

Oral intake of lingonberry and amla fruit extract improves skin conditions in healthy female subjects: A randomized, double-blind, placebo-controlled clinical trial

Taro Uchiyama ^{ID}*
Makoto Tsunenaga
Miho Miyanaga ^{ID}
Osamu Ueda
Masashi Ogo

Shiseido Global Innovation Center, Yokohama, Kanagawa-ken, Japan

Abstract

In this study, we examined the effect of ingestion of lingonberry and amla fruit extract (LAE) on several human skin conditions. To conduct a randomized, double-blinded, placebo-controlled study, we randomly divided 99 healthy female subjects into three groups; the first group received a drink containing 25 mg of lingonberry extract and 30 mg of amla fruit extract; the second group received a drink containing double the volume of extracts received by the first group; and the third group received a placebo drink. Each participant drank 50 mL of their assigned drink once daily for 12 weeks. The primary endpoint was skin elasticity, and the secondary endpoints included skin thickness, stratum

corneum water content, and degree of wrinkles around the eyes. After 12 weeks of LAE drink intake, skin elasticity showed significant, dose-dependent improvements ($P < 0.01$). Skin thickness, stratum corneum water content, and the degree of wrinkles also significantly improved ($P < 0.001$) in a dose-dependent manner. The improvements in skin elasticity and thickness, as well as in the stratum corneum water content and the degree of wrinkles, observed upon oral intake of LAE indicate that LAE may be considered a candidate anti-aging agent for preventing skin weakening. © 2019 International Union of Biochemistry and Molecular Biology, Inc. Volume 66, Number 5, Pages 870–879, 2019

Keywords: lingonberry and amla fruit extract, randomized double-blind placebo-controlled study, skin elasticity, skin tear, skin thickness

1. Introduction

The skin protects the body from the external environment and is comprised of the epidermis, dermis, and subcutaneous tissue. Between the epidermis and dermis, there is a basal membrane that contains type IV collagen, whereas the dermis is mainly composed of type I collagen, elastin, and hyaluronan, which are together known as the extracellular matrix [1, 2].

It has been reported that skin collagen [3, 4], skin elastin [5], and epidermal hyaluronan contents decrease with aging [6]. Moreover, it is known that the skin becomes thinner [3, 7] and loses elasticity with age [8]. Further, it has been confirmed

that dermal fibroblasts produce less collagen as people age [9], leading to a thinning of the extracellular matrix and poorer skin protection from the external environment. Thus, there is a tendency in people over 70 years of age to experience “skin tear” [10] because the skin becomes fragile and is easily ruptured. Prevention of this disease requires good skin care from a young age to maintain skin elasticity.

Ultraviolet (UV) rays are an external factor known to adversely affect the skin and have primarily been studied because of their association with aging [11–14]. In these studies, it was found that treatment with polyphenols improved the state of skin damaged by UV rays.

Lingonberry (*Vaccinium vitis-idaea*) and amla (*Phyllanthus emblica*) fruits have attracted significant attention as food ingredients containing high concentrations of polyphenols [15–18]. Lingonberry or mountain cranberry is a common fruit in Northern Europe and contains many polyphenols such as resveratrol, procyanidin, quercetin, catechin, epicatechin, and coumaric acid [15, 16]. It has been reported that lingonberries inhibited the production of advanced glycation end products [19] and the activation of activator protein 1 and nuclear factor- κ B in mouse epidermal cells irradiated with UV rays [20]. Lingonberry is also known to reduce cholesterol levels

Abbreviations: LAE, Lingonberry and amla fruit extracts; MMP, Matrix metalloproteinase; NMF, natural moisturizing factor; TEWL, transepidermal water loss; UV, ultraviolet.

*Address for correspondence: Taro Uchiyama, PhD, Shiseido Global Innovation Center, 2-2-1, Hayabuchi, Tsuzuki-ku, Yokohama, Kanagawa-ken 224-0025, Japan. Tel.: +81 70 3859 1323; Fax: +81 45 590 6019; e-mail: taro.uchiyama@shiseido.com

Received 5 March 2019; accepted 20 July 2019

DOI: 10.1002/bab.1800

Published online 16 August 2019 in Wiley Online Library (wileyonlinelibrary.com)

[21], which may explain its beneficial effect on lifestyle-related diseases.

The amla fruit, a therapeutic ingredient in Ayurvedic medicine, contains gallic acid, gallogen, vitamin C, β -glucogallin (tannin), and mucinous acid [17, 18]. It is reported that amla fruit promote the production of type I procollagen and inhibit that of matrix metalloproteinase-1 (MMP-1) in human skin fibroblasts [22–24]. Furthermore, some studies have reported that the active constituents of this fruit, such as resveratrol, catechin, and quercetin, exert strong antioxidant effects [25], while tannin inhibits MMP expression [26]. In addition, gallic acid has been reported to suppress collagenase activity [27], and the polyphenols resveratrol and quercetin showed a synergistic antioxidant effect [28].

In our previous study, we found that lingonberry and amla fruits promoted collagen production by dermal fibroblasts. Based on this result, together with the knowledge that lingonberry and amla fruits contain high concentrations of polyphenols, we further evaluated the collagen-promoting effect of combined lingonberry and amla fruit extracts (LAE). The results showed that lingonberry and amla fruits caused a synergistic collagen-promoting effect in human dermal fibroblasts [29].

Because the collagen content of the skin may affect skin elasticity, we performed a clinical trial to evaluate the effect of LAE juice on skin elasticity. The 8-week trial was a single-blinded study with healthy subjects who noticed a decline in skin elasticity, experienced skin dryness, and showed loss of skin barrier function. The results showed that skin elasticity improved after treatment with LAE [29]. Recently, another group reported that the degree of wrinkles improved in UV-irradiated mice treated with LAE. The findings revealed that the mechanism underlying this wrinkle-improving effect involved the suppression of MMP-2 and MMP-9 and an increase in type IV collagen content in the skin [30].

In this study, we examined whether regular LAE ingestion could be potentially beneficial for the skin by performing a 12-week clinical trial. The enrolled subjects were women who regularly performed skin care activities. The clinical trial was conducted as a randomized, double-blinded, placebo-controlled study. The primary endpoint was skin elasticity, and the secondary endpoints were skin thickness, the degree of wrinkles, and skin hydration levels.

2. Materials and Methods

2.1. Study design

This study was carried out from November 2015 to March 2016 by researchers at Infoward (Ebisu, Tokyo). It was performed in accordance with the principles of the Declaration of Helsinki and the Ethical Guidelines on Epidemiological Studies and was approved by the ethical review board of the Japan Aesthetic Dermatology Symposium (UMIN-CTR No. UMIN000031682). The study was retrospectively registered. All participants provided written informed consent.

The study was designed as a randomized, placebo-controlled, double-blinded, parallel-group comparative study. The randomization was done prior to the start of the study by an independent researcher who used computer-generated codes to assign subjects to one of three groups and kept the randomized sequence secret to the research team members. The detailed randomization method is provided below. The subjects were arranged in an increasing order according to their skin elasticity and then divided into blocks of three subjects in the ascending order of random numbers. Those with the middle random number were assigned to the LAE single group, and those with the largest random number were assigned to the LAE double group. The same researcher provided the blinded test beverages (identified only by sequential numbers) to Infoward.

The candidates (individuals who experienced skin dryness and decreased skin elasticity) were screened for enrollment in the study. From 201 healthy female candidates, subjects with skin abnormalities at the evaluation site or those who refused to participate were excluded. Cheek elasticity was then measured with a Cutometer, and 99 subjects with a low cheek elasticity were selected for enrollment in the study. These 99 subjects were randomly divided into three groups (33 subjects per group), as described above. After this grouping, the subjects were double blinded.

In each group, the subjects ingested the assigned beverage daily for 12 weeks. Each subject was instructed to visit Infoward (Ebisu Skin Research Center) immediately before beginning the intake (week 0) and at 4, 8, and 12 weeks after beginning the intake to evaluate the condition of their skin. To minimize environmental influences such as temperature and humidity on the skin condition at the time of evaluation, the subjects were first acclimated for 30 Min to the test laboratory environment. The temperature and relative humidity in the test laboratory were controlled at 21 ± 2 °C and $45\% \pm 5\%$, respectively.

2.2. Subject selection

Study candidates included 201 healthy females who were recruited under the inclusion criteria listed in Table 1 and were not excluded based on the exclusion criteria listed in Table 2. After screening, we chose 99 study subjects who had a low cheek elasticity (F3). After all data were acquired, a clinical conference was held before the blinding was revealed. Subjects who committed rule infractions (Table 3) or showed low compliance with the intake protocol were excluded from the evaluation. After these exclusions, 95 subjects were included in the final analysis (Fig. 1). Tables 1–3 show the inclusion criteria, exclusion criteria, and instructions to subjects during the study period, respectively, as provided in the previous report of the study [31].

2.3. Test beverages

The lingonberry extract was purchased from Oryza Oil & Fat Chemical (Aichi, Japan). The amla fruit extract was purchased

TABLE 1
Inclusion criteria [31]

- (1) Females aged 35–50 years
- (2) Individuals conscious of skin roughness due to drying
- (3) Individuals who felt an effect on their skin after intake of the supplement, which improves skin characteristics
- (4) Individuals who felt a decrease in skin elasticity
- (5) Individuals who do not use a supplement or a drug regularly at present
- (6) Individuals who abstained from drinking alcohol from the day before measurement to the end of measurement
- (7) Individuals who agreed to participate in the study
- (8) Individuals judged appropriate for the study by the attending physician

TABLE 2
Exclusion criteria [31]

- (1) Individuals with skin abnormalities in areas to be tested
- (2) Individuals considered likely to develop an allergy to the test substance (individuals with food allergy)
- (3) Individuals with chronic cutis symptoms such as atopic dermatitis
- (4) Patients receiving outpatient drug therapy
- (5) Individuals who have participated in other studies within the past 3 months
- (6) Habitual smokers
- (7) Patients with asthma
- (8) Pregnant women, women desiring to become pregnant, or lactating women
- (9) Individuals who have used cosmetics or other skin products that have moisture-retaining effects in skin areas to be tested within 1 month of the start of the study
- (10) Individuals taking or intending to take drugs against pollinosis
- (11) Individuals judged inappropriate for the study by the attending physician

from Taiyo Kagaku (Tokyo, Japan). The LAE single-dose test beverage (LAE single) was prepared from 25 mg of dry lingonberry extract and 30 mg of dry amla fruit extract and the following additives: a sweetener (erythritol, sucralose, a fructose/glucose solution, and acesulfame K), sour seasoning (citric acid and malic acid), and flavor (fruit mixture) in 50 mL water. The LAE double-dose test beverage (LAE double) was prepared from 50 mg of dry lingonberry extract and 60 mg of dry amla fruit extract and the same additives as LAE single in 50 mL water. The placebo beverage was prepared with the same amount of the sweetener, acidulant, and flavor as the test beverages so that all three beverages had the same taste.

TABLE 3
Instructions to subjects during the study period [31]

- (1) Only cosmetics that were being used before initiation of the study may be used during the study.
- (2) Cutting of hair or shaving of the test areas is prohibited for 2 weeks before each measurement.
- (3) The use of bathing agents or similar products is prohibited.
- (4) The use of medicines, quasimedicines, and herbal mixtures is prohibited.
- (5) Ingestion of food supplements or foods with health-promoting functions, other than those that were being used routinely before the study, is prohibited.
- (6) Excessive alcohol consumption (consumption beyond the customary level) is prohibited.
- (7) No alcohol may be consumed on the day before each measurement.
- (8) Outdoor sports, exposure to artificial ultraviolet ray lamps, and other acts that might cause sunburn are prohibited. Care needs to be taken during daily living to avoid direct exposure of the test skin areas to ultraviolet rays both indoors and outdoors. Concretely, it is advisable to protect the test skin areas with a cap or clothes, or by routine use of sun-screening agents or similar products.
- (9) Hard exercise is prohibited on the day of measurement until the measurement for that day is completed.
- (10) Stimulating foods such as hot food, curry, red pepper, and Tabasco may not be ingested on the day of measurement.
- (11) Use of thermal undershirts or similar products, which exert special heat-insulating effects, is prohibited.
- (12) A bath should be taken before going to bed on the day before measurement, and bathing on the day of measurement (prior to measurement) is prohibited.

2.4. Skin elasticity measurements

Elasticity was evaluated in the cheek and forearm using a Cutometer dual MPA 580 (Courage + Khazaka Electronic, Köln, Germany). We used 2- and 6-mm diameter measuring probes and applied a constant suction of 400 mbar for 1 Sec, followed by a relaxation time of 1 Sec for 30 repetitions [32]. The curves of skin deformation obtained were analyzed using the Cutometer Dual, Version 1.4.6.2 software. The regions R0, R1, R2, R3, R5, R6, R7, and R8 (R parameters) and F3 (region parameter) were chosen for analysis based on the results of a previous study [29]. Cutometer parameters are shown in Table 4. The main elasticity parameter was F3, and therefore, the screening and intergroup comparisons were performed using data from F3.

2.5. Measurement of skin thickness

Skin thickness in the cheek and forearm was measured using a 20-MHz ultrasound device (DermaLab; Cortex Technology,

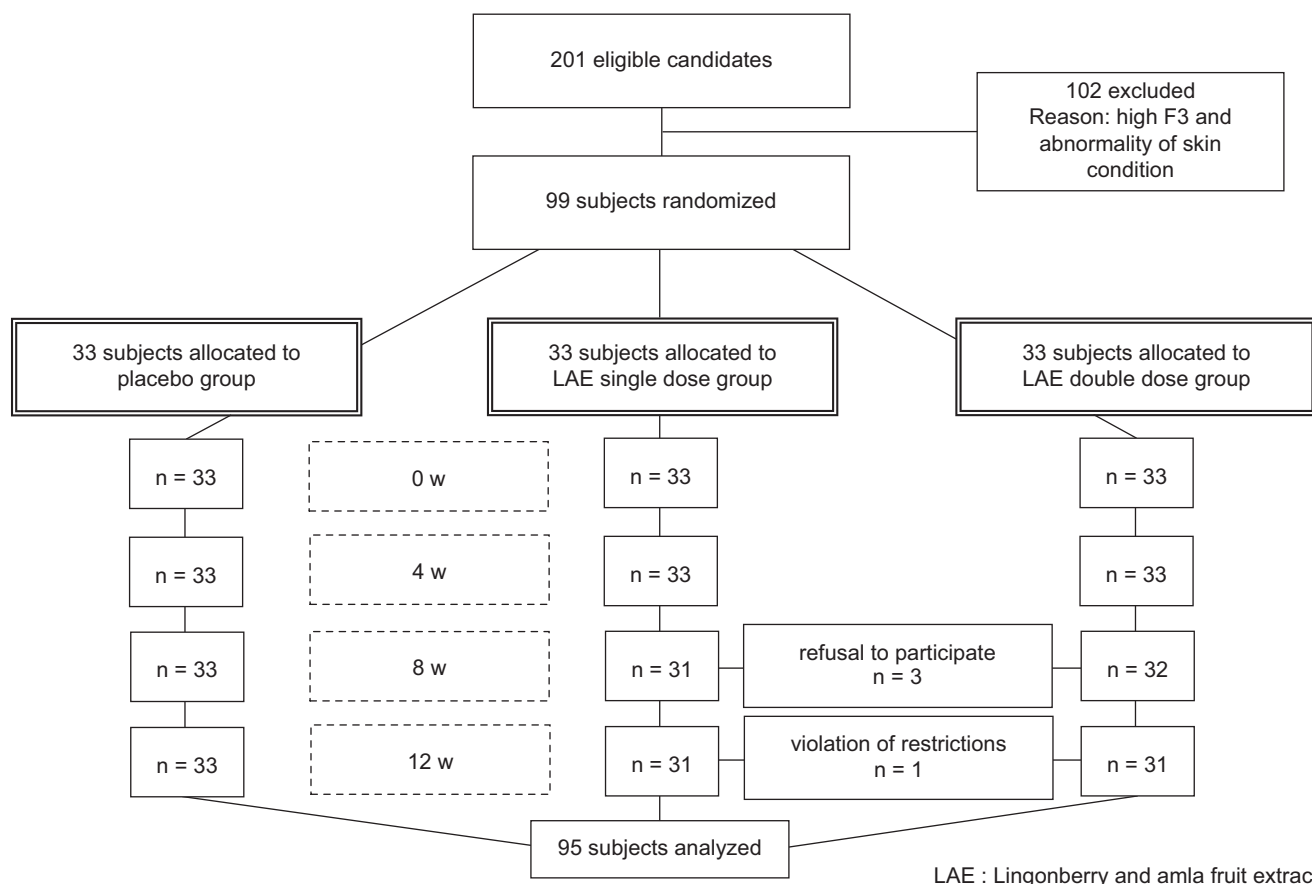


FIG. 1

Flow chart of subject selection.

Hadsund, Denmark). Skin thickness was measured at each site five times, and the mean was determined.

2.6. Evaluation of wrinkles at the corner of the eye

A dermatologist evaluated the degree of wrinkles at the edge of the eyes using a grading range, from grade 7 (bad) to grade 0 (good), according to the method described by Takahashi et al. [33].

2.7. Evaluation of stratum corneum hydration

Stratum corneum hydration was evaluated in the cheek and forearm using a Corneometer (CM 825; Courage + Khazaka Electronic). The variation in this parameter from the baseline was compared among the three groups.

2.8. Evaluation of epidermal and dermal hydration

Epidermal and dermal hydration were evaluated in the cheek and forearm using MoistureMeter-D (Delfin Technologies, Kuopio, Finland). An XS5 probe was used to measure hydration in the epidermis, and an S15 probe was used to measure hydration in the dermis. The variation in this parameter from the baseline was compared among the three groups.

2.9. Evaluation of transepidermal water loss

Transepidermal water loss (TEWL) was evaluated in the cheek and forearm using a Tewameter TW210 (Courage + Khazaka Electronic). TEWL was measured for 120 Sec, and the most stable value was adopted as the TEWL value. The variation in the TEWL value from the baseline was compared among the three groups.

2.10. Statistical analysis

The sample size was designed based on the elasticity data from a previous report [29]. It was estimated that a minimum sample of 33 subjects in each group would allow detection of the differences due to the effects of LAE with an 80% power, a 5% two-sided significance level, and a 20% dropout rate.

Data are expressed as the mean $\pm$ SD. Statistical analysis of the differences among the groups was performed using mixed models for repeated measures and Dunnett's test. *P*-values <0.05 were considered statistically significant. Statistical analysis was performed using the SAS 9.3 software (SAS Institute, Cary, NC, USA) and data analysis was performed with the advice of a specialist in statistical analysis.

3. Results

3.1. Subjects

We eliminated confounding factors that could potentially affect the elasticity of the skin by applying the inclusion criteria

TABLE 4 Definition of skin elasticity, as measured with a cutometer

Parameter	Good	Evaluation definition
R0	— ^a	Stretched length of the skin when suctioned
R1	Small value	The lowest point of the first curve
R2	1	Recovery ratio of the skin length
R3	— ^a	The highest length of the last curve in a series suction
R5	1	Ratio of elasticity when stretched and during constriction
R6	Small value	Ratio of viscosity and elasticity during the suction phase
R7	1	Ratio of the immediate recovery and the top value
R8	Big value	Length of the skin recovered
F3	Big value	Cumulative area value of repetition of pull and constriction (area within the envelope curves)

^aCaused by interpretation.

(Table 1) and exclusion criteria (Table 2). Thus, no difference in mean elasticity (F3) was observed among the groups (Table 5). In addition, the mean age affecting the elasticity of the skin was similar among the groups.

3.2. Effect of LAE on skin elasticity

Cheek elasticity was measured using the 2- and 6-mm probes after 12 weeks of LAE juice ingestion at both doses, and significant improvements were observed in cheek elasticity

($\Delta F3$) compared with that in the control group (2-mm probe of the cheek, single dose: $P < 0.01$, double dose: $P < 0.001$; 6-mm probe of the cheek, single dose: $P < 0.01$, double dose: $P < 0.001$). Significant improvements were also observed in forearm elasticity of the two test groups when measured with the 2-mm probe (single dose: $P < 0.01$, double dose: $P < 0.001$). No significant differences were observed in the elasticity of the forearm among the study groups when the 6-mm probe was used (Fig. 2). All other parameters evaluated in this trial were similar among the study groups.

3.3. Effect of LAE on skin thickness

As shown in Fig. 3, the changes in the cheek thickness at week 8 were $-15.1 \pm 138.6 \mu\text{m}$ in the control group, $109.8 \pm 100.0 \mu\text{m}$ in the LAE single-dose group ($P < 0.001$), and $94.0 \pm 71.9 \mu\text{m}$ in the LAE double-dose group ($P < 0.001$). The changes in the cheek thickness at week 12 were $-52.7 \pm 148.2 \mu\text{m}$ in the control group, $113.1 \pm 97.3 \mu\text{m}$ in the LAE single-dose group ($P < 0.001$), and $123.0 \pm 79.1 \mu\text{m}$ in the LAE double-dose group ($P < 0.001$). The changes in the forearm thickness were significantly higher in the LAE single-dose group ($46.7 \pm 49.8 \mu\text{m}$) and the LAE double-dose group ($55.9 \pm 54.7 \mu\text{m}$) than in the control group ($-9.5 \pm 51.1 \mu\text{m}$) after 12 weeks of ingestion (both $P < 0.001$).

3.4. Effect of LAE on wrinkles at the corner of the eyes

As shown in Fig. 4, the changes in wrinkles at week 8 were 0.05 ± 0.13 in the control group, -0.02 ± 0.09 in the LAE single-dose group, and -0.03 ± 0.09 in the LAE double-dose group ($P < 0.05$). The changes in wrinkles at week 12 were 0.05 ± 0.16 in the control group, -0.06 ± 0.14 in the LAE single-dose group ($P < 0.05$), and -0.07 ± 0.12 in the LAE double-dose group ($P < 0.01$).

3.5. Effects of LAE on skin hydration

3.5.1. Stratum corneum hydration

As shown in Fig. 5(I), the changes in corneum hydration in the cheek at week 8 were -2.35 ± 4.81 (au) in the control group and 2.12 ± 3.59 in the LAE double-dose group ($P < 0.01$).

TABLE 5 Average age and cheek elasticity of subjects at screening

	Lingonberry and amla fruit extract					
	Control group	Single-dose group		Double-dose group		Total
	Mean $\pm$ SD	Mean $\pm$ SD	P	Mean $\pm$ SD	P	Mean $\pm$ SD
Subjects	n = 33	n = 33	—	n = 33	—	n = 99
Age (years)	43.3 $\pm$ 3.6 (38–50)	43.6 $\pm$ 4.8 (35–50)	>0.2	43.6 $\pm$ 4.1 (35–50)	>0.2	43.5 $\pm$ 4.1 (35–50)
Cheek elasticity (cutometer F3)	36.7 $\pm$ 4.8 (26.3–45.6)	36.5 $\pm$ 4.9 (26.2–44.6)	>0.2	36.9 $\pm$ 5.0 (25.1–44.8)	>0.2	36.7 $\pm$ 4.8 (26.2–44.6)

n = no. of subjects; upper value shows the mean $\pm$ SD; lower values in parentheses represent the range.

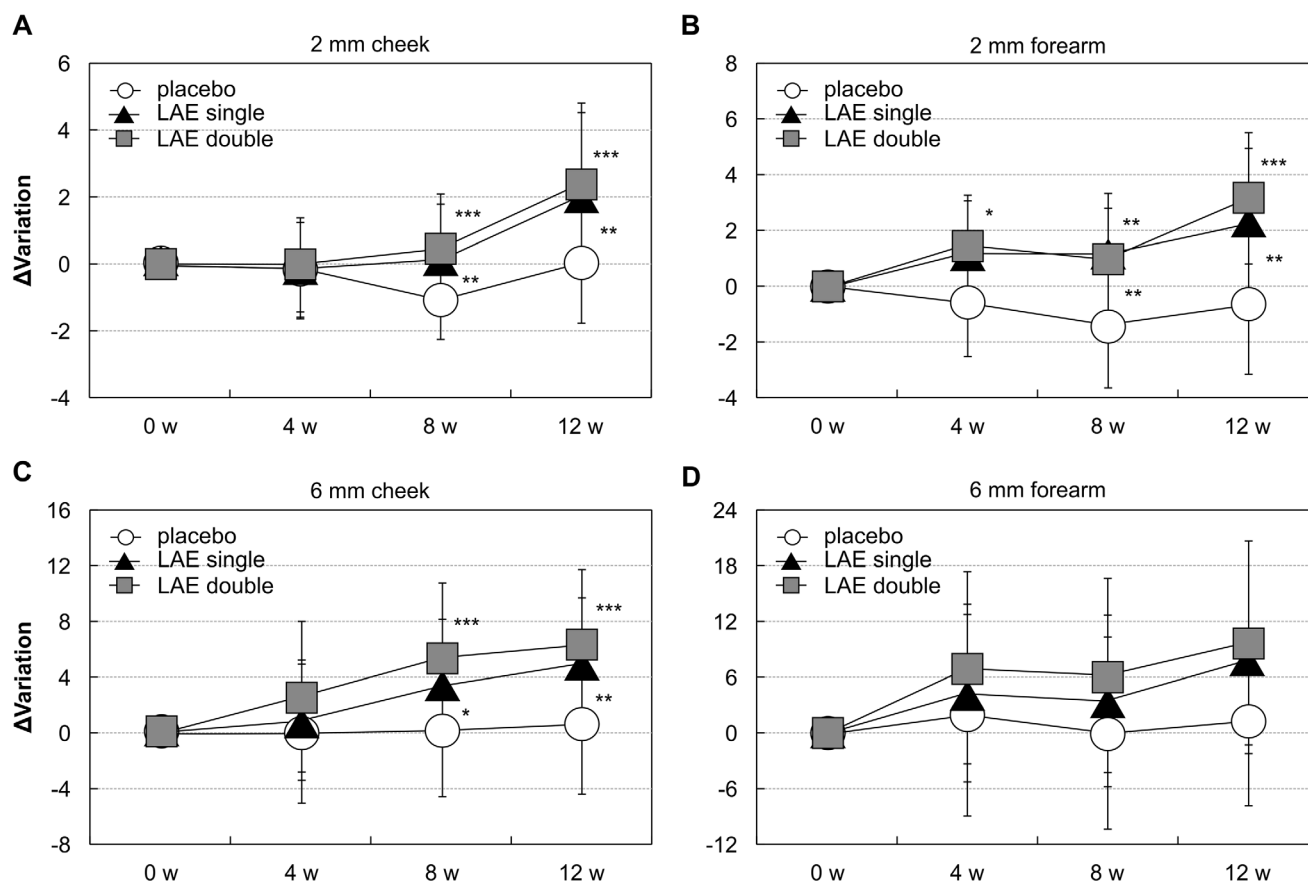


FIG. 2

Improvement in skin elasticity following ingestion of a beverage containing lingonberry and amla fruit extracts (LAE); variations in elasticity relative to the baseline level (F3) were measured with a 2-mm probe in (A) the cheek and (B) the forearm and with a 6-mm probe in (C) the cheek and (D) the forearm. Single-dose LAE: 25 mg of dry lingonberry extract + 30 mg of dry amla fruit extract; double-dose LAE: 50 mg + 60 mg of the same extracts, respectively. Data are presented as the mean $\pm$ SD, * P < 0.05, ** P < 0.01, and *** P < 0.001 versus the placebo group.

The changes in the corneum hydration at week 12 were -1.68 ± 4.62 in the control group, 4.81 ± 5.58 in the LAE single-dose group (P < 0.001), and 6.82 ± 5.50 in the LAE double-dose group (P < 0.001). The change in corneum hydration in the forearm was significantly higher in the LAE double-dose group (1.32 ± 4.84) than in the control group (-1.86 ± 5.47) after 12 weeks of ingestion (P < 0.05).

3.5.2. Epidermal hydration

As shown in Fig. 5-(II), changes in the epidermal hydration of the cheek were measured, but there were no significant differences among the three groups. The changes in the epidermal hydration of the forearm were significantly higher in the LAE double-dose group (2.61 ± 1.87) than in the control group (0.44 ± 2.11) after 12 weeks of ingestion (P < 0.001).

3.5.3. Dermal hydration

As shown in Fig. 5-(III), changes in the dermal hydration of the cheek were measured; however, there were no significant differences among the three groups. The change in the dermal hydration of the forearm was significantly higher in the LAE double-dose group (2.89 ± 2.94) than in the control group (0.92 ± 3.21) after 12 weeks of ingestion (P < 0.05).

3.5.4. TEWL

As shown in Fig. 5-(IV), TEWL in the cheek and forearm was measured, but no significant differences were noted among the three groups.

4. Discussion

In a previous study, we reported that skin elasticity improved after 8 weeks of LAE drink intake [29]. In the current study, multiple measurements were obtained via a randomized, placebo-controlled, double-blinded, parallel intergroup trial over 12 weeks of LAE intake. The effects of LAE on skin elasticity were reproducible, and the results also demonstrated improvements in eye wrinkles, skin thickness, and skin hydration, indicating that LAE ingestion exerted a strong anti-aging effect on the skin.

Skin elasticity (F3), which was the primary endpoint, was measured using a 2-mm probe (superficial part of the skin) and a 6-mm probe (deep part of the skin). LAE drink intake

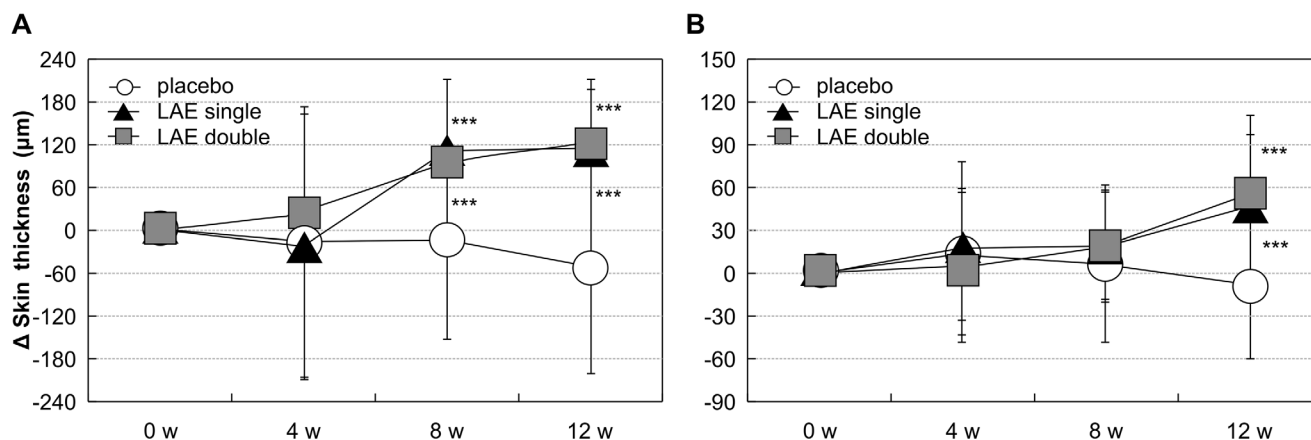


FIG. 3 Effects of the ingestion of beverages containing lingonberry and amla fruit extracts (LAE) on skin thickness; variations in skin thickness relative to the baseline level were measured in (A) the cheeks and (B) the forearms. Single-dose LAE: 25 mg of dry lingonberry extract + 30 mg of dry amla fruit extract; double-dose LAE: 50 mg + 60 mg of the same extracts, respectively. Data are presented as the mean $\pm$ SD, *** P < 0.001 versus the placebo group.

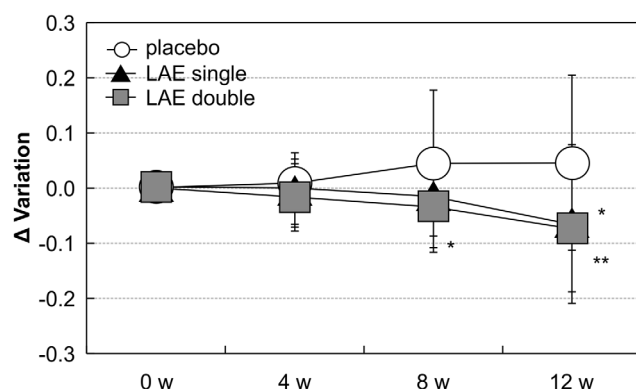


FIG. 4 Improvement in eye wrinkles following ingestion of a beverage containing lingonberry and amla fruit extracts (LAE); degrees of eye wrinkles were judged by a dermatologist, and variations are shown. Single-dose LAE: 25 mg of dry lingonberry extract + 30 mg of dry amla fruit extract; double-dose LAE: 50 mg + 60 mg of the same extracts, respectively. Data are presented as the mean $\pm$ SD * P < 0.05 and ** P < 0.01 versus the placebo group.

had a significant dose-dependent effect on skin elasticity, with the exception of the deep part of the arm, measured using the 6-mm probe, thus closely reproducing the previous results. It is likely that no significant differences were observed with the 6-mm probe because the results varied greatly and the effects of LAE on skin elasticity were similar in both superficial and deep parts of the skin.

Since skin elasticity improved with LAE intake and LAE is known to promote collagen production [29], the effect of LAE on skin thickness was also examined. The results showed significant improvement in the forearm and cheek skin thickness with LAE intake in a dose-dependent fashion. Therefore, it is possible that an increase in collagen partly contributed to the increase in skin thickness.

A previous study reported improvements in the state of UV-irradiated mouse skin after LAE ingestion [30], including increases in type IV collagen levels in the skin, decreases in the levels of collagen-degrading enzymes MMP-2 and MMP-9, and consequent improvements in wrinkle levels. Based on the findings of this report, we hypothesized that the wrinkle improvement observed in our present study may have been caused by similar mechanisms of action.

Significant changes in skin moisture of the cheek after LAE ingestion were observed only in the stratum corneum. In contrast, we observed significant differences in the hydration of the stratum corneum, epidermis, and dermis of the forearm after 12 weeks of ingestion of double-dose LAE. It is known that natural moisturizing factors (NMFs) are important for regulating the water content in the stratum corneum [34, 35]. It has been speculated that keratinocytes promote hyaluronan production. Therefore, our results are likely due to increased production of NMF or hyaluronan in keratinocytes. In addition, the increase in dermal hydration may have been due to the rise in hyaluronan production in dermal fibroblasts. However, both mechanisms should be clarified in future studies.

We have previously examined the efficacy of LAE and showed that the two extracts synergistically increased collagen production in human dermal fibroblasts [29], inhibited collagen glycation [36], and improved skin elasticity in a clinical trial [29]. From these results, we hypothesized that the improvement in skin elasticity after the ingestion of LAE was due to an increase in collagen levels and inhibition of collagen glycation, a mechanism that should only affect skin elasticity. However, in the present study, LAE intake not only improved elasticity in a dose-dependent manner but also improved eye wrinkles, skin thickness, and skin hydration. Thus, it was found that LAE intake significantly improved the overall state of subjects' skin.

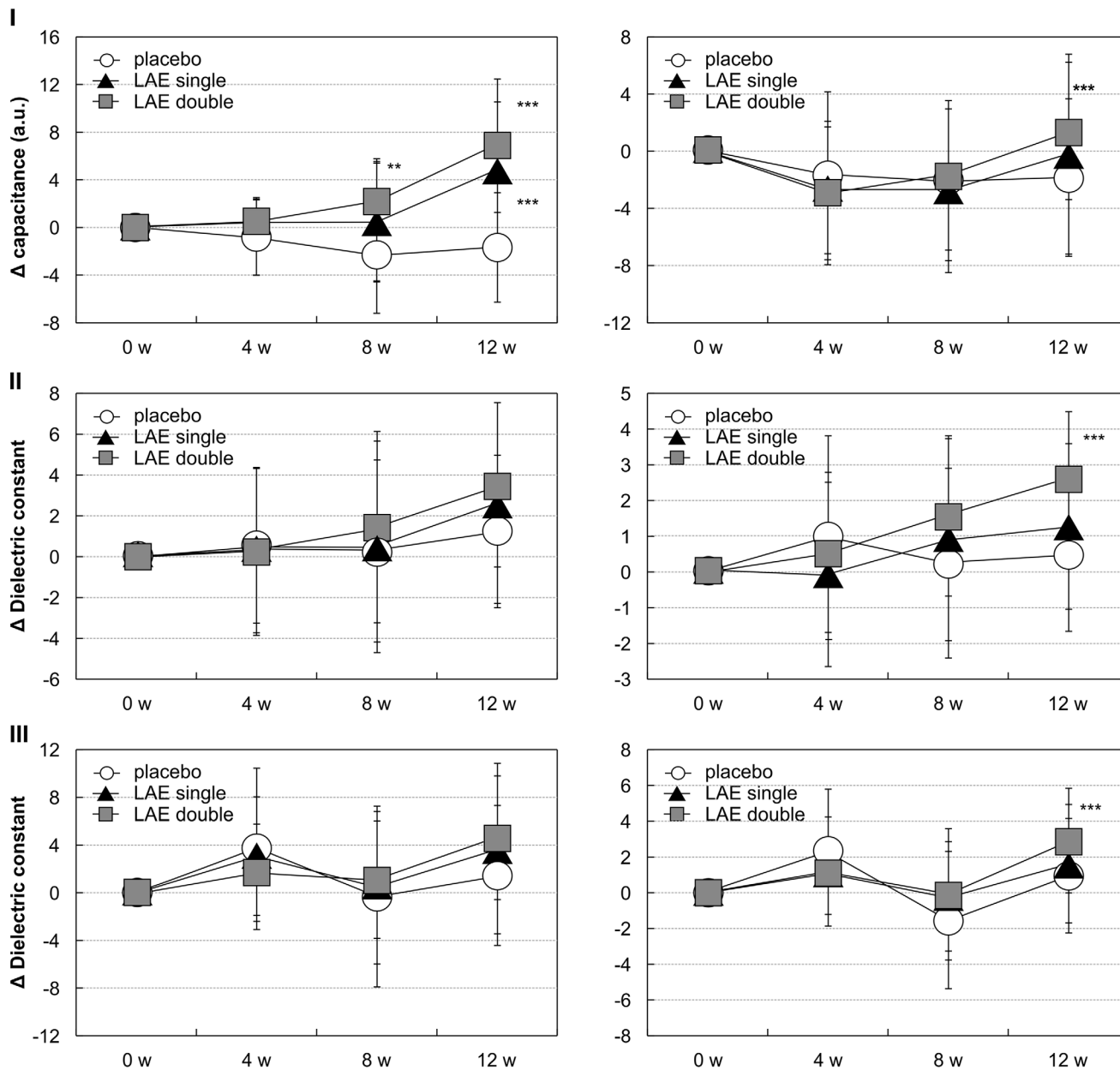


FIG. 5

Improvement in the skin moisture content following ingestion of a beverage containing lingonberry and amla fruit extracts (LAE); variations in moisture are shown relative to the baseline level in (I) the stratum corneum, (II) the epidermis, and (III) the dermis, along with (IV) TEWL values for (A) the cheeks and (B) the forearms. Single-dose LAE: 25 mg of dry lingonberry extract + 30 mg of dry amla fruit extract; double-dose LAE: 50 mg + 60 mg of the same extracts, respectively. Data are presented as the mean $\pm$ SD, * P < 0.05 and *** P < 0.001 versus the placebo group.

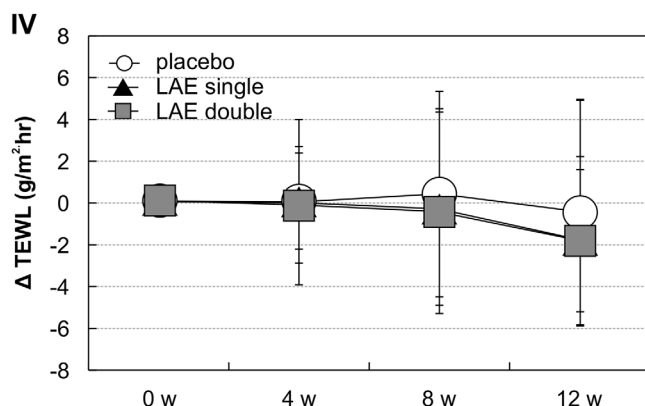


FIG. 5

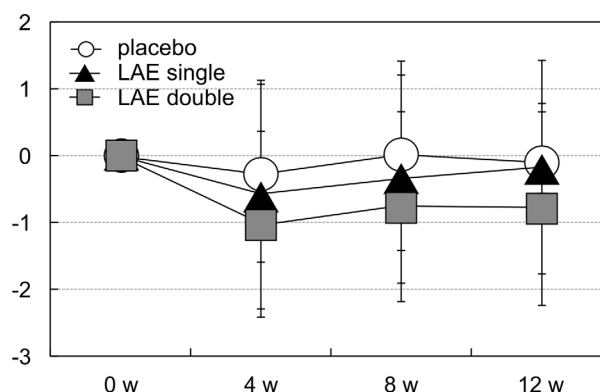
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Furthermore, we confirmed that increases in collagen and skin hydration contributed to improvements in skin elasticity and skin thickness.

The improvement in skin elasticity could have resulted from an increase and improvement in components of the skin intercellular matrix, such as collagen, which contributes to elasticity, and hyaluronan, which has a strong ability to absorb and retain moisture. We speculated that the increase in elasticity caused by the factors above led to a general improvement in skin texture.

As the skin becomes fragile with aging, skin tears may occur in hospitalized patients. Skin tears are an acute skin injury that occur primarily in the elderly; they are caused by normal friction in normal medical care settings [10]. The skin tears threaten the well-being of the patient as they cause strong pain. In addition, it might cause misunderstandings with the patient's family, as they might think that the injury was caused by inappropriate care by the healthcare worker and caregivers. Thus, skin tear is a serious problem in the medical scene. To solve this problem, different methods have been studied to understand and avoid skin tear. However, a definitive solution to this problem is not yet available. The skin of an average 90-year-old patient with skin tears, compared with that of a patient without skin tears, shows decreased levels of MMP-2 and type IV collagen, an increase in the tumor necrosis factor- α level, and reduced elasticity [37]. It is possible that the epidermis and dermis are easily separated in the skin of the elderly because the basal membrane becomes more fragile and the epidermis hardens. Furthermore, it is known that the skin becomes thin with age [3, 7] and the levels of skin elastin (in non-exposed skin) [5], skin collagen [3], and epidermal hyaluronan decrease [6], while glycation of collagen increases [38]. These data indicate that skin tissue becomes fragile with age, and if glycation of skin connective tissue is inhibited, skin tears may occur less frequently.

Another group study [28] suggested that combining the extracts of lingonberry and amla fruits provided a combinatorial



effect of certain polyphenols. This was evidenced by the observation that the extract combination had a synergistic effect that induced collagen production in a cell culture system. Therefore, we examined whether the effect could also be confirmed in a clinical trial, and the results of this study demonstrated that LAE had multiple positive effects on the skin.

However, as with the collagen production, it is unclear whether the synergistic effect obtained with the combination would be comparable to that of each separate extract. This should be investigated in future studies. In addition, the contribution of each LAE constituent needs to be elucidated. Resveratrol, procyanidin, quercetin, catechin, epicatechin, coumaric acid, and other polyphenols are found in lingonberries [15, 16], whereas gallic acid, gallogen, vitamin C, β -glucogallin, mucinous acid, and other polyphenols are found in amla fruit [17, 18]. We have already found that some of these components contribute to the effects of LAE on the skin, and detailed reports on these components will be provided in future publications.

5. Conclusions

The results of our study showed that regular ingestion of LAE drink was associated with a dose-dependent improvement in skin conditions, which typically worsen with age. Generally, women have better skin texture than men. Therefore, only women participated in this study. It was speculated that LAE would be effective in all people with poor skin conditions. Our findings indicated that LAE exerted strong beneficial effects on the skin. Because various skin conditions were improved by oral intake of LAE, the combination extract may act as a potential anti-aging agent by inhibiting the weakening of the skin structure caused by aging.

6. Acknowledgements

The authors express their gratitude to all study participants and to Infoward. They also thank Shigehisa Ibe who provided advice regarding statistical analysis. This study was funded by Shiseido Co., Ltd. (1510-BUMON-00415). The sponsor did not

have any influence on the performance of the trial, analysis of the data, writing of the manuscript, or publication of the results.

The authors declare that they have no competing interests.

7. References

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