Original Article 診療と新薬 2017; 54: 29–36

診療と新薬 Web

Efficacy on Breast Skin Tension of the Supplement Containing *Pueraria Mirifica* in Healthy Japanese Females:

A Randomized, Double-blind, Placebo-controlled Study

Masatomo NAJIMA[1] ／ **Akinobu MIYATA**[2] ／ **Hiroyuki SASAGAWA**[3]

● Abstract

Objective: The objective of this study is to examine how the ingestion of food containing *Pueraria mirifica* affects the condition of breast skin tension.
Methods: A randomized, placebo-controlled, double-blind study was conducted to verify breast skin tension. In this study we measured the angle between the inframammary fold (imf) and the highest point of the breast (nipple), tested female's hormones, and also carried out subjective reporting in form of a questionnaire. The blood and urine tests were evaluated for the assessment of product safety as a secondary endpoint.
Results: From all of 113 applicants, 86 were eliminated due to not meeting the inclusion criteria. Among 27 subjects, 14 were withdrawn due to trouble of gauge, and the remaining 13 (Test; 5, Placebo; 8) completed the study. 4（Test; 1, Placebo; 3）were withdrawn from the analysis due to menstruation. Data obtained with 9 subjects (Test; 4, Placebo; 5) was used for the analysis of efficacy. After the 12-week ingestion the test group showed an increasing tendency of the angle of imf and nipple on both breasts, and the angle of both sides of breast showed a significant difference compared to the Placebo. According to the result of the questionnaire, the test group showed a significant difference in tension in the upper breast after 12 weeks, compared to the Placebo. No adverse effects were observed after the ingestion of the test product.
Conclusion: We found out that the ingestion of the food containing *Pueraria mirifica* for 12 weeks resulted in the improvement of tension of upper breast area and the angle between imf and the nipple, which both suggest the improvement of breast shape. In addition, no safety-related matter occurred during the 12-week test period.

Key Words: *Pueraria mirifica*, phytoestrogens, miroestrol, breasts, breast skin tension

1. INTRODUCTION

Breasts are the symbol of femininity. Needless to say, breasts have an important function of breast-feeding, but it can be also said that beautiful breasts are a desire of women and they significantly influence the QOL (quality of life) of women. According to one study report, men tend to look more often and for longer at the breasts, regardless of the waist-to hip ratio of women[1]; considering this result, it may be no exaggeration to say that "the upward breasts with good shape" or "the resilient breasts" are an aspiration of all women. However, approximately 80 percent of Asian women under 50 years old (including Japanese) are said to have "dense breasts" with a large proportion containing the mammary gland[2], and the rest is made up of a small amount of breast tissue. In addition, women's breast potentially suffer from symptoms such as ptosis or thinning of breasts, which are caused by childbirth or aging. The ptosis of breasts causes a bad posture[3], and it also triggers problems such as chronic dermatitis at the lower border of the breasts.

Pueraria mirifica is a Thai plant with a very large tuber. In Thailand, the dried powder of the root has traditionally been used as a folk remedy for menopause-related disorders for centuries[4]. Also, *Pueraria mirifica* contains the phytoestrogens such as flavonoids (daidzin, puerarin, genistein, etc.) or miroestrol[5-7].

It is reported that the female hormone works on the formulation of breasts[8]. Also, another report shows that the phytoestrogen performs an estrogen-like effect once ingested into one's body[9], and this effect is considered to be effective for forming the breasts into better shape. Furthermore, a lot of supplement-foods containing *Pueraria mirifica* are being sold in modern society. However, there are few studies which actually examine how the *Pueraria mirifica* affects the shape of breasts of Japanese women. Therefore, in this study, we conducted a randomized, placebo-controlled, double-blind study on healthy Japanese women by using the food containing

1) JACTA (Japan Clinical Trial Association)
2) Nihonbashi M's Clinic
3) Hoshi corporation

Pueraria mirifica, to verify the efficacy of the *Pueraria mirifica* for the shape of breasts. Simultaneously, we also examined the safeness of ingesting the food.

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from Hoshi corporation (Tokyo) at two centers (OZ clinic, Tokyo and JACTA, Tokyo). The study period was 12 weeks, from April 11th to July 5th, 2016. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo), recruited in March, 2016.

2.2.1. Inclusion criteria

(1) Healthy females aged between 25 and 45 years;
(2) Premenopausal females;
(3) Individuals with Japanese bra cup size A, B, or C;
(4) Non-smoker.

2.2.2. Exclusion criteria

(1) Individuals undergoing treatment of chronic diseases such as atrial fibrillation, arrhythmia, rheumatism, diabetes, high blood pressure, lipid metabolism, and diseases of the liver, kidney, central nervous system, and circulatory system;
(2) Individuals who have experienced augmentation mammoplasty;
(3) Individuals on medication that may affect hormone secretion, including herbal medicines;
(4) Individuals with a pollen or food allergy;
(5) Individuals who are pregnant, nursing, or likely to become pregnant during the trial;
(6) Individuals judged to be ill by the principle investigator.

2.2.3. Efficacy eligibility

With respect to the analysis of efficacy, we set the following criteria of exclusion:
(1) Individuals who were or started menstruating within 5 days prior to the test day;
(2) Individuals who consumed less than 80% of the expected dose;
(3) Individuals without adequate records;
(4) Individuals who fell under the exclusion criteria after enrollment;
(5) Individuals who had justifiable reason for exclusion.

2.3. Randomization

From all of 113 applicants, 86 were eliminated according to exclusion criteria. The inclusion criteria was judged by the principle investigator. All subjects were sequentially allocated to group A (n = 13) and group B (n = 14) using a random number table. In the process of subject assignment, back ground factors such as age and bra cup size were taken into consideration to avoid biased distribution. Subjects in Group A ingested the test sample, and subjects in Group B ingested the placebo for 12 weeks.

2.4. Description of test foods and blinding

The test product was prepared by Hoshi corporation. The amount of daily intake was 4 capsules (1 capsule weighs 230 mg, therefore 4 capsules weigh 920 mg). The composition of the test product was *Pueraria mirifica*, collagen, hyaluronan etc., while the placebo product was mainly consisted of indigestible dextrin and did not include *Pueraria mirifica*. Both tablets were indistinguishable in shape, color, or taste, and were managed by an identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 4 capsules of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of their physical condition and lifestyle factors such as amount of meals, exercise, and sleep for the day during the test period.

2.5.2. Outcome

The objective of this study was to verify breast skin tension by ingesting food containing *Pueraria mirifica*. For this purpose, we measured the angle between the inframammary fold (imf) and the highest point of the breast (nipple), tested female's hormones, and had them report their experience subjectively. Moreover, the blood and urine test was evaluated for the assessment of product safety as a secondary endpoint. Furthermore adverse events were collected by means of a written questionnaire during the study.

According to the schedule shown in **Table 1**, we measured parameters on efficacy and safety. These assessments were conducted upon pre-intervention and post-intervention.

2.5.2.1. Angle between imf and nipple

The investigator took 3D photos[10] of the breast using 3D sccanner (Artec Europe S.a.r.l) and measured the angle between imf and nipple by 3D photos (**Appendix 1**).

2.5.2.2. Female hormones

To evaluate female's hormones, DHEA-S, Progesteron, and Estradiol in the plasma was measured.

2.5.2.3. Subjective reporting

Further, subjective reporting was observed as the

Table 1　Schedule for the study

Item \ Term	Screening	Pretrial test	Test period 12 w
Informed consent	●		
Selection and/or allocation	●		
Angle between imf and nipple		●	●
Female hormone		●	●
Subjective reporting		●	●
Blood and urine test		●	●
Ingestion of test foods			↔
Log			↔

● : Implementation
↔ : Daily practice during the test period

primary outcome. The questionnaire (3 items) was designed by the conducting doctor. Responses to each question were rated on an ordinal scale of 0 to 4, with higher scores indicating a better result.

2.5.2.4. Blood and urine test

Blood biochemical parameters and urine parameters were recorded to evaluate the safety of the test food as a secondary outcome.

2.6. Data analysis

A per protocol set was adopted in the present study and no sample size design was used. All statistics were expressed as mean ± standard deviation (SD). For the angle between imf and the nipple, female's hormones, subjective repotting, and biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using Wilcoxon signed-rank test. Mann-Whitney U test was used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject backgrounds between groups. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis.

Statistical analyses were performed using Statcel 4 (Yanai, 2015). The results were considered significant at a ＜5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 27 subjects were randomly assigned to intervention groups and made a start with ingestion. 14 were withdrawn due to trouble of gauge, and the remaining 13 (Test; 5, Placebo; 8) completed the study. 4（Test; 1, Placebo; 3）were out of analysis due to the time point of the menstruation. Thus, data obtained with 9 subjects (Test; 4, Placebo; 5) was used for the analysis of efficacy (**Fig. 1**). There were no significant differences in, weight, and bra cup size between groups (**Table 2**).

3.2. Angle between imf and nipple

Table 3 shows the results of test analyses. Each side angle tended to increase by week 12 in Test, moreover each side angle of Test increased more significantly than Placebo in the comparison of ⊿ 0-12 w.

3.3 Female's hormones

The results of female's hormones are shown in **Table 4**. Progesterone and Estradiol tended to decrease at week 12 in Test, whereas no significant difference was found in the intergroup analysis.

3.4 Questionnaire analyses

The result of subjective condition assessments are shown in **Table 5**. Question #3 (Do you feel any tension in the upper breast part?) showed a significant difference in the between-group comparison of ⊿ 0-12 w.

3.5. Blood and urine test

Table 6 show the blood biochemical and urine parameters. With respect to the blood biochemical and urine test, no significant difference was observed in Test after 12 weeks of ingestion. In Placebo, γ-GT and chloride showed significant difference. However, the principle investigator judged it as the range of physiological variation (or clinically safe).

3.6. Adverse event

No adverse effects associated with the test product were observed in the course of the reporting.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of the food containing *Pueraria mirifica*. The objective of this study is to verify whether the ingestion of the food containing *Pueraria mirifica* affects the condition of female breast skin tension. As the primary outcome, after the 12-week ingestion the test group showed an increasing tendency of the angle of imf and nipple on both breasts, and the angle of the left side of the breast showed a significant difference compared to the Placebo. In addition, according to the result of the questionnaire, the test group showed a significant difference in the item "Do you feel any tension in the upper breast part?" after 12 weeks, compared to the Placebo. On the other hand, no change was observed in the hormone level among the

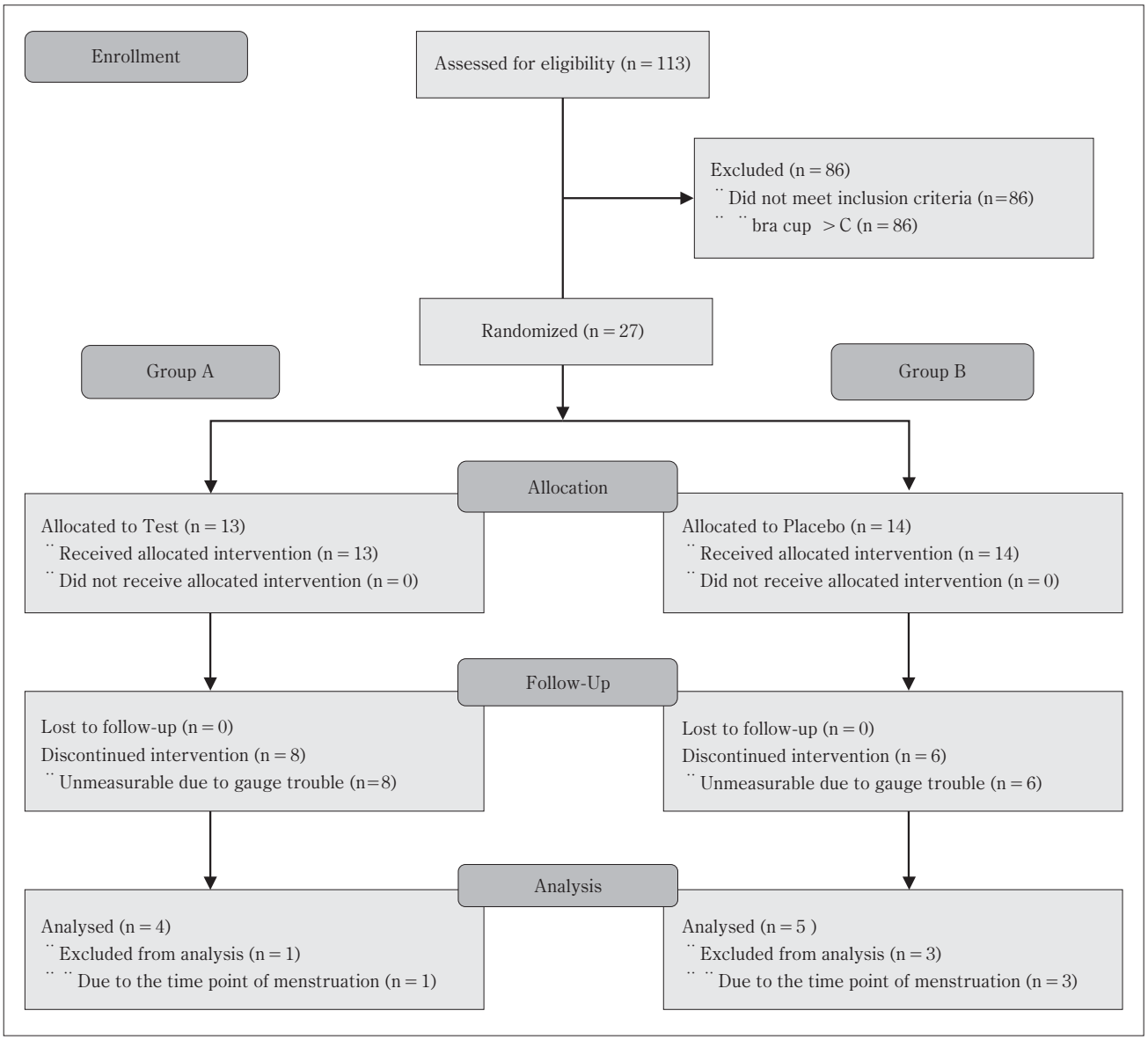

Fig. 1 Flow diagram of subject disposition

test group. In addition, as the secondary outcome, the safety of ingesting the test product for 12 weeks was suggested by the results of blood test, urine test, diary, and medical interview during the test period.

Main Findings

This study showed a significant difference in the tension in the upper breast part as a result of the ingestion of the test product containing *Pueraria mirifica*. Also, regarding the angle of imf and nipple, both sides of the breasts showed a significant improvement compared to the Placebo, and the within-group data also showed an improvement tendency. On the other hand, the female and male hormone level did not show any significant change.

Women's breast consist of eight tissues: rib, pectoralis major muscle, Cooper's ligament, mammary gland, adipose tissue, lactiferous duct, papilla and dermis. The breasts themselves consist of the mammary gland and adipose tissue, and these tissues are distributed in the breasts, surrounded by the connective tissue called Cooper's ligament; all of them are connected to the foundation consisting of ribs and the pectoralis major muscle [11)12)]. The composition ratio between mammary gland and adipose tissue is regarded as 1: 1, based upon

Table 2 Subject demographics

Item	Unit	Test	Placebo
Subjects	numbers	4	5
Bra cup *	numbers	B; 2, C; 2	B; 4, C; 1
Weight *	kg	53.0 ± 2.0	54.0 ± 3.5

mean ± SD

* No significant difference

Table 3　Results of angle between imf and nipple

Item	Time points	Values		P-value[2)]
		Test (n=4)[1)]	Placebo (n=5)[1)]	
Angle between imf and nipple (right)	Baseline	61.10 ± 5.85	60.10 ± 6.88	
	12-week	64.48 ± 4.92 †	58.24 ± 9.81	
	⊿ 0-12w	3.38 ± 2.12	− 1.86 ± 3.17	0.014 #
Angle between imf and nipple (left)	Baseline	60.00 ± 6.61	54.74 ± 17.71	
	12-week	63.40 ± 4.91 †	53.00 ± 20.90	
	⊿ 0-12w	3.40 ± 1.83	− 1.74 ± 4.13	0.037 #

Values are expressed as the mean ± SD.
1) † $p < 0.1$ against baseline.
2) # $p < 0.05$ between-group difference in change from baseline.

Table 4　Results of female hormone

Item	Unit	Time points	Values		P-value[2)]
			Test (n = 4)[1)]	Placebo (n = 5)[1)]	
DHEA-S	μg/dL	Baseline	176.3 ± 33.6	254.2 ± 128.2	
		12-week	149.3 ± 44.1	216.0 ± 119.3	
		⊿ 0-12 w	− 27.0 ± 34.4	− 38.2 ± 63.4	0.624
Progesteron	ng/mL	Baseline	9.33 ± 8.21	7.90 ± 5.28	
		12-week	2.70 ± 4.67 †	5.92 ± 7.75	
		⊿ 0-12 w	− 6.63 ± 5.21	− 1.98 ± 5.98	0.221
Estradiol	pg/mL	Baseline	390.3 ± 412.6	176.2 ± 117.3	
		12-week	195.5 ± 151.2 †	134.0 ± 87.3	
		⊿ 0-12 w	− 194.8 ± 262.9	− 42.2 ± 132.9	0.142

Values are expressed as the mean ± SD.
1) † $p < 0.1$ against baseline.
2) between-group difference in change from baseline.

Table 5　Results of questionnaire analyses

Item		Time points	Scores		P-value[2)]
			Test (n = 4)[1)]	Placebo (n = 5)[1)]	
1	Do your breasts feel perky?	Baseline	0.3 ± 0.5	0.2 ± 0.4	
		12-week	1.3 ± 0.5	0.4 ± 0.5	
		⊿ 0-12 w	1.0 ± 0.8	0.2 ± 0.4	0.142
2	Can you feel the suppleness increasing?	Baseline	0.3 ± 0.5	0.2 ± 0.4	
		12-week	1.3 ± 0.5	0.6 ± 0.5	
		⊿ 0-12 w	1.0 ± 0.8	0.4 ± 0.5	0.270
3	Do you feel any tension in the upper breast part?	Baseline	0.0 ± 0.0	0.2 ± 0.4	
		12-week	1.5 ± 0.6 †	0.4 ± 0.5	
		⊿ 0-12 w	1.5 ± 0.6	0.2 ± 0.4	0.027#

Scores are expressed as the mean ± SD.
1) † $p < 0.1$ against baseline.
2) # $p < 0.05$ between-group difference in change from baseline.

Table 6 Changes in biochemical blood and urine test

Item	Unit	Std. Value	Time point	Values[1)2)]	
				Test（n＝4）	Placebo（n＝5）
Total Bilirubin	mg/dL	0.2–1.2	Baseline	0.48 ± 0.13	0.70 ± 0.48
			12–week	0.40 ± 0.14	0.54 ± 0.33
			⊿ 0–12 w	− 0.08 ± 0.21	− 0.16 ± 0.19
Total Protein	g/dl	6.5–8.3	Baseline	7.5 ± 0.3	7.5 ± 0.1
			12–week	7.4 ± 0.2	7.3 ± 0.2
			⊿ 0–12 w	− 0.1 ± 0.3	− 0.2 ± 0.3
Albumen	ratio	3.8–5.3	Baseline	4.6 ± 0.1	4.7 ± 0.2
			12–week	4.4 ± 0.1	4.5 ± 0.4
			⊿ 0–12 w	− 0.2 ± 0.2	− 0.2 ± 0.3
AST（GOT）	U/L	8–38	Baseline	18.8 ± 2.1	29.8 ± 26.6
			12–week	20.3 ± 5.9	21.4 ± 6.1
			⊿ 0–12 w	1.5 ± 5.8	− 8.4 ± 23.0
ALT（GPT）	U/L	4–43	Baseline	12.3 ± 2.1	21.4 ± 15.8
			12–week	13.3 ± 3.3	16.0 ± 6.9
			⊿ 0–12 w	1.0 ± 3.6	− 5.4 ± 12.3
ALP	U/L	110–354	Baseline	162.5 ± 35.6	158.8 ± 36.5
			12–week	176.3 ± 51.1	142.6 ± 27.3 †
			⊿ 0–12 w	13.8 ± 32.7	− 16.2 ± 16.1 ‡
LD（LDH）	U/L	121–245	Baseline	171.0 ± 30.0	184.0 ± 48.0
			12–week	180.0 ± 35.8	176.8 ± 29.8
			⊿ 0–12 w	9.0 ± 12.1	− 7.2 ± 28.0
γ-GT（γ GTP）	U/L	48 and under	Baseline	20.5 ± 2.6	39.2 ± 28.2
			12–week	18.0 ± 2.6	27.8 ± 19.1 *
			⊿ 0–12 w	− 2.5 ± 3.7	− 11.4 ± 9.9
CK（CPK）	U/L	30–172	Baseline	100.5 ± 34.8	95.8 ± 33.4
			12–week	97.8 ± 37.7	144.8 ± 105.0
			⊿ 0–12 w	− 2.8 ± 9.5	49.0 ± 113.8
Total Cholesterol	mg/dL	130–219	Baseline	201.0 ± 19.3	211.4 ± 27.7
			12–week	193.5 ± 21.7	206.2 ± 35.0
			⊿ 0–12 w	− 7.5 ± 16.5	− 5.2 ± 30.4
Neutral Fat（TG）	mg/dL	30–149	Baseline	57.8 ± 10.1	101.8 ± 85.3
			12–week	74.8 ± 21.4	116.8 ± 72.0
			⊿ 0–12 w	17.0 ± 28.5	15.0 ± 81.2
Sodium	mEq/L	135–150	Baseline	140.8 ± 2.1	140.4 ± 1.1
			12–week	142.5 ± 1.0	142.2 ± 1.1 †
			⊿ 0–12 w	1.8 ± 1.5	1.8 ± 1.3
Chlorid	mEq/L	98–110	Baseline	104.8 ± 1.5	103.0 ± 2.0
			12–week	106.0 ± 2.7	105.4 ± 2.7*
			⊿ 0–12 w	1.3 ± 2.5	2.4 ± 1.3
Potassium	mEq/L	3.5–5.3	Baseline	4.2 ± 0.1	4.3 ± 0.3
			12–week	3.8 ± 0.2 †	4.0 ± 0.3
			⊿ 0–12 w	− 0.3 ± 0.3	− 0.3 ± 0.5
Calcium	mg/dL	8.4–10.2	Baseline	9.5 ± 0.2	9.6 ± 0.4
			12–week	9.3 ± 0.2	9.4 ± 0.2
			⊿ 0–12 w	− 0.2 ± 0.4	− 0.2 ± 0.5
Inorganic Phosphorus	mg/dL	2.5–4.5	Baseline	3.6 ± 0.2	3.7 ± 0.7
			12–week	4.1 ± 0.3	3.4 ± 0.6
			⊿ 0–12 w	0.5 ± 0.5	− 0.3 ± 0.7
Urea Nitrogen	mg/dL	8.0–22.0	Baseline	12.5 ± 1.1	14.6 ± 2.2
			12–week	12.6 ± 1.0	14.1 ± 2.4
			⊿ 0–12 w	0.2 ± 0.3	− 0.5 ± 2.7
Creatinine	mg/dL	0.47–0.79	Baseline	0.57 ± 0.04	0.68 ± 0.06
			12–week	0.61 ± 0.04	0.69 ± 0.09
			⊿ 0–12 w	0.04 ± 0.03	0.01 ± 0.05
Blood Sugar（Serum）	mg/dL	60–109	Baseline	76.5 ± 18.1	86.6 ± 26.4
			12–week	69.0 ± 11.0	72.8 ± 8.0
			⊿ 0–12 w	− 7.5 ± 14.3	− 13.8 ± 21.4
Specific Gravity	—	1.010–1.025	Baseline	1.018 ± 0.012	1.015 ± 0.004
			12–week	1.016 ± 0.011	1.017 ± 0.010
			⊿ 0–12 w	− 0.001 ± 0.005	0.002 ± 0.012
pH	—	4.5–8.0	Baseline	6.0 ± 0.4	6.1 ± 1.1
			12–week	6.9 ± 0.8	6.5 ± 0.8
			⊿ 0–12 w	0.9 ± 0.6	0.4 ± 1.1

Values are expressed as the mean ± SD.
1) † $p < 0.1$, * $p < 0.05$ against baseline.
2) ‡ $p < 0.1$ between-group differences in change from baseline.

the estimated data from mammography[13]. However, this ratio of adipose tissue decreases with advancing age[14] or enlargement of breast size[15], and the condition of breast changes from "breasts with tension" to "shriveled and pendulous breasts". The laxity of Cooper's ligament holding the breasts is also thought to be the cause of ptosis. It is believed that the mammary gland development occurs mainly before the lactation stage[16], and involves a complex process regulated by various steroid and polypeptide hormones such as estrogen, progesterone, or prolactin[17)18)]. There is also a study which reports that the administration of estrogen or progesterone triggers the development of mammary gland or blood vessel in mice[19]. Furthermore, it is reported that estrogen increases the expression of FGF (the fibroblast growth factors) 7 and FGF10 (which stimulate the cell growth or cell differentiation), whereas prolactin increases the expression of FGF7 significantly in pregnancy and lactation; however, on the other hand, prolactin and progesterone reduce the expression of FGF10 significantly in a virgin[20].

Pueraria mirifica contains, as its active ingredient, five major isoflavonoids (puerarin, daidzin, genistin, daidzein, and genistein), miroestrol and deoxymiroestrol. Deoxymiroestrol easily changes to miroestrol when exposed to the oxygen in the air[21]. These ingredients are phytoestrogens which mimic the biological activity of the female hormone estrogen[22] and are thought of as a causal substance of the function of *Pueraria mirifica*[23]. The phytoestrogen contained in *Pueraria mirifica* performs an estrogen-like effect internally[24]. It is reasonable to speculate that this effect activated the expression of FGF and accelerated the development of the mammary gland, in conclusion contributed to the improvement of the tension of upper breast part and the angle of imf and the nipple. In addition, since estrogen has a function of supporting the collagen production in the dermic layer[25], it is considered that the pectoralis major muscle has been strengthened and the dermis enfolding the breasts has been fixed by the collagen production, which eventually led to the improvement of the tension of the upper breast area. Furthermore, the fact that the values of estradiol (estrogen), progesterone and DHEA-S did not change during the test supports the hypothesis that the test results were not produced from hormones secreted inside the body, but were yielded in consequence of the ingestion of the test product.

Secondary Findings

In this study, it was observed that based upon clinical findings such as the blood and urine test, no abnormal change was triggered by ingestion of the test product. Although γ-GT and chloride showed significant a difference in Placebo, the difference was within range of the baseline, and the investigator judged it as the range of physiological variation (or clinically safe). In addition, the test was interrupted due to trouble of gauge and menstruation. The former interruption was caused by the gauge trouble during the test which disabled the measurement of part of test subjects and forced us to use only the data of subjects who had completed the measurement before the gauge trouble. The latter problem was related to the criteria 2.2.3 (Individuals who were or started menstruating within 5 days prior to the test day). Both had no harmful influence against biochemical and/or physiological matters of the test subjects which seemed to have a causal relationship with the test product.

These results indicate the safety of the ingestion of the test product for the 12-week test period.

General Information

The ptosis or the loss of tension are generally seen in the breasts of many women as a result of childbirth or aging. The deterioration of the shape of breasts not only leads to visual change, it also triggers many problems such as the degradation of QOL, difficulty in breast-feeding, bad posture, shoulder stiffness[3], or dermatitis due to the friction on the lower border of the breasts. Cosmetic surgery such as lifting or implanting is often used in order to arrange the shape of breasts. However, it involves high expense, scars on breasts, and reportedly has risks of side-effects such as blood circulation disorder or cutaneous necrosis of the breasts[26)27)]. Therefore, if it is possible to improve ptosis or the loss of tension by the ingestion of a plant-derived food, without undergoing any surgery, it should be quite beneficial for women troubled by the conditions of their breasts in terms of both physical (such as improvement of posture, shoulder stiffness or dermatitis) and mental aspects (improvement of QOL).

Limitations

For women, the menstrual cycle significantly affects the hemodynamic status of body and the condition of nerves[28]. Therefore, it is reasonable to say that the shape, tension/suppleness or tightness of breasts are greatly influenced by this cycle. In this study, we excluded any individuals who were or started menstruating within 5 days prior to the test day in order to be clear of the effects caused by menstruation. However, it is undeniable that the physical condition varies widely from person to person depending upon the timing, since human body changes on a daily basis just like the phases of the moon. In addition, due to the trouble of gauge during the test, a number of test subjects had to withdraw from the test during the process, and as a result of this trouble the number of test subjects who completed the entire test process became lower than originally expected. Since the test items significantly involve the individual variability, it is desirable to scrutinize the matter further with a larger test sample.

5. CONCLUSION

In conclusion, we found out that the ingestion of the food containing *Pueraria mirifica* for 12 weeks resulted in the improvement of the tension of upper breast part and the angle of imf and nipple, which both suggest the improvement of breast shape. In addition, no safety-related matter occurred during the 12-week test period.

CONFLICT OF INTEREST

All parts of this study were funded by Hoshi corporation. Hiroyuki Sasagawa is a director. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

REFERENCES

1) Dixson BJ, Grimshaw GM, Linklater WL, et al. Eye-tracking of men's preferences for waist-to-hip ratio and breast size of women. Arch Sex Behav. 2011; **40**: 43-50.
2) Kolb TM, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. Radiology. 2002; **225**: 165-75.
3) Yamaguchi M, Tsutsui H. Investigation of the effects of changes of the Breast's Position in the Function of the Shoulder Complex. Katakansetsu. 2000-2001; **25**: 475-7.
4) FDA.Dockets. Premarket Notification for Pueraria candollei var. mirifica root extract as a New Dietary Ingredient.
5) Cherdshewasart W, Subtang S, Dahlan W. Major isoflavonoid contents of the phytoestrogen rich-herb Pueraria mirifica in comparison with Pueraria lobata. J Pharm Biomed Anal. 2007; **43**: 428-34.
6) Schoeller W, Dohrn M, Hohlweg W. Über eine oestrogene Substanz aus der Knolle der siamesischen Schlingpflanze Butea superba. Naturwissenschaften. 1940; **28**; 532.
7) Okada Y. Potentialrisks and benefits of phytoestrogen in foods. Bull. Aichi Pref. Univ. School . Bull. Aichi Pref. Univ. School of Nurs&Health. **21**; 1-10: 2015.
8) Eriksson N, Benton GM, Do CB, et al. Genetic variants associated with breast size also influence breast cancer risk. BMC Med Genet. 2012; **13**: 53.
9) Franco OH, Chowdhury R, Troup J, et al. Use of Plant-Based Therapies and Menopausal Symptoms: A Systematic Review and Meta-analysis. JAMA. 2016; **315**: 2554-63.
10) Chen D, Chittajallu DR, Passalis G, et al. Computational tools for quantitative breast morphometry based on 3D scans. Ann Biomed Eng. 2010; **38**: 1703-18.
11) AP Cooper. On the anatomy of the breast. Longman, Orme, Green, Brown, and Longmans, London. 1840
12) Ramsay DT, Kent JC, Hartmann RA, et al. Anatomy of the lactating human breast redefined with ultrasound imaging. J Anat. 2005; **206**: 525-34.
13) Geddes DT. Anatomy of the human period of lactation breast. PERINATAL CARE. 2007; **26**; 446-455.
14) Jamal N, Ng KH, McLean D, et al. Mammographic Breast Glandularity in Malaysian Women: Data Derived from Radiography. AJR Am J Roentgenol. 2004; **182**: 713-7.
15) Cruz-Korchin N, Korchin L, González-Keelan C, et al. Macromastia: how much of it is fat? Plast Reconstr Surg. 2002; **109**: 64-8.
16) Russo J, Russo IH. Development of the human breast. Maturitas. 2004; **49**: 2-15.
17) Hovey RC, Trott JF, Vonderhaar BK. Establishing a framework for the functional mammary gland: from endocrinology to morphology. J Mammary Gland Biol Neoplasia. 2002; **7**: 17-38.
18) Oka T, Yoshimura M, Lavandero S. Control of growth and differentiation of the mammary gland by growth factors. Journal of Dairy Science. 1991; **74**: 2788-800.
19) Matsumoto M, Nishinakagawa H, Kurohmaru M, et al. Effects of estrogen and progesterone on the development of the mammary gland and the associated blood vessels in ovariectomized mice. J Vet Med Sci. 1992; **54**: 1117-24.
20) Cui Y, Li Q. Effect of mammogenic hormones on the expression of FGF7, FGF10 and their receptor in mouse mammary gland. Sci China C Life Sci. 2008; **51**: 711-7.
21) Chansakaow S, Ishikawa T, Seki H, et al. Identification of deoxymiroestrol as the actual rejuvenating principle of "Kwao Keur", Pueraria mirifica. The known miroestrol may be an artifact. J Nat Prod. 2000; **63**; 173-5.
22) Yildiz F. Phytoestrogens in functional foods. Taylor and Francis. Limited. 2005; p.3-5, p.210-211.
23) CAIN JC. Miroestrol: an oestrogen from the plant Pueraria mirifica. Nature. 1960; **188**: 774-7.
24) Hajirahimkhan A, Dietz BM, Bolton JL. Botanical modulation of menopausal symptoms: mechanisms of action? Planta Med. 2013; **79**: 538-53.
25) Takahashi N, Iwama M, Matsumoto H, et al. Effect of Vitamin E Deficiency and/or Oral Female Sex Hormones on Aortic Connective Tissue Components in Female Rat. Food Hygiene and Safety Science. 1990; **31**: 409-13.
26) Farace F, Faenza M, Bulla A, et al. s mammary reconstruction with the anatomical Becker expander a simple procedure? Complications and hidden problems leading to secondary surgical procedures: a follow-up study. J Plast Reconstr Aesthet Surg. 2013; **66**: 741-6.
27) Fryzek JP, Signorello LB, Hakelius L, et al. Local complications and sukbsequent symptom reporting among women with cosmetic breast implants. Plast Reconstr Surg. 2001; **107**: 214-21.
28) Gordon JL, Girdler SS, et al. Mechanisms underlying hemodynamic and neuroendocrine stress reactivity at different phases of the menstrual cycle. Psychophysiology. 2014; **51**: 309-18.

Appendix 1. Angle between the inframammary fold (imf) and the highest point of the breast (nipple)