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Effects of oligomeric proanthocyanidins (OPCs) of red wine to improve skin whitening and moisturizing in healthy women – a placebo-controlled randomized double-blind parallel group comparative study

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Abstract. – OBJECTIVE: This study was undertaken to evaluate the effects of red wine from grapes oligomeric procyanidins (OPCs) intake on skin color and skin moisture in Japanese healthy women. The purpose of this study was to improve skin condition, with the primary endpoint set to improve sunburn by ultraviolet (UV) and the secondary endpoint set to improve dryness.

PATIENTS AND METHODS: A randomized, placebo-controlled, double-blind, parallel-group study was conducted on 100 subjects (30 to 59 years of age). They were administered a test beverage, including 200 mg of the red wine OPCs (the test beverage group) or a placebo beverage (the control beverage group) once a day for 12 weeks. The properties of facial skin were measured at 0 (start value), 4th, 8th, and 12th week of the test period.

RESULTS: After 12 weeks of administration, the pigmentation scores and melanin index values of the OPC group were significantly reduced from the start value and were lower than the control group ($p<0.05$). In addition, the OPC group showed a significant increase in water content of the stratum corneum compared to the start value, while that of the control group significantly decreased.

CONCLUSIONS: The red wine OPCs showed the effects of skin whitening and moisturizing, and it is suggested that OPCs may improve the skin condition of healthy women.

Key Words:
Wine, Grape, OPCs (oligomeric procyanidin), Skin whitening effect, Skin moisturizing effect.

Introduction

Red wine contains various types of polyphenols. Among them, anthocyanins and proanthocyanidins are the major compounds. Proanthocyanidins are polymerized polyphenols with flavan-3-ol units (catechin, epicatechin, and other flavan-3-ols) linked by C4-C8 or C4-C6. Therefore, there are many compounds with various degree of polymerization. It has been reported that the proanthocyanidins contained in red wine contribute to astringency in wine by binding with salivary proteins[1] and also contribute to the stability of color in red wine by interacting with anthocyanins[2]. In addition, it has been observed that among proanthocyanidins, oligomeric proanthocyanidins (OPCs) with a low degree of polymerization have various physiological activities. For example, the OPCs containing procyanidin C1 derived from immature apples (*Rosaceae, Malus spp.*) show antiallergic activity in mice when given orally[3]. Furthermore, pycnogenol®, which is the OPC derived from pine wood bark, shows immunostimulatory activity in mice infected with retroviruses or loaded with ethanol[4]. Moreover, the OPCs were evaluated for various health claims in humans. The antihypertensive effect in humans orally receiving hawthorn (*Crataegus monogyna*) extract containing OPCs[5] and the antioxidant effects in serum of humans receiving Leucoselect® which is the OPC derived from grape seed[6] were reported. Additionally, in humans orally receiving pine wood bark extract (flavangenol®), the effects of improvement of photoaged skin condition was detected[7]. In the recent years, natural food ingredients are expected to have the effect of skin whitening for sunspots, dullness, and other problems, or moisturizing effect for dryness. It has been showed that red wine OPCs have an effect of

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inhibiting melanin production in B16 melanoma cells[8]. Red wine OPCs are expected to show this effect in humans too but this is not yet confirmed. Therefore, in this placebo-controlled randomized double-blind parallel group comparative study, we investigated the effect of long-term ingestion of beverages containing red wine OPCs on skin color and water content of stratum corneum in women.

Patients and Methods

Study Organization

This study operated by Suntory Global Innovation Center Ltd. (Tokyo, Japan) was outsourced to KSO Corporation by Suntory Global Innovation Center Ltd. (Tokyo, Japan) and was conducted at Inforward, Inc. EBISU skin research center (Tokyo, Japan). The principal investigator was Dr. Kayoko Numano of Queen's Square Medical Facilities.

This study was conducted in accordance with the Declaration of Helsinki (revised October 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (2014 Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare Ministerial Notification No. 3) under deliberation and approval from the Institutional Review Board (IRB number: 11001096) of "a specified non-profit organization of Japan Aesthetic Dermatology Symposium". A summary of the study was registered in UMIN (University Hospital Medical Information Network) Clinical Trials Registry (UMIN ID: UMIN000024478). This study was conducted after the written informed consent was obtained from all subjects.

Subjects

250 subjects were screened and 100 subjects who met all inclusion criteria and none of the exclusion criteria were selected to participate in this study. The inclusion criteria were as follows: (1) healthy women aged at least 30 years and less than 60 years; (2) women with measurable sunspots on cheeks; (3) women who received full explanation of the purpose and contents of this study with ability to consent, voluntarily applied to participate in this study based on full understanding of above explanation, and consent to participate in this study by providing written informed consent. The exclusion criteria were as follows: (1) women using medicines for the treatment of various skin disorders; (2) women with atopic dermatitis; (3) women with marked skin abnormalities at the test site (face); (4) women with food allergies and hay fever; (5) pregnant women, women who wish to be pregnant during the study, and breastfeeding women; (6) women participating, or intending to participate in the study using other foods or medicines or in the study applying cosmetics and medicines or who completed the study within 4 weeks; (7) women drinking a lot of red wine daily; (8) women using cosmetics containing wine extracts; (9) women assessed as ineligible by the investigator.

Before starting the study, we obtained informed consent with signature after providing the following explanation: (1) the contents of the study, (2) voluntary decision to participate in the study, (3) not suffering any disadvantages even if they did not give consent.

Study Beverages

The study beverages were prepared by Suntory Spirits Ltd. The test beverage (200 mL) contained 200 mg of red wine OPCs as the functional substance and the control beverage (200 mL) did not contain red wine OPCs. The control beverage was prepared not to be differentiated from the test beverage by appearance, taste, color. Table I shows the composition of both beverages. The study beverage was packed by the testing laboratory to preclude identification by appearance and the identification was converted randomly to the A group and the B group by the testing laboratory. The allocation manager prepared the allocation table of the study beverage (key code) and confirmed the indistinguishability of the study beverage before strictly storing the allocation table of the study beverage (key code) sealed until database finalization to maintain the blinding.

Red wine (*Vitis vinifera*, Cv. Ancellotte grapes) was concentrated in vacuo to remove ethanol to prepare extract. The extract containing red wine OPCs was blended to make a beverage to be 1000 ppm after measurement of the OPC concentration.

Analysis of red wine OPCs as functional substances was performed as shown below. One mL of the test beverage was loaded with Sep-pak-C18 Environmental cartridge (2 cc, waters) and washed with 6 mL of water before the fraction eluted with 6 mL of 70% ethanol. All eluent was concentrated and subjected to constant volume in a 2 mL volumetric flask of which 1 mL was dried in a test tube. For the control beverage, all fractions eluted with 70% ethanol with solid phase

Table I. Functional substances and nutrient contents per test beverage (200 mL).

	Test beverage	Control beverage
Functional substances: red wine OPCs	200 mg	0 mg
Energy	14 kcal	14 kcal
Protein	0 g	0 g
Lipid	0 g	0 g
Carbohydrate	3.4 g	3.4 g
Sodium	40 mg	0 mg
Dietary fiber	0.04 g	0.64 g

extraction were dried in the same manner. The dried fractions were dissolved with 1 mL of butanol containing 0.6 N hydrochloric acid and heated at 90°C for two hours. As a reference material, 0.5 mg procyanidinB1 (Funakoshi Co., Ltd., Bunkyo-Ku, Tokyo, Japan) was heated with 1 mL of butanol containing 0.6 N hydrochloric acid and used in the same manner. After heating, the analysis was performed using high performance liquid chromatography (HPLC) and the resulting cyanidin was compared.

HPLC conditions:

- Column: YMC-Pack ODS-A, 6 mmφ × 150 mm (YMC.CO., LTD., Shimogyo-ku, Kyoto, Japan);
- Mobile phase: acetic acid: Methanol: H_2O = 15: 17.5: 67.5;
- Flow rate: 1 mL/min;
- Detection: A520 nm;
- Analysis time: 12 min;
- Column temperature: 40°C;
- Injection volume: 5 µL.

Cyanidin concentration in the solutions used for analysis was calculated with the calibration curve plotted using cyanidin chloride (Funakoshi Co., Ltd., Bunkyo-Ku, Tokyo, Japan) and the OPC concentration in beverage was calculated using the following formula: OPC concentration (µg/mL) = 500 × (cyanidin concentration of HPLC sample × 2) / (cyanidin concentration of procyanidin B1).

As a result, the OPC mean concentration of the test beverage analyzed 3 times consecutively was 1042 µg/mL, which indicated that the concentration of OPCs per bottle was 208 mg/bottle. In addition, the concentration of procyanidin B1, procyanidin B2, procyanidin B3, and procyanidin B4 analyzed using reversed-phase liquid chromatography-mass spectrometry (LC/MS) was 2.26 mg/bottle, 1.19 mg/bottle, 0.46 mg/bottle, and 0.20 mg/bottle, respectively[8]. It was found that the control beverage did not contain OPCs.

Human Study Design and its Schedule

The study design was a randomized double-blind parallel group comparative study using control beverages. The study consisted of a screening test, a 3-week pre-observation period, a 12-week ingestion period, and a 4-week post-observation period, and lasted from the start day of screening test to 29 days after the last day of a 12-week ingestion period. The test beverage was ingested for 12 weeks from November 24, 2016 to February 19, 2017. Subjects ingested the test beverage or the control beverage (200 mL) within 1 hour once daily any time. The primary endpoints were sunspot score diagnosed by the dermatologist, melanin index value in sunspots, and skin-color by a CIE 1976 lightness (L* value), and the secondary endpoints were the water content of the stratum corneum, skin viscoelasticity, wrinkle depth, and visual analog scale (VAS) questionnaire (sunspots, dullness and moisture). The safety endpoints included the occurrence of adverse events and adverse reactions (adverse event that cannot be denied in relation to the study beverage) based on medical interview, blood pressure, pulse rate, and clinical laboratory values. A laboratory test was conducted at screening, week 0, week 4, week 8, and week 12 of ingestion. Table II shows the test items and schedule.

The allocation manager randomly divided the subjects assessed as eligible at screening into the two groups based on the following background factors at significance level of 15% to eliminate bias between the two groups and prepared the allocation table of the study beverage (key code): age, diagnostic sunspot score by the dermatologist, and body mass index (BMI). The allocation manager sealed the allocation table of

Table II. Laboratory test schedule.

Item	Pre-observation period	Start date of ingestion	Ingestion period		
Timing	Before 2-4 weeks	Week 0	Week 4	Week 8	Week 12
Height	●				
Blood-pressure measurement (Sitting position)	●	●			●
Heart rate	●	●			●
Hematology	●	●			●
Blood biochemistry	●	●			●
Urinalysis	●	●			●
Water content of the stratum corneum		●	●	●	●
Skin viscoelasticity		●	●	●	●
Wrinkle (Replica analysis)		●	●	●	●
Melanin index value	●	●	●	●	●
lightness (L* value)		●	●	●	●
VAS questionnaire		●	●	●	●

the study beverage after ensuring no problems and described the study ID and the sealed date on this envelope. The allocation manager stored strictly the allocation table of the study beverage sealed until database finalization to maintain the blinding before opening the envelope.

The subjects were instructed to record the subject diary (absence or presence of ingestion of the study beverage, changes in physical conditions, changes in living conditions, ingestion of alcohol, ingestion of health foods, use of medications, and changes in cosmetics use) every day from the start date of the study beverage ingestion to study completion. The subjects were also instructed to perform the following activities during the study period: (1) ingest the study beverage according to the instruction of the investigator or sub-investigator; (2) record the subject diary every day during the designated period; (3) continue to use cosmetics that they currently use and do not use cosmetics they rarely use or new cosmetics. In addition, do not use a new beauty essence for eyes at the part of examination by dermatologists (tail of the eye); (4) do not ingest or use new supplements or cosmetics that can improve skin sunspots; (5) do not ingest beverages containing red grape extract or polyphenol components and beverages with description of effects on the skin as product characteristics; (6) do not use treatment (surgical treatment or beauty treatment) or medications that can significantly affect the skin; (7) do not sun-tan by direct exposure to sun.

Measurement Method

Measurement was conducted at the site free from skin symptoms, such as scratching or dermatitis during the study period. Skin evaluation was performed on the face. The subjects removed makeup and washed their faces. Then, the facial skin was acclimatized for 20 min under stable conditions in a temperature-controlled and humidity-controlled room (temperature: 21±1°C, humidity: 50%±5%) before the conduct of the test.

Diagnostic sunspot score by the dermatologist was determined by objective quantitation (from 1 to 5 by increments of 0.25) through visual comparison between the score and the chart with which the dermatologist evaluated the cheeks. Water content of the stratum corneum was measured at

the junction between the tail of the eye and the wings of nose by using Corneometer CM825 (manufactured by Courage+Khazaka electronic GmbH, Mathias-Brüggen-Str. 91, Köln, Germany). The water content value was obtained as a mean value of three of five times measured value by eliminating the lowest and highest values. Skin viscoelasticity was obtained by using the mean of three measurements after the junction between the tail of the eye and the wings of nose was measured using Cutometer MPA580 (manufactured by Courage+Khazaka electronic GmbH, Mathias-Brüggen-Str. 91, Köln, Germany) in the same manner. The melanin index value was obtained by using the mean after measuring sunspots and the peripheral regions twice respectively using Mexameter MX18 (manufactured by Courage+Khazaka electronic GmbH, Mathias-Brüggen-Str. 91, Köln, Germany). The coloring and lightness, L* value was obtained by using the mean after measuring sunspots and the peripheral regions twice respectively using the Chroma Meter CR-400 (manufactured by Konica Minolta inc., Chiyoda-Ku, Tokyo, Japan). The VAS questionnaire was conducted after measuring 10 cm scale by increments of 0.01 cm in the item of sunspots, dullness, and moisture.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). The significance level was 5% and the two-sided test was used. Continuous variables were calculated by rounding the mean and standard deviation to 1 digit lower than the number of significant digits as basic statistics. For categorical variables, the frequency and proportion (%) were calculated and the proportion (%) was rounded to one decimal place. In addition, the missing data was not entered.

For the diagnostic sunspot score by the dermatologist, the intragroup difference was compared using Wilcoxon signed rank test and the intergroup difference was compared using Wilcoxon rank sum tests in terms of changes from the start value (week 0). Regarding the melanin index value, a coloring and L* value, water content of the stratum corneum, skin viscoelasticity, wrinkle depth evaluated by replica of eye area and VAS questionnaire, the intragroup difference was compared using paired *t*-test and the intergroup difference was compared using non-paired *t*-test.

The efficacy analysis population consisted of subjects who did not meet any exclusion criteria from analysis. The subjects excluded from analysis were determined by the investigator before unblinding. Exclusion criteria from analysis were shown in the following (1) to (5). (1) subjects who were found to violate the exclusion criteria during the study, (2) subjects who had less than 90% of the study beverage ingestion rate, (3) subjects who were found to have problems in health and living conditions by the investigator, (4) subjects who could not follow the instructions of the investigator, (5) Subjects who were considered inappropriate as analysis population for other reasons by the investigator.

Results

Subject Characteristics and Analysis Population

Subject disposition is shown in Figure 1 with a flow chart. All the subjects completed the study (50 subjects in the test beverage group and 50 subjects in the control beverage group).

A total of 97 subjects (50 subjects in the test beverage group and 47 subjects in the control beverage group) excluding 3 subjects (all in the control beverage group) who met exclusion criteria from analysis were included in efficacy analysis. All the subjects who ingested the study beverage (50 subjects in the test beverage group and 50 subjects in the control beverage group) were included in the safety analysis.

Table III shows the general background of subjects. No bias was observed between the two groups in terms of age, diagnostic sunspot score, and BMI.

Diagnostic Sunspot Score by the Dermatologist

Table IV and Figure 2 show the results of diagnostic sunspot score by the dermatologist. When the intragroup difference was compared, it was revealed that the score in the test beverage group was significantly lower at week 8 and week 12 than at week 0 while in the control beverage group it was significantly lower at week 12 than at week 0 (Table IV). When changes from the start value were compared between groups, it was found that the changes were significantly reduced at week 8 and week 12 in the test beverage group than in the control beverage group (Figure 2).

Melanin Index Value

Table V and Figure 3 show the results of analysis of melanin index value in sunspots and the

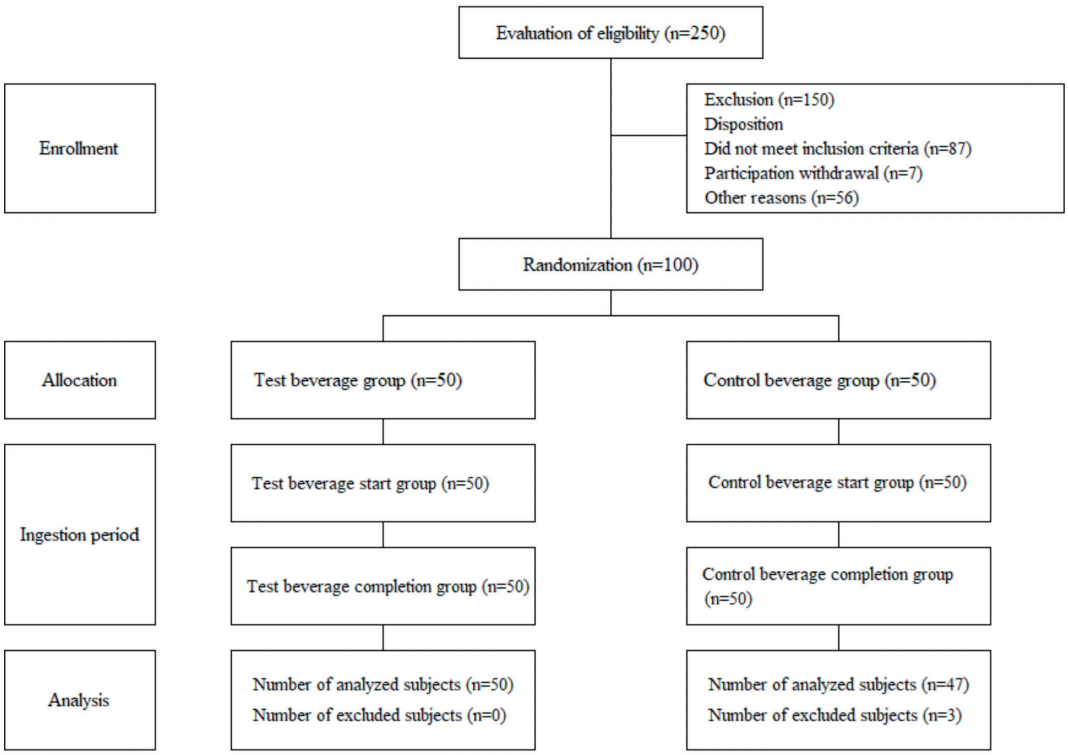

Figure 1. Subject disposition and flow chart.

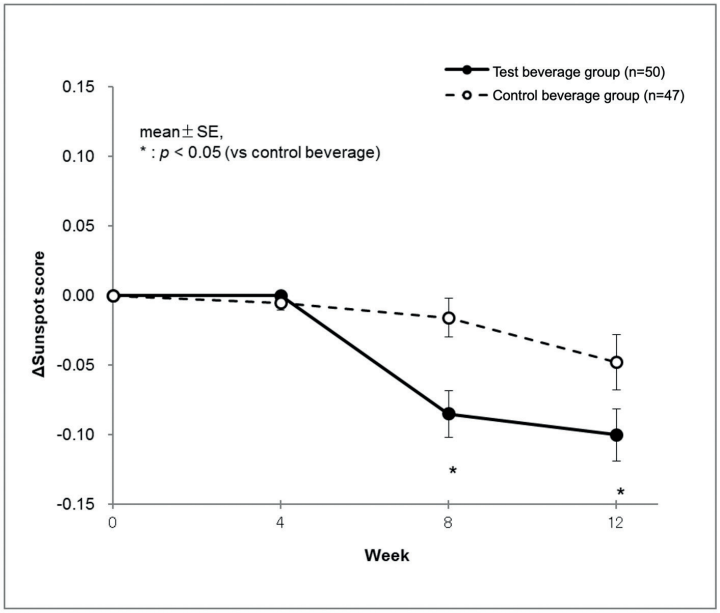

Figure 2. Diagnostic sunspot score by the dermatologist (Changes).

peripheral regions (skin free from sunspots). The melanin index value was significantly lower at week 4, week 8, and week 12 than at week 0 both in the test beverage group and the control beverage group (Table V). When changes from the start value between groups were compared, there was a significant reduction in sunspots at week 4, week 8, and week 12 in the test beverage group than in

Table III. General background.

Parameter	Unit	Test beverage group (n=50)						Control beverage group (n=50)						
		Mean	±	SD	Minimum value	Maximum value	Median value	Mean	±	SD	Minimum value	Maximum value	Median value	*p*-value
Age	Age	44.28	±	6.50	30.0	57.0	45.00	44.66	±	5.93	33.0	57.0	44.50	0.761
BMI	-	21.07	±	1.78	17.9	26.5	20.95	21.08	±	2.59	17.3	31.0	20.80	0.982
Diagnostic sunspot score	-	2.22	±	0.70	1.25	4.00	2.25	2.22	±	0.69	1.00	4.50	2.00	0.992

p-value: Wilcoxon rank sum tests (*vs* Control beverage group). The intragroup statistical difference compared to the start value is reported as * ($p < 0.05$). The intergroup statistical difference at each time point is reported as *p*-value.

Table IV. Results of analysis of diagnostic sunspot score by the dermatologist.

Parameter	Group	n	0 W				4 W				8 W				12 W			
			Mean	±	SD	*p*-value	Mean	±	SD	*p*-value	Mean	±	SD	*p*-value	Mean	±	SD	*p*-value
Sunspot Score	Test beverage	50	2.24	±	0.66	0.807	2.24	±	0.66	0.841	2.15	±	0.68*	0.427	2.14	±	0.69*	0.445
	Control beverage	47	2.29	±	0.70		2.29	±	0.71		2.28	±	0.70		2.24	±	0.72*	

The intragroup statistic difference compared to the start value is reported as * ($p < 0.05$). The intergroup statistical difference at each time point is reported as *p*-value.

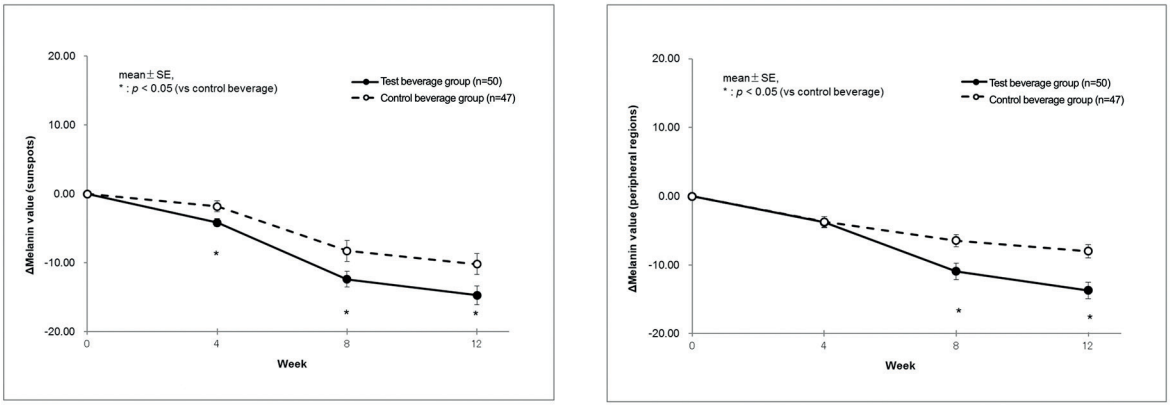

Figure 3. Melanin index value in sunspots and the peripheral regions (changes). Figure **A** and **B** show the time course of the changes in melanin index value in sunspots and the peripheral regions, respectively. Figure **A** and **B** show the changes (Δ) from the start value (week 0) at week 4, week 8, and week 12.

the control beverage group while in the peripheral regions the sunspots were significantly reduced at week 8 and week 12 in the test beverage group than in the control beverage group (Figure 3).

Skin-Color by Coloring and Lightness

Table VI and Figure 4 show the L* value (lightness) in sunspots and the peripheral regions.

The L* value in sunspots was significantly higher at week 4, week 8 and week 12 than at week 0 in the test beverage group, while in the control beverage group it was significantly higher at week 8 and week 12 than at week 0. When the changes from the start value were compared between groups, the changes were higher at week 12 in the test beverage group than in the control beverage group. However, this difference was not significant ($p = 0.059$).

The L* value in the peripheral regions was significantly higher at week 4, week 8, and week 12 than at week 0 in the test beverage group, while in the control beverage group it was significantly higher at week 8 and week 12 than at week 0. However, the changes from the start value were not significantly different between groups at any observation time points.

Water Content of the Stratum Corneum and Other Secondary Endpoints

Table VII and Figure 5 show the results of analysis of water content of the stratum corneum. The water content of the stratum corneum was significantly lower at week 4, week 8, and week 12 in the control beverage group than at week 0, while in the test beverage group it was significantly higher at week 4, week 8, and week 12 than at week 0. The

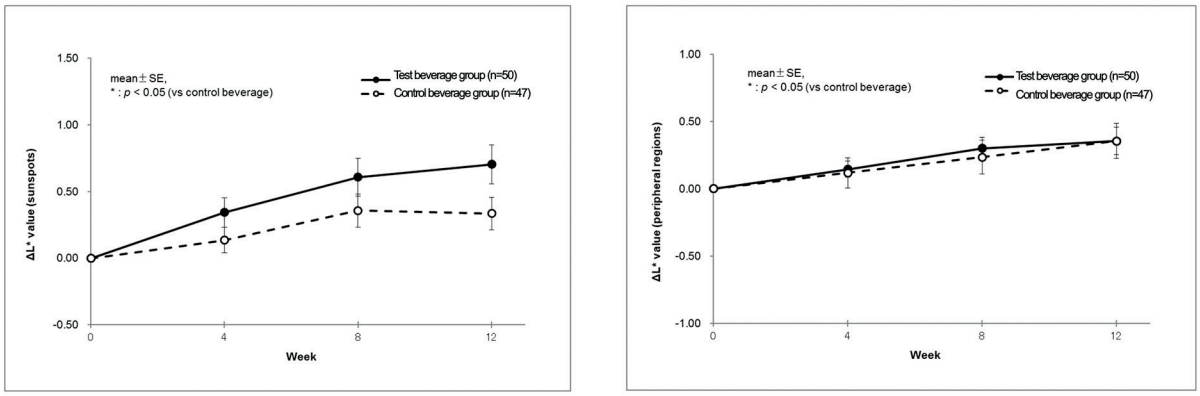

Figure 4. L* value (lightness) in sunspots and the peripheral regions (changes). Figure **A** and **B** show the time course of the changes in L* value in sunspots and the peripheral regions, respectively. Figure **A** and Figure **A** and **B** show the changes (Δ) from the start value (week 0) at week 4, week 8, and week 12.

Table V. Results of analysis of melanin index value.

Parameter	Group	n	0 W			4 W			8 W			12W		
			Mean ± SD	*p*-value		Mean ± SD	*p*-value		Mean ± SD	*p*-value		Mean ± SD	*p*-value	
Melanin index value (sunspots)	Test beverage	50	211.70 ± 35.97	0.624		207.54 ± 35.9*	0.866		199.30 ± 36.5*	0.934		196.98 ± 36.27*	0.888	
	Control beverage	47	208.17 ± 34.64			206.34 ± 33.97*			199.88 ± 32.12*			197.98 ± 33.23*		
Melanin index value (peripheral regions)	Test beverage	50	146.63 ± 24.15	0.615		142.85 ± 25.08*	0.635		135.69 ± 24.21*	0.697		132.91 ± 23.21*	0.519	
	Control beverage	47	144.12 ± 24.94			140.40 ± 25.55*			137.67 ± 25.67*			136.11 ± 25.47*		

The intragroup statistical difference is reported as follows: when compared with the start value: * ($p < 0.05$); when observed at each time point: *p*-value.

Table VI. Results of analysis of a coloring and L* value.

Parameter	Group	n	0 W			4 W			8 W			12W		
			Mean ± SD	*p*-value		Mean ± SD	*p*-value		Mean ± SD	*p*-value		Mean ± SD	*p*-value	
L* value in sunspots	Test beverage	50	61.44 ± 2.53	0.605		61.78 ± 2.47*	0.901		62.04 ± 2.45*	0.966		62.14 ± 2.56*	0.853	
	Control beverage	47	61.71 ± 2.62			61.84 ± 2.59			62.07 ± 2.53*			62.04 ± 2.58*		
L* value in the peripheral regions	Test beverage	50	65.96 ± 2.04	0.324		66.11 ± 2.10*	0.359		66.27 ± 2.00*	0.403		66.32 ± 2.05*	0.341	
	Control beverage	47	66.40 ± 2.27			66.52 ± 2.24*			66.63 ± 2.31*			66.75 ± 2.41*		

The intragroup statistical difference is reported as follows: when compared with the start value: * ($p < 0.05$); when observed at each time point: *p*-value.

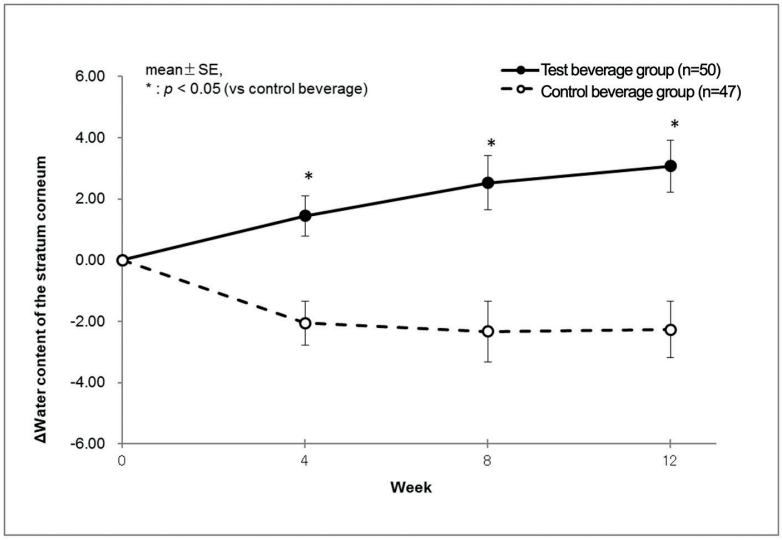

Figure 5. Water content of the stratum corneum (changes). Figure 5 shows the changes (Δ) from the start value (week 0) at week 4, week 8, and week 12.

measured value was significantly higher at week 12 in the test beverage group than in the control beverage group. In addition, the changes from the start value, 3.07 points increased, were significantly higher at week 4, week 8, and week 12 in the test beverage group than in the control beverage group.

Table VIII shows the measured value and the changes from the start value of skin viscoelasticity, wrinkle depth, and VAS questionnaire as other secondary endpoints. The measured value of skin viscoelasticity, wrinkle depth and VAS questionnaire was significantly lower at week 12 in both the groups than at week 0. However, there were no significant differences between the groups at any observation time points.

Safety Endpoints

Table IX and Table X show the number of subjects who presented adverse event and adverse reactions, respectively, as safety endpoints. No serious adverse events were reported during the study period. Although 23 subjects presented adverse events (13 events in the test beverage group, 10 events in the control beverage group) during the study period, all events were mild and resolved within several days. There were no clinical laboratory changes due to the test and the test food. One subject in test beverage group presented an adverse event (abdominal bloating) but it was resolved and confirmed 4 days later. As a result of the Chi-square test, no significant difference occurred in the occurrence of adverse events and adverse reactions between the test beverage group and the control beverage group.

Discussion

It was revealed that long-term ingestion of a beverage containing red wine OPCs improved the brightening of sunspots and skin moisturization in healthy women.

It is known that melanin is involved in skin color and sunspots. The melanin pigment in epidermal cells (keratinocytes) gives a brown color. Therefore, it is greatly involved in skin color. Sunspots are the site where melanin is accumulated and looks darker than skin color. Therefore, it is known that melanin is excessively produced and accumulated to form sunspots by activation of melanocytes with ultraviolet (UV) radiation. It has been confirmed that red wine OPCs showed the activity to inhibit tyrosinase in *in vitro* study and exhibited the ability to inhibit the melanin production in B16 melanoma cells[8]. In addition, the OPCs derived from pine wood bark or the OPCs derived from grape seed had the effect to improve blood flow[7]. Therefore, they are expected to brighten sunspots by reducing melanin by promoting skin turnover effect.

This study evaluated the effect of red wine OPCs to improve sunspots based on the diagnostic sunspot score by the dermatologist, melanin index value in sunspots, and lightness of skin col-

Table VII. Results of analysis of water content of the stratum corneum.

Parameter	Group	n	0 W				4 W				8 W				12 W			
			Mean	±	SD	*p*-value	Mean	±	SD	*p*-value	Mean	±	SD	*p*-value	Mean	±	SD	*p*-value
Water content of the stratum corneum	Test beverage	50	40.21	±	7.78	0.344	41.65	±	9.12	0.255	42.73	±	10.35*	0.093	43.28	±	9.47*	0.040
	Control beverage	47	41.78	±	8.44		39.72	±	7.37		39.44	±	8.59		39.51	±	8.25*	

The intragroup statistical difference compared to the start value is reported as * ($p < 0.05$). The intergroup statistical difference at each time point is reported as *p*-value.

Table VIII. Measurement of secondary endpoints using skin measuring instruments.

Parameter	Group	n	Measured value 0 W Mean	±	SD	p-value	12W Mean	±	SD	p-value	Changes (Δ) 12 W Mean	±	SD	p-value
Water content of the stratum corneum	Test beverage	50	40.21	±	7.78	0.344	43.28	±	9.47*	0.040	3.07	±	5.99	0.000
	Control beverage	47	41.78	±	8.44		39.51	±	8.25*		-2.27	±	6.30	
Skin viscoelasticity														
R2	Test beverage	50	0.66	±	0.06	0.572	0.64	±	0.06*	0.158	-0.02	±	0.06	0.463
	Control beverage	47	0.65	±	0.07		0.63	±	0.05*		-0.03	±	0.06	
R7	Test beverage	50	0.35	±	0.05	0.320	0.31	±	0.05*	0.288	-0.04	±	0.05	0.995
	Control beverage	47	0.34	±	0.05		0.30	±	0.04*		-0.04	±	0.04	
Wrinkle depth														
Wrinkle area ratio (%)	Test beverage	50	4.16	±	3.59	0.062	4.79	±	3.93*	0.045	0.63	±	2.09	0.422
	Control beverage	47	5.66	±	4.23		6.73	±	5.29*		1.07	±	3.12	
Mean wrinkle depth (mm)	Test beverage	50	52.48	±	3.90	0.070	53.30	±	4.05*	0.027	0.82	±	2.18	0.322
	Control beverage	47	54.02	±	4.38		55.30	±	4.70*		1.28	±	2.34	
Total wrinkle volume (mm^3)	Test beverage	50	0.23	±	0.22	0.073	0.27	±	0.23*	0.040	0.04	±	0.13	0.335
	Control beverage	47	0.32	±	0.27			±	0.34*		0.07	±	0.20	
VAS questionnaire														
Dullness	Test beverage	50	6.68	±	2.44	0.511	5.36	±	2.17*	0.526	-1.33	±	2.31	0.211
	Control beverage	47	6.98	±	1.90		5.06	±	2.45*		-1.92	±	2.32	
Sunspots	Test beverage	50	8.31	±	2.12	0.183	6.41	±	2.48*	0.978	-1.90	±	2.47	0.324
	Control beverage	47	8.81	±	1.46		6.40	±	2.66*		-2.41	±	2.62	
Moisture	Test beverage	50	6.69	±	1.57	0.088	5.11	±	1.97*	0.224	-1.58	±	2.41	0.804
	Control beverage	47	6.10	±	1.81		4.64	±	1.81*		-1.46	±	2.43	

The intragroup statistical difference is reported as follows: when compared with the start value: * ($p < 0.05$); when observed at each time point: p-value.

Table IX. Occurrence of adverse events.

Adverse events	Test beverage	Control beverage	Total
Absent	37	40	77
Present	13	10	23
Total	50	50	100
Occurrence	26%	20%	23%
p-value	0.476		

The intergroup statistical difference is reported as *p*-value.

Table X. Occurrence of adverse reactions.

Adverse events	Test beverage	Control beverage	Total
Absent	49	50	99
Present	1	0	1
Total	50	50	100
Occurrence	2%	0%	1%
p-value	0.315		

The intergroup statistical difference is reported as *p*-value.

or measured by the Chroma Meter. For the diagnostic sunspot score by the dermatologist, it was found that the score was significantly reduced at week 8 and week 12 in the test beverage group (the red wine OPC ingesting group) than in the control beverage group. On the other hand, for the intragroup difference, the score was significantly lower at week 8 and week 12 in the group ingesting red wine OPCs than at week 0, while in the control beverage group it was significantly reduced at week 12 than at week 0. Since the melanin in the skin generally changes depending on sunshine duration, it was suggested that the melanin decreased in both groups because of a shortening of sunshine duration, and a decrease in UV radiation in this study conducted from November to February. The effect associated with a decrease in UV radiation was also represented in melanin index value and skin color, which indicated that the melanin index value in sunspots was significantly lower in both groups at week 4, week 8, and week 12 than at week 0. A decrease in the melanin index value in sunspots was significantly larger at week 4, week 8, and week 12 in the group ingesting red wine OPCs than in the control beverage group. Therefore, the effect of reduction in sunspots by ingesting red wine OPCs was confirmed in addition to the reduction of melanin by a decrease in UV radiation. In addition, lightness, the L* value as an indicator of skin color, was higher at week 12 in the group ingesting red wine OPCs than in the control beverage group in terms of changes in sunspots. Therefore, it was confirmed that red wine OPCs had the effect of skin whitening for sunspots. This value in the peripheral regions was significantly higher at week 4 thereafter in the group ingesting red wine OPCs than at week 0, while in the control beverage group it was significantly higher at week 8 thereafter than at week 0. The lightness of skin increased in both the groups with no significant intergroup difference. These results suggested that red wine OPCs improved the brightening of sunspots by the ability to inhibit the melanin production through the action of inhibiting tyrosinase and activating skin turnover by improving blood flow in skin sunspots.

On the other hand, skin moisturization was improved as indicated by an increase in water content of the stratum corneum only in the group ingesting red wine OPCs although the study was conducted during the dry season. The water content of the stratum corneum was significantly reduced at week 4, week 8, and week 12 in the control beverage group than at week 0, while in the group ingesting red wine OPCs it was significantly increased at week 4, week 8, and week 12 than at week 0. Also, in the analysis of changes, the water content of the stratum corneum was significantly increased at week 4, week 8, and week 12 in the test beverage group than in the control beverage group. The water content of the stratum corneum was lower in the control beverage group because the study was conducted during the dry season. Meanwhile, it could be detected that the water content of the stratum corneum was increased by ingestion of red wine OPCs. Therefore, it was demonstrated that the ingestion of red wine OPCs contributed to the prevention of skin dryness, as well as improvement of skin moisturization. This result indicates that red wine OPCs may have a strong effect of improving skin moisturization as compared with previous reports on cosmetics.

An increase in expression of aquaporin 3 (AQP3) can be attributed to the mechanism to increase the water content of the stratum corneum by red wine OPCs. The epidermis consists of stratum corneum, granular stratum, stratum spinosum, and germinative stratum. Stratum corneum functions to prevent water evaporation into air. In addition, it has the ability to absorb water from the atmosphere or from inner skin, as well as to retain the water. There is no blood

vessel as supply sources of water in stratum corneum. However, water supplied through AQP3 expressed on cell membrane of the epidermis can be distributed to the stratum corneum. As it has been reported that the water content of the stratum corneum and skin viscoelasticity were markedly reduced on the skin that has no AQP3, it has been suggested that AQP3 has played an important role in maintaining skin moisturization[9]. Since it is known that AQP3 is also involved in cell differentiation to greatly affect the ability to cure wounds such as surface roughening, it is suggested that various skin troubles can be improved by promoting the production of AQP3[10]. It has been reported that the OPCs contained in red wine, particularly dimer and trimer OPCs, are potent to promote the expression of AQP3[11]. It has been speculated that dimer and trimer OPCs increased skin moisturization and the water content of the stratum corneum in humans because they contain procyanidin B1 (PB1) as a major compound in the red wine OPC and promoted the expression of AQP3 in the skin particularly by PB1.

Conclusions

It was revealed that long-term ingestion of a beverage containing red wine OPCs (200 mg/day as OPCs) significantly reduced the diagnostic sunspot score evaluation by the dermatologist and melanin index value, and increased the water content of the stratum corneum in healthy women and contributed to the lightness of sunspots, and improvement of skin moisturization. Red wine is useful for improving skin color and skin moisturization as health and beauty materials. Among them, it was suggested that red wine OPCs, which were detected to have a whitening effect and the ability to enhance AQP3 in *in vitro* study, contributed to the lightness of sunspots and improvement of skin moisturization.

In addition, since this study used a beverage from red wine from which alcohol had been removed, no problems related to safety of the beverage were seen based on the results of this study.

Conflicts of interest

The test beverage was prepared by Suntory Spirits Ltd. (Tokyo, Japan). The cost related to implementation of the study was paid by Suntory Global Innovation Center Ltd. (Tokyo, Japan).

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References

1) Ma W, Waffo-Teguo P, Jourdes M, Li H, Teissedre PL. Chemical affinity between tannin size and salivary protein binding abilities: implications for wine astringency. PLoS One 2016; 11: e0161095.
2) Singleton VL, Trousdale EK. Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines. Am J Enol Vitic 1992; 43: 63-70.
3) Nakano N, Nishiyama C, Tokura T, Nagasako-Akazome Y, Ohtake Y, Okumura K, Ogawa H. Procyanidin C1 from apple extracts inhibits Fc epsilon RI-mediated mast cell activation. Int Arch Allergy Immunol 2008; 147: 213-221.
4) Cheshier JE, Ardestani-Kaboudanian S, Liang B, Araghiniknam M, Chung S, Lane L, Castro A, Watson RR. Immunomodulation by pycnogenol in retrovirus-infected or ethanol-fed mice. Life Sci 1996; 58: PL 87-96.
5) Asher GN, Viera AJ, Weaver MA, Dominik R, Caughey M, Hinderliter AL. Effect of hawthorn standardized extract on flow mediated dilation in prehypertensive and mildly hypertensive adults: a randomized, controlled cross-over trial. BMC Complement Altern Med 2012; 29: 12-26.
6) Nuttall SL, Kendall MJ, Bombardelli E, Morazzoni P. An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect. J Clin Pharm Ther 1998; 23: 385-389.
7) Furumura M, Sato N, Kusaba N, Takagaki K, Nakayama J. Oral administration of French maritime pine bark extract (Flavangenol®) improves clinical symptoms in photoaged facial skin. Clin Interv Aging 2012; 7: 275-86.
8) Fujimaki T, Mori S, Horikawa M, Fukui Y. Isolation of proanthocyanidins from red wine, and their inhibitory effects on melanin synthesis *in vitro*. Food Chem 2018; 248: 61-69.
9) Hara-Chikuma M, Verkman AS. Roles of aquaporin-3 in the epidermis. J Invest Dermatol 2008; 128: 2145-51.
10) Dumas M, Sadick NS, Noblesse E, Juan M, Lachmann-Weber N, Boury-Jamot M, Sougrat R, Verbavatz JM, Schnebert S, Bonté F. Hydrating skin by stimulating biosynthesis of aquaporins. J Drugs Dermatol 2007; 6: s20-24.
11) Nakamura Y, Fukui Y, Tsuchiya T, Izumi R. Composition for promoting expression of aquaporin 3. WO 2019/066031 A1.