THE SWELLING-REDUCING EFFECTS OF "*Sururu no Omegumi*" — A DOUBLE-BLIND, RANDOMIZED CROSSOVER STUDY —

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Abstract

Background: Red vine leaf is considered effective in reducing swelling of the lower limbs, but no studies have investigated the effectiveness of products containing these ingredients on lower limb swelling.
Methods: A randomized crossover study was conducted in 20 women with symptoms of swelling as the subjects. The study participants took part in the study under two conditions, ingesting a test food containing red vine leaf and ingesting a placebo food, with a washout period of 20 days between each condition. The primary outcomes were maximal calf circumference and the circumference 5 cm under the maximal calf, which were measured on days 1 and 5 of test food ingestion. On each measurement day, each circumference was measured after study participants ingested distributed food, followed by ingestion of the test food. Thereafter, participants were asked to sit in a chair for 6 hours with their knees bent at a 90° angle. After ingesting the test food, each circumference was measured at 2, 4 and 6 hours.
Results: Five days after ingestion, maximal region of calf circumference had increased with time, but this increase was suppressed significantly during ingestion of the test food as compared with during ingestion of the placebo. The circumference 5 cm under the maximal calf had also increased with time, and while this increase was reduced under the test food condition, significant difference was seen partly with the placebo condition.
Conclusion: Food containing red vine leaf extract tended to suppress swelling of the maximal region of calf circumference.

Key words: Red vine leaf, swelling, flavonoids

1. INTRODUCTION

Lower limb swelling is a symptom seen in occupations that involve a lot of work performed standing[1)2)]. Accompanying symptoms include lower limb languor, aches and cramps. The main cause of swelling is considered a build-up of fluid associated with reduced lower limb circulation, which is typically treated with lower limb compression using compression stockings, oral diuretics, and vascular surgical treatment[3)]. In cases where these treatments are inappropriate or ineffective, however, improvement of venous blood flow is attempted through ingestion of foods and so forth. Particularly in Germany, ingestion of flavonoids is recommended in cases where elastic bandages or compression stockings cannot be indicated[4)5)].

Red vine leaf extract can be counted among the functional foods containing flavonoids effective in suppressing swelling of the lower limbs. Red vine leaf is a medicinal herb traditionally used in Europe that is rich in flavonoids such as anthocyanin and quercetin[3)]. It is listed in official European compendiums (French herbal remedy guidelines, European Scientific Cooperative on Phytotherapy Monographs), and in Japan, products with red vine leaf as the main ingredient are certified as direct over-the-counter drugs. The effects of red vine leaf on lower limb swelling have also been verified using methods such as the water tank discharge method, and red vine leaf has been reported to reduce the amplitude of the lower limbs and alleviate subjective symptoms such as lower limb heaviness and aches[3)6)7)].

While red vine leaf can be expected to have an inhibitory effect on lower limb swelling, no study to date has examined the effects of taking this extract in humans. The present study therefore investigated the effects of this functional ingredient using the functional food "*Sururu no Omegumi*," which contains red vine leaf extract.

2. METHODS

Study Design

This study had a double-blind, randomized crossover

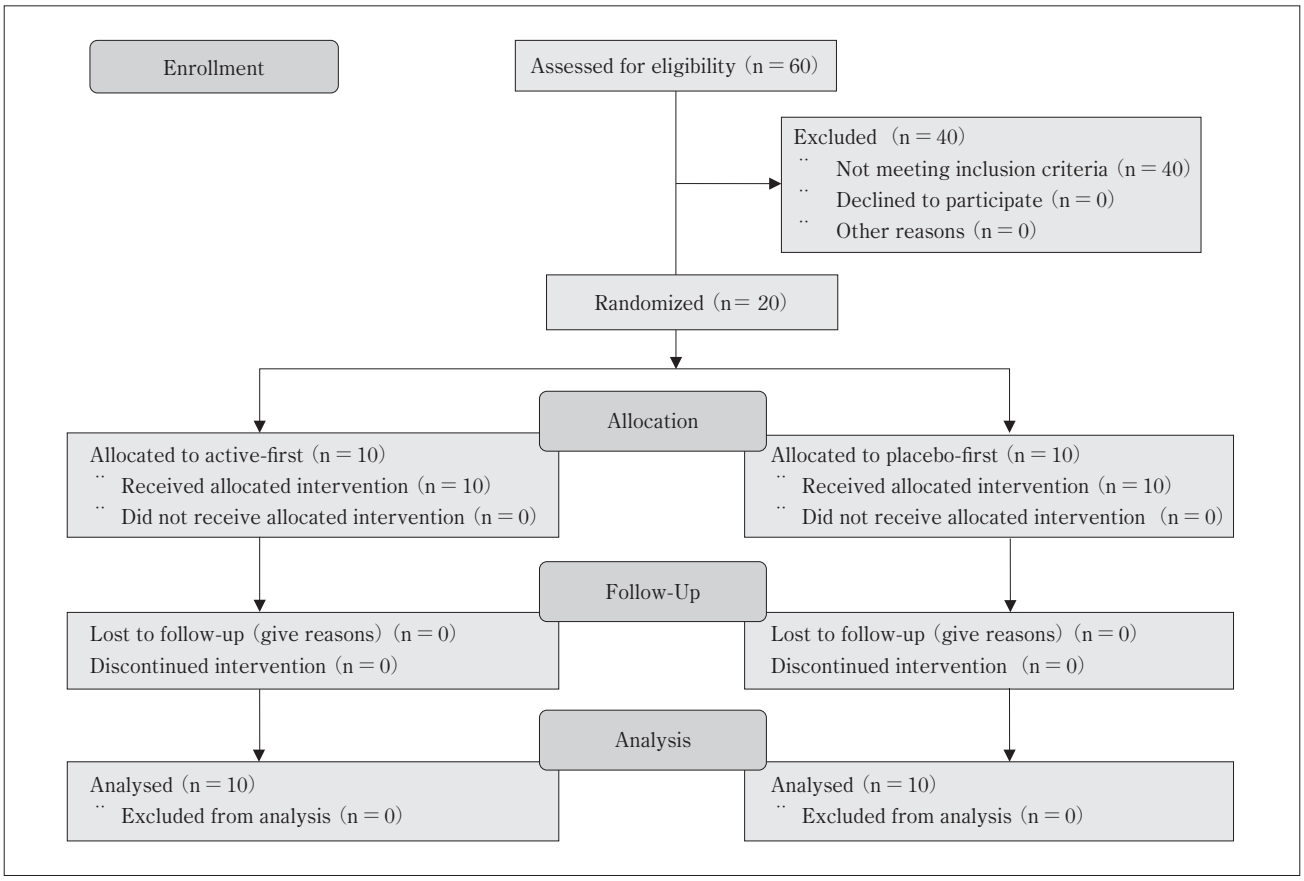

Fig. 1 The flow-diagram of this trial

design. Two study conditions were set: Condition A, in which study participants first ingested the test food, and Condition B, in which participants first ingested the placebo food. The study was conducted in a facility in the Tokyo metropolitan area (Japan Clinical Trial Association [JACTA]). The study implementation period was from September 8 to October 16, 2015.

This study was implemented in compliance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board. Pharmaceutical Low Wisdoms (Tokyo) before starting the study. In addition, all study participants gave their written informed consent to participate.

Study Participants

Healthy women registered in the monitor bank of Stephens & Associates,Inc. and who met the following eligibility criteria were recruited as study participants. Inclusion criteria were (1) women aged 20 to 39 and (2) those aware of swelling in their lower limbs. Exclusion criteria were (1) those in the process of receiving treatment for atrial fibrillation, arrhythmia, rheumatism, diabetes, hypertension, liver disease, kidney disease, diseases of the central nervous system, cardiovascular disease and/or dyslipidemia, (2) those taking some kind of oral therapy such as Chinese herbal remedies, (3) those who are pregnant, breastfeeding or who might fall pregnant during the study period, and (4) those deemed ineligible to participate in this study by the study doctor. A flow chart of the course of study participants is presented in **Fig. 1**

Table 1 Nutritional content of the sample per 100 g

Item	active	placebo
energy	387 kcal	386 kcal
protein	4.4 g	less than 0.1 g
lipid	2.9 g	0.5 g
carbohydrates	85.7 g	95.3 g
salt equivalent	0.290 g	0.394 g

Test Food and Study Procedure

The test food was "*Sururu no Omegumi*", which was provided by Fanfare.inc. The placebo food was also provided by them. 4 tablets of the supplement are consumed with hot or cold water everyday (1 tablet weighs 360 mg, therefore 4 tablets weigh 1440 mg). The test food included red vine leaf powdered extract as active ingredient. The placebo food included crystalline cellulose instead of red vine leaf powdered extract. **Table 1** shows the nutritional content of the sample.

The study participants took part in Session 1 for 5 days, followed by a 20-day washout period before taking part in Session 2 for 5 days. Participants made clinic visits to undergo examinations on days 1 and 5 of each

Table 2　Result of physical measurement and Urinary frequency

Item	Unit	Condition	Day-1	Day-5
systolic blood pressure	mmHg	active placebo	109.8 (15.0) 113.0 (14.0)	109.7 (11.5) 112.0 (14.1)
diastolic blood pressure	mmHg	active placebo	68.5 (9.4) 71.9 (9.6)	68.0 (8.7) 69.8 (8.5)
pulse rate	/minute	active placebo	83.7 (10.9) 81.3 (11.3)	80.6 (11.8) 84.7 (13.2)
body weight	kg	active placebo	57.0 (8.3) 57.0 (7.9)	57.0 (8.1) 56.9 (7.8)
body mass index	kg/m^2	active placebo	22.3 (3.4) 22.4 (3.1)	22.4 (3.2) 22.3 (3.1)
percent body fat	%	active placebo	32.5 (6.4) 32.8 (5.7)	32.5 (6.0) 33.1 (5.7)
the maximal region of calf circumference in hour-0	cm	active placebo	36.3 (2.6) 35.8 (2.3)	36.1 (2.7) 35.9 (2.4)
5 cm under the maximal region of calf circumference in hour-0	cm	active placebo	34.1 (2.9) 32.2 (2.8)	32.5 (3.2) 33.2 (2.4)
urinary frequency	times	active placebo	1.15 (0.88) 1.35 (0.99)	1.35 (0.88) 1.30 (0.98)

n = 20×2

Data are shown in Mean (SD).

session. On the day of each visit, participants underwent an interview, physical examination and lifestyle check. They were then given the same food (one commercially available rice ball) and 100 mL of water, which they were asked to ingest. Thereafter, participants were asked to sit on a chair for 6 hours and 30 minutes to induce lower limb swelling. The chair stood a height of 40 cm above the floor. During sitting, participants were requested to stay as still as possible with their knees bent almost at right angles. Thirty minutes after starting sitting, participants were asked to ingest the test food. Lower limb swelling was evaluated before ingestion of the test food and 2, 4 and 6 hours after ingestion.

Study participants were instructed to adhere to the following three requirements for compliance during the study period: (1) ingest the test food as instructed, (2) lead a usual lifestyle, and (3) avoid over-eating and drinking, excessive intake of alcohol and excessive exercise.

Randomization, Allocation and Ensuring Blinding

The study doctor determined eligibility criteria. Thereafter, the person in charge of allocation randomly allocated participants who met the eligibility criteria into two test conditions using an allocation table. The allocation table was created by the person in charge of allocation. Until completion of the study, the allocation table was securely locked away in and managed from a safety box located at JACTA. Information on allocation was not disclosed to the study participants, the study doctor, or those in charge of examinations during the study implementation period. In addition, the test food and placebo food had the same shape, color, smell and taste.

Endpoints

The primary endpoint of this study was the diurnal variation in the maximal region of calf circumference 5 days after starting test food ingestion, in accordance with Morita et al[8]. The secondary endpoint was diurnal variation in the circumference 5 cm under the maximal calf after 5 days. In accordance with an earlier study[8], the maximal region of the left calf circumference and the circumference 5 cm under the maximal region were evaluated using a fiberglass tape measure (Hi Ace, hoechstmass, Germany). Upon measurement, participants placed their heel in the default position while seated and a set square was used to determine if the region below the knee was perpendicular to the floor. Diurnal variation was calculated by subtracting the circumference before test food ingestion from each circumference 2, 4 and 6 hours after ingestion.

The physical examination involved measurements of systolic blood pressure, diastolic blood pressure, pulse rate, body weight, body mass index and percent body fat.

Table 3 Changes from hour-0 in calf circumference in Day-5.

Item	Condition	hour-2	hour-4	hour-6
maximal region	active	0.3 (0.3)	0.5 (0.4)	0.7 (0.3)
	placebo	0.7 (0.3)	1.3 (0.5)	1.7 (0.3)
	p-value	< 0.001**	< 0.001**	< 0.001**
5 cm under maximal region	active	0.3 (0.3)	0.7 (0.3)	0.8 (0.4)
	placebo	0.7 (0.7)	1.1 (0.7)	1.1 (0.7)
	p-value	0.051 †	0.034*	0.091 †

n = 20×2

Data are shown in Mean (SD).

†: $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ between-group differences in change from baseline.

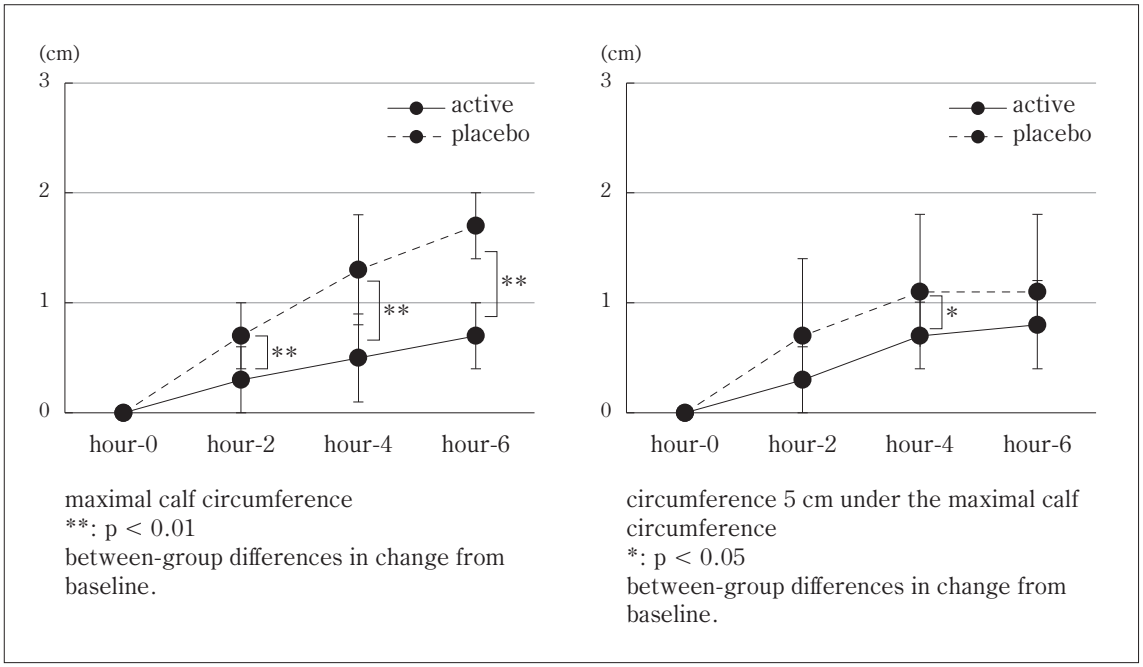

Fig. 2 Mean changes of calf circumferences from hour-0 to hour-6, in Day-5. Error bars represent SD.

Statistical Analysis

The Intention-to-treat principal was adopted in the present study. The sample size was calculated as 15 participants with the following settings: statistical power of 80%, significance level of $p < 0.05$.

We used a paired-t test to assess intragroup change and intergroup comparison at each observation point. The level of significance was set at $p < 0.05$ in the two-sided test. Statcel 3 (Yanai, 2011) was used for statistical analysis.

3. RESULTS

A total of 20 participants took part in this study and all 20 completed the study. The course of study participants is presented in **Fig. 1**, while background information and urinary frequency during the 6 hours of sitting are presented in **Table 2** respectively. Measurement results for the maximal region of calf circumference and the circumference 5 cm under the maximal calf are presented in **Table 3** and **Fig. 2.** No significant difference in urinary frequency was seen between test food and placebo food ingestion times on both days 1 and 5.

The maximal region of calf circumference increased with time, but this increase was significantly suppressed under the test food condition as compared with the placebo food condition (after 2 hours: $p < 0.001$, after 4 hours: $p < 0.001$, after 6 hours: $p < 0.001$). The circumference 5 cm under the maximal calf similarly increased with time, and this increase was smaller under the test food condition than under the placebo food condition; however, the difference between conditions was partly significant (after 2 hours: $p = 0.051$, after 4 hours: $p = 0.034$, after 6 hours: $p = 0.091$).

Adverse Events

Throughout the entire period of the study, no adverse events directly related to test food intake were reported.

4. DISCUSSION

The objective of this study was to verify the effects of functional foods containing red vine leaf extract on lower limb swelling. Five days of ingesting the test food resulted in an increase in calf circumference following 6 hours of sitting, although this increase was suppressed as compared with ingestion of the placebo. On the other hand, the increase in the calf circumference 5 cm under the maximal region from day 1 to day 5 tended to be suppressed after ingesting the test food, although significant partly.

While different results were obtained for the maximal region of calf circumference and the region 5 cm under the maximal calf, the changes in both circumferences shared the same trend, which makes it valid to assume that the results obtained for 5 cm under the maximal region were smaller. The reason for this appears that, statistically, the standard deviation at 5 cm under the maximal region under the placebo condition after 5 days is large. **Table 3** shows that under the placebo condition alone, the standard deviation for the circumference at 5 cm under the maximal region of calf circumference after 5 days is specifically larger, and that this trend is not seen on day 1 or under other measurement conditions. This specific standard deviation for the region at 5 cm under the maximal calf after 5 days under the placebo condition may have reduced the amount of effect between the test food and placebo conditions. However, it is not clear from the study data why this specific standard deviation arose. It could be the effect of the measurer, or the effect of the specific climate on one day under the placebo condition. Alternatively, it could be the effect of factors such as the presence or absence of participant exercise habits, muscle mass, or the shape of the calf muscles on measurement accuracy. Furthermore, no sample size was set in this study, therefore it is impossible to determine if the fact that significant results for the region 5 cm under the maximal calf was obtained partly was due to the fact that the statistical power was insufficient. Further study is thus required.

The present study had several limitations. The first limitation was that no indicator other than calf circumference was measured. In swelling of the lower limbs and its cause, chronic venous insufficiency of the lower limbs, is accompanied by unpleasant symptoms including leg heaviness, aches and cramps and so forth other than swelling. By measuring these symptoms through the use of self-administered questionnaires and interviews, it may become possible to more widely demonstrate the further effects of "*Sururu no Omegumi*". However, the significance probability after 2, 4 and 6 hours of sitting was $p < 0.001$ for maximal region of calf circumference, and partly $p < 0.05$ for the region 5 cm under the maximal region of calf circumference, which suggests that the issue of redundancy does not have a large impact on the interpretation of results.

5. CONCLUSION

In this study, the effectiveness of functional food containing red vine leaf extract on lower limb swelling was verified. The test food appeared to have a significant improving effect on the maximal region of calf circumference.

CONFLICT OF INTEREST

Kazuki Shimazaki is an employee of Fanfare.inc. All remaining authors have declared no conflicts of interest.

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