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Efficacy on Menopausal Discomfort of the Supplement Containing *Pueraria Mirifica* in Healthy Japanese Females:

A Randomized, Double-blind, Placebo-controlled Study

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● Abstract

Objective: The objective of this study is to verify the improvement of women's menopausal discomfort by ingesting food containing *Pueraria mirifica*.
Methods: A randomized, placebo-controlled, double-blind study was conducted to evaluate various conditions related to menopausal discomfort. The study used the method of subjective reporting (questionnaire) for evaluating the above conditions.
Results: From all of 89 applicants, 63 were eliminated due to not meeting the inclusion criteria. Among 26 subjects, 1 was withdrawn due to personal reasons and the remaining 25 subjects completed the study (Test; 13, Placebo; 12). After 12 weeks of ingestion, the study showed significant difference in the items related to night sweating, difficulty sleeping and unstable feeling. These results suggest that the ingestion of the test product contributed to improving parts of symptoms concerning menopausal discomfort. No adverse effects were observed after the ingestion of the test product.
Conclusion: We found out that the ingestion of the test product containing Pueraria mirifica for 12 weeks contributed to the improvement of symptoms of menopausal discomfort (night sweating, urination, discomfort of skin, unstable feeling, and loss of interest in sex). In addition, no safety-related matter occurred during the 12-week test period.

Key Words: *Pueraria mirifica*, menopause, climacteric age, menopausal discomfort, estrogen

1. INTRODUCTION

Generally, "climacteric age" refers to the 5 years before and 5 years after menopause in the life cycle of women. Since the median age of menopause of Japanese women is around 50 years old, the age-group between 45 and 55 years old is described as the climacteric age. During the climacteric age, about 80% of women suffer from symptoms such as emotional stress, hot flushes, palpitations, or perspiration (sweating) due to a decrease of estrogen, a type of female hormone. Among these symptoms (menopausal discomfort), the clinical conditions that hinder women's our daily lives are defined as "postmenopausal syndrome"[1]. *Pueraria mirifica* is a Thai plant with a very large tuber. The dried powder of the root has traditionally been used as a folk remedy for menopause-related disorders for centuries[2]. Also, *Pueraria mirifica* contains the flavonoids such as daidzin, puerarin or genistein[3] and the phytoestrogens such as miroestrol[4].

1) JACTA (Japan Clinical Trial Association)
2) OZ Clinic
3) Hoshi corporation

The decrease in the amount of estrogen with aging is regarded as one of the causes of menopausal discomfort. It is reported that the phytoestrogen shows an estrogen-like effect when ingested internally[5], therefore it is considered to be effective for menopausal discomfort. There is also a report that verifies the efficacy of the phytoestrogen for menopausal discomfort[6]. Furthermore, a lot of supplement-foods containing *Pueraria mirifica* are being sold recently. However, there are few studies which actually examine how the ingestion of *Pueraria mirifica* affects the condition of menopausal discomfort of Japanese people. Therefore in this study, we conducted a randomized, placebo-controlled, double-blind study to verify the efficacy of *Pueraria mirifica* for menopausal discomfort of healthy Japanese and its safeness.

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 JACTA (Tokyo). The study period was 12 weeks, from April 8th, to July 1st, 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 by the person in charge of allocation and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subjects

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 from February through March, 2016.

2.2.1. Inclusion criteria

(1) Healthy females aged between 45 and 55 years;
(2) Individuals concerned by menopausal discomfort;
(3) 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 on medication, including herbal medicines;
(3) Individuals with a pollen or food allergy;
(4) Individuals who are pregnant, nursing, or likely to become pregnant during the trial;
(5) Individuals judged to be ill by the principle investigator.

2.3. Randomization

From all of 89 applicants, 63 were eliminated according to the 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＝13) using a random number table. In the process of subject assignment, background factors such as age were taken into consideration to avoid biased distribution. Subjects in Group A ingested the placebo, and subjects in Group B ingested the test sample 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 P*ueraria 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 lifestyle factors such as amount of food, exercise, and sleep for the day during the test period.

2.5.2. Outcome

The objective of this study was to verify the improvement of women's menopausal discomfort by ingesting food containing *Pueraria mirifica*. To evaluate this objective, subjective reporting of menopausal discomfort was observed as the primary outcome. The questionnaire (45 items) is designed by the conducting doctor. The details are illustrated in **Appendix 1.**

To evaluate the safety of the test foods, 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.

2.6. Data analysis

A full analysis set was adopted in the present study and no sample size design was used. All statistics were expressed as mean ± standard deviation (SD).

With respect to scores of subjective reporting, changes from baseline in the same group were assessed using the paired t-test. Student's t-test was used for intergroup comparisons of changes from 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 the ＜5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 26 subjects were randomly assigned to intervention groups and made a start with ingestion. 1 was withdrawn due to personal reasons, and the remaining 25 subjects completed the study. Thus, data obtained with 25 subjects (Test; 13, Placebo; 12) was used for the analysis of efficacy (**Fig. 1**). There was no significant difference in age between groups (**Table 2**).

3.2. Subjective condition assessments

The result of subjective condition assessments are shown in **Table 3**. The intergroup comparison of 6 weeks illustrated a significant difference regarding questions; #35 (feeling tingly skin), #38 (feeling virginal dryness), #41 (feeling sexual disinterest), #42 (uncontrolled urination), #43 (frequent urination), and #45 (anxious about urination). In addition, in the between-group comparison there was a significant difference after 12-weeks of ingestion, in #3 (sudden sweating at night), #8 (nervousness), #9 (feeling isolated), #12 (feeling unstable), #13 (weeping), #14 (sleeping light), #15 (difficulty falling asleep), #29 (becoming cold waist), #35

Table 1　Schedule for the study.

Item \ Term	Screening	Pretrial test	Test period	
			6 w	12 w
Informed consent	●			
Selection and/or allocation	●			
Subjective reporting		●	●	●
Ingestion of test foods			↔	↔
Log			↔	↔

● : Implementation
↔ : Daily practice during the test period

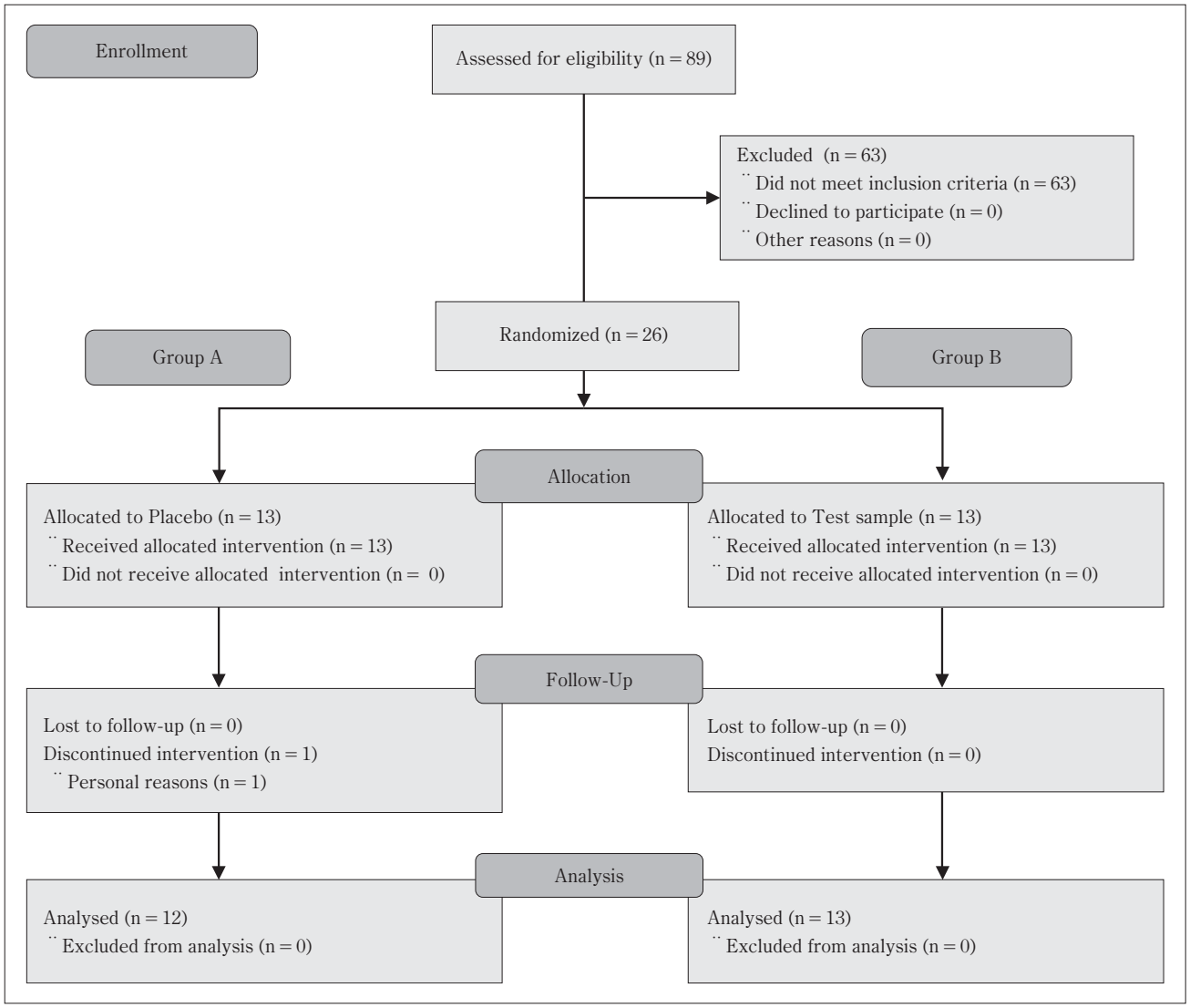

Fig. 1　Flow diagram of subject disposition

(feeling tingly skin), #41 (feeling sexual disinterest), #42 (uncontrolled urination), and #45 (anxious about urination).

3.3. Safety

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

Table 2　Subject demographics

Item	Unit	Test	Placebo
Subjects	numbers	13	12
Age*	years	50.9 ± 2.4	51.8 ± 2.5

mean ± SD
* No significant difference

Table 3 Results of questionnaire analyses (1)

No.	Time point	Scores		P-value[2)]
		Test (n = 13)[1)]	Placebo (n = 12)[1)]	
#1	Baseline	2.5 ± 1.4	2.2 ± 1.2	
	6-week	1.4 ± 1.2*	2.0 ± 1.4	
	⊿ 0-6 w	− 1.1 ± 1.3	− 0.2 ± 1.3	0.092‡
	12-week	1.5 ± 1.5*	1.3 ± 1.2†	
	⊿ 0-12 w	− 1.0 ± 1.3	− 0.9 ± 1.4	0.880
#2	Baseline	2.1 ± 1.6	2.3 ± 1.6	
	6-week	1.4 ± 1.4	2.0 ± 1.3	
	⊿ 0-6 w	− 0.7 ± 1.7	− 0.3 ± 1.0	0.521
	12-week	1.5 ± 1.5	2.4 ± 1.4	
	⊿ 0-12 w	− 0.6 ± 1.3	0.1 ± 0.7	0.114
#3	Baseline	1.9 ± 1.6	1.3 ± 1.5	
	6-week	0.8 ± 1.2†	1.2 ± 1.3	
	⊿ 0-6 w	− 1.2 ± 2.0	− 0.2 ± 0.7	0.126
	12-week	0.6 ± 1.3*	1.5 ± 1.4	
	⊿ 0-12 w	− 1.3 ± 1.6	0.2 ± 0.6	0.006‡‡
#4	Baseline	1.1 ± 1.0	1.8 ± 1.3	
	6-week	0.5 ± 0.8†	1.3 ± 1.2*	
	⊿ 0-6 w	− 0.5 ± 1.1	− 0.5 ± 0.7	0.915
	12-week	0.8 ± 1.2	1.8 ± 1.2	
	⊿ 0-12 w	− 0.2 ± 1.4	− 0.1 ± 0.3	0.717
#5	Baseline	1.2 ± 1.2	1.2 ± 1.3	
	6-week	0.9 ± 1.2	1.1 ± 1.1	
	⊿ 0-6 w	− 0.2 ± 1.0	− 0.1 ± 0.5	0.655
	12-week	1.1 ± 1.0	1.3 ± 1.3	
	⊿ 0-12 w	− 0.1 ± 1.0	0.1 ± 0.3	0.611
#6	Baseline	0.5 ± 1.2	1.3 ± 1.3	
	6-week	0.4 ± 1.0	1.6 ± 1.4	
	⊿ 0-6 w	− 0.2 ± 1.3	0.3 ± 0.9	0.386
	12-week	0.3 ± 0.9	1.7 ± 1.4*	
	⊿ 0-12 w	− 0.2 ± 1.5	0.3 ± 0.5	0.222
#7	Baseline	2.1 ± 1.2	2.3 ± 1.2	
	6-week	1.9 ± 1.3	2.2 ± 1.1	
	⊿ 0-6 w	− 0.2 ± 1.2	− 0.1 ± 1.0	0.876
	12-week	2.0 ± 1.0	2.3 ± 1.0	
	⊿ 0-12 w	− 0.1 ± 1.0	0.0 ± 0.7	0.825
#8	Baseline	2.8 ± 1.2	1.8 ± 1.4	
	6-week	1.8 ± 1.3*	1.8 ± 1.4	
	⊿ 0-6 w	− 0.9 ± 1.3	0.1 ± 1.4	0.075‡
	12-week	1.6 ± 1.2**	2.1 ± 1.2	
	⊿ 0-12 w	− 1.2 ± 1.2	0.3 ± 0.7	< 0.001‡‡
#9	Baseline	1.9 ± 1.4	1.2 ± 1.2	
	6-week	1.6 ± 1.3	1.3 ± 1.2	
	⊿ 0-6 w	− 0.3 ± 0.9	0.2 ± 0.7	0.148
	12-week	1.0 ± 1.2*	1.6 ± 1.3†	
	⊿ 0-12 w	− 0.9 ± 1.2	0.4 ± 0.8	0.003‡‡
#10	Baseline	1.6 ± 1.4	1.6 ± 1.1	
	6-week	1.3 ± 1.2	1.8 ± 1.2	
	⊿ 0-6 w	− 0.3 ± 0.6	0.3 ± 1.1	0.119
	12-week	1.2 ± 1.1	2.0 ± 1.2	
	⊿ 0-12 w	− 0.5 ± 1.5	0.4 ± 0.9	0.085‡
#11	Baseline	1.8 ± 1.0	2.1 ± 1.2	
	6-week	1.7 ± 1.4	2.2 ± 1.3	
	⊿ 0-6 w	− 0.1 ± 0.9	0.1 ± 0.8	0.634
	12-week	1.2 ± 0.9	2.3 ± 1.2	
	⊿ 0-12 w	− 0.5 ± 1.2	0.3 ± 0.6	0.053‡
#12	Baseline	1.6 ± 1.0	1.1 ± 0.7	
	6-week	1.5 ± 1.1	1.3 ± 0.8	
	⊿ 0-6 w	− 0.1 ± 0.6	0.3 ± 0.8	0.253
	12-week	0.8 ± 1.0*	1.4 ± 0.7	
	⊿ 0-12 w	− 0.8 ± 0.9	0.3 ± 0.7	0.002‡‡
#13	Baseline	1.4 ± 1.0	1.4 ± 1.1	
	6-week	1.2 ± 1.3	1.6 ± 1.4	
	⊿ 0-6 w	− 0.2 ± 1.4	0.2 ± 0.8	0.408
	12-week	0.8 ± 1.2*	2.0 ± 1.3*	
	⊿ 0-12 w	− 0.6 ± 1.0	0.6 ± 0.8	0.003‡‡
#14	Baseline	2.5 ± 1.5	1.8 ± 1.3	
	6-week	2.0 ± 1.3	1.8 ± 1.5	
	⊿ 0-6 w	− 0.5 ± 1.1	0.0 ± 1.0	0.282
	12-week	1.3 ± 1.5**	2.3 ± 1.4	
	⊿ 0-12 w	− 1.2 ± 1.2	0.5 ± 1.0	0.001‡‡
#15	Baseline	2.1 ± 1.5	1.3 ± 1.1	
	6-week	1.7 ± 1.3	1.3 ± 1.2	
	⊿ 0-6 w	− 0.4 ± 1.6	0.1 ± 1.2	0.407
	12-week	1.1 ± 1.3**	1.8 ± 1.1†	
	⊿ 0-12 w	− 1.0 ± 0.8	0.6 ± 1.1	< 0.001‡‡

Scores are expressed as the mean ± SD.
1) † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
2) ‡ $p < 0.1$, ‡‡ $p < 0.01$ between-group difference in change from baseline.

Table 3　Results of questionnaire analyses (2)

No.	Time point	Scores		P-value[2)]
		Test (n = 13)[1)]	Placebo (n = 12)[1)]	
#16	Baseline	2.6 ± 1.3	2.3 ± 1.4	
	6-week	2.2 ± 1.1	2.3 ± 1.4	
	⊿ 0-6 w	− 0.5 ± 1.1	− 0.1 ± 1.2	0.418
	12-week	2.3 ± 1.3	2.8 ± 1.3	
	⊿ 0-12 w	− 0.3 ± 1.0	0.4 ± 1.1	0.100
#17	Baseline	2.6 ± 1.4	2.3 ± 1.7	
	6-week	1.8 ± 1.3**	2.3 ± 1.3	
	⊿ 0-6 w	− 0.8 ± 0.6	0.0 ± 1.3	0.074 ‡
	12-week	2.2 ± 1.2	2.2 ± 1.6	
	⊿ 0-12 w	− 0.5 ± 1.0	− 0.1 ± 1.1	0.366
#18	Baseline	2.0 ± 1.2	2.3 ± 1.3	
	6-week	1.5 ± 1.0	2.3 ± 1.4	
	⊿ 0-6 w	− 0.5 ± 1.0	− 0.1 ± 0.8	0.299
	12-week	1.5 ± 1.3	2.6 ± 1.4 †	
	⊿ 0-12 w	− 0.5 ± 1.3	0.3 ± 0.5	0.092 ‡
#19	Baseline	2.0 ± 1.4	2.2 ± 1.2	
	6-week	1.7 ± 1.1	2.3 ± 1.5	
	⊿ 0-6 w	− 0.3 ± 0.9	0.1 ± 0.8	0.277
	12-week	1.8 ± 1.4	2.5 ± 1.3	
	⊿ 0-12 w	− 0.2 ± 1.3	0.3 ± 0.9	0.222
#20	Baseline	2.0 ± 1.2	2.3 ± 1.1	
	6-week	1.7 ± 1.0	2.3 ± 1.1	
	⊿ 0-6 w	− 0.3 ± 1.1	0.1 ± 0.7	0.302
	12-week	1.7 ± 1.2	2.2 ± 1.4	
	⊿ 0-12 w	− 0.3 ± 1.8	− 0.1 ± 1.0	0.701
#21	Baseline	0.9 ± 1.3	1.5 ± 1.2	
	6-week	1.2 ± 1.4	1.4 ± 1.2	
	⊿ 0-6 w	0.3 ± 1.0	− 0.1 ± 0.8	0.302
	12-week	1.2 ± 1.7	1.7 ± 1.2	
	⊿ 0-12 w	0.2 ± 1.1	0.2 ± 1.2	0.890
#22	Baseline	1.3 ± 1.5	2.3 ± 1.4	
	6-week	1.2 ± 1.5	1.9 ± 1.2	
	⊿ 0-6 w	− 0.1 ± 1.0	− 0.4 ± 1.4	0.478
	12-week	1.4 ± 1.7	2.5 ± 1.2	
	⊿ 0-12 w	0.1 ± 1.2	0.2 ± 0.6	0.815
#23	Baseline	2.1 ± 1.6	2.1 ± 1.2	
	6-week	2.0 ± 1.3	1.9 ± 1.2	
	⊿ 0-6 w	− 0.1 ± 0.9	− 0.2 ± 1.3	0.837
	12-week	1.6 ± 1.6	2.3 ± 1.2	
	⊿ 0-12 w	− 0.5 ± 1.4	0.3 ± 1.1	0.177
#24	Baseline	0.6 ± 1.2	1.6 ± 1.5	
	6-week	0.5 ± 0.8	1.4 ± 1.4	
	⊿ 0-6 w	− 0.2 ± 0.8	− 0.2 ± 1.0	0.972
	12-week	0.4 ± 0.7	1.9 ± 1.6	
	⊿ 0-12 w	− 0.2 ± 0.9	0.3 ± 0.8	0.115
#25	Baseline	0.5 ± 1.0	1.3 ± 1.4	
	6-week	0.3 ± 0.9	1.1 ± 1.4	
	⊿ 0-6 w	− 0.2 ± 1.0	− 0.2 ± 0.6	0.849
	12-week	0.2 ± 0.6	1.6 ± 1.5	
	⊿ 0-12 w	− 0.3 ± 0.9	0.3 ± 0.8	0.063 ‡
#26	Baseline	2.0 ± 1.6	3.3 ± 1.0	
	6-week	2.1 ± 1.8	3.2 ± 1.3	
	⊿ 0-6 w	0.1 ± 1.4	− 0.1 ± 0.9	0.744
	12-week	1.8 ± 1.6	3.2 ± 1.1	
	⊿ 0-12 w	− 0.2 ± 1.3	− 0.1 ± 1.0	0.884
#27	Baseline	2.2 ± 1.5	1.9 ± 1.4	
	6-week	1.8 ± 1.3	1.6 ± 1.6	
	⊿ 0-6 w	− 0.4 ± 1.4	− 0.3 ± 0.7	0.908
	12-week	1.1 ± 1.3*	1.8 ± 1.5	
	⊿ 0-12 w	− 1.1 ± 1.6	− 0.2 ± 0.4	0.069 ‡
#28	Baseline	2.2 ± 1.5	2.5 ± 1.3	
	6-week	1.8 ± 1.5	2.2 ± 1.9	
	⊿ 0-6 w	− 0.3 ± 1.3	− 0.3 ± 0.8	0.952
	12-week	1.4 ± 1.5 †	2.4 ± 1.4	
	⊿ 0-12 w	− 0.8 ± 1.5	− 0.1 ± 0.3	0.142
#29	Baseline	1.5 ± 1.6	2.2 ± 1.3	
	6-week	1.5 ± 1.5	1.9 ± 1.5	
	⊿ 0-6 w	0.0 ± 1.6	− 0.3 ± 0.6	0.614
	12-week	0.8 ± 1.3 †	2.4 ± 1.1	
	⊿ 0-12 w	− 0.7 ± 1.3	0.3 ± 0.6	0.034 #
#30	Baseline	0.5 ± 1.0	1.3 ± 1.1	
	6-week	0.7 ± 1.2	1.2 ± 1.3	
	⊿ 0-6 w	0.2 ± 0.9	− 0.1 ± 0.7	0.465
	12-week	0.8 ± 1.2	1.3 ± 1.1 †	
	⊿ 0-12 w	0.3 ± 0.9	0.1 ± 0.3	0.440

Scores are expressed as the mean ± SD.
1) † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
2) ‡ $p < 0.1$, # $p < 0.05$ between-group difference in change from baseline.

Table 3 Results of questionnaire analyses (3)

No.	Time point	Scores		P-value[2)]
		Test (n = 13)[1)]	Placebo (n = 12)[1)]	
#31	Baseline	1.1 ± 1.2	1.0 ± 1.1	
	6-week	1.2 ± 1.1	0.9 ± 0.9	
	⊿ 0-6 w	0.2 ± 1.2	− 0.1 ± 0.5	0.538
	12-week	0.6 ± 1.0	1.3 ± 1.1	
	⊿ 0-12 w	− 0.5 ± 1.3	0.3 ± 0.7	0.064 ‡
#32	Baseline	0.2 ± 0.4	0.4 ± 0.9	
	6-week	0.2 ± 0.4	0.3 ± 0.5	
	⊿ 0-6 w	− 0.1 ± 0.5	− 0.2 ± 0.7	0.717
	12-week	0.1 ± 0.3	0.2 ± 0.4	
	⊿ 0-12 w	− 0.2 ± 0.4	− 0.3 ± 0.9	0.718
#33	Baseline	1.8 ± 1.4	2.8 ± 0.8	
	6-week	1.2 ± 1.0 *	2.4 ± 1.0 †	
	⊿ 0-6 w	− 0.6 ± 1.0	− 0.4 ± 0.8	0.580
	12-week	1.2 ± 1.0 †	2.8 ± 0.8	
	⊿ 0-12 w	− 0.7 ± 1.3	0.0 ± 0.0	0.082 ‡
#34	Baseline	1.3 ± 1.2	2.6 ± 1.2	
	6-week	0.5 ± 0.8 †	2.4 ± 1.2	
	⊿ 0-6 w	− 0.8 ± 1.3	− 0.2 ± 0.7	0.170
	12-week	0.8 ± 1.1	2.7 ± 1.2	
	⊿ 0-12 w	− 0.5 ± 1.5	0.1 ± 0.3	0.215
#35	Baseline	0.8 ± 1.2	1.5 ± 1.6	
	6-week	0.2 ± 0.4 *	1.9 ± 1.2	
	⊿ 0-6 w	− 0.7 ± 1.1	0.4 ± 0.9	0.012 #
	12-week	0.3 ± 0.6 *	1.9 ± 1.4 †	
	⊿ 0-12 w	− 0.5 ± 0.8	0.4 ± 0.8	0.006 ##
#36	Baseline	1.1 ± 1.3	1.4 ± 1.0	
	6-week	0.4 ± 0.9	1.3 ± 1.0	
	⊿ 0-6 w	− 0.7 ± 1.4	− 0.2 ± 0.6	0.250
	12-week	0.8 ± 1.2	1.1 ± 1.2	
	⊿ 0-12 w	− 0.3 ± 1.4	− 0.3 ± 0.9	0.958
#37	Baseline	1.5 ± 1.3	1.8 ± 1.8	
	6-week	1.0 ± 1.5	1.9 ± 1.6	
	⊿ 0-6 w	− 0.5 ± 1.2	0.2 ± 0.8	0.145
	12-week	0.9 ± 1.2	1.9 ± 1.4	
	⊿ 0-12 w	− 0.5 ± 1.4	0.2 ± 0.8	0.142
#38	Baseline	1.4 ± 1.0	0.9 ± 0.8	
	6-week	0.8 ± 1.0 *	1.0 ± 0.7	
	⊿ 0-6 w	− 0.5 ± 0.9	0.1 ± 0.5	0.043 #
	12-week	0.9 ± 1.0 †	0.8 ± 1.1	
	⊿ 0-12 w	− 0.5 ± 0.8	− 0.2 ± 0.6	0.296
#39	Baseline	0.9 ± 1.0	1.1 ± 1.2	
	6-week	0.5 ± 0.8 †	1.0 ± 1.2	
	⊿ 0-6 w	− 0.5 ± 0.8	− 0.1 ± 0.5	0.145
	12-week	0.5 ± 0.8 †	0.7 ± 0.0 †	
	⊿ 0-12 w	− 0.5 ± 0.8	− 0.4 ± 0.8	0.802
#40	Baseline	1.4 ± 1.2	1.2 ± 1.1	
	6-week	1.0 ± 1.2	1.0 ± 0.7	
	⊿ 0-6 w	− 0.4 ± 1.3	− 0.2 ± 1.1	0.619
	12-week	0.8 ± 0.9	1.1 ± 0.8 †	
	⊿ 0-12 w	− 0.6 ± 1.2	− 0.1 ± 0.9	0.200
#41	Baseline	3.0 ± 1.2	1.9 ± 1.4	
	6-week	1.3 ± 1.3 **	2.2 ± 1.4	
	⊿ 0-6 w	− 1.7 ± 1.5	0.3 ± 0.8	< 0.001 ##
	12-week	1.3 ± 1.5 **	2.1 ± 1.3	
	⊿ 0-12 w	− 1.7 ± 1.6	0.2 ± 0.6	< 0.001 ##
#42	Baseline	1.6 ± 1.3	0.3 ± 0.7	
	6-week	0.6 ± 0.9 *	0.3 ± 0.5	
	⊿ 0-6 w	− 1.0 ± 1.2	0.0 ± 0.6	0.014 #
	12-week	0.6 ± 1.0 **	0.3 ± 0.7	
	⊿ 0-12 w	− 1.0 ± 0.9	0.0 ± 0.7	0.005 ##
#43	Baseline	2.0 ± 1.4	1.8 ± 1.7	
	6-week	1.2 ± 1.1 **	1.8 ± 1.3	
	⊿ 0-6 w	− 0.8 ± 0.9	0.1 ± 1.0	0.018 #
	12-week	1.5 ± 1.4	2.0 ± 1.4	
	⊿ 0-12 w	− 0.5 ± 1.3	0.3 ± 0.9	0.080 ‡
#44	Baseline	0.8 ± 1.1	0.6 ± 0.7	
	6-week	0.5 ± 0.9	0.6 ± 0.9	
	⊿ 0-6 w	− 0.3 ± 0.6	0.0 ± 0.7	0.253
	12-week	0.3 ± 0.6 †	0.3 ± 0.5 †	
	⊿ 0-12 w	− 0.5 ± 0.8	− 0.3 ± 0.5	0.360
#45	Baseline	1.8 ± 1.3	0.8 ± 0.9	
	6-week	1.2 ± 1.1 *	0.9 ± 0.8 †	
	⊿ 0-6 w	− 0.6 ± 0.9	0.2 ± 0.7	0.020 #
	12-week	1.0 ± 1.1 †	1.0 ± 1.0	
	⊿ 0-12 w	− 0.8 ± 1.4	0.3 ± 1.0	0.030 #

Scores are expressed as the mean ± SD.
1) † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
2) ‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group difference in change from baseline.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of the test product containing *Pueraria mirifica*. The objective of this study was to verify whether the ingestion of the food containing *Pueraria mirifica* affects the conditions of menopausal discomfort.

As the primary outcome, the questionnaire about menopausal discomfort showed a significant difference in the items related to functions of skin, vagina and urination, after 6-weeks of ingestion of the test product. In addition, the results after 12 weeks showed a significant difference in the items related to sweating at night, difficulty sleeping and unstable feeling. These results suggest that the ingestion of the test product contributed to improving some symptoms concerning menopausal discomfort. In addition, as the secondary outcome, no adverse effects associated with the test product were observed in the course of the reporting, and the safety of ingesting the test product was suggested.

Main Findings

This study showed the improvement of several symptoms of menopausal discomfort, as a result of the ingestion of the test product containing *Pueraria mirifica*. According to the result data of the questionnaire, there was a significant difference in the items related to sweating at night such as "#3 (sudden sweating at night)", the items related to unstableness or nervousness such as #8, #9, #12, and #13, the items related to sleeping such as #14, and #15, the items related to physical disorder such as #29, #35, and #38, the items related to sexual disinterest such as #41, and the items related to urinary function such as #42, #43, and #45, after the ingestion of the test product. On the other hand, we could not find a significant difference in the items related to physical symptoms such as dizziness or headache, and the items related to the cognitive functions such as memory or forgetfulness. The questionnaire used for this test is prepared by the doctor in charge of carrying out the test, who consulted several menopausal indexes such as the Kupperman (menopausal) index [7)8)] or the Greene Climacteric Scale [9)10)] for diagnosing menopausal discomfort [11)]. Thanks to this preparation, this test enabled us to evaluate not only whether the test subjects suffer from menopausal discomfort or not, but also how the test product shows an influence against some symptoms of the menopausal discomfort.

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 [12)]. These ingredients are phytoestrogens which mimic the biological activity of female hormone estrogen [13)] and are thought as a causal substance of the functions of *Pueraria mirifica* [14)]. In women's bodies, gonadotropins are secreted from hypothalamus at the time of ovulation, and this secretion stimulates the pituitary to secrete follicle stimulating hormone (FSH) which encourages the secretion of female hormones by the ovary. The stimulation of the ovary by the hormone lets the ovarian follicle start its maturation, and during this maturation, estrogen is secreted [15)]. When an adequate amount of estrogen is secreted, ovulation occurs with the secretion of the corpus luteum hormone. On the other hand, once the women face climacteric symptoms and the secretion of estrogen becomes inadequate, the hypothalamus or the pituitary continuously stimulates the ovary in order to activate the secretion of estrogen (negative feedback). However, the adequate secretion of the estrogen is not achieved due to a decline in the function of the ovary, and as a result the hypothalamus becomes a continuous hyper-function. Eventually, since the hypothalamus is the nerve center of automatic nerves, the function of automatic nerves gets worse and this disorder has various negative impacts on the body; this is often regarded as one of the causes of menopausal discomfort [16)17)]. It is thought that since the phytoestrogen contained in *Pueraria mirifica* perform an estrogen-like effect internally, it inhibits the hyper-function state of hypothalamus and fixes up the dysfunction of automatic nerve [18)]. The automatic nerve controls the functions of organs or blood vessels and prepares the body's internal environment. Therefore, the symptoms which showed the tendency of improvement in this study are all thought to have a relationship with the automatic nerve functions. The affective disorders such as difficulty sleeping or unstable feeling may have been caused by the decrease of estrogen, which causes the inhibition of degradation of monoamine oxidase and the fall in the density of serotonin [19)], but is recovered and improved by the estrogen-like effect of *Pueraria mirifica*. As for the skin tingle, it is likely that the estrogen-like effect also brought an improvement of the condition, since estrogen is equipped with the function of supporting the collagen formation of dermic layer [20)]. In addition, the sexual factors such as an interest in sex or virginal dryness directly involve the estrogen (female hormone), so the improvement tendency has been possibly seen. On the other hand, the symptoms that did not show significant improvements are thought to be the symptoms the estrogen does not have a direct involvement in, or the symptoms which have a strong relationship with social or environmental factors of women.

Based on the discussion above, it is suggested that the ingestion of the test product containing *Pueraria mirifica* contributes to improving several disorders of climacteric age.

Secondary Findings

In this study, adverse events were collected by means of a written questionnaire during the study, and no

abnormal change caused by the test product was observed during the ingesting period. During the test period one test subject discontinued the test. The reason of discontinuance was personal one and it has nothing to do with the ingestion of the test product. These results indicated the safety of the ingestion of the test product for the 12-week test period

General Information

Menopausal discomfort tends to significantly deteriorate the quality of life (QOL) of women, due to its unpleasant symptom. It is a fact, however, that the appropriate information or understanding about menopausal discomfort is not well-known in Japan[21)22)]. Therefore, a lot of women are inclined to regard the symptoms as a naturally occurring change caused by aging, and relieve them. In addition, Japanese women of that age (around 50 years old) often get caught up in businesses such as nursing care of their parents, which may mean they have limited time to see doctors[23)]. For these reasons, it can be said that almost all Japanese women of climacteric age do not regularly receive medical support for menopausal discomfort. It is reported that seeking help to relieve unpleasant symptoms leads to losing a sense of well-being and encouraging a negative feeling[24)]. Based upon the above discussion, it is contemplated that if the unpleasant feeling of climacteric symptoms can be alleviated by the ingestion of the food of supplement type (which is easily prepared), it may contribute to improving the QOL of middle-aged women.

Limitations

Menopausal discomfort is also known as "climacteric complaints". It displays several physical symptoms, even though there may not be any organic disease. In other words, in many cases we cannot identify a cause of the discomfort since a medical examination does not show any numerical anomaly. Therefore, although a variety of objective examinations are being used to check up and diagnose menopausal discomfort[25)26)], it is still inevitable to depend on the subjective evaluation such as the questionnaire for a comprehensive diagnosis[11)27)]. In addition, since the onset of the symptom has a strong a relationship with many factors such as whether they have a job or not, their dietary habits or sports habits[28)], and the type of their job or life style they have[29)], the degree of the symptom differs substantially between individuals, and this fact makes it difficult to judge whether the test results are surely yielded by the ingestion of the test product. Therefore, it is necessary to examine with more test subjects. Also, excessive ingestion of the phytoestrogen in *Pueraria mirifica* reportedly brings on an endocrine-disrupting effect[5)], it is important to ingest a moderate amount of it.

5. CONCLUSION

In conclusion, we found out that the ingestion of the test product containing *Pueraria mirifica* for 12 weeks contributed to the improvement of symptoms of menopausal discomfort (sweating at night, urination, discomfort of skin, unstable feeling, and loss of interest in sex). 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.

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Appendix 1. Subjective condition assessments

Scale of 0 to 4, with lower scores indicating a better result.

#1 becoming facial redness
#2 becoming sweaty
#3 sudden sweating at night
#4 suddenly becoming heart beating in daily life
#5 feeling dizziness
#6 feeling tinnitus
#7 feeling anxious
#8 nervousness
#9 feeling isolated
#10 becoming tense
#11 feeling maddening
#12 feeling unstable
#13 weeping
#14 sleeping light
#15 difficulty falling asleep
#16 feeling tired
#17 feeling malaise
#18 lack of concentration
#19 difficult to memory
#20 becoming forgetful
#21 feeling headache
#22 feeling lower back discomfort
#23 becoming swelling
#24 becoming numbness in hand
#25 becoming numbness in foot
#26 becoming stiff shoulder
#27 becoming cold hand
#28 becoming cold foot
#29 becoming cold waist
#30 becoming abdominal pain
#31 becoming constipation
#32 feeling vomiting
#33 becoming dry skin
#34 becoming itchy skin
#35 feeling tingly skin
#36 feeling skin eczema
#37 feeling hair falling
#38 feeling virginal dryness
#39 becoming painful sex
#40 feeling sexual discomfort
#41 feeling sexual disinterest
#42 uncontrolled urination
#43 frequent urination
#44 feeling residual urine
#45 anxious about urination