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® Gliagenox 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 “glaucomatic” optic neuropathy and visual field loss, is a leading cause of irreversible blindness worldwide,(1–6) including Japan.(5) 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.(5) 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).(5) 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.(5–8)

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 (IOPgat). 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 IOPgat 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) (IOPrim).
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 supplements (e.g., extracts of pine bark and bilberry fruits; flavonoids such as catechin, anthocyanin, and procyanidins; 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; the possibility of becoming pregnant 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 Pharmacopoeia 9.0 on “Fresh bilberry fruit dry extract, refined and standardized” (information provided by the manufacturer). The IOP$_{\text{GAT}}$ 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.

| Case | 18 | ± 2.0 | 1–3 |
| Eyes | 29 | ± 0.8 | 1–3 |
| Age (years) | 57.7 ± 8.0 | ± 2.0 | 1–3 |
| Sex | | | |
| Male | 7 | ± 0.8 | 1–3 |
| Female | 11 | ± 0.8 | 1–3 |
| Number of glaucoma medications (bottle) | | | |
| 1 | 6 | ± 0.8 | 1–3 |
| 2 | 6 | ± 0.8 | 1–3 |
| 3 | 6 | ± 0.8 | 1–3 |
| Glaucoma medication | | | |
| Prostaglandin | 18 | ± 0.8 | 1–3 |
| β blocker | 16 | ± 0.8 | 1–3 |
| CAI | 10 | ± 0.8 | 1–3 |
| α2 stimulator | 7 | ± 0.8 | 1–3 |
| Pilocarpine | 1 | ± 0.8 | 1–3 |
| Best-corrected visual acuity (logMAR) | –0.03 | ± 0.8 | 1–3 |
| Spherical equivalent refractive error (D) | –5.7 | ± 0.8 | 1–3 |
| Visual field MD (dB) | –5.1 | ± 0.8 | 1–3 |
| Lens status | | | |
| Phakic | 22 | ± 0.8 | 1–3 |
| Pseudophakic | 7 | ± 0.8 | 1–3 |
| Corneal thickness (um) | 540 | ± 0.8 | 1–3 |

n, number; CAI, carbonic anhydrase inhibitor; logMAR, logarithm of the minimum angle of resolution; MD, mean deviation; dB, decibels; D, diopters.
IOP measurement. At the week –2, 0, and 4 visits, one of the two examiners (KM or MT) measured the IOP \( \text{GAT} \). During periods 1 and 2, the subjects were instructed to perform self-tonometry to record the IOP \( \text{RBT} \) 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 \( \text{RBT} \) 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 \(-80^\circ \text{C}\) 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 \(4^\circ \text{C}\). All blood analyses were performed using a free radical analyzer system (FREE Carpe Diem, Wismerrl 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 System, according to the manufacturer's instructions. To analyze 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 alkoxo and peroxy radicals. These newly formed radicals oxidize an additive aromatic amine (\(N,N\)-diethyl-\(para\)-phenylenediamine) 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 \(\mu\)mol/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 \(\mu\)mol/L of the SH groups.

**Statistical analysis.** The IOP \( \text{GAT} \), dROM, BAP, and SH were compared between weeks 0 and 4 using the paired \( t \) test. The IOP \( \text{RBT} \) measured at any time, and the IOP \( \text{RBT} \) 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 \( \text{RBT} \). For each subject, the IOP \( \text{RBT} \) pooled data were compared between periods 1 and 2 using the unpaired \( t \) test. All statistical analyses other than the pooled IOP \( \text{RBT} \) 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 \( \text{RBT} \) 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 \( \text{GAT} \) values that were measured by the examiner are summarized in Table 2. The IOP \( \text{GAT} \) of 17.1 mmHg at week –2 (i.e., the day of study inclusion) was equivalent to the IOP \( \text{GAT} \) of 17.2 mmHg at week 0 (i.e., the day before the start of supplement intake). In contrast, the IOP \( \text{GAT} \) 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 \( \text{RBT} \) values that were measured by the subjects are summarized in Table 3. In the morning, the IOP \( \text{RBT} \) 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 \( \text{RBT} \) 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 \( \text{RBT} \) values during period 1, the pooled IOP \( \text{RBT} \) 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...
pine bark extract and 90mg of bilberry fruit extract for 4 weeks. Significantly between the week 0 and 4 visits.

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 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 of 25.2 mmHg to 22.0 mmHg at 3 months and 22.0 mmHg at 6 months. 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. 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 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. In that study, simultaneous intake of the supplement potentiated the IOP-lowering effect of topical latanoprost. 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 exceeded 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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**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 AM–9 AM) | 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 PM–3 PM) | 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 (6 PM–9 PM) | 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* |
|------|------------------------|----------------------|-------------------------------|----------|
|      | Mean ± SD Range        | Mean ± SD Range      | Mean ± SD Range               |          |
| 1    | 15.4 ± 2.6 14.8–16.1  | 15.3 ± 2.1 14.8–14.7 | –1.0 ± 0.2 –2.6–0.1          | <0.0001  |
| 2    | 11.6 ± 2.3 10.9–12.4  | 11.2 ± 2.9 10.5–12   | 0.0 ± 0.6 –1.5–0.9          | 0.5026   |
| 3    | 13.2 ± 1.6 12.4–13.9  | 12.9 ± 2.4 12.5–14   | –0.3 ± 0.3 –1.9–0.6          | 0.0002   |
| 4    | 12.4 ± 3 11.5–13.3     | 9.8 ± 1.9 9.3–10.2   | +2.5 ± 0.7 3.6–1.8          | <0.0001  |
| 5    | 13.2 ± 2.3 12.4–13.6  | 12.9 ± 2.1 12.7–13.7 | +0.1 ± 0.4 0.6–0.9          | 0.6987   |
| 6    | 11.6 ± 3 10.6–12.5     | 11.2 ± 2.9 10.5–12   | –0.3 ± 0.6 –1.5–0.9          | 0.5026   |
| 7    | 14.2 ± 1.9 13.7–14.7  | 12.9 ± 2.4 12.5–13.4 | –1.3 ± 0.3 –1.9–0.6          | 0.0002   |
| 8    | 12.5 ± 1.9 11.9–13     | 11.4 ± 1.8 11–11.8   | –1.1 ± 0.3 –1.7–0.4          | 0.0021   |
| 9    | 10.1 ± 3 9.2–11.1     | 12 ± 2.4 11.4–12.5   | +1.9 ± 0.5 0.9–2.8          | 0.0003   |
| 10   | 14.2 ± 2.7 13.4–15     | 14 ± 2.3 14–14.5     | +0.7 ± 0.4 0.2–1.6          | 0.1213   |
| 11   | 12.1 ± 1.9 11.5–12.6  | 10.9 ± 2.3 10.4–11.5 | –1.2 ± 0.4 –2.0–0.3          | 0.0065   |
| 12   | 14.1 ± 4.3 13–15.3     | 15.3 ± 1.9 14.8–15.7 | +1.2 ± 0.5 0.1–2.2          | 0.0336   |
| 13   | 13.8 ± 2.1 13.3–14.3  | 13 ± 1.9 12.5–13.4   | –0.8 ± 0.3 –1.5–0.2          | 0.0114   |
| 14   | 22.8 ± 3.9 21.7–23.9   | 22.6 ± 3.2 21.8–23   | –0.2 ± 0.6 –1.5–1.0          | 0.7111   |
| 15   | 17.9 ± 2.2 17.3–18.5  | 16.9 ± 2.6 16.3–17.5 | –1.0 ± 0.4 –1.9–0.2          | 0.0181   |
| 16   | 14.6 ± 2.7 13.8–15.3   | 12.9 ± 2.5 12.3–13.4 | –1.7 ± 0.5 –2.6–0.7          | 0.0005   |
| 17   | 9.9 ± 2.2 9.3–10.5     | 11 ± 2 10.5–11.4    | +1.0 ± 0.4 0.3–1.8          | 0.0075   |
| 18   | 12.7 ± 2.8 11.9–13.6   | 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 Mean ± SD Range | Week 4 Mean ± SD Range | Difference (Week 4–Week 0) Mean ± SD Range | p value* |
|------------------|------------------------|------------------------|-------------------------------------------|----------|
| 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                         | 0.7902   |
| 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.
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\(_{\text{RBT}}\) 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\(_{\text{RBT}}\) level was the highest in the morning in the current subjects. Previous studies of subjects with ocular hypertension with higher baseline IOPs\(^{(12,22,23)}\) have reported much larger reductions of IOP after supplementation than in the current study; thus, the lower baseline IOP\(_{\text{RBT}}\) 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,\(^{(14,22,23)}\) 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\(_{\text{GAT}}\) might include a measurement bias derived from the examiners. In this respect, including the IOP\(_{\text{RBT}}\) in the study should eliminate such bias.

Nucci \textit{et al.}\(^{(26)}\) described significantly lower total antioxidant capacity in the aqueous humor and blood samples from patients with POAG compared with controls. Sorkhabi \textit{et al.}\(^{(34)}\) 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,\(^{(29)}\) 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\(^{(32,35)}\) 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.\(^{(29,30)}\) Mirtoselect\(^{(1)}\) improved several retinal microcirculatory and perfusional parameters.\(^{(15)}\) Pycnogenol\(^*(\) increased endothelium-dependent vasodilation\(^{(24)}\) and decreased systolic and diastolic blood pressure\(^{(15)}\) 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 weaknesses 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 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.

\textbf{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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\textbf{Abbreviations}

| Abbreviation | Description |
|--------------|-------------|
| BAP          | biologic antioxidant potential |
| BCVA         | best-corrected visual acuity |
| CCT          | central corneal thickness |
| dROM         | Diacron reactive oxygen metabolite |
| EX           | exfoliation syndrome |
| IOP          | intraocular pressure |
| IOP\(_{\text{GAT}}\) | IOP measured by Goldmann applanation tonometer |
| IOP\(_{\text{RBT}}\) | 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           | sulfhydril |
| TM           | trabecular meshwork |

\textbf{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.
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