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Tomato Seed Extract Containing Lycoperoside H Improves Skin Elasticity in Japanese Female Subjects: A Randomized, Placebo-Controlled, Double-Blind Trial

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Abstract

Background and Objective: Tomato seeds are edible seeds unconsciously ingested with the fruit. However, there are few reports regarding the constituents and biological activities of tomato seed extract (TSE). Recently, we found that saponins are major constituents of TSE including lycoperoside H. Previous reports have described that several plant-derived saponins improve skin diseases such as wounds and microangiopathy. Therefore, to discover the effect of TSE on the skin condition, we conducted a clinical trial of TSE (Tomato Seed Extract-P) standardized with lycoperoside H when orally ingested. **Methods:** The study was performed as a randomized, double-blind, placebo-controlled study. TSE (200 mg daily) containing 1 mg of lycoperoside H was used as the active sample. We enrolled 44 Japanese women who have concerns about facial elasticity and relatively low facial skin elasticity. All subjects were randomly allocated into either the active group (n = 22) or the placebo group (n = 22) using a computerized random-number generator. Capsules containing either the active sample or a placebo were administered for 8 weeks between October 12, 2020, and January 16, 2021. Facial elasticity, specifically the R7 value, was evaluated as the primary outcome. The remaining facial R parameters, upper arm R parameters, and other skin parameters including epidermal moisture, trans epidermal water loss, dermal parameters, and advanced glycation end products (AGEs) parameters were measured at 0, 4, and 8 weeks of ingestion. Blood, urine, and body parameters were also evaluated for safety. **Results:** Forty-three subjects completed the trial, and the

per protocol set comprised 21 subjects in the TSE group and 22 subjects in the placebo group. After ingesting TSE for 8 weeks, the R7 value was significantly higher in the TSE group compared to the placebo group. Furthermore, the change in R7 values from the baseline at 4 and 8 weeks were also higher in the TSE group. Among the secondary outcomes, facial elasticity parameters including R2, R5, R1, and R4 at 4 weeks and facial R5, R1, and R4 and upper arm R2 at 8 weeks were higher in the TSE group. In addition, plasma pentosidine significantly decreased in the TSE group after 8 weeks of ingestion. There were no significant differences in moisture, DermaLab® parameters and AGEs parameters except plasma pentosidine. Laboratory tests revealed no abnormalities suggesting adverse effects of TSE. **Conclusions:** TSE (200 mg/day) standardized with lycoperoside H improved the facial elasticity parameters. Thus, daily ingestion of TSE was suggested to be beneficial for maintaining the facial skin elasticity. However, the relationship between the reduction of pentosidine and skin elasticity by TSE ingestion should be clarified through further studies. **Trial Registration:** UMIN-CTR: UMIN000041881. **Foundation:** Oryza Oil & Fat Chemical Co., Ltd.

Keywords

Tomato Seed, Saponin, Lycoperoside, Skin Elasticity, Cutometer, Pentosidine

1. Introduction

Ingestion of tomato fruit is recognized to be beneficial to maintain a healthy skin condition [1] because it contains many types of phytochemicals [2]. Lycopene is a principal, well-known carotenoid in tomato [3] which can reduce the risks of reactive oxygen species [4]. Its photoprotective activity can prevent UV-induced erythema after 10 to 12 weeks of ingestion [5] [6]. The amount of lycopene in daily tomato ingestion is sufficient to protect the skin from photodamage [7]. As well as the photoprotective effects of tomato carotenoids, tomatoes also contain several types of polyphenols such as naringenin [8] and naringenin chalcones [9]. There are no clinical trial reports of naringenin when orally ingested, however, topical application of naringenin micro sponges [10], liposomes [11], and nanoparticles [12] were reported to improve the skin condition such as wound scars [13] and atopic dermatitis [10]. These applications of naringenin were based on anti-inflammatory [14] and anti-allergy [15] [16] effects proved by *in vivo* models. Furthermore, naringenin protects cultured human keratinocytes [17] and mouse skin [18] [19] from UV damage through anti-photoaging effects [20] and exhibits whitening effects in B16 melanoma cells [21].

As well as lycopene and polyphenols, saponins are a third type of phytochemical found in tomato [22]. Several types of saponins, such as lycoperosides [23] [24], esculeosides [25] [26] [27] [28] [29], and tomatine [30], have been identified in tomatoes. Regarding the biological activities of tomato saponins on the skin condition, Zhou *et al.* [31] demonstrated that the oral administration of esculeo-

side B isolated from tomato juice attenuated 2,4-dinitrochlorobenzene-induced type IV allergic dermatitis in mice. On the other hand, esculeoside A was found to inhibit hyaluronidase inhibitory activity and suppress itching in a mouse dermatitis model [32]. Generally, tomato seeds are discarded after squeezing juice or oil extraction. Thus, we performed a constituent study of tomato seeds to find effective use of waste resources and found that lycoperosides are major saponins in the seeds. Furthermore, lycoperoside H was found to suppress atopic dermatitis-like skin inflammation in IL-33 deficient mice [33].

Despite the above chemical and preclinical studies of tomato saponins, there are no reports of a clinical study of saponins especially on the skin condition. However, several plant extracts containing saponins have been found to have improving effects on skin diseases. For example, *Centella asiatica* extract containing asiaticosides or centella saponins were found to improve wound appearance after topical [34] and oral [35] application. Moreover, the topical application of escin, a horse chestnut seed saponin, ameliorates diabetic microangiopathy [36] and superficial vein thrombosis [37]. These efficacies were caused by improvement of micro blood circulation [38]. From these reports, TSE was suggested to have beneficial effects on the skin condition. Therefore, we conducted a clinical trial of tomato seed extract (TSE) standardized with lycoperoside H content on the skin condition.

2. Materials and Methods

2.1. TSE and Lycoperoside H

Tomato Seed Extract-P (Lot. N-027) manufactured by Oryza Oil & Fat Chemical Co. Ltd. was used as the TSE for the study. The content of lycoperoside H was 0.5%. The content was determined by HPLC equipped with a charged aerosol detector (Corona™ Veo™ CAD detector, Thirmo Scientific, USA) [39] and a revered phase HPLC column (CAPCELL PAK C18 SG120, 4.6 mm *i.d.* × 250 mm, Osaka Soda Co., Japan). Sixty percent MeOH containing 0.1% trifluoro acetic acid was used as a solvent. The injection volume was 10 μL and the flow rate was 1 mL/min. The evaporation temperature and filter setting of the charged aerosol detector were 35˚C and 5.0 seconds, respectively. The N_2 gas pressure was 62.9 psi and the charger voltage was 1.8 kV. The range scale was set to 200 pA. Lycoperoside H standard was prepared at Oryza Oil & Fat Chemical Co. Ltd. Standard lycoperoside H was dissolved in MeOH and TSE was dissolved in 60% MeOH. The approximate analyzing time was 40 min.

2.2. Participants and Grouping

All subjects were recruited between September 17 and October 24, 2020, through the Go106 website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. The inclusion criteria were healthy Japanese female adults (20 years old or more) with concerns about facial skin elasticity. The exclusion criteria were as follows:

1) Current or previous cancer, heart failure, or myocardial infarction.

2) Subjects with a heart pacer or an implantable cardioverter defibrillator.

3) Current treatment for arrhythmia, hepatitis, nephritis, rheumatoid arthritis, cerebrovascular disease, diabetes, hyperlipidemia, hypertension, or other chronic diseases.

4) Current use of medications or dietary supplements or beverages.

5) Subjects with allergic reactions to medicines or foods related to tomatoes.

6) Pregnancy, lactation, or expected/planned pregnancy during the study period.

7) Subjects currently participating in another clinical trial or who had participated in one within the previous 3 months.

8) Subjects who have had plastic surgeries.

9) Subjects who have daily skin care therapy such as esthetic treatments or using facial beauty appliances.

10) Subjects who use skin care products daily except creams, serums, all-in-one cosmetics, facial packs, face lotions, face emulsions, and sunscreens.

11) Subjects who have been diagnosed as having atopic dermatitis before.

12) Subjects determined to be inappropriate for the study for other reasons by the attending physician.

13) Subjects who have the risk of disrupting their normal daily routine such as shift work or long trips.

14) Subjects who cannot avoid sunburn or exposure to sunlight for a long time.

The selection criteria were persons who have relatively low facial skin elasticity and were determined to be appropriate for the study by the physician. We chose subjects who had cheeks with relatively lower ratio (R7) of elasticity among candidates at the screening periods. And we regarded them as those who have relatively low facial skin elasticity.

Forty-four female subjects with concerns about facial skin elasticity were selected after they were confirmed to be suitable for the study by a physician (**Figure 1**). The participants were asked to take test capsules according to the designated method and avoid excessive eating and drinking. In addition, the subjects were requested to refrain from taking dietary supplements and supplemental beverages and maintain a regular lifestyle during the study period. In terms of skin care, excessive sunburn, usage of ceramide cream, and having esthetic treatments were prohibited and the subjects were asked not to change their usual make up style.

One day before testing, the subjects were required to avoid excessive drinking of alcoholic beverages and intensive exercise, and they fasted for 6 hours prior to blood collection except for drinking water. On the screening day, using any type of cosmetics and taking a bath or shower were prohibited.

2.3. Test Samples and Allocation

The test samples (indistinguishable brown capsules containing either TSE or a placebo were provided by Oryza Oil & Fat Chemical Co., Ltd. as hard capsules.

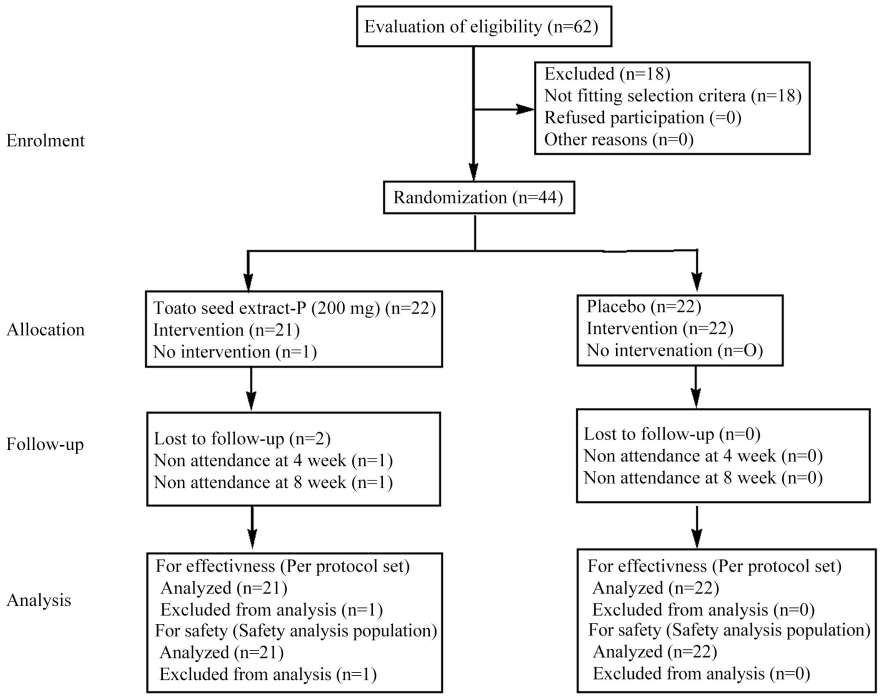

Figure 1. Flowchart showing the characteristics of the subjects.

The active capsules contained 100 mg of Tomato Seed Extract-P (containing 0.5 mg of lycoperoside H) and 50 mg of maltodextrin. Tomato Seed Extract-P consisted of 5% absolute tomato seed extract and 95% maltodextrin. The placebo capsules contained 150 mg of maltodextrin.

Oryza Oil & Fat Chemical Co. Ltd. provided the test samples with red or blue markings on the packages. They strictly kept the sample information until the study period was over.

When the number of registered subjects reached 44, an allocation controller at ORTHOMEDICO Inc. ordered the test capsules according to the provided identification markers and made an allocation sheet and an emergency key. To prepare random numbers for the allocation sheet, Statlight #11 (Ver. 2.10, Yukms Inc., Japan) was used. The allocation sheet was only provided to the test sample distributers and then strictly concealed with the emergency key by the allocation controller. Then, the test capsules were allocated by class randomization to equalize the allocation ratio (1:1). Allocation was required in order for the means and standard deviations (SD) of cheek elasticity and age not to differ between the groups. Information about allocation was not disclosed to anyone until the subjects for analysis were determined at a clinical conference after study completion.

2.4. Study Protocol and Skin Parameters

This randomized, placebo-controlled, double-blind, parallel-group study was carried out at Hiroo Dermatology Clinic & Mentors Inc. (Tokyo, Japan), and statistical analysis was performed by ORTHOMEDICO Inc. The protocol was

registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN000041881). The study was performed between October 12, 2020, and January 16, 2021. The subjects took 2 appropriate capsules (either TSE or a placebo) daily after breakfast for 8 weeks. All the subjects recorded a daily report, including capsule ingestion and menstruation, and answered a questionnaire at 4 and 8 weeks from a physician. Also, the subjects were asked to record a calorie and nutrition diary from 3 days before to the day of screening.

The following parameters were examined at baseline and at 4 and 8 weeks after intake. The R7 value of the cheek at 8 weeks was measured using a cutometer dual MPA580 (Courage + Khazaka Electronic GmbH, Köln, Germany), and was set as the primary outcome. The other elasticity parameters (R1 to R6, R8 and R9) of the cheek at the other periods and R1 to R9 of other parts were set as the secondary outcomes. Each R value was calculated from the deformation length (mm) of Uv (delayed distention), Ue (immediate distention), Uf (total elongation), Ur (immediate retraction), and Ua (total recovery) on the chart recorded by a cutometer [40]. R1 is calculated by the difference between Uf and Ua. R2, R5, R6, and R7 are the ratios of Ua/Uf, Ur/Ue, Uv/Ue, and Ur/Uf, respectively. R3 and R4 are the last maximal amplitude and last minimal amplitude, respectively. The cross point of the line drawn vertically from the outer corner of the left eye and the drawn horizontally from the center of the nose was chosen for the evaluation site. The measurement site for the upper arm was defined as the inside of mid-point of the line drawn between the tip of the right shoulder and the elbow. We measured the parameters on the same site. Measurement was performed 3 times and then the average was calculated.

As the other secondary outcomes, the following items were evaluated. The transepidermal water loss (TEWL) was measured using a Tewameter® TM300 (Courage + Khazaka Electronic GmbH). The epidermal moisture was measured using a Corneometer CM825 (Courage + Khazaka Electronic GmbH). Skin moisture content and TEWL of each individual site were measured 3 times and once, respectively. DermaLab® (Cortex Technology, Denmark) [41] was used to measure cheek dermal parameters such as intensity, skin thickness, and low echologenic band (LEB). An advanced glycation end products (AGEs) sensor (Air Water Biodesighn Inc, Kobe, Japan) was used for evaluation of finger AGEs. Plasma pentosidine and (carboxylmethyl)lysine (CML) were determined using a VersaMax Microplate Reader (Molecular Devices, LLC., USA) and CircuLex CML/Nε-(Carboxymethyl)lysine ELISA kit (Medical & Biological Laboratories Co. Ltd., Japan).

2.5. Laboratory Tests

The bodyweight, body mass index (BMI), body fat ratio, blood pressure, and pulse rate were measured at all test periods.

Blood and urine were analyzed by LSI Medience Corporation (Tokyo, Japan). All items were examined at baseline and at 4 and 8 weeks after intake. A venous

blood sample was collected from an arm vein and the following tests were performed for safety assessment.

The hematology components were as follows: counts of red blood cells, leukocytes, platelets, lymphocytes, monocytes, eosinophils and basophils, hemoglobin, and hematocrit. The biochemical components were as follows: total protein, total bilirubin, urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, hemoglobin (Hb) A1c, blood glucose, glycoalbumin, amylase, creatine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), chorine esterase, cholecystokinin, Na, K, Cl, Ca, Fe, inorganic phosphorus, and IgE.

In addition, urine samples were collected for qualitative evaluation, including protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood.

2.6. Ethics, Adherence, and Compliance

This study was performed according to the Declaration of Helsinki (2013 revision) and was carried out in conformity with ethical considerations. This protocol was approved by the ethics committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) on September 15, 2020 (Approved ID: 2009-00023-0048-21-TC), and substantial deviation from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before consenting to participation. No subject was part of the sponsoring or funding companies. This study was conducted in accordance with the consort statement.

2.7. Statistical Analysis

PPS was chosen as the analysis data set for the primary and secondary outcomes. The results are reported as means and SD. For statistical analysis, one-way analysis of variance (ANOVA) was performed, followed by Dunnett's test. Two-way repeated measures analysis of covariance (ANCOVA) or ANOVA followed by post hoc analysis was performed to detect significant differences between the two groups. The results of the physical examination and blood tests are reported as means and SD. The Student's *t*-test was used to evaluate the significance of differences between before and after ingestion of the test sample. The χ^2-test was used for urinalysis parameters, with normal and abnormal values being coded as "1" and "0", respectively. We set the significance level at 5% with no adjustment for multiple comparisons. SPSS (Ver. 23.0, Japan IBM) or Microsoft Excel 2013 was used for statistical evaluation.

3. Results

3.1. Study Performance

During the study period, one subject in the TSE group refrained to have inter-

vention after allocation for personal reasons (Figure 1) and was excluded from the analysis in the TSE group from this period. Accordingly, 21 subjects (52.0 ± 9.2 years) were available for analysis in the TSE group, whereas 22 subjects (51.2 ± 10.0 years) were available for the placebo group. The physical profile of the subjects included in analysis is shown in Table 1. After 8 weeks of ingestion of TSE, the systolic and diastolic blood pressure significantly decreased compared to the placebo group.

3.2. Elasticity Parameters

Table 2 shows the elasticity parameters of the cheek skin. As the primary outcome of the study, the R7 value, meaning skin firmness at 8 weeks in the TSE group, was significantly higher than that of the placebo group. Furthermore, regarding the changes in R7 from the baseline, the values for the TSE group were higher than the placebo group at 4 and 8 weeks. As the secondary outcomes, R2 (gross elasticity) at 4 weeks and R5 (net elasticity) at 4 and 8 weeks in the TSE group were significantly higher than the placebo group. In contrast, R1 (return to original skin) and R4 (last minimal amplitude) at 4 and 8 weeks in the TSE group were lower than the placebo group. Table 3 shows the elasticity parameters of the upper skin. In contrast to the cheek skin, only a few parameters have changed. The R2 value at 8 weeks was higher than the placebo group.

Table 1. Profile of the participants.

	Baseline		8 W	
	TSE	Placebo	TSE	Placebo
Age	52.0 ± 9.2	51.2 ± 10.0	-	-
Height (cm)	157.0 ± 5.9	158.4 ± 6.1	-	-
Body weight (kg)	52.6 ± 10.0	54.2 ± 10.8	53.1 ± 10.2	54.5 ± 10.8
BMI (kg/m^2)	21.4 ± 4.4	21.5 ± 3.6	21.6 ± 4.5	21.7 ± 3.6
Body fat ratio (%)	27.4 ± 9.6	30.1 ± 7.3	27.5 ± 9.2	30.3 ± 7.3
Systolic blood pressure (mmHg)	113.7 ± 14.6	117.6 ± 8.3	108.4 ± 14.1**	122.1 ± 12.6
Diastolic blood pressure (mmHg)	73.3 ± 11.3	75.2 ± 7.2	69.1 ± 11.2*	76.9 ± 8.3
Pals rate (bpm)	76.2 ± 10.9	71.0 ± 10.1	72.2 ± 8.7	69.8 ± 10.7
Body temperature (˚C)	32.9 ± 0.7	32.9 ± 1.1	32.8 ± 1.1	32.9 ± 1.0
IgE (IU/mL)	361.1 ± 897.0	375.2 ± 780.1	-	-

Data are shown as the mean ± SD (n = 21 for TSE, n = 22 for placebo). Student's *t*-test was used for evaluation of significant differences except age (χ-squared test). Asterisks indicate significant differences from the placebo at *: $p < 0.05$ and **: $p < 0.01$.

Table 2. Changes in facial skin elasticity parameters measured using a cutometer.

	Baseline		4 W		8 W	
	TSE	Placebo	TSE	Placebo	TSE	Placebo
R7 (Skin firmness)	0.359 ± 0.044	0.365 ± 0.048	0.416 ± 0.091 (0.056 ± 0.090*)	0.370 ± 0.100 (0.004 ± 0.108)	0.356 ± 0.068* (−0.003 ± 0.057*)	0.308 ± 0.069 (−0.058 ± 0.075)
R1 (Return to original skin, mm)	0.052 ± 0.013	0.049 ± 0.014	0.030 ± 0.014* (−0.022 ± 0.020*)	0.042 ± 0.017 (−0.007 ± 0.022)	0.044 ± 0.021* (−0.008 ± 0.014*)	0.056 ± 0.014 (0.007 ± 0.021)
R2 (Gross elasticity, ratio)	0.641 ± 0.065	0.064 ± 0.093	0.739 ± 0.121* (0.098 ± 0.126)	0.665 ± 0.142 (0.020 ± 0.198)	0.712 ± 0.180 (0.069 ± 0.099)	0.642 ± 0.096 (−0.003 ± 0.138)
R3 (Last maximal amplitude, mm)	0.143 ± 0.029	0.144 ± 0.035	0.115 ± 0.026 (−0.028 ± 0.038)	0.125 ± 0.026 (−0.019 ± 0.037)	0.148 ± 0.053 (0.004 ± 0.047)	0.160 ± 0.027 (0.016 ± 0.049)
R4 (Last minimal amplitude, mm)	0.052 ± 0.013	0.049 ± 0.014	0.030 ± 0.014* (−0.022 ± 0.020*)	0.042 ± 0.017 (−0.007 ± 0.022)	0.044 ± 0.021* (−0.008 ± 0.014*)	0.056 ± 0.014 (0.007 ± 0.021)
R5 (Net elasticity, ratio)	0.533 ± 0.071	0.539 ± 0.080	0.572 ± 0.110* (0.039 ± 0.127)	0.507 ± 0.124 (−0.032 ± 0.129)	0.481 ± 0.145* (−0.049 ± 0.083*)	0.413 ± 0.089 (−0.125 ± 0.103)
R6 (Viscoelasticity, ratio)	0.483 ± 0.095	0.473 ± 0.143	0.381 ± 0.095 (−0.103 ± 0.139)	0.384 ± 0.096 (−0.089 ± 0.169)	0.348 ± 0.111 (−0.126 ± 0.112)	0.349 ± 0.074 (−0.124 ± 0.149)
R8 (Total recovery, mm)	0.092 ± 0.021	0.095 ± 0.030	0.086 ± 0.024 (−0.006 ± 0.028)	0.083 ± 0.029 (−0.012 ± 0.039)	0.105 ± 0.040 (0.012 ± 0.042)	0.104 ± 0.029 (0.009 ± 0.045)
R9 (Skin fatigue, mm)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n = 21 for TSE and n = 22 for Placebo). Baseline data were analyzed using Student's *t*-test. After the intervention data, the actual scores were analyzed using the linear mixed model, with the baseline data utilized as covariates and with time, group, group-time interaction, and subject as factors, and changes from baseline were analyzed using the linear mixed model, with time, group, group-time interaction, and subject as factors. An asterisk indicates a significant difference from the placebo at *: $p < 0.05$.

Table 3. Changes in upper arm skin elasticity parameters measured using a cutometer.

	Baseline		4 W		8 W	
	TSE	Placebo	TSE	Placebo	TSE	Placebo
R7 (Skin firmness)	0.656 ± 0.093	0.663 ± 0.098	0.641 ± 0.082 (−0.016 ± 0.107)	0.626 ± 0.094 (−0.037 ± 0.073)	0.622 ± 0.154 (−0.034 ± 0.095)	0.596 ± 0.078 (−0.068 ± 0.079)
R1 (Return to original skin, mm)	0.028 ± 0.010	0.027 ± 0.009	0.020 ± 0.008 (−0.008 ± 0.012)	0.021 ± 0.006 (−0.006 ± 0.011)	0.027 ± 0.010 (−0.002 ± 0.013)	0.029 ± 0.006 (0.002 ± 0.009)
R2 (Gross elasticity, ratio)	0.824 ± 0.079	0.835 ± 0.056	0.837 ± 0.047 (0.013 ± 0.094)	0.825 ± 0.048 (−0.009 ± 0.062)	0.826 ± 0.185* (0.001 ± 0.070)	0.798 ± 0.047 (−0.036 ± 0.056)
R3 (Last maximal amplitude, mm)	0.178 ± 0.044	0.170 ± 0.034	0.123 ± 0.033 (−0.054 ± 0.054)	0.119 ± 0.023 (−0.050 ± 0.029)	0.151 ± 0.046 (−0.028 ± 0.052)	0.147 ± 0.031 (−0.023 ± 0.046)
R4 (Last minimal amplitude, mm)	0.028 ± 0.010	0.027 ± 0.009	0.020 ± 0.008 (−0.008 ± 0.012)	0.021 ± 0.006 (−0.006 ± 0.011)	0.027 ± 0.010 (−0.002 ± 0.013)	0.029 ± 0.006 (0.002 ± 0.009)
R5 (Net elasticity, ratio)	0.869 ± 0.106	0.877 ± 0.104	0.834 ± 0.099 (−0.035 ± 0.116)	0.818 ± 0.102 (−0.059 ± 0.088)	0.787 ± 0.191 (−0.079 ± 0.109)	0.749 ± 0.076 (−0.128 ± 0.070)
R6 (Viscoelasticity, ratio)	0.323 ± 0.070	0.331 ± 0.088	0.308 ± 0.078 (−0.016 ± 0.105)	0.314 ± 0.073 (−0.017 ± 0.086)	0.269 ± 0.091 (−0.049 ± 0.057)	0.265 ± 0.072 (−0.067 ± 0.104)
R8 (Total recovery, mm)	0.149 ± 0.040	0.143 ± 0.034	0.103 ± 0.029 (−0.046 ± 0.048)	0.098 ± 0.020 (−0.044 ± 0.026)	0.125 ± 0.039 (−0.026 ± 0.044)	0.118 ± 0.028 (−0.025 ± 0.042)
R9 (Skin fatigue, mm)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n = 21 for TSE and n = 22 for Placebo). Baseline data were analyzed using Student's *t*-test. After the intervention data, the actual scores were analyzed using the linear mixed model, with the baseline data utilized as covariates and with time, group, group-time interaction, and subject as factors, and changes from baseline were analyzed using the linear mixed model, with time, group, group-time interaction, and subject as factors. An asterisk indicates a significant difference from the placebo at *: $p < 0.05$.

3.3. Moisture and DermaLab® Parameters

Table 4 shows the moisture parameters of the cheek and upper arm. However, there were no significant differences in either group for skin moisture or TEWL. Furthermore, the DermaLab® parameters did not change such as LEB, skin thickness, and intensity.

3.4. AGEs Parameters

As shown in Table 5, there were no significant differences in the AGEs scores and serum CML. However, serum pentosidine significantly decreased in the TSE group at 8 weeks.

Table 4. Changes in skin moisture and TEWL of the cheek and upper arm and DermaLabo® parameters of the cheek.

	Baseline		4 W		8 W	
	TSE	Placebo	TSE	Placebo	TSE	Placebo
Cheek						
Skin moisture	49.6 ± 10.0	54.3 ± 15.2	44.1 ± 15.6 (−5.6 ± 15.4)	45.5 ± 11.4 (−8.8 ± 14.6)	46.9 ± 14.7 (−2.8 ± 16.9)	44.8 ± 12.7 (−9.5 ± 15.5)
TEWL (g/m^2·hr)	17.9 ± 6.5	14.0 ± 4.7	18.0 ± 4.9 (0.1 ± 4.7)	16.4 ± 3.6 (2.4 ± 4.0)	15.8 ± 5.9 (−2.2 ± 3.0)	15.1 ± 5.3 (1.1 ± 4.8)
Upper arm						
Skin moisture	34.7 ± 9.6	35.1 ± 10.4	31.4 ± 12.6 (−3.3 ± 11.7)	26.0 ± 7.0 (−9.2 ± 12.6)	32.3 ± 8.2 (−2.9 ± 10.7)	29.4 ± 9.8 (−5.8 ± 12.2)
TEWL (g/m^2·hr)	9.6 ± 4.8	8.7 ± 2.1	9.6 ± 2.9 (0.0 ± 4.8)	10.3 ± 3.4 (1.6 ± 3.1)	9.6 ± 4.0 (−0.1 ± 6.9)	10.1 ± 3.5 (1.3 ± 3.4)
LEB (μm)	0.0 ± 0.0	9.0 ± 42.2	0.0 ± 0.0 (0.0 ± 0.0)	0.0 ± 0.0 (−9.0 ± 42.2)	0.0 ± 0.0 (0.0 ± 0.0)	0.0 ± 0.0 (−9.0 ± 42.2)
Skin thickness (μm)	1291.0 ± 237.6	1259.8 ± 216.6	1302.9 ± 245.7 (12.0 ± 243.2)	1373.6 ± 276.5 (113.8 ± 251.5)	1326.5 ± 282.9 (64.5 ± 266.5)	1363.9 ± 253.3 (104.1 ± 184.1)
Intensity	26.3 ± 12.3	26.4 ± 10.0	22.4 ± 9.3 (−3.9 ± 10.0)	21.7 ± 7.6 (−4.7 ± 9.8)	27.9 ± 14.5 (0.8 ± 13.0)	23.6 ± 8.5 (−2.7 ± 8.2)

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n = 21 for TSE and n = 22 for placebo). Baseline data were analyzed using Student's *t*-test. After the intervention data, the actual scores were analyzed using the linear mixed model, with the baseline data utilized as covariates and with time, group, group-time interaction, and subject as factors, and changes from baseline were analyzed using the linear mixed model, with time, group, group-time interaction, and subject as factors. No significant differences were detected between the TSE and placebo groups.

Table 5. Changes in AGEs parameters.

	Baseline		4 W		8 W	
	TSE	Placebo	TSE	Placebo	TSE	Placebo
AGEs score in middle finger (a.u.)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1 (0.0 ± 0.1)	0.5 ± 0.1 (0.0 ± 0.1)	0.5 ± 0.1 (0.0 ± 0.1)	0.5 ± 0.1 (0.0 ± 0.1)
Serum pentosidine (μg/mL)	0.055 ± 0.006	0.054 ± 0.010	0.054 ± 0.009 (−0.001 ± 0.008)	0.053 ± 0.011 (−0.001 ± 0.011)	0.048 ± 0.007* (−0.007 ± 0.007*)	0.053 ± 0.009 (0.000 ± 0.009)
Serum CML (μg/mL)	4.18 ± 4.60	2.98 ± 0.63	4.04 ± 4.50 (−0.13 ± 0.28)	2.84 ± 0.60 (−0.14 ± 0.31)	4.05 ± 4.91 (−0.16 ± 0.35)	2.82 ± 0.53 (−0.16 ± 0.35)

Actual scores and changes from baseline (in parentheses) are shown as the mean and SD (n = 21 for TSE and n = 22 for placebo). Baseline data were analyzed using Student's *t*-test. After the intervention data, the actual scores were analyzed using the linear mixed model, with the baseline data utilized as covariates and with time, group, group-time interaction, and subject as factors, and changes from baseline were analyzed using the linear mixed model, with time, group, group-time interaction, and subject as factors. An asterisk indicates a significant difference from the placebo at *: $p < 0.05$.

3.5. Laboratory Data and Adverse Effects

The blood pressure, pulse rate, body temperature, and serum IgE are listed in Table 1 and the blood hematology parameters are shown in Table 6. Except for blood pressure, no significant changes were observed between the 2 groups (Table 1). The biochemical parameters are shown in Table 7. After 8 weeks of intervention, significant differences were observed in the total protein between the 2 groups. However, this change was within the reference range. The urinalysis parameters did not change in either group (Table 8).

Table 6. Changes in hematology parameters.

	Baseline		After 8 weeks of ingestion		Standard value
	TSE	Placebo	TSE	Placebo	
Red blood cells ($\times 10^4$ cells/μL)	430 ± 30	446 ± 38	431 ± 35	447 ± 37	380 - 500
Leukocytes (cells/μL)	5333 ± 1333	5055 ± 1371	4765 ± 1252	5059 ± 1452	3300 - 9000
Hemoglobin (g/dL)	13.0 ± 1.1	13.4 ± 0.9	13.4 ± 1.1	13.6 ± 0.8	11.5 - 15.0
Hematocrit (%)	41.7 ± 2.8	42.7 ± 2.6	41.7 ± 2.9	42.7 ± 2.5	34.8 - 45.0
Platelets ($\times 10^4$ cells/μL)	31.3 ± 5.4	27.4 ± 5.0	29.4 ± 5.0	27.0 ± 5.2	14.0 - 34.0
Neutrophils (cells/μL)	3152 ± 1091	2927 ± 929	2731 ± 1026	2929 ± 940	
Lymphocytes (cells/μL)	1783 ± 604	1738 ± 586	1620 ± 457	1697 ± 560	
Monocytes (cells/μL)	254 ± 88	234 ± 67	260 ± 77	264 ± 78	
Eosinophils (cells/μL)	111 ± 93	121 ± 78	114 ± 55	125 ± 66	
Basophils (cells/μL)	32.6 ± 14.5	34.5 ± 18.9	39.5 ± 17.0	44.9 ± 12.4	

Each value is shown as the mean and SD (n = 21 for TSE and n = 22 for placebo). Student's *t*-test was employed for the statistical analysis. No significant difference was detected between the placebo and TSE groups.

Table 7. Changes in biochemical parameters.

	Baseline		After 8 weeks of ingestion		Standard value
	TSE	Placebo	TSE	Placebo	
Total protein (g/dL)	7.2 ± 0.3	7.3 ± 0.4	7.0 ± 0.4	7.3 ± 0.4*	6.7 - 8.3
Total bilirubin (mg/dL)	0.96 ± 0.55	0.80 ± 0.25	0.88 ± 0.31	0.86 ± 0.32	0.2 - 1.2
Urea N (mg/dL)	12.7 ± 3.1	12.6 ± 3.1	12.4 ± 3.0	13.0 ± 2.8	8 - 20
Creatinine (mg/dL)	0.61 ± 0.07	0.62 ± 0.08	0.59 ± 0.08	0.61 ± 0.08	0.47 - 0.79
Uric acid (mg/dL)	4.1 ± 1.0	4.2 ± 0.9	3.9 ± 1.0	4.2 ± 1.0	2.5 - 7.0
Total cholesterol (mg/dL)	235 ± 27	228 ± 34	239 ± 24	238 ± 35	120 - 219
LDL cholesterol (mg/dL)	141 ± 25	133 ± 33	145 ± 24	140 ± 34	65 - 139
HDL cholesterol (mg/dL)	75 ± 15	80 ± 21	78 ± 15	83 ± 21	40 - 95
Triglyceride (mg/dL)	109 ± 81	78 ± 30	86 ± 41	78 ± 34	30 - 149
HbA1c (%)	5.3 ± 0.3	5.4 ± 0.3	5.3 ± 0.3	5.4 ± 0.3	4.6 - 6.2
Glycoalbumin (%)	14.2 ± 1.4	14.2 ± 1.6	13.7 ± 1.5	13.7 ± 1.3	12.3 - 16.5
Blood glucose (mg/dL)	85 ± 10	84 ± 6	88 ± 15	83 ± 8	70 - 109

Continued

Amylase (U/L)	88 ± 25	86 ± 31	91 ± 30	85 ± 24	40 - 122
CK (U/L)	96 ± 52	108 ± 53	88 ± 45	116 ± 50	40 - 150
AST (U/L)	20.7 ± 7.8	21.3 ± 4.5	20.6 ± 6.5	21.8 ± 5.7	10 - 40
ALT (U/L)	16.5 ± 10.0	17.0 ± 9.7	16.7 ± 8.8	17.1 ± 10.1	5 - 45
γ-GTP (U/L)	15.6 ± 4.0	17.0 ± 7.2	15.6 ± 4.3	17.7 ± 7.9	<30
ALP (U/L)	173 ± 56	201 ± 61	165 ± 52	201 ± 65	100 - 325
LAP (U/L)	47 ± 7	49 ± 6	46 ± 6	50 ± 5	37 - 61
LDH (U/L)	187 ± 32	191 ± 23	183 ± 31	197 ± 24	120 - 240
Na (mEq/L)	140 ± 2	140 ± 2	141 ± 2	140 ± 1	137 - 147
K (mEq/L)	4.1 ± 0.3	4.2 ± 0.3	4.2 ± 0.2	4.2 ± 0.3	3.5 - 5.0
Cl (mEq/L)	100 ± 2	100 ± 2	101 ± 2	101 ± 1	98 - 108
Ca (mg/dL)	9.1 ± 0.4	9.3 ± 0.3	9.3 ± 0.3	9.4 ± 0.3	8.4 - 10.4
Fe (μg/dL)	102 ± 28	103 ± 25	90 ± 33	102 ± 33	40 - 180
Inorganic P (mg/dL)	3.8 ± 0.5	3.5 ± 0.5	3.7 ± 0.3	3.4 ± 0.4	2.5 - 4.5

Each value is shown as the mean and SD (n = 21 for TSE and n = 22 for placebo). Student's *t*-test was employed for statistical analysis. An asterisk indicates a significant difference from the placebo at *: $p < 0.05$.

Table 8. Changes in urine parameters.

	Week	TSE	Placebo	Standard value
Protein	0	(nor):19, (ab):2	(nor):21, (ab):1	(−)
	8	(nor):19, (ab):1	(nor):20, (ab):2	
Glucose	0	(nor):21, (ab):0	(nor):22, (ab):0	(−)
	8	(nor):20, (ab):0	(nor):22, (ab):0	
Urobilinogen	0	(nor):21, (ab):0	(nor):22, (ab):0	(±)
	8	(nor):20, (ab):0	(nor):22, (ab):0	
Bilirubin	0	(nor):21, (ab):0	(nor):22, (ab):0	(−)
	8	(nor):20, (ab):0	(nor):22, (ab):0	
pH	0	(nor):20, (ab):1	(nor):22, (ab):0	(5.0 - 7.5)
	8	(nor):18, (ab):2	(nor):21, (ab):1	
Occult blood	0	(nor):21, (ab):0	(nor):18, (ab):4	(−)
	8	(nor):19, (ab):1	(nor):17, (ab):5	
Ketone bodies	0	(nor):20, (ab):1	(nor):21, (ab):1	(−)
	8	(nor):19, (ab):1	(nor):22, (ab):0	

Data are presented as the number of subjects with normal values (nor) or abnormal values (ab). The χ^2-test was used for urinalysis parameters. No significant difference was detected between the placebo and TSE groups.

4. Discussion

Complaints about facial wrinkles and skin sagging from women increase with aging [42]. In healthy skin, the extracellular matrix (ECM) and dermal fibrob-

lasts maintain the skin elasticity [43]. ECM is composed of elastin and collagen fibers [44], and elastin fibers consist of elastin and micro fibrils [45]. Decreases or denaturation of ECM or elastin fibers may cause deep wrinkles and skin sagging [42] [46]. In the normal metabolic process of collagen fibers, old collagen fibers are decomposed and then taken up by fibroblasts to be decomposed into amino acids, which are recycled as materials for new collagen synthesis [47] [48]. Thus, deterioration of collagen metabolism with aging is thought to change the skin's appearance [42]. Furthermore, the number of dermal fibroblasts decreases with skin senescence especially during the postmenopausal period, and collagen and elastin productions decline [49]. Therefore, recovering of collagen and elastin fiber production is promising to delay skin aging. For this purpose, ingestion of several food ingredients has been reported to be effective such as collagen peptides [50] and coenzyme Q10 [51].

Another factor for skin senescence is aging-related glycation of collagen [52]. Accumulation of AGEs in the dermis gives glycation stress to ECM [53]. Since the structure of elastin and collagen that form ECM become fragile with glycation, the skin is not able to maintain the structure and elasticity [52] [53]. To minimize the influence of dermal AGEs, oral supplementation of a cherry blossom extract [54] or mangosteen peel extract [55] are used.

In our current study, we examined the effect of continuous intake of TSE on the skin elasticity. The skin elasticity was evaluated by the value of viscoelasticity measured using a cutometer. The cutometer changes the tip of the probe into a negative pressure state, sucks the skin, and then opens it in the measurement process. R parameters including R1 to R9 represent the elasticity of the skin [56]. Regarding the R parameters in the study, significant differences were confirmed by TSE ingestion. R1 shows the ability to return to the original state after the first suction. When the value is closer to 0, it means that elasticity is higher [57]. R1 and R4 were the same values in this study because R4 showed the ability to return to the original state after the final suction and we only sucked 1 time [57]. On the other hand, R2 shows the total elasticity over the measurement time and R5 shows the net elasticity at the time of suction. Moreover, R7 shows the ability of recovery corresponding to the maximum dented depth. A previous study on Japanese female subjects showed that the R2, R5, and R7 parameters correlated to age (29 to 55 years old or 31 to 59 years) [58] [59]. Thus, these parameters are suggested to reflect skin aging. In our study, the cheek R7 value of the TSE group at 8 weeks was higher than the placebo group (**Table 2**). The averaged ages of the subjects were 51 to 52 years old and the difference in R7 between the placebo and TSE groups was approximately 0.05. A previously described study [59] showed that R7 decreased 0.008 per each year of age calculated from the approximate expression formula. Therefore, TSE is suggested to maintain cheek elasticity while cheek R7 value in placebo group changed toward aging about 6 to 7 years.

In addition, similarly, we applied the approximate expression [59] of R1, R2, and R5 to our study. As a result, R1 increases 0.002 per year of age, R2 decreases

by 0.003, and R5 decreases by 0.010 per year. When these trends are applied to our results, R1 and R5 are considered to prevent aging by about 6 to 7 years, and R2 is considered to be rejuvenated by about 23 to 25 years after 8 weeks of ingestion of TSE. Among these parameters, the tendency to deterioration were more common in the placebo group than TSE group. Therefore, it is considered that the systemic effect of TSE ingestion tended to suppress deterioration in the several parameters. From these results, it was shown that TSE intake is useful for maintaining cheek elasticity.

In terms of elasticity of the upper arms, a significant difference between the groups was confirmed only for R2 at 8 weeks (**Table 3**). The R2 of the upper arm in the TSE group was higher than that of the placebo group. The following are possible reasons why consistent results were not obtained in the upper arm in comparison to the cheek. Firstly, the subjects who were chosen had trouble with facial skin elasticity. However, it was unclear whether they had a similar problem with their upper arms. Another factor is the lack of randomization based on the elasticity of the upper arm. Therefore, further study is required to evaluate the effect of TSE on the elasticity of the upper arm by recruiting appropriate subjects.

As AGEs parameters in our study, the middle finger AGEs score and plasma pentosidine and CML were evaluated and no significant differences were found between the groups, however, the actual value and changed value of plasma pentosidine at 8 weeks were significantly lower in the TSE group compared to the placebo group (**Table 5**). Pentosidine is a cross-linkable AGE and is known to promote the formation of crosslinks by collagen glycation to cause a decrease in skin viscoelasticity [55]. In a study regarding the anti-glycation effect of mangosteen peel extract, healthy female subjects aged 32 to 48 years ingested the extract [55]. As a result, an improvement in the skin elasticity was observed with a decrease in pentosidine. Similarly, there was an improvement effect of TSE on the facial elasticity accompanied with pentosidine reduction.

There is still a question remaining. Regarding the fact that there was no change in the AGEs score of the middle finger, which is an index of glycation stress. The AGEs scores of the subjects of this study were in the range of healthy people aged 20 to 80 years old and the same as the averaged values for men and women [60]. Thus, it seems to be difficult to detect the influence of TSE. Moreover, CML is a non-crosslinking AGE and serum CML increases in a diabetic condition [61]. Since our study was conducted in healthy Japanese women, serum CML was in normal range. Thus, it is probable that the effect of TSE was not detected.

Finally, in the safety assessment, no side effects were identified during the study period under the conditions of this study, though adverse events were identified in some participants. However, based on the criteria set before the study, the investigator determined that there was no causal relationship with TSE. Physical measurements and serum parameters showed significant differences between the groups in terms of blood pressure throughout the study pe-

riod, but the average values were within the standard values. In the biochemical parameters, there were some items that showed significant differences between the groups. However, the average value was within the standard value in all cases. Therefore, TSE was found to be safe to ingest under the conditions of the study.

In conclusion, TSE (200 mg) standardized lycoperoside H ingestion was found to improve cheek elasticity. This effect seems to be beneficial for skin elasticity. Furthermore, the effect may suppress skin aging. Thus, further studies are required.

5. Conclusion

This study demonstrated that TSE (200 mg/day for 8 weeks) containing 1 mg of lycoperoside H ameliorated skin elasticity in healthy female subjects with anxiety regarding facial skin elasticity. In addition, serum pentosidine was decreased by TSE. Therefore, TSE ingestion may contribute to skin elasticity. Furthermore, the intake of TSE was safe under the conditions of this study.

Abbreviations

AGEs, advanced glycation end products; ANCOVA, analysis of covariance; ANOVA, one-way analysis of variance; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CK, creatinine kinase; CML, (carboxymethyl) lysine; ELSD, evaporative light scattering detector; ECM, extracellular matrix; GTP, glutamyltransferase; HDL, high-density lipoprotein; Hb, hemoglobin; LAP, leucine aminopeptidase; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LEB, low echologenic band; SD, standard deviation; TEWL, trans epidermal water loss; TSE, tomato seed extract.

Author Contributions

The sponsor of the present study, Oryza Oil & Fat Chemical Co., Ltd., assigned ORTHOMEDICO Inc. to conduct the study. S.T and H.S. (Ph.D.) are affiliated with Oryza Oil & Fat Chemical Co., Ltd., and K.Y., N.S., S.Y., S.I., H.N., T.K., and A.S. are members of ORTHOMEDICO Inc. This study was conducted by both Oryza Oil & Fat Chemical Co., Ltd. and Hiroo Dermatology Clinic & Mentors Inc. I.T. (MD) was the principal investigator who monitored all the subjects' conditions. Furthermore, S.T., W.Y., and H.S. isolated and identified the chemical structure of lycoperoside.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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