Clinical Interventions in Aging

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ORIGINAL RESEARCH

Oral administration of French maritime pine bark extract (Flavangenol®) improves clinical symptoms in photoaged facial skin

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Background: French maritime pine bark extract (PBE) has gained popularity as a dietary supplement in the treatment of various diseases due to its polyphenol-rich ingredients. Oligometric proanthocyanidins (OPCs), a class of bioflavonoid complexes, are enriched in French maritime PBE and have antioxidant and anti-inflammatory activity. Previous studies have suggested that French maritime PBE helps reduce ultraviolet radiation damage to the skin and may protect human facial skin from symptoms of photoaging. To evaluate the clinical efficacy of French maritime PBE in the improvement of photodamaged facial skin, we conducted a randomized trial of oral supplementation with PBE.

Methods: One hundred and twelve women with mild to moderate photoaging of the skin were randomized to either a 12-week open trial regimen of 100 mg PBE supplementation once daily or to a parallel-group trial regimen of 40 mg PBE supplementation once daily.

Results: A significant decrease in clinical grading of skin photoaging scores was observed in both time courses of 100 mg daily and 40 mg daily PBE supplementation regimens. A significant reduction in the pigmentation of age spots was also demonstrated utilizing skin color measurements.

Conclusion: Clinically significant improvement in photodamaged skin could be achieved with PBE. Our findings confirm the efficacy and safety of PBE.

Keywords: polyphenols, pine bark extract, skin photoaging, antioxidants, antiaging

Introduction

French maritime pine bark extract (*Pinus maritima*, PBE) is a complex mixture of bioflavonoids, with oligometric proanthocyanidins (OPCs) as the major constituents. OPCs are dimers or oligomers of catechin, epicatechin, and their gallic acid esters. The major OPCs in PBE are proanthocyanidin B_1 (epicatechin-(4β→8)-catechin), catechin, and epicatechin.[1]

The process of concentrative extraction of OPCs from pine bark was established by Masquelier et al in 1948.[2] OPCs are found in a wide range of other plants, including common foods in an ordinary diet,[3,4] and are widely consumed as antioxidant supplements. Recent research has focused primarily on the clinical efficacy of PBE, including a wide range of cardiovascular benefits.[5,6] In addition to their antioxidant properties, PBE has been reported to lower blood pressure and to improve glycemic control, the lipid profile, fatty acid synthesis, and the peripheral circulation.[7]

Skin aging is accelerated on sun-exposed areas.[8] Solar ultraviolet radiation is a key factor in the photoaging process of human skin. Ultraviolet radiation generates reactive oxygen species (ROS) and thus leads to oxidative stress. To counteract

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the harmful effects of oxidative damage by ROS, the skin is naturally equipped with ultraviolet inducible/adaptive antioxidant systems that have evolved to quench intracellular ROS, and consist of a variety of low molecular weight antioxidants (vitamins C and E) and intrinsic antioxidant enzymes for ROS-scavenging activity.[9,10] However, antioxidant mechanisms are not completely efficient and their limitations become gradually more pronounced during aging. The end result of this process is an imbalance in cellular redox homeostasis, causing indiscriminate oxidative damage to a wide range of biomolecules.[11] Oxidative stress induces proinflammatory cytokines, which in turn increase intracellular levels of ROS.[12] Consequently, there is an age-associated augmentation in both ROS production and in levels of oxidized/degraded proteins.[13,14]

Localized hyperpigmented lesions recognized as "age spots" are the most visible alterations in photoaged Asian skin.[15] They are clinically diagnosed as solar lentigines, and are frequently associated with mottled pigmentation and slight keratosis.[16] To date, almost all standard protocols for treating age spots are topical therapies.[17,18] Taking photoaging and ultraviolet defense mechanisms into consideration, an oral antioxidant treatment is thought to be an effective option. Orally administered vitamins or botanical polyphenols demonstrate ultraviolet-protective properties in the skin.[19–21] OPCs have been reported to be the strongest quenchers of ROS among the various antioxidants.[22] Therefore, PBE is expected to prevent photoaging and to improve the appearance of photodamaged skin.

PBE has been used as a food ingredient for thousands of years because of its safety and astringent taste. PBE and other OPC products, such as grape seed extracts, are now widely used as dietary supplements. Extensive research conducted with various formulations of PBE has already established its safety and tolerability for long-term human consumption.[23] However, an aqueous green tea extract supplement, which is another popular food source of polyphenols, has recently been reported to have the potential to induce liver failure.[24,25] Such adverse reactions appear to happen more often in European countries and in Canada, when high-dose green tea extracts are taken orally for a long time,[26–29] although green tea is consumed widely in Japan, China, and other Asian nations, and is becoming more popular in Western nations.

Taking these findings into consideration, in addition to dermatological testing of the efficacy of PBE, we characterized its hepatic safety and efficacy in the improvement of glycemic control and the plasma lipoprotein profile, as was recently shown in animal studies.[30] We performed total blood assays, including liver function and cholesterol blood tests. The primary purpose of this study was to evaluate the clinical efficacy and safety of French maritime PBE for improvement of photodamaged facial skin in a randomized trial of oral supplementation with PBE.

Materials and methods

French maritime PBE

French maritime PBE (Flavangenol®) was provided by Toyo Shinyaku Inc, Tosu City, Saga, Japan. Flavangenol is a registered trademark in Japan, and the trademark Toyo-FVG® has been registered in the US.

Patients

We enrolled 112 healthy women younger than 60 years with age spots, mostly diagnosed as solar lentigines, and multiple symptoms of photodamaged skin, including mottled pigmentation, roughness (including dry flaky skin), wrinkles, and swelling. All women enrolled in this study had mild to moderate facial photodamage graded on the Glogau scale between II and III and Fitzpatrick skin phototypes III to IV. After approval by the institutional ethics committee of Fukuoka University, which adheres to the principles of the Declaration of Helsinki, informed consent was obtained from all participants in the study. None of the subjects took topical/systemic retinoids, health food supplements, oral medications such as hormone replacement therapy, or topical medications, or were pregnant 4 weeks prior to enrolling in this study.

Study design

Because of the seasonal fluctuation in daily doses of ultraviolet radiation, all clinical trials were started in December to minimize the influence of seasonal skin changes, such as sun tanning and the darkening of age spots due to environmental ultraviolet light exposure. In order to assess the time course of changes in photoaging scores, clinical assessments were conducted at baseline (study day 1) and every 4 weeks thereafter.

Twenty-four women were enrolled in an open-label, high-dose PBE trial and were treated with 100 mg/day PBE for 12 weeks, while a further 88 women were enrolled in part 1 of a separate low-dose trial and treated with PBE 40 mg/day for a total of 24 weeks in an open-label, randomized, parallel-group comparative fashion. The time course of the low-dose PBE trial is shown in Figure 1, in which randomization of subjects into groups 1 and 2 was performed using a computer-generated random number table. Allocation to

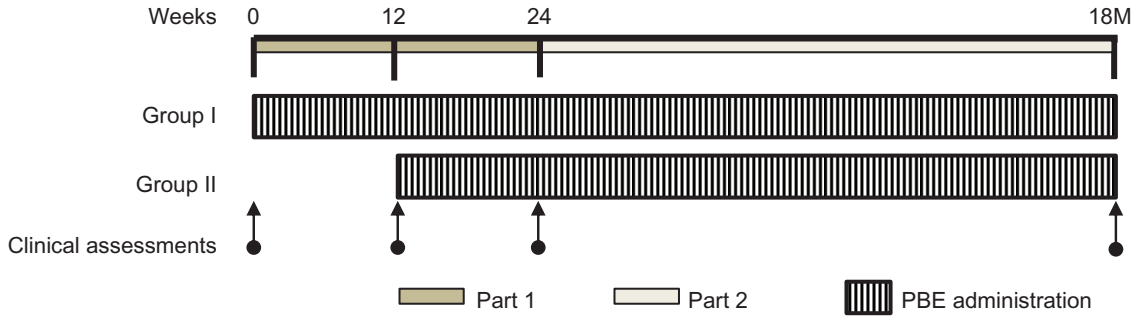

Figure 1 Schematic outline of part 1 and part 2 of study using low-dose pine bark extract.
Abbreviation: PBE, pine bark extract.

group 1 or group 2 was done by opening the next sequentially numbered, sealed, opaque envelope. Group 1 participants were asked to take PBE 40 mg/day once daily, and to use a cleanser and sunscreen for 24 weeks throughout part 1 of the study. Group 2 participants were merely placed under observation without taking PBE for the first 12 weeks before starting oral treatment with PBE 40 mg/day once daily for the next 12 weeks, and were instructed to use a cleanser and sunscreen for 24 weeks throughout part 1 of the study.

We compared the improvement between subjects who received oral PBE for 24 weeks (group 1) and those who received only 12 weeks of treatment (group 2), corresponding to the latter half of the 24-week treatment period for group 1. At the end of part 1 of the low-dose study, 24 subjects were instructed to take PBE for a further 12 months (part 2 of the study) to evaluate the long-term efficacy and safety of PBE (Figure 1).

Subjective assessments

The subjects completed a self-assessment questionnaire concerning their facial skin on a scale of 0 to 5 of increasing severity for the following criteria: pigmentation, wrinkles, roughness, dryness, and overall improvement. Each patient was asked to complete a self-assessment questionnaire at the final visit of each study, ie, after 12 weeks of treatment in the high-dose PBE trial or 24 weeks after completing part 1 of the low-dose trial. If a patient checked the "unknown" option, their results were excluded from statistical analysis.

Three board-certified dermatologists subjectively graded the subjects for clinical symptoms, ie, solar lentigines, mottled pigmentation, roughness (including dry/flaky skin), wrinkles, and swelling, based on a standardized assessment scale using photographs, after which an overall evaluation of the results was performed. All assessors remained unaware of subject treatment assignment throughout the trial.

Objective measurements

At each visit, the melanin index of the age spots was evaluated using a method described in a previous report.[31] A colorimeter (CR-13, Konica-Minolta, Tokyo, Japan) was used to record facultative forehead skin color by calculating individual typology angle (ITA) degrees, as described elsewhere.[32] Fluctuation of facultative skin color was quantified in groups 1 and 2 during part 1 of the low-dose trial. In order to assess for any age-related decrease in epidermal keratinocyte turnover, we planimetrically measured the mean area of tape-stripped, basic fuchsin crystal violet-stained corneocytes by digital image analysis.

Safety assessments

Fasting (10 hours) blood samples were collected from all enrolled subjects on day 0 and after 12 weeks (high-dose trial only), and at 24 weeks and 18 months from the start of the trials for routine hematology, blood chemistry, and urine investigations. A lipid panel measuring high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and free fatty acids was used to assess the plasma lipoprotein cholesterol profile. Routine hematology tests and examination of serum iron/ferritin levels were used to detect iron deficiency anemia. Aspartate aminotransferase and alanine aminotransferase levels were used to assess the safety of PBE with respect to its potential liver toxicity. Assessment of these tests was conducted by an outside laboratory (BML Inc, Tokyo, Japan). Body weight measurements and body fat percentage assessments were performed for all subjects in the low-dose trial.

Data analysis

All statistical calculations were performed using StatView for Windows, version 5.0.1 (SAS Institute Inc, Cary, NC). The data are presented as the mean ± standard deviation, unless otherwise specified. An unpaired *t*-test or Mann–Whitney U test was used to compare subjects when equal variance was shown by the F-test or when equal variance was not shown,

respectively. For clinical assessments, the Wilcoxon signed-rank test for related samples was used in a time course of trials, and the Mann–Whitney U test was used for comparing the two treatment groups. A two-tailed, independent *t*-test was performed for grading of clinical assessments, with *P* values noted. Either Dunnett's test or the Tukey–Kramer method was used to compare the means of the melanin index at each point during the observational study, with *P* values noted. Clinical laboratory test results were analyzed statistically using a Student's two-tailed paired *t*-test.

Results

Demographics

Twenty-four subjects aged 31–59 (mean 36.9 ± 6.6) years completed 12 weeks of treatment with PBE 100 mg/day in the high-dose trial. Eighty-eight subjects enrolled into part 1 of the 24-week, low-dose, comparative study, and 77 (n = 38 in group 1, n = 39 in group 2) completed the 24-week treatment program and had data available for statistical analysis. The mean age at study entry was 36.7 ± 7.9 years. The background details of the subjects are shown in Table 1. There was no significant difference in background features (age or facultative skin color) at study entry between the two groups, suggesting adequate randomization. There were no clinically meaningful differences in laboratory tests or body measurements at study entry. Eleven withdrawals occurred because of poor compliance, amongst which no adverse events were reported. Twenty-four subjects participated in the 18-month part 2 phase of the study, consisting of 15 subjects from group 1 and nine subjects from group 2 who completed the 24-week study and had long-term data available for statistical analysis. Their mean age at study entry was 37.2 ± 4.4 years. Three dropouts occurred because of poor compliance, for whom no adverse events were reported.

Subjective efficacy assessment

In the high-dose trial, the questionnaire survey about subjective facial symptoms showed that a relatively large number of subjects felt that their facial skin roughness, including dry skin, had improved (Table 2). Multiple digital image assessments made by the dermatologists showed that 71% of participants had significant improvement of their age spots.

Table 1 Baseline demographics of randomized groups (phase I, low-dose PBE trial)

Characteristics	Group I N = 38	Group II N = 39	*P* value
Age (yrs)	39.6 (8.3)	33.8 (6.5)	0.66
ITA (degrees)	30.9 (8.3)	28.6 (5.4)	0.44

Note: Mean (SD).
Abbreviations: ITA, individual topology angle; PBE, pine bark extract.

Table 2 Score assessments of questionnaire survey (high-dose PBE trial, N = 24)

Symptoms	Improved	Unchanged	Aggravated
Age spots	7 (29)	16 (67)	1 (4)
Wrinkles	7 (29)	17 (71)	0 (0)
Roughness (dry skin)	9 (38)	15 (63)	0 (0)
Roughness (other than dry skin)	13 (54)	9 (38)	2 (8)

Notes: Number of patients (%). The score changes were evaluated as compared with baseline of symptoms before PBE administration.
Abbreviation: PBE, pine bark extract.

The percentage of group 1 subjects in part 1 of the low-dose trial who answered that their skin symptoms generally became better after starting oral PBE was around 30%, and this number was much larger than that for subjects who felt that their condition was aggravated after 24 weeks (Table 3A). A similar trend was seen for group 2 patients in the questionnaire, especially those related to facial photoaging symptoms after 24 weeks (Table 3B). In part 2 of the low-dose trial, over 40% of participants in groups 1 and 2 answered that their photoaging symptoms, including solar lentigines and mottled pigmentation, had improved after 18 months as compared with baseline (Table 4).

In part 1 of the low-dose trial, photoaging scores in group 1 as assessed by dermatologists after 12 weeks of PBE were compared with those at the beginning of the study. Scores for solar lentigines, mottled pigmentation, roughness, wrinkles, and swelling showed significant improvement at 12 weeks. Scores for solar lentigines, mottled pigmentation, wrinkles, and swelling were significantly improved (Table 5). Representative clinical images of a typically improved case before and after PBE are shown in Figure 2. In Group 2, there were no significant improvements in scores seen 12 weeks after initial observation as compared with scores before treatment. However, after 24 weeks of treatment, which consisted of a 12-week PBE treatment period following a

Table 3A Score assessments of questionnaire survey for group I (phase I, low-dose PBE trial, N = 38)

Symptoms	Improved	No change	Aggravated
Solar lentigines	12 (32)	24 (65)	1 (3)
Mottled pigmentation	14 (38)	22 (59)	1 (3)
Roughness (dry skin)	8 (23)	26 (74)	1 (3)
Roughness (other than dry skin)	5 (14)	28 (78)	3 (8)
Wrinkles	1 (3)	33 (97)	0 (0)

Notes: Number of patients (%). The score changes were evaluated as compared with baseline of symptoms before PBE administration.
Abbreviation: PBE, pine bark extract.

Table 3B Score assessments of questionnaire survey for group II (phase I, low-dose PBE trial, N = 39)

Symptoms	Improved	Unchanged	Aggravated
Solar lentigines	13 (33)	25 (64)	1 (3)
Mottled pigmentation	10 (26)	27 (71)	1 (3)
Roughness (dry skin)	11 (30)	24 (65)	2 (5)
Roughness (other than dry skin)	7 (18)	26 (68)	5 (13)
Wrinkles	4 (11)	31 (84)	2 (5)

Notes: Number of patients (%). The score changes were evaluated as compared with baseline of symptoms before PBE administration.
Abbreviation: PBE, pine bark extract.

12-week observation-only period, scores for solar lentigines, mottled pigmentation, and roughness had significantly improved (Table 5).

By comparing the scores between the two groups in part 1 of the low-dose trial, 12 weeks after the initial observation, scores for solar lentigines and skin roughness in part 1 showed significant improvement over those in group 2 (Table 5) during part 2 of the low-dose trial. All 21 cases showed significantly better scores for solar lentigines, mottled pigmentation, roughness, wrinkles, and swelling compared with those at the end of part 1 (Table 6). For part 1 of the low-dose trial, the overall rating for the therapeutic index achieved with low-dose PBE is summarized in Table 7A. An efficacy rating of slightly improved or better was recorded for 87% of subjects in group 1 and 72% in group 2. In part 2 of the trial, significant overall improvements were recorded for all 21 subjects (Table 7B).

Objective measurements

Time course of melanin pigmentation in age spots

The melanin index was calculated from the L*a*b* values for each digital image of an age spot. In the high-dose trial, the average melanin index of the age spots gradually decreased from baseline over the 12 weeks. A significant decrease in average melanin index scores at weeks 4 and 12 was confirmed using the Tukey–Kramer method (Figure 3). Twenty-one of the 88 subjects were followed in the low-dose trial. A significant decrease in average melanin index was observed in both groups, with the melanin index scores in group 1 being significantly lower than at baseline between weeks 8 and 24 ($P < 0.05$), while in group 2, mean melanin index scores were significantly decreased between weeks 16 and 24 (week 16 corresponds to 4 weeks after starting PBE in group 2, Figure 4A and B).

Table 4 Score assessment of questionnaire survey for 18-month PBE administration (phase II, low-dose PBE trial, N = 21)

Symptoms	Improved	Unchanged	Aggravated
Solar lentigines	9 (43)	12 (57)	0 (0)
Mottled pigmentation	9 (43)	10 (48)	1 (5)
Roughness (dry skin)	8 (38)	10 (48)	1 (5)
Roughness (other than dry skin)	4 (19)	12 (48)	2 (10)
Wrinkles	4 (19)	16 (76)	0 (0)

Note: Number of patients (%).
Abbreviation: PBE, pine bark extract.

Table 5 Physical examination scores (phase I, low-dose PBE trial)

Symptoms	Group	Baseline	12 weeks	24 weeks
Solar lentigines	I	2.8 (0.8)	2.3 (0.6)[c,d]	2.3 (0.5)[c]
	II	2.7 (0.7)	2.7 (0.7)	2.4 (0.8)[b]
Mottled pigmentation	I	2.5 (0.6)	2.1 (0.5)[c]	1.9 (0.4)[c]
	II	2.2 (0.7)	2.2 (0.6)	1.9 (0.6)[a]
Roughness	I	2.2 (0.7)	1.8 (0.6)[a,d]	1.9 (0.6)
	II	1.9 (0.8)	2.1 (0.8)	1.5 (0.6)[a]
Wrinkles	I	2.2 (0.7)	2.0 (0.6)[a]	1.9 (0.6)[b]
	II	2.1 (0.5)	2.1 (0.5)	2.0 (0.5)
Swelling	I	1.4 (0.8)	1.3 (0.5)[a]	1.2 (0.4)[a]
	II	1.4 (0.6)	1.4 (0.6)	1.3 (0.4)

Notes: Mean score (SD). The efficacy of PBE was assessed by analyzing the change from baseline scores. Reliability was assessed by Wilcoxon signed rank test. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$. Reliability was assessed by Mann–Whitney U test between group I and group II. [d]$P < 0.05$ (group I vs group II).
Abbreviation: PBE, pine bark extract.

Effects of PBE on facultative facial skin color

During parts 1 and 2 of the low-dose trial, the whitening effects of PBE on facultative facial skin color were evaluated by average ITA degrees chronologically obtained from a colorimetric assay of forehead skin color. Although the ITA values fluctuated in groups 1 and 2 throughout the study, the average ITA values did not show an overall trend towards lighter values with respect to facial complexion (Figure 5).

Effects of PBE on corneocyte size

We found a statistically significant reduction in the size of corneocytes in the surface layers of the skin during PBE

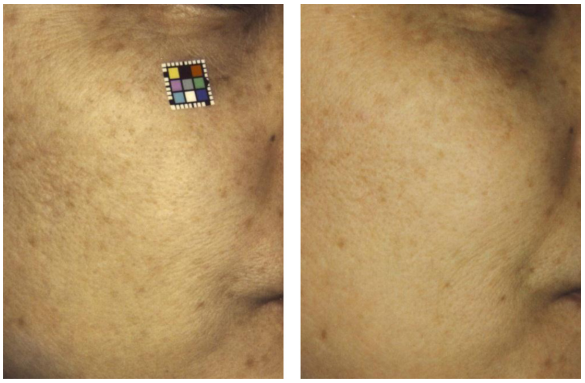

Figure 2 Before (left) and 6 months after (right) treatment of photodamaged skin with oral pine bark extract.

Table 6 Physical examination scores (phase II, low-dose PBE trial, N = 21)

Symptoms	Baseline	24 weeks	18 months
Solar lentigines	2.8 (0.8)	2.1 (0.4)[b]	1.9 (0.3)[c]
Mottled pigmentation	2.3 (0.6)	1.8 (0.4)[b]	1.6 (0.5)[b]
Roughness	2.0 (0.9)	1.5 (0.6)[a]	1.3 (0.5)[b]
Wrinkles	1.6 (0.7)	1.4 (0.5)	1.3 (0.5)[a]
Swelling	2.0 (0.8)	1.7 (0.7)[a]	1.6 (0.5)[a]

Notes: Mean score (SD). The efficacy of PBE was assessed by analyzing the change from baseline scores. Reliability was assessed by Wilcoxon signed rank test. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.0001$.
Abbreviation: PBE, pine bark extract.

Table 7B Overall efficacy rate after 18-month low-dose PBE administration (phase II, low-dose PBE trial)

Outcome	
Markedly improved	1 (5)
Moderately improved	15 (71)
Slightly improved	5 (24)
Unchanged	0 (0)
Aggravated	0 (0)
Total	21

Note: Number of patients (%).
Abbreviation: PBE, pine bark extract.

administration for longer than 5 months, corresponding to the latter periods of parts 1 and 2 in the low-dose trial. This suggests that PBE helped to repair existing skin damage, considering that corneocyte size usually increases with aging (Figure 6).

Safety and tolerability

In the high-dose trial, mean corpuscular volume demonstrated a significant change of values after 12 weeks, but the change was within the normal range. No other hematological parameters showed significant changes (Table 8A). In the low-dose trial, mean corpuscular hemoglobin showed a significant change of values in group 2 after 24 weeks, but this value change was within the normal range. No other data showed significant changes (Table 8B).

In the high-dose trial, blood chemistry tests demonstrated significant changes in serum sodium and chloride after 12 weeks of treatment with PBE. There were no other significant changes in blood chemistry parameters (Table 9A).

Significant changes were observed in aspartate aminotransferase in group 1 in the low-dose trial, 24 weeks after oral administration. In group 2, after 15–18 months of treatment with PBE, serum potassium concentrations changed significantly. However, these were both minor fluctuations within the normal range. Other test values, including glycemic control and plasma lipoprotein profile, did not show any significant changes at 18 months (Table 9B). Urine examination was entirely normal (data not shown).

Table 7A Overall efficacy rate after 24-week low-dose PBE administration (phase I, low-dose PBE trial)

Outcome	Group I	Group II
Markedly improved	1 (3)	1 (3)
Moderately improved	16 (42)	9 (23)
Slightly improved	16 (42)	18 (46)
Unchanged	5 (13)	11 (28)
Aggravated	0 (0)	0 (0)
Total	38	39

Note: Number of patients (%).
Abbreviation: PBE, pine bark extract.

No significant changes in body weight or body fat percentage were observed during the test period (data not shown). No adverse events were reported during the study, and the tolerability of PBE was deemed to be acceptable. PBE was considered to be systemically safe based on hematology and biochemistry. Although slight but significant fluctuations in the data were observed for some parameters, all values were within the normal biological ranges.

Discussion

We examined the efficacy of PBE in the treatment of photodamaged facial skin, and significant improvement was suggested from multiple dermatological score assessments during this study. A subject questionnaire concerning subjective facial symptoms demonstrated that a relatively large number of subjects felt that the roughness of their facial skin had improved in the high-dose trial. Although improvement in age spots was only recognized by a relatively small number of subjects in the high-dose trial, detailed evaluation of digital images revealed that 71% of participants had improvement of their age spots, albeit to a varying extent. In part 1 of the

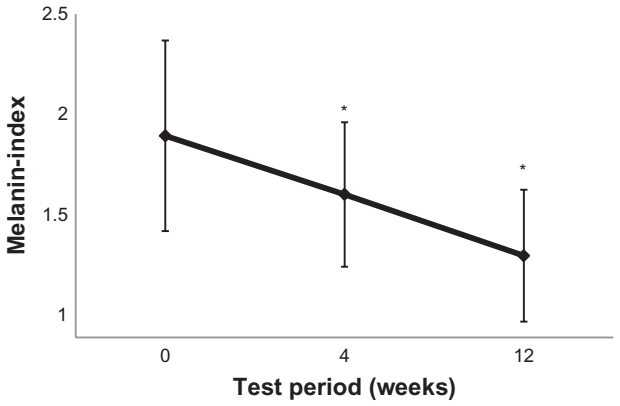

Figure 3 The melanin index was significantly decreased after 4 and 12 weeks of oral administration of pine bark extract 100 mg/day.
Notes: The melanin index was calculated according to the method reported by Yamamoto et al.[31] Values are expressed as the mean ± standard deviation. *$P < 0.05$ (Tukey–Kramer method), n = 24.

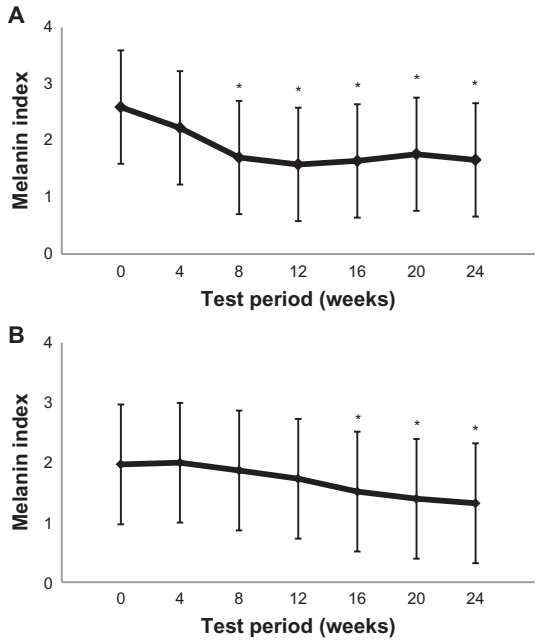

Figure 4 Time course of mean melanin index changes in age spots in group 1 (**A**) and group 2 (**B**).
Notes: A significant decrease in melanin index was seen after 4–8 weeks of oral pine bark extract 40 mg/day. Melanin index scores were calculated according to the method reported by Yamamoto et al.[31] Values are expressed as the mean ± standard deviation. *$P < 0.05$ (Tukey–Kramer method), n = 38 (group 1), n = 39 (group 2).

low-dose trial, there was significant improvement in scores for solar lentigines, mottled pigmentation, skin roughness, and swelling only when subjects were on treatment with PBE. Further, subjects treated with PBE had significantly lower scores for solar lentigines and skin roughness when compared with the untreated patients. Therefore, we consider that both the high-dose and low-dose arms in this study demonstrate a similar trend of improvement in symptoms of photodamaged facial skin. Further significant improvements were seen during the long-term 18-month study (part 2 of the low-dose trial) in almost every photoaging score. This improvement was maintained and enhanced by continuous administration of PBE over a long period. Finally, 72% of the subjects receiving PBE for 12 weeks (group 2) showed improvement versus 87% of those receiving PBE for 24 weeks (group 1). All subjects who completed treatment with PBE for 15–18 months showed improvement in symptoms. In line with the score assessment results, objective biophysical measurements demonstrated a significant gradual decrease in average melanin index during treatment with PBE in both trials.

In an earlier safety evaluation, PBE showed excellent overall tolerability without any side effects.[33] Health concerns about prolonged administration of OPCs, such as iron and/or methionine deficiency,[34] were ruled out after it was found that there was no abnormal nutritional sequelae, including iron deficiency anemia, elevated cholesterol readings, or liver damage.

In an earlier study of treatment of photoaged skin with oral polyphenols,[35] a popular polyphenol-rich green tea extract containing (−)-epigallocatechin gallate (EGCG) was used. Although facial photoaging scores improved on treatment with the green tea extract for the first 12 months, there was no significant antiphotoaging effect after 24 months of

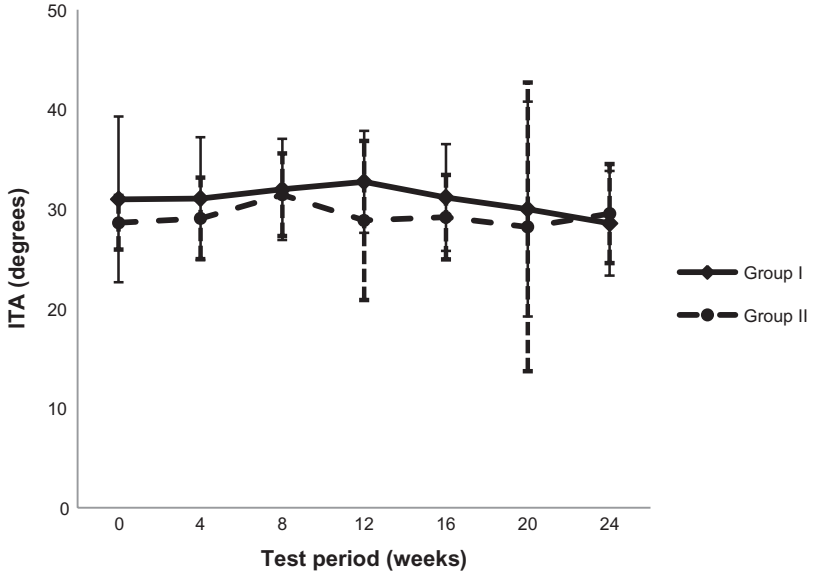

Figure 5 Facultative skin color was assessed using a colorimeter followed by individual typology angle value calculation.
Notes: The Mann–Whitney U test was used to determine differences between baseline and after treatment with pine bark extract. There are no significant differences between baseline and following treatment with pine bark extract ($P > 0.05$), n = 21.
Abbreviation: ITA, individual typology angle.

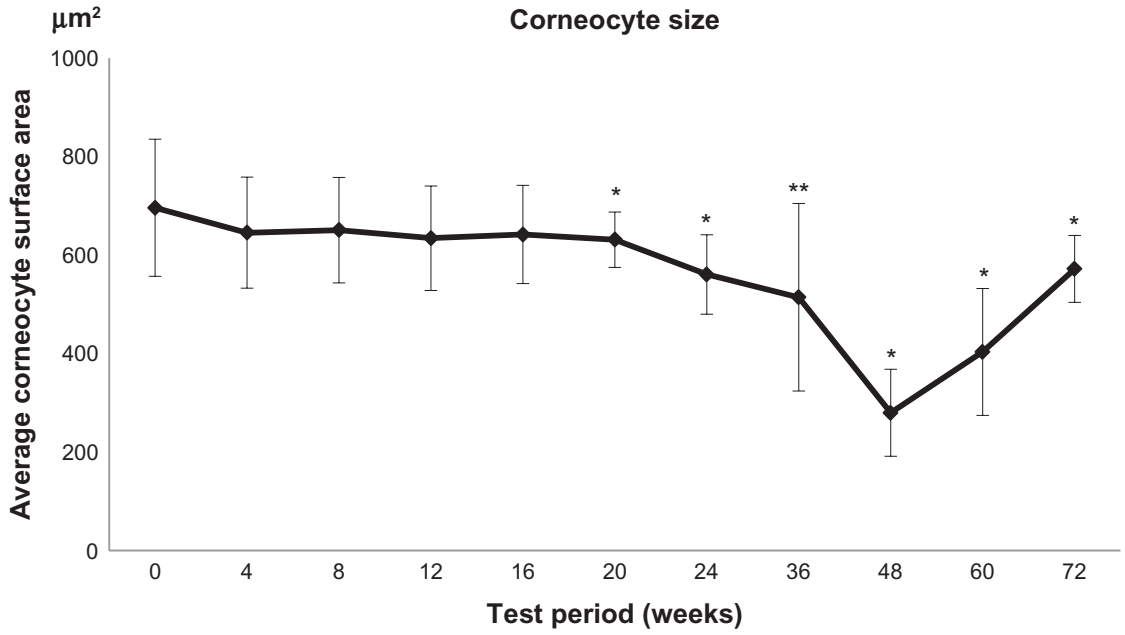

Figure 6 Time course of corneocyte size changes measured by a planimetric method during pine bark extract administration.
Notes: The mean surface size of the corneocytes decreased significantly after 5 months of treatment with pine bark extract. Values are expressed as the mean ± standard deviation of corneocyte surface area. *$P < 0.05$ (unpaired *t*-test); **$P < 0.05$ (Mann–Whitney U test), n = 21.

treatment. In contrast, gradual improvement of photoaging scores even at 18 months was confirmed in our PBE trial. Demographic diversity in subject age and race might account for the different results seen in these two studies. The mean age of the subjects in the previous study was around 12 years older than in our study, so a less favorable outcome would be expected because of the exponential decline of intrinsic antioxidative potency in the elderly. Our findings in Japanese women might be positively biased by a racial difference, ie, age spots in East Asians often appear as early as in the 20 s and 30 s, while age spots in Caucasians tend to become apparent between the ages of 50 and 60 years.[36]

Table 8A Results of hematology in 24 evaluated cases (high-dose PBE trial, 12-week administration)

Parameter (units)	Reference ranges	Baseline	12 weeks
Red blood cells (10^4/µL)	370–510	420.0 ± 31.2	420.2 ± 42.4
Hemoglobin (g/dL)	11–15.5	13.0 ± 0.7	12.8 ± 1.0
Hematocrit (%)	34–46	37.9 ± 2.9	38.1 ± 5.1
MCV (fL)	80–103	90.6 ± 6.1	90.9 ± 6.8[a]
MCH (pg)	26–35	30.6 ± 2.9	30.5 ± 3.3
MCHC (%)	31–36	33.7 ± 1.4	33.5 ± 1.5
Platelets (10^4/µL)	12–34	23.9 ± 5.7	24.8 ± 5.5
White blood cells (µL)	3500–9100	5276 ± 1780	5375 ± 1990
Neutrophil (%)	41–73	59.8 ± 9.3	60.5 ± 9.2
Lymphocyte (%)	20–50	32.0 ± 7.0	31.4 ± 7.5
Eosinophil (%)	0–9	2.3 ± 1.4	2.4 ± 1.5

Notes: Mean ± SD. [a]$P < 0.05$, Student's *t*-test for paired values, 2-tailed.
Abbreviations: PBE, pine bark extract. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

The EGCG used in the previous trial was a prodelphinidin-type flavonoid that also acts as a pro-oxidant, with production of active superoxide radical anions.[37] EGCG is beneficial in chemoprevention because of its pro-oxidant nature, and it works as a PI3K/AKT/mTOR pathway inhibitor, triggering apoptosis of cancer cells.[38,39] However, this action might cause a pro/antioxidant cellular imbalance and aggravate photoaging of the skin. In contrast, such undesirable effects on the skin are much less likely to occur with (−)-epicatechin, (+)-catechin, or procyanidin-type polymers, that are the main constituents of the OPCs in PBE.

In the skin, PBE has been found to protect capillary walls[40] and to inhibit matrix metalloproteinases.[41] Direct assessment of the antioxidant effects of PBE by electron spin resonance spectroscopy showed that PBE had significant antioxidant effects on the facial skin of ultraviolet B-irradiated hairless mice in vivo.[1] OPCs have also been reported to be effective inhibitors of tyrosinase in skin-derived melanocytes and in the hyperpigmented skin of ultraviolet-irradiated mice and guinea pigs.[42–45] Oral OPC supplements are expected to have desirable effects on photoaging because they promote tissue elasticity, help heal microinjuries, reduce bruising and swelling by strengthening blood vessels, prevent postinflammatory skin pigmentation, restore dermal collagen, and improve the

Table 8B Results of hematology in 21 evaluated cases (phase II, low-dose PBE trial, 18-month administration)

Parameter (units)	Reference range	Group	Baseline	24 weeks	18 months
Red cell ($10^4/\mu L$)	370–510	I	434.1 ± 24.4	426.8 ± 32.4	428.7 ± 34.1
		II	422.7 ± 29.1	425.5 ± 23.3	425.5 ± 22.4
Hemoglobin (g/dL)	11–15.5	I	13.0 ± 0.7	12.8 ± 1.0	12.8 ± 1.1
		II	12.7 ± 0.7	13.0 ± 0.7	12.9 ± 0.7
Hematocrit (%)	34–46	I	40.6 ± 2.0	39.3 ± 2.9	39.7 ± 3.4
		II	40.7 ± 2.6	40.0 ± 2.1	40.5 ± 2.7
MCV (fL)	80–103	I	93.7 ± 4.5	92.2 ± 4.1	92.6 ± 4.8
		II	96.3 ± 2.5	94.1 ± 2.5	95.2 ± 4.5
MCH (pg)	26–35	I	30.1 ± 1.4	30.1 ± 1.5	29.9 ± 1.8
		II	30.0 ± 1.3	30.6 ± 1.1[a]	30.3 ± 0.9
MCHC (%)	31–36	I	32.2 ± 1.1	32.6 ± 1.3	32.3 ± 0.9
		II	31.2 ± 1.1	32.6 ± 0.5	31.9 ± 0.7
Platelet ($10^4/\mu L$)	12–34	I	27.5 ± 8.0	27.0 ± 5.3	27.3 ± 5.3
		II	26.4 ± 3.6	26.7 ± 3.8	26.4 ± 4.1
White cell (μL)	3500–9100	I	6193 ± 1876	6060 ± 1558	6253 ± 1799
		II	5883 ± 1222	5916 ± 1052	5283 ± 768
Neutrophil (%)	41–73	I	58.9 ± 6.9	58.7 ± 9.8	61.1 ± 10.3
		II	59.0 ± 7.1	59.2 ± 4.7	58.5 ± 4.5
Lymphocyte (%)	20–50	I	31.9 ± 5.7	32.8 ± 9.4	30.7 ± 9.6
		II	31.8 ± 5.9	32.2 ± 6.1	34.3 ± 2.4
Eosinophil (%)	0–9	I	3.3 ± 2.5	2.7 ± 1.8	2.9 ± 1.9
		II	3.8 ± 2.6	3.5 ± 2.5	2.7 ± 2.4

Notes: Mean ± SD. [a]$P < 0.01$, Student's *t*-test for paired values, 2-tailed.
Abbreviations: PBE, pine bark extract; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

peripheral circulation.[46,47] In fact, OPCs from grape seeds have previously been reported to improve melasma to a significant extent.[48]

A white complexion is a highly desirable symbol of beauty among Asian women, who believe that it is powerful enough to hide a number of faults. PBE did not modify facultative skin color in our trial, suggesting that the skin lightening elicited by PBE is confined to solar lentigines that appear with chronic inflammation, and can persist long after exposure to ultraviolet light. Recent profiling of solar lentigines with cDNA microarrays and immunohistochemical assays revealed a number of upregulated genes for the enzymes that synthesize arachidonic acid, as well as melanogenic and inflammatory genes in those lesions.[49]

Nuclear factor E2-related factor 2 (Nrf2)/antioxidative response element-mediated phase 2 detoxifying/antioxidant enzymes are induced by OPCs,[50] so OPC-activated Nrf2 confers protection against ultraviolet-induced skin inflammation.[51] OPCs are expected to be potent suppressors of expression or activity of genes in the melanogenic, inflammatory, and arachidonic acid-synthesizing categories downstream of Nrf2.[52,53] Interestingly, the arachidonate 12-lipoxygenase gene (*ALOX*), which is locally upregulated in solar lentigines,[49] may be downregulated by treatment with oral OPCs in mice.[54]

Another possible lightening mechanism, ie, acceleration of epidermal turnover, is suggested by the significant reduction in corneocyte size by PBE. PBE induces a gentle yet sufficiently effective exfoliating action in the skin to promote release of excess epidermal melanin deposition.[55,56]

Table 9A Results of blood chemistry in 24 evaluated cases (high dose PBE trial, 12-week administration)

Parameter (units)	Reference range	Baseline	12 weeks
Total cholesterol (mg/dL)	150–219	186.4 ± 39.2	185.1 ± 30.6[a]
HDL-C (mg/dL)	40–96	70.0 ± 14.3	63.1 ± 12.3
Triglycerides (mg/dL)	50–149	78.4 ± 40.1	82.3 ± 56.1
Aspartate aminotransferase (IU/l)	10–40	17.4 ± 3.0	16.6 ± 1.8
Alanine aminotransferase (IU/l)	5–40	13.9 ± 6.4	15.9 ± 8.3
Blood urea nitrogen (mg/dL)	6–20	11.7 ± 3.3	11.0 ± 2.8
Na^+ (mEq/l)	136–147	140.5 ± 1.7	139.1 ± 1.0[b]
K^+ (mEq/l)	3.6–5	4.2 ± 0.4	4.1 ± 0.4
Cl^- (mEq/l)	98–109	104.2 ± 1.9	103.5 ± 1.6[a]

Notes: Mean ± SD. [a]$P < 0.05$, [b]$P < 0.01$, Student's *t*-test for paired values, 2-tailed.
Abbreviations: PBE, pine bark extract; HDL-C, high-density lipoprotein cholesterol.

Table 9B Results of blood chemistry in 21 evaluated cases (phase II, low-dose PBE trial, 18-month administration)

Parameter (units)	Reference range	Group	Baseline	24 weeks	18 months
Total cholesterol (mg/dL)	150–219	I	198.1 ± 33.2	192.9 ± 29.2	192.9 ± 32.5
		II	178.0 ± 36.0	171.5 ± 39.9	180.2 ± 40.3
HDL-C (mg/dL)	40–96	I	68.1 ± 15.8	65.9 ± 10.9	68.7 ± 14.6
		II	75.2 ± 11.4	72.2 ± 13.8	73.8 ± 14.2
LDL-C (mg/dL)	70–139	I	113.0 ± 23.8	113.1 ± 27.0	108.4 ± 23.3
		II	88.7 ± 35.9	89.8 ± 32.9	92.2 ± 28.4
Triglycerides (mg/dL)	50–149	I	87.9 ± 69.6	88.3 ± 41.0	82.3 ± 41.6
		II	54.5 ± 13.2	69.8 ± 37.0	58.5 ± 20.3
Free fat acid (mEq/l)	0.14–0.85	I	0.35 ± 0.18	0.39 ± 0.31	0.44 ± 0.34
		II	0.44 ± 0.22	0.32 ± 0.15	0.34 ± 0.26
Aspartate aminotransferase (IU/l)	10–40	I	19.3 ± 4.5	16.6 ± 1.8[a]	17.2 ± 3.6
		II	18.8 ± 2.4	19.5 ± 7.7	15.2 ± 2.2
Alanine aminotransferase (IU/l)	5–40	I	14.3 ± 6.8	11.3 ± 3.8	12.5 ± 4.8
		II	14.7 ± 4.4	16.2 ± 6.8	11.5 ± 2.7
Blood urea nitrogen (mg/dL)	6–20	I	12.6 ± 2.8	13.4 ± 4.2	13.2 ± 3.7
		II	12.8 ± 2.1	12.3 ± 1.3	12.1 ± 2.5
Na^+ (mEq/l)	136–147	I	139.9 ± 2.2	140.1 ± 1.2	139.5 ± 1.6
		II	138.7 ± 1.5	139.3 ± 1.8	139.7 ± 0.5
K^+ (mEq/l)	3.6–5	I	4.3 ± 0.7	4.3 ± 0.5	4.1 ± 0.6
		II	4.4 ± 0.5	4.3 ± 0.6	3.9 ± 0.3[b]
Cl^- (mEq/l)	98–109	I	104.0 ± 3.4	104.9 ± 1.8	104.6 ± 2.4
		II	103.8 ± 1.8	104.8 ± 2.0	104.3 ± 3.2
Iron (μg/dL)	48–154	I	81.5 ± 41.0	91.5 ± 40.1	86.9 ± 54.8
		II	84.0 ± 50.8	98.7 ± 19.0	88.8 ± 34.8
Ferritin (ng/mL)	7–110	I	20.2 ± 18.0	15.1 ± 18.7	19.5 ± 17.0
		II	29.5 ± 15.4	28.7 ± 15.7	33.4 ± 22.9

Notes: Mean ± SD. [a]$P < 0.05$, [b]$P < 0.01$, Student's *t*-test for paired values, 2-tailed.
Abbreviations: PBE, pine bark extract; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

Conclusion

Oral administration of French maritime PBE effectively reduces facial symptoms of photoaging. Long-term administration of PBE is safe and useful for improving the appearance of photodamaged facial skin.

Disclosure

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