study; current smoking; possible lifestyle change during the study period (e.g., extended trip, fasting, or dieting for weight control); pregnancy, lactation; the possibility of becoming pregnant during the study; current participation in another clinical trial or other clinical trial within 3 months before inclusion; possible allergy to extracts of French maritime pine bark and bilberry fruits (Sante® Glagenox, Santen Pharmaceutical Co., Ltd., Osaka, Japan); and severe allergy to foods and drugs.

Study design and scheduled examinations. This was a multicenter, open-label, prospective, single-arm, before/after comparison study. During the study, the subjects were scheduled to visit the study site three times at weeks –2 (allowed range, –21 days to –7 days from the day of week 0), 0, and 4 (21 days to 35 days from the day of week 0). The period between week –2 and 0 was defined as period 1 and between week 0 and 4, period 2. All subjects continued antiglaucoma medication(s) during periods 1 and 2. Any change in antiglaucoma medication or other eye drops during the study periods was regarded as study dropout. The subjects were instructed to take one tablet/day of the study supplement (Sante Glagenox, Santen Pharmaceutical Co., Ltd., Osaka, Japan) during period 2 (from the day after the week 0 visit to the day of the week 4 visit). Each tablet contained 40 mg of French maritime pine bark extract (Pycnogenol®) and 90 mg of bilberry fruit extract (Mirtoselect®). Pycnogenol® is comprised of organic acids, taxifolin, and flavan-3-ols standardized to 70 ± 5% procyanidins that conform to United States Pharmacopeia 42 on "Maritime pine extract" (information provided by the manufacturer) . Mirtoselect®, *Vaccinium myrtillus* L. extract, is standardized to contain 36% anthocyanins, and conforms to the European Pharmacopeia 9.0 on "Fresh bilberry fruit dry extract, refined and standardized" (information provided by the manufacturer). The IOP_{GAT} was recorded at every visit, and any systemic and ocular adverse events were assessed by interview and ocular examinations. At the weeks 0 and 4 visits, the interviewer assessed the adherence to glaucoma therapy. The adherence was categorized into four grades, with 1 indicating 100% adherence; 2, less than total adherence sometimes (75–99% adherence); 3, less than total adherence by approximately half (25–74% adherence); and 4, almost no use of topical medication (less than 25% adherence) of antiglaucoma medication use during both periods 1 and 2. At the week 4 visit, the interviewer assessed the adherence to supplementation; the adherence was categorized into 4 grades, with 1 indicating total adherence (100% adherence); 2, adherence absent one to two times (90–99% adherence); 3, adherence absent three to five times (80–89% adherence); and 4, adherence absent more than 6 times (less than 80% adherence) of supplementation during period 2. At the week –2 visit, the demographic subject data were recorded that included gender, age, concomitant disease, number of antiglaucoma medications, BCVA, spherical equivalent refractive error (SERE), visual field mean deviation (MD), lens status (phakic or pseudophakic), and central corneal thickness (CCT). The BCVA was measured using a Landolt ring chart in decimal notation and converted to the logarithm of the minimal angle of resolution for statistical analyses. The SERE was measured using an auto-refract-keratometer (RC5000, Tomey Corporation, Nagoya, Japan). The visual field MD was assessed using the Swedish Interactive Threshold Algorithm Standard 30-2 program of the Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Dublin, CA). The CCT was measured by anterior-segment optical coherence tomography (Casia 2, Tomey Corporation). The subjects' demographic data are summarized in Table 1.

Table 1. Demographic data

	Mean ± SD or *n*	Range or %
Case	18	
Eyes	29	
Age (years)	57.7 ± 8.0	40–69
Sex		
Male	7	39%
Female	11	61%
Number of glaucoma medications (bottle)		
1	6	
2	6	
3	6	
	2.0 ± 0.8	1–3
Glaucoma medication		
Prostaglandin	18	62%
β blocker	16	55%
CAI	10	34%
α2 stimulator	7	24%
Pilocarpine	1	3%
Best-corrected visual acuity (logMAR)	–0.03	–0.08––0.2
Sperical equivalent refractive error (D)	–5.7	–12.8––1.3
Visual field MD (dB)	–5.1	–13.6–+0.2
Lens status		
Phakic	22	76%
Pseudophakic	7	24%
Corneal thickness (um)	540	496–572

n, number; CAI, carbonic anhydrase inhibitor; logMAR, logarithm of the minimum angle of resolution; MD, mean deviation; dB, decibels; D, diopters.

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IOP measurement. At the week –2, 0, and 4 visits, one of the two examiners (KM or MT) measured the IOP_{GAT}. During periods 1 and 2, the subjects were instructed to perform self-tonometry to record the IOP_{RBT} three times daily at prescheduled times, i.e., morning (6:00–9:00), noon (12:00–15:00), and evening (18:00–21:00). Other than those times, the subjects also were allowed to measure the IOP_{RBT} more than three times daily. The day of the week –2 visit, one author (KM) or an experienced examiner trained the subjects in the use of the iCare Home device. At the week 0 and 4 visits, the obtained IOP data were exported from the tonometer device using iCare Link Software (ver. 1.88, iCare Finland, Vantaa, Finland); other than the IOP and eye (i.e., right or left eye), the exported data included the date and time of the measurement. To assure adherence to self-tonometry, two or more IOP recordings within the prescheduled timings/day were the minimal requirements for the data to be included in the statistical analyses; otherwise, the subjects were regarded as drop-outs from the study.

Measurements of oxidative stress markers. At the week 0 and 4 visits, venous blood specimens were collected from the antecubital vein into evacuated tubes. Serum samples obtained by centrifugation of the collected venous blood were stored at –80°C until the time of the measurements of redox markers. During all handling procedures, including transportation from the clinical setting to the laboratory and centrifugation, the temperature was maintained at 4°C. All blood analyses were performed using a free radical analyzer system (FREE Carpe Diem, Wismerll Company Ltd., Tokyo, Japan) that included a spectrophotometric device reader and a thermostatically regulated mini-centrifuge; the measurement kits were optimized to the FREE Carpe Diem System, according to the manufacturer's instructions. To analyze the serum levels of reactive oxygen metabolites, antioxidant capacity, and thiol-antioxidant capacity, diacron reactive oxygen metabolite (dROM), biologic antioxidant potential (BAP), and sulfhydryl (SH) tests were performed, respectively.

The dROM test reflects the amount of organic hydroperoxides that is related to the free radicals from which they are formed. When the samples are dissolved in an acidic buffer, the hydroperoxides react with the transition metal (mainly iron) ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals. These newly formed radicals oxidize an additive aromatic amine (*N,N*-diethyl-*para*-phenylendiamine) and cause formation of a relatively stable colored cation radical that is spectrophotometrically detectable at 505 nm.[16,17] The results of dROM testing were expressed in arbitrary units (U.Carr), one unit of which corresponds to 0.8 mg/L of hydrogen peroxide.[16,17]

The BAP test provides an estimate of the global antioxidant capacity of blood plasma, measured as its reducing potential against ferric ions. When the sample is added to the colored solution obtained by mixing a ferric chloride solution with a thiocyanate derivative solution, decoloration results. The intensity of the decoloration is spectrophotometrically detectable at 505 nm and is proportional to the ability of plasma to reduce ferric ions.[18,19] The results of the BAP test were expressed in μmol/L of the reduced ferric ions.

The SH test provides an estimate of the total thiol groups in the biologic samples, using a modified Ellman method.[20,21] When the sample is added to the solution, the SH groups in the sample react with 5,5-dithiobis-2-nitrobenzoic acid, which is followed by development of a stained complex that is spectrophotometrically detectable at 405 nm and is proportional to their concentration according to the Beer-Lambert law.[16,19] The results were expressed as μmol/L of the SH groups.

Statistical analysis. The IOP_{GAT}, dROM, BAP, and SH were compared between weeks 0 and 4 using the paired *t* test. The IOP_{RBT} measured at any time, and the IOP_{RBT} measured in the morning (6:00–9:00), at noon (12:00–15:00), and in the evening (18:00–21:00) were compared between periods 1 and 2 using the paired *t* test. For these comparisons, the mean IOP during the indicated time periods was regarded as the subject's IOP_{RBT}. For each subject, the IOP_{RBT} pooled data were compared between periods 1 and 2 using the unpaired *t* test. All statistical analyses other than the pooled IOP_{RBT} comparisons were performed by an externally outsourced company (Kondo Photo Process Co., Ltd., Osaka, Japan) using IBM SPSS Statistics 25.0 (IBM Corp, Armonk, NY). One author (MT) performed all pooled IOP_{RBT} comparisons using JMP ver. 11 statistical software (SAS Institute, Inc., Cary, NC). The data are expressed as the means ± SD (or ± SE) or frequency and percent. *P* values <0.05 were considered statistically significant.

Results

All the subjects completed the study. Regarding adherence to the glaucoma medications, at the week 0 visit,16 subjects (89%) were categorized as having 100% adherence and two subjects (11%) were categorized as having 75% to 99% adherence; at the week 4 visit, 17 subjects (94%) were categorized as having 100% adherence and one subject (6%) was categorized as having 75% to 99% adherence. Regarding adherence to the supplementation, at the week 4 visit, 17 subjects (94%) were categorized has having 100% adherence and one subject (6%) was categorized as having 90% to 99% adherence. No adverse events were reported during the study periods.

The IOP_{GAT} values that were measured by the examiner are summarized in Table 2. The IOP_{GAT} of 17.1 mmHg at week –2 (i.e., the day of study inclusion) was equivalent to the IOP_{GAT} of 17. 2 mmHg at week 0 (i.e., the day before the start of supplement intake). In contrast, the IOP_{GAT} of 15.7 mmHg at week 4 (i.e., the visit after the supplement intake for 4 weeks) was significantly lower (–1.5 mmHg, 8.7% reduction) than that of week 0 ($p = 0.0046$).

The IOP_{RBT} values that were measured by the subjects are summarized in Table 3. In the morning, the IOP_{RBT} of 14.1 mmHg during period 1 (i.e., without supplement intake) decreased to 13.3 mmHg (–0.8 mmHg, 5.7% reduction) during period 2 (i.e., with supplement intake); the difference was significant ($p = 0.0291$). At the other time points, a decrease in the IOP_{RBT} during period 2 from period 1 was observed; however, the difference did not reach significance. The intra-subject comparisons showed that compared to the pooled IOP_{RBT} values during period 1, the pooled IOP_{RBT} values during period 2 were significantly lower in nine subjects (50%), unchanged in six subjects (33%), or higher in three subjects (17%) (Table 4).

The dROM, BAP, SH are summarized in Table 5. No oxidative

Table 2. Intraoucular pressure measured by Goldmann applanation tonometer ($n = 18$)

	Week –2	Week 0	Week 4	Difference (Week 4–Week 0)	*p* value*
Mean ± SD	17.1 ± 2.1	17.2 ± 2.3	15.7 ± 1.9	–1.5 ± 1.9	0.0046
Range	15.0–24.0	14.0–23.0	13.5–20.0	–5.5–+1.0	

**p* values are calculated between weeks 0 and 4 using the paired *t* test.

Table 3. Intraocular pressure measured by rebound tonometry (n = 18)

	Period 1 (Weeks –2–0)		Period 2 (Weeks 0–4)		Difference (Period 2–Period 1)		p value*
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Morning (6:00–9:00)	14.1 ± 3.1	12.5–15.7	13.3 ± 2.9	11.8–14.7	–0.8 ± 1.3	–1.5––0.1	0.0291
Noon (12:00–15:00)	14.1 ± 3.7	12.3–15.9	13.5 ± 3.2	11.9–15.1	–0.6 ± 1.8	–1.5–+0.3	0.1810
Night (18:00–21:00)	13.2 ± 2.9	11.8–14.7	13.0 ± 3.0	11.5–14.5	–0.2 ± 1.5	–1.0–+0.5	0.5119
All times	13.7 ± 3.0	12.2–15.2	13.2 ± 2.9	11.8–14.6	–0.5 ± 1.2	–1.1–+0.1	0.0862

*p values are calculated using the paired t test.

Table 4. Intraocular pressure measured by rebound tonometry in each subject

Case	Period 1 (Weeks –2–0)			Period 2 (Weeks 0–4)			Difference (Period 2–Period 1)		p value*
	n	Mean ± SD	Range	n	Mean ± SD	Range	Mean ± SE	Range	
1	57	15.4 ± 2.6	14.8–16.1	106	13.6 ± 2.1	13.2–14	–1.8 ± 0.4	–2.6–-1.1	<0.0001
2	41	11.6 ± 2.3	10.9–12.4	85	11.8 ± 2.9	11.2–12.4	+0.2 ± 0.5	–0.9–+1.2	0.7492
3	47	13.2 ± 2.6	12.4–13.9	87	13.2 ± 2	12.8–13.6	+0.1 ± 0.4	–0.7–+0.8	0.9050
4	44	12.4 ± 3	11.5–13.3	70	9.8 ± 1.9	9.3–10.2	–2.7 ± 0.5	–3.6–-1.8	<0.0001
5	57	13 ± 2.3	12.4–13.6	72	13.2 ± 2.1	12.7–13.7	+0.1 ± 0.4	–0.6–+0.9	0.6987
6	38	11.6 ± 3	10.6–12.5	64	11.2 ± 2.9	10.5–12	–0.3 ± 0.6	–1.5–+0.9	0.5826
7	66	14.2 ± 1.9	13.7–14.7	78	12.9 ± 2	12.5–13.4	–1.3 ± 0.3	–1.9–-0.6	0.0002
8	48	12.5 ± 1.9	11.9–13	79	11.4 ± 1.8	11–11.8	–1.1 ± 0.3	–1.7–-0.4	0.0021
9	44	10.1 ± 3	9.2–11	77	12 ± 2.4	11.4–12.5	+1.9 ± 0.5	0.9–2.8	0.0003
10	45	14.2 ± 2.7	13.4–15	102	14.9 ± 2.3	14.4–15.3	+0.7 ± 0.4	–0.2–+1.6	0.1213
11	45	12.1 ± 1.9	11.5–12.6	67	10.9 ± 2.3	10.4–11.5	–1.2 ± 0.4	–2.0–-0.3	0.0065
12	54	14.1 ± 4.3	13–15.3	81	15.3 ± 1.9	14.8–15.7	+1.2 ± 0.5	0.1–2.2	0.0336
13	71	13.8 ± 2.1	13.3–14.3	81	13 ± 1.9	12.5–13.4	–0.8 ± 0.3	–1.5–-0.2	0.0114
14	53	22.8 ± 3.9	21.7–23.9	67	22.6 ± 3.2	21.8–23.3	–0.2 ± 0.6	–1.5–+1.0	0.7111
15	51	17.9 ± 2.2	17.3–18.5	85	16.9 ± 2.6	16.3–17.5	–1.0 ± 0.4	–1.9–-0.2	0.0181
16	50	14.6 ± 2.7	13.8–15.3	86	12.9 ± 2.5	12.3–13.4	–1.7 ± 0.5	–2.6–-0.7	0.0005
17	50	9.9 ± 2.2	9.3–10.5	72	11 ± 2	10.5–11.4	+1.0 ± 0.4	0.3–1.8	0.0075
18	46	12.7 ± 2.8	11.9–13.6	87	11.4 ± 2.3	11–11.9	–1.3 ± 0.5	–2.2–-0.4	0.0051

*p values are calculated using the unpaired t test. n, number.

Table 5. Blood redox markers (n = 18)

	Week 0		Week 4		Difference (Week 4–Week 0)		p value*
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
dROM (U.Carr)	324 ± 53	209–403	328 ± 60	214–401	4 ± 33	–81–+69	0.5813
BAP (μmol/L)	2,347 ± 472	1,577–2,986	2,376 ± 547	1,531–3,202	29 ± 323	–978–+480	0.7092
SH (μmol/L)	663 ± 56	546–745	659 ± 54	563–768	–4–+35	–79–+68	0.6205

*p values are calculated using the paired t test. dROM, diacron reactive oxygen metabolites; BAP, biologic antioxidant potential; SH, sulfhydryl.

stress markers measured in the blood samples differed significantly between the week 0 and 4 visits.

Discussion

In this study, Japanese patients with POAG who were using glaucoma medications (mean age, 57.7 years) and taking 1 tablet daily of a supplement that contained 40 mg of French maritime pine bark extract and 90 mg of bilberry fruit extract for 4 weeks had reductions in the baseline IOP_{GAT} of 17.2 mmHg to 15.7 mmHg at 4 weeks (Table 2). Previously, Italian patients with ocular hypertension without glaucoma (mean age, 45 years, and no previous use of glaucoma medication), dietary supplementation of two tablets daily of Mirtogenol containing 40 mg of pine tree bark and 80 mg of bilberry fruit extracts, for 6 months had a reduction of the baseline IOP_{GAT} of 25.2 mmHg to 22.0 mmHg at 3 months and 22.0 mmHg at 6 months.[22] In the supplementation group, compared to baseline, the systolic and diastolic components of the ocular blood flow including the central retinal, ophthalmic, and posterior ciliary arteries improved when measured by color Doppler imaging.[22] In Italian subjects with ocular hypertension (mean age, 49 years, and no previous use of glaucoma medication) supplemented with one tablet daily of Mirtogenol for 24 weeks, the IOP_{GAT} of 38.1 mmHg at baseline decreased to 34.1 mmHg at 4 weeks and decreased significantly to 33.3 mmHg at 6 weeks; the reduction of IOP was significant thereafter for up to 24 weeks.[23] In that study, simultaneous intake of the supplement potentiated the IOP-lowering effect of topical latanoprost.[23] In Italian subjects with ocular hypertension (mean age, 49 years, and no previous use of glaucoma medication) supplemented with two tablets daily of Mirtogenol for 12 weeks, the IOP_{GAT} exceeding 30 mmHg at baseline decreased to around 20 mmHg at 6 weeks and decreased further to less than 20 mmHg at 12 weeks with supplementation plus latanoprost, while no

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further reduction of IOP during the 6- to 12-week period was observed in the group taking latanoprost only.[15] Thus, the IOP-reducing effect of oral French maritime pine bark/bilberry fruit extracts in subjects with glaucoma seen in the current study is unique in the literature. The baseline IOP in this study was much lower than in the previous studies; IOP reduction seen even in subjects with such a lower baseline IOP seems especially important to Japanese subjects since more than two-thirds of the Japanese glaucomatous eyes develop glaucoma within the normal range of IOP.[24]

Among the various time points at which the IOP was measured, the significant IOP_{RBT} reduction was recorded only in the morning (Table 3). The IOP in patients with glaucoma generally is higher in the morning because of the 24-h IOP rhythm.[25] In fact, among the time points, the baseline IOP_{RBT} level was the highest in the morning in the current subjects. Previous studies of subjects with ocular hypertension with higher baseline IOPs[15,22,23] have reported much larger reductions of IOP after supplementation than in the current study; thus, the lower baseline IOP_{RBT} might explain the fact that there were no significant IOP reductions at the measurement time points other than in the morning. Compared to previous studies,[15,22,23] the duration of supplementation was shorter and the dose of supplementation was smaller in the current study; accordingly, optimization of the duration and dose may be required to detect the IOP-lowering effect of this supplementation in eyes with POAG with low IOP. Use of self-tonometry enabled us to increase the number of IOP measurements, which should be associated with the reduction of effect of a measurement error. Since this was an open-label study, the IOP_{GAT} might include a measurement bias derived from the examiners. In this respect, including the IOP_{RBT} in the study should eliminate such bias.

Nucci *et al.*[26] described significantly lower total antioxidant capacity in the aqueous humor and blood samples from patients with POAG compared with controls. Sorkhabi *et al.*[27] reported a correlation between a higher aqueous humor 8-hydroxy-2'-deoxyguanosine (8-OHdG) level, a marker of oxidative stress-induced DNA damage, and between a higher 8-OHdG level and lower antioxidant capacity levels in serum samples obtained from patients with glaucoma. Oral intake of astaxanthin, an antioxidant phytochemical,[28] reduced total hydroperoxide in aqueous humor.[29] Thus, the systemic antioxidant capacity can reflect the local ocular redox status. Hydrogen peroxide treatment affects the cytoskeletal structure and cell-matrix interactions in TM cells;[30] depletion of glutathione and treatment with hydrogen peroxide decrease the TM outflow facility.[31] Increased 8-OHdG levels in human TM specimens are associated with higher IOP[7] and more severe visual field loss[32,33] in OAG; increased aqueous humor oxidative stress is associated with higher IOP in patients with EX.[34] Considering all those results, changes in systemic redox status such as decreased antioxidant capacity can be associated with increased IOP via its roles in the redox status of intraocular components such as the aqueous humor and TM cells. In a previous study, use of two tablets daily of Mirtogenol for 12 weeks significantly decreased the plasma dROM level in subjects with ocular hypertension,[15] although we could not reproduce the results in the current subjects (Table 5). Accordingly, although the mechanism of IOP reduction by French maritime pine bark/bilberry fruit extracts can be explained by the effects on systemic redox status, this mechanism is inconclusive in our study and requires further confirmation.

Previous studies have reported a significant association between IOP elevation and high systolic blood pressure[35,36] or both high systolic and diastolic blood pressures.[37,38] The proposed mechanisms of the roles of blood pressure in IOP elevation are that increased blood pressure leads to an increased filtration fraction of the aqueous humor through elevated ciliary artery pressure, and increased serum corticoids and sympathetic tone result in elevated IOP.[39,40] Mirtoselect® improved several retinal microcirculatory and perfusional parameters.[15] Pycnogenol® increased endothelium-dependent vasodilation[41] and decreased systolic and diastolic blood pressure[42] in hypertensive patients. Accordingly, decreased sympathetic tone and blood pressure may be involved in the IOP reduction caused by French maritime pine bark/bilberry fruit extracts, and, therefore, blood pressure should be measured in future studies.

Although we conducted this study as an initial pilot study in Japan, the small number of subjects and lack of a control group were the major weakness of this study. The times of the site visits and supplementation intake were not uniform among the subjects in this study, and blood samples were not obtained after fasting; thus, these variations might have weakened the power of statistical detection. Other than lower baseline IOP, racial and age differences of the subjects also may have been associated with the lower magnitude of IOP reduction in this study compared with previous studies.[15,22,23]

In conclusion, we found that administration of French maritime pine bark/bilberry fruit extracts for 4 weeks can further reduce IOP even in Japanese patients with POAG who have controlled IOP with use one bottle of glaucoma medication or more. Individual analyses have suggested that some patients do not respond to French maritime pine bark/bilberry fruit extracts (Table 4). Thus, further studies that confirm the IOP-lowering effects of this supplementation and determined the mechanisms of the reductions are needed to enhance the use of French maritime pine bark/bilberry fruit extracts in glaucoma management.

Author Contributions

KM, SK, YT, EM, and MT conceived and designed the study. KM, AT, MM, YM, and MT collected the clinical data and blood samples. SK performed the laboratory experiments. KM and MT analyzed the data. KM and MT wrote the manuscript. SK, AT, MM, YM, YT, and EM reviewed the manuscript.

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Abbreviations

BAP	biologic antioxidant potential
BCVA	best-corrected visual acuity
CCT	central corneal thickness
dROM	diacron reactive oxygen metabolite
EX	pseudoexfoliation syndrome
IOP	intraocular pressure
IOP_{GAT}	IOP measured by Goldmann applanation tonometer
IOP_{RBT}	IOP measured by iCare Home rebound tonometer
MD	visual field mean deviation
OAG	open-angle glaucoma
8-OHdG	8-hydroxy-2'-deoxyguanosine
POAG	primary OAG
RGC	retinal ganglion cell
SERE	spherical equivalent refractive error
SH	sulfhydryl
TM	trabecular meshwork

Conflict of Interest

MT received lecture fees, consultant fees, and research donations from Santen Pharmaceutical Co., Ltd. YT and EM are employees of Santen Pharmaceutical Co., Ltd.

References

1 Foster A, Resnikoff S. The impact of Vision 2020 on global blindness. *Eye (Lond)* 2005; **19**: 1133–1135.
2 Iwase A, Araie M, Tomidokoro A, Yamamoto T, Shimizu H, Kitazawa Y; Tajimi Study Group. Prevalence and causes of low vision and blindness in a Japanese adult population: the Tajimi Study. *Ophthalmology* 2006; **113**: 1354–1362.
3 Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004; **363**: 1711–1720.
4 Alvarado JA, Murphy CG. Outflow obstruction in pigmentary and primary open angle glaucoma. *Arch Ophthalmol* 1992; **110**: 1769–1778.
5 Lütjen-Drecoll E, Shimizu T, Rohrbach M, Rohen JW. Quantitative analysis of 'plaque material' in the inner- and outer wall of Schlemm's canal in normal- and glaucomatous eyes. *Exp Eye Res* 1986; **42**: 443–455.
6 Izzotti A, Bagnis A, Saccà SC. The role of oxidative stress in glaucoma. *Mutat Res* 2006; **612**: 105–114.
7 Izzotti A, Longobardi M, Cartiglia C, Saccà SC. Mitochondrial damage in the trabecular meshwork occurs only in primary open-angle glaucoma and in pseudoexfoliative glaucoma. *PLoS One* 2011; **6**: e14567.
8 Takai Y, Tanito M, Ohira A. Multiplex cytokine analysis of aqueous humor in eyes with primary open-angle glaucoma, exfoliation glaucoma, and cataract. *Invest Ophthalmol Vis Sci* 2012; **53**: 241–247.
9 Tanito M, Kaidzu S, Takai Y, Ohira A. Status of systemic oxidative stresses in patients with primary open-angle glaucoma and pseudoexfoliation syndrome. *PLoS One* 2012; **7**: e49680.
10 Umeno A, Tanito M, Kaidzu S, Takai Y, Horie M, Yoshida Y. Comprehensive measurements of hydroxylinoleate and hydroxyarachidonate isomers in blood samples from primary open-angle glaucoma patients and controls. *Sci Rep* 2019; **9**: 2171.
11 Umeno A, Tanito M, Kaidzu S, Takai Y, Yoshida Y. Involvement of free radical-mediated oxidation in the pathogenesis of pseudoexfoliation syndrome detected based on specific hydroxylinoleate isomers. *Free Radic Biol Med* 2019; **147**: 61–68.
12 Tanito M, Kaidzu S, Takai Y, Ohira A. Correlation between systemic oxidative stress and intraocular pressure level. *PLoS One* 2015; **10**: e0133582.
13 Tanito M, Kaidzu S, Takai Y, Ohira A. Association between systemic oxidative stress and visual field damage in open-angle glaucoma. *Sci Rep* 2016; **6**: 25792.
14 Yamada E, Himori N, Kunikata H, *et al*. The relationship between increased oxidative stress and visual field defect progression in glaucoma patients with sleep apnoea syndrome. *Acta Ophthalmol* 2018; **96**: e479–e484.
15 Gizzi C, Torino-Rodriguez P, Belcaro G, Hu S, Hosoi M, Feragalli B. Mirtogenol® supplementation in association with dorzolamide-timolol or latanoprost improves the retinal microcirculation in asymptomatic patients with increased ocular pressure. *Eur Rev Med Pharmacol Sci* 2017; **21**: 4720–4725.
16 Carratelli M, Porcaro L, Ruscica M, De Simone E, Bertelli AA, Corsi MM. Reactive oxygen metabolites and prooxidant status in children with Down's syndrome. *Int J Clin Pharmacol Res* 2001; **21**: 79–84.
17 Cornelli U, Terranova R, Luca S, Cornelli M, Alberti A. Bioavailability and antioxidant activity of some food supplements in men and women using the D-Roms test as a marker of oxidative stress. *J Nutr* 2001; **131**: 3208–3211.
18 Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL. Analytical performances of d-ROMs test and BAP test in canine plasma. Definition of the normal range in healthy Labrador dogs. *Vet Res Commun* 2008; **32**: 137–143.
19 Martinovic J, Dopsaj V, Dopsaj MJ, *et al*. Long-term effects of oxidative stress in volleyball players. *Int J Sports Med* 2009; **30**: 851–856.
20 Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**: 70–77.
21 Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968; **25**: 192–205.
22 Steigerwalt RD, Gianni B, Paolo M, Bombardelli E, Burki C, Schönlau F. Effects of Mirtogenol on ocular blood flow and intraocular hypertension in asymptomatic subjects. *Mol Vis* 2008; **14**: 1288–1292.
23 Steigerwalt RD Jr, Belcaro G, Morazzoni P, Bombardelli E, Burki C, Schönlau F. Mirtogenol potentiates latanoprost in lowering intraocular pressure and improves ocular blood flow in asymptomatic subjects. *Clin Ophthalmol* 2010; **4**: 471–476.
24 Yamamoto T, Iwase A, Araie M, *et al*.; Tajimi Study Group, Japan Glaucoma Society. The Tajimi Study report 2: prevalence of primary angle closure and secondary glaucoma in a Japanese population. *Ophthalmology* 2005; **112**: 1661–1669.
25 Konstas AG, Kahook MY, Araie M, *et al*. Diurnal and 24-h intraocular pressures in glaucoma: monitoring strategies and impact on prognosis and treatment. *Adv Ther* 2018; **35**: 1775–1804.
26 Nucci C, Di Pierro D, Varesi C, *et al*. Increased malondialdehyde concentration and reduced total antioxidant capacity in aqueous humor and blood samples from patients with glaucoma. *Mol Vis* 2013; **19**: 1841–1846.
27 Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A, Rashtchizadeh N, Moharrery M. Oxidative DNA damage and total antioxidant status in glaucoma patients. *Mol Vis* 2011; **17**: 41–46.
28 Shimokawa T, Yoshida M, Fukuta T, Tanaka T, Inagi T, Kogure K. Efficacy of high-affinity liposomal astaxanthin on up-regulation of age-related markers induced by oxidative stress in human corneal epithelial cells. *J Clion Biochem Nutr* 2019; **64**: 27–35.
29 Hashimoto H, Arai K, Takahashi J, Chikuda M. Effects of astaxanthin on VEGF level and antioxidation in human aqueous humor: difference by sex. *J Clin Biochem Nutr* 2019; **65**: 47–51.
30 Zhou L, Li Y, Yue BY. Oxidative stress affects cytoskeletal structure and cell-matrix interactions in cells from an ocular tissue: the trabecular meshwork. *J Cell Physiol* 1999; **180**: 182–189.
31 Kahn MG, Giblin FJ, Epstein DL. Glutathione in calf trabecular meshwork and its relation to aqueous humor outflow facility. *Invest Ophthalmol Vis Sci* 1983; **24**: 1283–1287.
32 Izzotti A, Saccà SC, Cartiglia C, De Flora S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. *Am J Med* 2003; **114**: 638–646.
33 Saccà 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**: 458–463.
34 Beyazyildiz E, Cankaya AB, Beyazyıldiz O, *et al*. Disturbed oxidant/antioxidant balance in aqueous humour of patients with exfoliation syndrome. *Jpn J Ophthalmol* 2014; **58**: 353–358.
35 Lee MK, Cho SI, Kim H, *et al*. Epidemiologic characteristics of intraocular pressure in the Korean and Mongolian populations: the Healthy Twin and the GENDISCAN study. *Ophthalmology* 2012; **119**: 450–457.
36 Lin CP, Lin YS, Wu SC, Ko YS. Age- and gender-specific association between intraocular pressure and metabolic variables in a Taiwanese population. *Eur J Internal Med* 2012; **23**: 76–82.
37 Chang YC, Lin JW, Wang LC, Chen HM, Hwang JJ, Chuang LM. Association of intraocular pressure with the metabolic syndrome and novel cardiometabolic risk factors. *Eye (Lond)* 2010; **24**: 1037–1043.
38 Park SS, Lee EH, Jargal G, Paek D, Cho SI. The distribution of intraocular pressure and its association with metabolic syndrome in a community. *J Prevent Med Pub* 2010; **43**: 125–130.
39 Bulpitt CJ, Hodes C, Everitt MG. Intraocular pressure and systemic blood pressure in the elderly. *Br J Ophthalmol* 1975; **59**: 717–720.
40 Shiose Y, Kawase Y. A new approach to stratified normal intraocular pressure in a general population. *Am J Ophthalmol* 1986; **101**: 714–721.
41 Nishioka K, Hidaka T, Nakamura S, *et al*. Pycnogenol, French maritime pine bark extract, augments endothelium-dependent vasodilation in humans. *Hypertension Res* 2007; **30**: 775–780.
42 Pourmasoumi M, Hadi A, Mohammadi H, Rouhani MH. Effect of pycnogenol supplementation on blood pressure: a systematic review and meta-analysis of clinical trials. *Phytother Res* 2020; **34**: 67–76.

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