Effect of *Apocynum venetum* Leaf Extract (VENETRON®) on Unidentified Complaints Relating Menstruation in Healthy Female Subjects

—A Randomized, Double-blind, Placebo-controlled Parallel-group Comparison Study—

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ABSTRACT

Background *Apocynum venetum* L. is a traditional herb distributed in northwestern China. Previous research suggests that *A. venetum* leaf extract (VENETRON®) improves sleep quality. However, there is little information on improvement of unidentified complaints related to menstruation in women.

Objectives This study aimed to investigate the efficacy of VENETRON® on unidentified complaints relating menstruation in healthy female subjects.

Methods A randomized, double-blind, placebo-controlled parallel-group comparison study was performed on 44 healthy Japanese women. The subjects were randomly allocated and ingested either the placebo ($n=22$) or 50 mg/day VENETRON® as test food ($n=22$) for one menstrual cycle. Menstrual Distress Questionnaire (MDQ), Oguri-Shirakawa-Azumi Sleep Inventory for Middle-age and Aged version (OSA-MA), Profile of Mood States 2nd Edition (POMS2), Uchida-Kraepelin (UK) performance test, as well as the autonomic nervous system, cognitive functions, salivary biomarkers of stress, skin, anthropometric examination, original questionnaire, and female hormones were assessed.

Results Forty-two subjects completed the trial, and 21 in the test food group and 21 in the placebo group were analyzed. Results indicated that the test food significantly reduced total scores in MDQ before menstruation and during menstruation than the placebo. The scores of Autonomic Reactions, Negative Affect, Concentration, and Behavioral Change before menstruation and scores of Pain, Concentration, and Control during menstruation were significantly decreased in the test food group after ingestion of VENETRON® than in the placebo group. However, no significant changes were observed after menstruation. In OSA-MA, the results showed significant improvement in 'Initiation and maintenance of sleep' before menstruation in the test food group than in the placebo group. No significant differences were observed in other outcomes. No medically problematic changes occurred with the ingestion of VENETRON®.

Conclusions VENETRON® improves unidentified complaints related to menstruation and appeared to be safe under the conditions employed in the present study.

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KEY WORDS *Apocynum venetum* L., VENETRON, MDQ, OSA-MA, Hyperoside, isoquercitrin, Menstrual cycle

INTRODUCTION

Rafuma（*Apocynum venetum* L., *A. venetum*）is a perennial plant of the Apocynaceae family that grows in the wild in northwestern China. The leaves of *A. venetum* are listed in the Chinese Pharmacopoeia, and used for insomnia and stress relief. *A. venetum* leaves contain flavonoids such as isoquercitrin and hyperoside, which have been reported to have hepatoprotective and antioxidant activities.[1)]

Animal studies have shown that *A. venetum* leaf extract（hereafter referred to as VENETRON®）has antidepressant and anxiolytic effects.[2,3)] It has been reported that hyperoside and isoquercitrin are metabolized into quercetin and its glucuronide, which inhibit the metabolism of serotonin, a precursor of melatonin that regulates the sleep-wake rhythm.[4)] Moreover, administration of VENETRON® has been reported to increase the amount of serotonin in the brains of rats.[5)] In previous clinical studies on healthy subjects, VENETRON® increased the non-REM sleep ratio[6)] and improved the score of 'Initiation and maintenance of sleep' in the Oguri-Shirakawa-Azumi Sleep Inventory for Middle-age and Aged version（OSA-MA）,[7)] suggesting that VENETRON® improved the quality of sleep. Moreover, VENETRON® reduced the fluctuating rate of performance[7)] and salivary chromogranin A in the Uchida-Kraepelin（UK）performance test,[8)] suggesting a relief effect on the induced temporary psychological stress.

Women are prone to experience various physical and mental symptoms due to variations in hormone balance associated with the menstrual cycle, and unidentified complaints are especially likely to occur before menstruation. Premenstrual complaints that interfere in daily activities such as social activities, schoolwork, and work are referred to as Premenstrual Syndrome（PMS）. More severe symptoms are classified as Premenstrual Dysphoric Disorder（PMDD）.[9)] The main symptoms include irritability, anger, depressed mood, tearfulness, anxiety, tension, mood swings, lack of concentration, confusion, forgetfulness, restlessness, loneliness as psychologic symptoms; and fatigue, insomnia, dizziness, overeating, headaches, breast tenderness, back pain, abdominal pain and bloating, weight gain, swelling of extremities, water retention, nausea as physical symptoms.[9)] Moreover, several symptoms may occur not only before menstruation, but also during menstruation. The symptoms occurring during menstruation are called dysmenorrhea and includes abdominal pain, back pain, headache, diarrhea, fatigue, nausea, or vomiting.[10)] These symptoms associated with menstruation result in a decrease in the productivity of women[11-13)] and also affect their social activities.

The menstrual cycle is regulated by hormones such as estrogen（follicular hormone）and progesterone（progestin）. Imbalance in the levels of estrogen and progesterone is suggested to be the cause of menstrual complaints. Excess estrogen is implicated to cause a decrease in serotonin levels and increase in prolactin and aldosterone levels, thereby leading to depressed mood, breast pain, and breast tension, respectively.[14)] Decreased progesterone is involved in the reduction of serotonin secretion, which leads to symptoms related to mental stress.[15)] Although several etiologies have been presumed, the cause of symptoms related to menstruation is still unclear. At present, selective serotonin reuptake inhibitors（SSRIs）, which increases serotonin levels by inhibiting reuptake of serotonin into presynaptic terminals, are used for the treatment of psychological symptoms relating menstruation.

VENETRON® has been reported to improve the quality of sleep and relieve temporary psychological stress; however, there are no reports on its effects on unidentified complaints associated with menstruation. Hence, the purpose of our study is to evaluate the effects of VENETRON® on improvement of menstrual complaints in healthy women.

MATERIAL AND METHODS

1 Study design

This study was designed as a randomized, double-blind, placebo-controlled, parallel-group comparison study on the efficacy of VENETRON® on unidentified complaints related to menstruation in healthy female subjects, and was carried out from November 2022 to March 2023 at the Medical Corporation Seishinkai Takara Clinic（Tokyo, Japan）. This study was per-

formed in compliance with the ethical principles of the Declaration of Helsinki（amended in 2013）and the Ethical Guidelines for Medical and Health Research Involving Human Subjects（Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, Japan）. The Ethical Committee of the Medical Corporation Seishinkai, Takara Clinic in Tokyo, Japan, approved this study（Approval No. 2209-01216-0031-0F-TC）on September 2022. All the procedures employed in this study were fully explained to the subjects, and informed consent was documented and signed by all the subjects.

2 Subjects

The recruitment of subjects was performed on the website operated by ORTHOMEDICO Inc.（Tokyo, Japan）. The subjects who met the inclusion criteria and did not meet the exclusion criteria participated in the present study. The inclusion and exclusion criteria are listed below.

Inclusion criteria:

i. Japanese
ii. Women
iii. Aged between 20 and 40
iv. Healthy subjects
v. Subjects whose menstrual cycle was between 25 and 38 days
vi. Subjects whose lasting period of the menstruation was between three and seven days
vii. Subjects whose total score mentioned in the Medical Distress Questionnaire（MDQ）was relatively high

Exclusion criteria:

i. Subjects who were undergoing medical treatment or had a medical history of malignant tumor, heart failure, or myocardial infarction
ii. Subjects who had a pacemaker or an implantable cardioverter defibrillator（ICD）
iii. Subjects undergoing treatment for chronic diseases such as, cardiac arrhythmia, liver disorder, kidney disorder, cerebrovascular disorder, rheumatism, diabetes mellitus, dyslipidemia, hypertension, or any other chronic diseases
iv. Subjects who were undergoing medical treatment or had a medical history of gynopathy such as, premenstrual syndrome（PMS）, premenstrual dysphoric Disorder（PMDD）, secondary amenorrhea, dysmenorrhea, endometriosis, hysteromyoma, breast cancer, cervical cancer, endometrial carcinoma, or ovarian cancer
v. Subjects who did not have menstrual pain or symptoms associated with menstruation at all
vi. Subjects who had severe menstrual pain that could not be controlled with analgesics
vii. Subjects who were undergoing medical treatment or had a medical history of psychiatric disorder
viii. Subjects who were receiving hormonal therapy
ix. Subjects who were taking low dose pill
x. Postmenopausal subjects
xi. Subjects who consumed 'Foods for Specified Health Uses,' or 'Foods with Functional Claims' on a daily basis
xii. Subjects who were taking medications（including herbal medicines）and supplements
xiii. Subjects who were allergic to medicines and/or the test food-related products
xiv. Subjects who were pregnant, lactating, or planning to become pregnant during this trial
xv. Subjects who had suffered from COVID-19
xvi. Subjects who had been enrolled in other clinical trials within the last 28 days before the agreement to participate in this trial or had planned to participate another trial during this trial
xvii. Subjects who were judged to be ineligible to participate in this study by the principal investigator

3 Description of the test food

VENETRON® was provided by Tokiwa Phytochemical Co., Ltd.（Chiba, Japan）, and was standardized to contain no less than 2.0% of hyperoside and no less than 2.0% of isoquercitrin. The test food included 25 mg of VENETRON® per tablet in addition to starch hydrolysate, crystalline cellulose, lactose, oil, licorice extract, guar gum, shellac, glycerin fatty acid ester, and carnauba wax. In the case of placebo tablets, VENETRON® was replaced with caramel coloring. After the enrollment, the subjects took two tablets of either the placebo or test food per day. The daily ingestion of VENETRON® in the test food was 50 mg/day, which included no less than 1 mg/day hyperoside and no less than 1 mg/day isoquercitrin. The total ingestion period was one menstrual cycle, from the 7th day after the start of menstruation until the 7th

day after the start of the next menstruation. The test food was indistinguishable in appearance, shape, color, odor, and taste from the placebo.

4 Randomization

The subjects were allocated randomly, taking into account that the total score of the MDQ before menstruation, menstrual cycle duration, and age were not significantly different among groups. The allocation was conducted using Statlight #11 ver. 2.10（Yukms Co., Ltd., Kanagawa, Japan）by an operator who did not participate in the study. All members, who were related to this study, including the sponsors, principal investigator, sub-investigators, entire contract research organization staff（i. e., the director of the trial, the director of trial conduction, the person in charge of monitoring, the director and staff of statistical analysis, and the person in charge of shipping）, medical institution staff, institutional review board members, contract laboratory, and others were blinded in this study.

5 Evaluation methods

The subjects visited the hospital on the 3rd day before menstruation before（visit 1）and after（visit 2）the ingestion. Visit 1 was conducted from November 2022 to December 2022, and visit 2 was conducted from February 2023 to March 2023.

Evaluation of unidentified complaints relating menstruation was performed using the MDQ Japanese version.[16] Total score and eight subscales（Pain, Water Retention, Autonomic Reactions, Negative Affect, Concentration, Behavioral Change, Arousal, Control）were assessed. Higher scores indicated more severe symptoms. The subjects answered the questionnaire on the 3rd day before menstruation（before menstruation）, 2nd day after the start of menstruation（during menstruation）, and 8th day after the start of menstruation（after menstruation）. The outcomes other than those mentioned in the MDQ were evaluated only on the 3rd day before menstruation（before menstruation）.

The quality of sleep was evaluated by OSA-MA.[17] Five items including Sleepiness on rising, Initiation and maintenance of sleep, Frequent dreaming, Refreshing on rising, and Sleep length were assessed. Mood was evaluated using the Profile of Mood States 2nd Edition（POMS2 Japanese version）.[18] The autonomic nervous system was evaluated using VM302（Fatigue Science Laboratory Inc., Osaka, Japan）. Low frequency（LF）, high frequency（HF）, LF/HF, average heart rate, maximum heart rate, minimum heart rate, total power（TP）, and coefficient of component variance total power（ccvTP）were assessed. Tolerance to temporary psychological stress was evaluated using the UK performance test.[19] Salivary biomarkers of stress, chromogranin A and cortisol were determined. The salivary chromogranin A levels were measured via services provided by the COSMO BIO Co., Ltd.（Tokyo, Japan）, and salivary cortisol levels were measured via services provides by the LSI Medience Corporation（Tokyo, Japan）. Cognitive functions were evaluated using Cognitrax（Health Solution, Inc., Tokyo Japan）.[20] Skin moisture content was measured using Corneometer® CM 825（Courage+Khazaka Electronic GmbH, Cologne, Germany）, and transepidermal water loss was measured using Tewameter® TM 300（Courage+Khazaka Electronic GmbH, Cologne, Germany）. Anthropometric examination was performed to evaluate the swelling in different body parts of the subjects. Volume of the face was measured by VECTRA®（Canfield Scientific, Inc., New Jersey, USA）to assess swelling of the face. Calf circumference of the right leg, left leg and average of right and left legs were measured to assess the swelling of the calf. Body water content was measured using Multi-frequency Body Composition Meter MC-780A-N（TANITA Corporation, Tokyo, Japan）. Subjective symptoms related to menstruation were evaluated by means of original questionnaires using Visual Analogue Scale（VAS）. Female hormones in blood, estradiol（E2）, follicle stimulating hormone（FSH）, luteinizing hormone（LH）, progesterone（P4）in blood were analyzed by services provided by the LSI Medience Corporation.

At the time of recruitment, the previous menstrual start date, menstrual cycle duration, menstrual duration, and any other changes in physical condition experienced before menstruation were investigated. During the study, the subjects were instructed to record the test food ingestion, menstruation, frequency of defecation, and basal body temperature in a diary designated by the contracted research organization. The subjects were instructed to adhere to the following points during the study.

Compliance instructions to the subjects:

a. Follow the prescribed dosage;
b. Ingestion rate should be over 80%;
c. Avoid overeating and overdrinking and do not change lifestyle from the date of signing the consent to the end of the study;
d. Do not drink alcohol or overexercise from the

day before each examination to the end of examination;

e. Do not drinking or ingest food, including the test food, functional water and tea, 6 h before blood collection (except water);

f. Measure body temperature every day with the thermometer lent by the contract research organization and record it in the diary;

g. If there is any change in physical condition during the study, contact the contract research organization immediately and follow their instructions;

h. Avoid taking 'Foods for Specified Health Uses', 'Foods for Functional Claims' and other foods/beverages considered to have some functions during the study;

i. During the study, take precautions to prevent infections such as coronavirus by thorough hand washing, hand disinfection, wearing a mask, etc. If there is any suspicion of infection, contact the contract research organization immediately and follow their instructions.

6 Outcomes

The primary outcome was total score in the MDQ before, during and after menstruation. The secondary outcomes were assessment of the following criteria: the eight subscales of MDQ before, during and after menstruation, OSA-MA, POMS2, autonomic nervous system test, UK performance test, cognitive functions, saliva test, skin test, anthropometric examination, original questionnaire, and measurement of female hormones.

7 Safety evaluations

Anthropometric examination, urinalysis, and blood biochemical tests were performed at visit 1 and 2. Urinalysis and blood biochemical measurements were conducted by LSI Medience Corporation.

For the anthropometric examination, the following parameters were recorded: height, body weight, BMI, body fat ratio, muscle mass, fat mass, lean mass, and systolic blood pressure and diastolic blood pressure. Protein, glucose, pH, and occult blood were recorded as urinalysis parameters. The following parameters were recorded in the blood biochemical examination: white blood cell count, red blood cell count, hemoglobin levels, hematocrit levels, platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), bilirubin, total protein, urea nitrogen, creatinine, uric acid, sodium (Na), potassium (K), chlorine (Cl), serum amylase, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride (TG), glucose, and HbA1c levels.

8 Statistical analysis

In the efficacy and the safety evaluation studies, the baseline was the value before the start of ingestion. In addition, comparison of the main interventions was performed between the groups at the time of visit 2. Data of the baseline were compared between groups using Welch's t-test. Data after ingestion were compared between groups using ANCOVA with the baseline as a covariate and group and presence or absence of heavy alcohol consumption habit as fixed factors. Since it has been reported that alcohol consumption affects menstrual complaints, it was added as a factor in the statistical analysis to remove the effect of heavy alcohol consumption habit.[21] All values were presented as mean±standard deviation or estimated marginal mean (EMM)±standard error.

Full analysis set (FAS) was used as the analysis data set for evaluating the efficacy. FAS is the data obtained by excluding subjects who met at least one of the following conditions from all the subjects who provide informed consent and enrolled in the study:

a. Subjects who had not been provided the interventions to which they were allocated;

b. Subjects who did not meet the criteria of the target population (including cases with any diseases determined by a definitive diagnosis or cases that conflicted with clearly defined and objectively determinable important inclusion/exclusion criteria);

c. Subjects who had never received an intervention after allocation;

d. Subjects on whom no data was available after allocation.

In the safety evaluation, the side-effects and adverse events observed were recorded for each subject. The incidence of side effects and adverse events was aggregated for each group, and the 95% confidence interval related to the difference between incidence within each group and incidence between groups was also calculated. In addition, the incidence of side effects and adverse events in each group was compared using Fisher's exact test. The percentage of cases in which each measured value of urinalysis and blood biochemical test within the reference value at

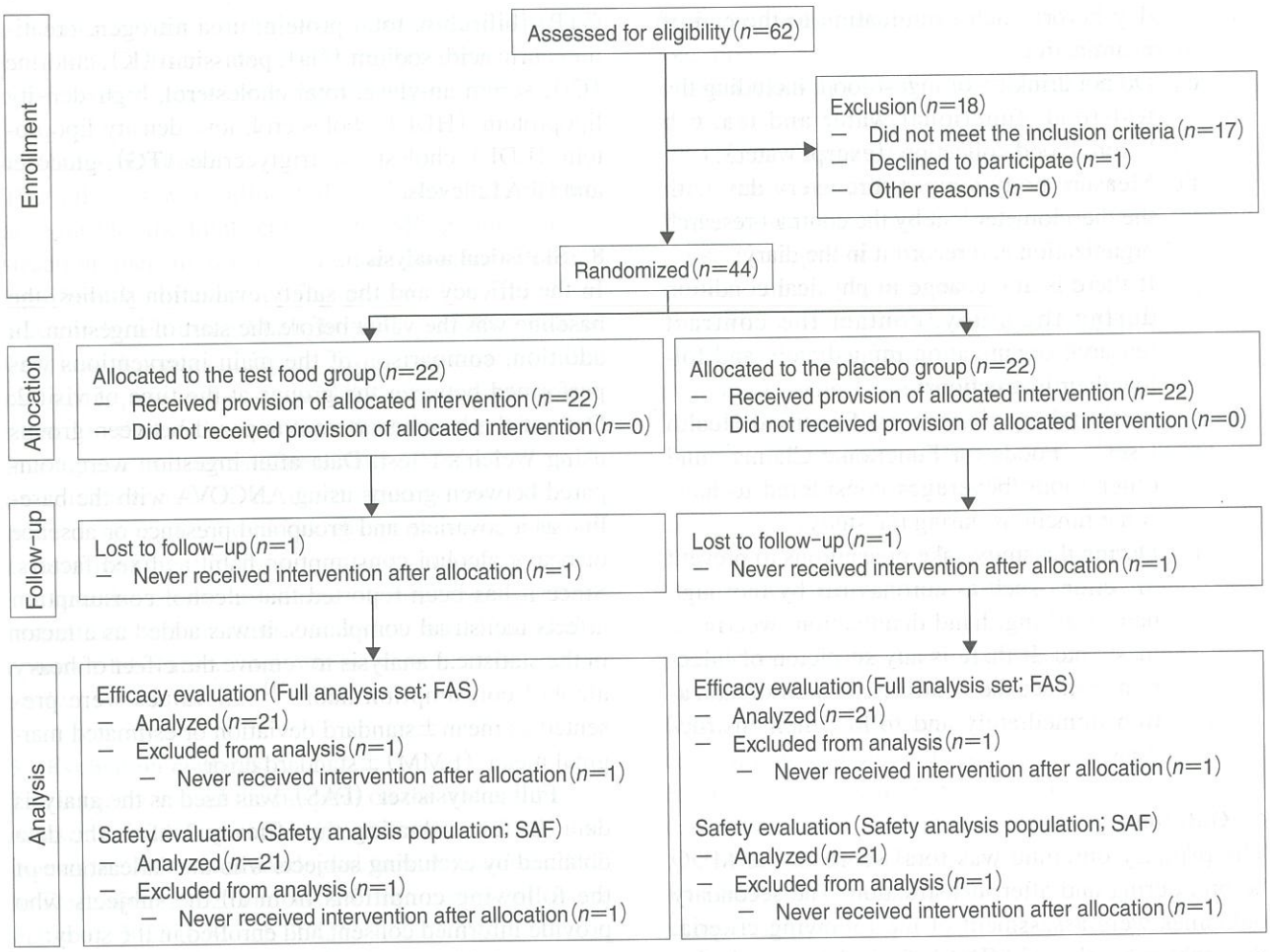

Fig. 1 Flowchart of the study

the start of ingestion, and which had altered and were outside the reference value after the intervention, were calculated, and the comparison between the groups at each time-point was conducted by Fisher's exact test. Other safety evaluation items were calculated for each measured value. Safety analysis population (SAF) was used as the analysis data set in the safety evaluation. SAF is data obtained by excluding subjects who met at least one of the following conditions from all the enrolled subjects:

a. Subjects who had not been provided the interventions to which they were allocated;
b. Subjects who had never received an intervention after allocation;
c. Subjects in whom the safety evaluation parameters had never been measured after allocation.

All statistical analyses were performed using two-sided tests, and statistical significance was set at $P<0.05$; however, P-values were adjusted using the Bonferroni correction to account for statistical multiplicity in the primary outcome, the MDQ total scores before, during, and after menstruation.

SPSS Statistics ver. 23.0, Windows version (IBM Japan, Ltd., Tokyo, Japan) was used for statistical analysis. In addition, the analysis in this study emphasizes upon the primary outcome, and does not consider the multiplicity that occurs in the multi-hypothetical secondary outcomes.

RESULTS

1 Baseline characteristics of subjects

Subjects were recruited from September to November 2022, and the study was conducted from November 2022 to March 2023. Of the 62 healthy Japanese women who agreed to participate in the study, 44 subjects were enrolled in the intervention study in accordance with the inclusion and exclusion criteria and were allocated to either the test food group or placebo

Table 1　Baseline characteristics of subjects

Parameters	Test food group (n=21)	Placebo group (n=21)
Age	31.6±5.3	31.9±5.0
Menstrual cycle duration（days）	29.1±2.6	29.1±2.9
MDQ total score（before menstruation）	56.0±26.0	57.2±21.2

All values are expressed as mean±standard deviation.

Table 2　MDQ total score and 8 subscales before menstruation（3rd day before menstruation）, during menstruation（2nd day after the start of menstruation）, and after menstruation（8th day after the start of menstruation）

Menstruation	Parameters	Before ingestion		P-value	After ingestion		P-value
		Test food group (n=21)	Placebo group (n=21)		Test food group (n=21)	Placebo group (n=21)	
Before	MDQ total score	56.0±26.0	57.2±21.2	0.88	38.9±16.3	50.0±24.7	0.049*
	Pain	7.7±4.9	8.5±4.0	0.56	5.5±3.8	6.5±4.0	0.38
	Water retention	5.6±3.0	6.6±3.1	0.29	4.6±2.8	5.3±2.9	0.50
	Autonomic reactions	1.9±2.0	2.1±2.2	0.66	1.3±2.1	2.5±2.6	0.04*
	Negative affect	13.1±7.2	12.0±6.1	0.57	6.9±4.8	9.1±6.9	0.03*
	Concentration	12.1±7.7	11.7±6.1	0.84	8.0±4.4	10.0±6.6	0.049*
	Behavioral change	8.9±5.5	8.9±3.9	1.00	5.4±2.8	7.1±3.8	0.02*
	Arousal	4.2±3.5	4.5±3.3	0.82	5.2±3.0	7.0±4.7	0.21
	Control	2.5±2.1	3.0±2.6	0.56	2.0±2.3	2.4±2.6	0.73
During	MDQ total score	68.2±30.2	54.0±25.2	0.10	42.9±24.5	44.0±22.7	0.048*
	Pain	11.3±4.8	9.9±4.1	0.29	7.2±4.2	8.2±4.8	0.049*
	Water retention	6.7±3.9	5.5±2.4	0.26	5.5±3.8	4.4±2.0	0.56
	Autonomic reactions	4.2±3.0	2.4±2.0	0.03*	1.7±2.6	1.7±1.7	0.20
	Negative affect	13.9±7.2	11.4±7.8	0.29	8.1±7.0	7.2±7.1	0.45
	Concentration	14.1±7.7	10.5±6.3	0.10	5.5±1.1†	8.0±1.0†	0.04*
	Behavioral change	10.5±4.3	8.5±3.8	0.13	6.3±5.1	6.7±4.3	0.18
	Arousal	4.0±3.4	3.0±2.5	0.33	4.5±4.0	5.6±3.5	0.12
	Control	3.5±4.1	2.7±3.3	0.49	1.1±1.6	1.9±2.7	0.04*
After	MDQ total score	33.3±28.3	21.1±11.2	0.08	31.0±22.8	23.6±13.3	1.00
	Pain	3.5±2.9	3.6±3.5	0.96	3.6±2.7	3.8±3.1	0.74
	Water retention	3.2±3.7	1.7±1.2	0.09	2.9±3.0	1.8±1.5	0.65
	Autonomic reactions	1.1±2.7	0.3±0.7	0.20	0.9±1.8	0.3±0.6	0.70
	Negative affects	7.6±7.2	3.0±2.7	0.01*	5.7±5.3	3.7±4.1	0.64
	Concentration	6.1±7.7	3.0±3.0	0.10	6.2±6.5	5.0±3.9	0.52
	Behavioral change	4.2±4.9	2.7±2.1	0.19	4.5±5.6	3.8±2.6	0.80
	Arousal	6.1±3.3	6.3±3.7	0.86	6.1±3.8	4.8±3.1	0.17
	Control	1.4±3.0	0.5±1.2	0.21	1.1±1.4	0.4±1.0	0.16

All values except "Concentration during menstruation" are expressed as mean±standard deviation
† Concentration is expressed as EMM±standard error, * $P<0.05$ vs. the placebo group

group. Twenty-two subjects were assigned to each group. Two subjects were excluded because they had never received the intervention after the allocation. Therefore, the data set used for the analysis of efficacy and safety evaluation were 21 in the test food group and 21 in the placebo group（**Fig. 1**）. No significant difference was observed between the test food and placebo groups before ingestion（**Table 1**）.

Table 3 Score of OSA-MA before menstruation（3rd day before menstruation）

Items	Before ingestion		*P*-value	After ingestion		*P*-value
	Test food group (n=21)	Placebo group (n=21)		Test food group (n=21)	Placebo group (n=21)	
Sleepiness on rising	15.4±4.9	15.3±3.6	0.95	14.3±5.3	16.4±5.4	0.31
Initiation and maintenance of sleep	17.2±7.3	16.8±5.9	0.85	18.5±5.7	15.3±5.1	0.048*
Frequent dreaming	19.7±8.2	23.8±6.5	0.08	21.2±8.5	23.5±7.5	0.72
Refreshing on rising	16.0±4.7	14.5±4.5	0.31	14.4±6.2	16.3±4.7	0.26
Sleep length	18.5±6.0	18.2±7.4	0.89	17.7±6.4	19.4±6.1	0.33

All values are expressed as mean±standard deviation, *P<0.05 vs. the placebo group.

2 MDQ

MDQ scores before menstruation, during menstruation, and after menstruation were determined before and after ingestion to evaluate unidentified complaints relating menstruation. Total score in the MDQ observed before menstruation regarding the test food group showed significant reduction after ingestion than that observed the placebo group（**Table 2**, P=0.049）. Of the 8 subscales, Autonomic Reactions, Negative Affect, Concentration, Behavioral Change were significantly decreased in the test food group after ingestion than in the placebo group（**Table 2**; P=0.04, 0.03, 0.049, and 0.02, respectively）. Moreover, the MDQ total score during menstruation was significantly lower in the test food group than in the placebo group after ingestion（**Table 2**, P=0.048）. Significant reduction was also observed in Pain, Concentration, Control in the test food group after ingestion than in the placebo group（**Table 2**; P=0.049, 0.04 and 0.04, respectively）. In the case of after menstruation, other than Negative Affect before ingestion, no significant difference was observed between the test food and placebo groups（**Table 2**; P=0.01）. Negative Affect before ingestion was significantly higher in the test food group than in the placebo group but significant difference disappeared after ingestion.

3 OSA-MA

To evaluate efficacy on quality of sleep, OSA-MA was performed before menstruation. The results showed significant improvement related to 'Initiation and maintenance of sleep' in the test food group than in the placebo group（**Table 3**, P=0.048）.

No significant differences were observed in the parameters such as, POMS2, UK performance test, autonomic nervous system, cognitive functions, salivary biomarkers of stress, skin test, anthropometric examination, original questionnaire, and female hormones（data not shown）.

4 Safety evaluation

Although some study subjects were observed to have a few adverse events, they were not related to the study food based on the judgement of the principal investigator. There were no significant differences between groups in the percentage of cases in which urinalysis and blood biochemical examination values changed from within the normal range at the pre-treatment test to outside the normal range after the intervention. Furthermore, there were no medically problematic changes associated with consumption of the test food observed in physical and physical measurements, urinalysis, and blood biochemical examination.

DISCUSSION

Due to religious reasons and other reasons, women were not able to speak out about the complaints associated with menstruation for a long time. However, due to scientific progress and increase in social activities of women, health issues related to menstruation have been focused upon. Furthermore, the frequency of menstruation experienced by women has significantly increased with a decrease in the rate of pregnancy and childbirth; consequently, the incidence of symptoms related to menstruation have been on the rise.[22] The symptoms relating menstruation result from an increased sensitivity of the central nervous system to hormonal fluctuations in the menstrual cycle,[23,24] and the symptoms occur periodically before menstruation and decrease with the onset of menstruation. The mechanism behind these symptoms is considered to be dysregulation of the serotonergic pathway and progesterone metabolism.[23,24] The symptoms

relating menstruation are experienced by almost all sexually mature women,[25] and there is a large demand related to the improvement of their quality of life through the ingestion of supplements and not by medical intervention.

VENETRON® has been reported to have antidepressant and anxiolytic effects in animal studies,[2,3] and has been observed to improve sleep quality and alleviate temporal psychological stress in human clinical studies,[6-8] thus suggesting that it may reduce unidentified complaints associated with the menstrual cycle. In our study, we examined the effect of VENETRON® on unidentified complaints related to menstruation in healthy 20- to 40-year-old women.

The MDQ consisted of 46 questions on physical and mental changes associated with the menstrual cycle, rated on a 5-point scale and classified into 8 subscales.[16] In the current clinical study, test food was ingested by the subjects during one menstrual cycle, and parameters in the MDQ were assessed before, during, and after menstruation. The results showed that total score in the MDQ was significantly lower in the test food group than in the placebo group before menstruation, thus suggesting that ingestion of VENETRON® reduced the complaints associated with menstruation. In addition, MDQ subscales based on Negative Affect, Concentration, Behavioral Change, and Autonomic Reactions were significantly lower in the test food group after ingestion than in the placebo group. Furthermore, during menstruation, the MDQ total score was significantly lower in the test food group than in the placebo group. In addition, the subscales based on Pain, Concentration, and Control were significantly lower in the test food group than in the placebo group after ingestion. However, no significant differences were observed in either the MDQ total score or subscales after menstruation.

The four subscales of mental disorders, including Negative Affect, Concentration, Behavioral Change, and Autonomic Reactions, showed significant improvement before menstruation, which was attributable to the antidepressant and anxiolytic effect of VENETRON®. Results of the MDQ during menstruation showed significant improvement in Pain and Control as well as in Concentration. Both Pain and Control consisted of questions regarding physical symptoms, and improvement was observed mainly in physical discomfort felt during menstruation compared to that before menstruation. This is consistent with the fact that the main physical complaints during menstruation, such as menstrual cramps, occur more frequently than before menstruation.[26] Except for Negative Affect, there were no significant differences in MDQ total score or subscale scores after menstruation, and the baseline MDQ total score after menstruation was lower than that before and during menstruation. Negative Affect before ingestion also had lower score than those of before and during menstruation, although significant differences were found. These results imply that complaints associated with menstruation generally appear before and during menstruation and disappear at the end of menstruation.

The U. S. Food and Drug Administration（FDA）recommends the use of SSRIs as the first-line of treatment for PMDD.[27] Studies have shown that SSRIs are effective in treating various symptoms of PMDD, including depressed mood, anxiety, emotional instability, and irritability.[28] Previous studies have reported that serotonin levels were increased in the hypothalamus of rats that were fed VENETRON®.[5] Since a decreased activity of serotonergic nerves is thought to be involved in stress, anxiety, and depression,[28] the ingestion of VENETRON® may alleviate these symptoms by increasing serotonin levels in the brain. Therefore, the alleviation of mental symptoms associated with the menstrual cycle is considered to be a result of the serotonin-increasing effect of VENETRON®.

In addition to various complaints associated with menstruation, it has been reported that many women experience insomnia before menstruation.[29,30] Because previous human clinical studies have indicated that VENETRON® improves 'Initiation and maintenance of sleep' of OSA-MA,[7] we investigated the quality of sleep using OSA-MA before menstruation to assess the effect of VENETRON® on premenstrual insomnia. The results showed a significant improvement in 'Initiation and maintenance of sleep' in the test food group than in the placebo group. Serotonin is a precursor of melatonin,[31] and melatonin has been reported to be a key compound associated with sleep rhythm and an increase in non-REM sleep, a state of deep sleep.[32,33] Therefore, the serotonin-increasing effect of VENETRON® may be attributable to the improvement in 'Initiation and maintenance of sleep'. During the luteal phase of the premenstrual period, progesterone has been reported to increase both minimum and maximum core body temperatures than during the follicular phase,[34] this phenomenon is implicated to be associated with nocturnal awakenings. Melatonin has been reported to decrease core body temperature at night,[35] and may be another

mechanism for the significant improvement in 'Initiation and maintenance of sleep' in the present study.

POMS2, which assesses mood profiles, and an original questionnaire designed by the authors, in addition to the MDQ, were used in this clinical trial to assess unidentified complaints associated with menstruation. However, none of the differences were found to be significant. As POMS2 and the original questionnaire were not developed to specifically evaluate unidentified complaints associated with menstruation, it was suggested that these methods may not be scientifically appropriate to assess unidentified complaints associated with menstruation.

We also investigated whether the ingestion of VENETRON® affected tolerance to temporal psychological stress using the UK performance test. The results showed no significant changes between the test food and placebo groups. There were also no significant differences in the levels of salivary chromogranin A and salivary cortisol, which are biomarkers of stress. In addition, the results of premenstrual autonomic nervous system and cognitive functions showed no significant changes between the test food group and placebo group. The subjects in this study were all healthy women with some complaints associated with menstruation, and their premenstrual symptoms were not severe enough to cause abnormalities in the autonomic nervous system or cognitive function. By contrast, a significant improvement was observed in Autonomic Reactions observed in the MDQ subscale before menstruation, suggesting that this MDQ subscale, which was designed specially to assess symptoms associated with menstruation, is a scientifically appropriate method to detect changes in the symptoms associated with menstruation.

It has been reported that swelling and roughness of the skin is experienced before menstruation.[9,36] In our study, we examined whether the ingestion of VENETRON® improved premenstrual swelling of the face and calves, body water content, skin moisture content, and the transepidermal water loss. The results suggested that there were no significant changes observed in either the test food group or the placebo group, and no significant changes were found in the Water Retention subscale of MDQ, which includes questions regarding swelling and skin roughness. Premenstrual swelling and skin roughness is thought to be related to hormonal changes.[37,38] In this clinical study, no significant effects on female hormones were observed with the ingestion of VENETRON®. As a result, no effect on swelling or skin roughness associated with hormonal changes was observed.

No side-effects were observed during the study period, and no adverse events attributable to the test food were observed under the conditions of the present study. Moreover, there was no significant difference in the parameters related to safety evaluation between the test food group and the placebo group, and the principal investigator confirmed that there were no medically problematic changes attributable to the test food.

Changes in female hormones have been reported to play a major role in unidentified complaints associated with menstruation. Preventing adverse effects on female hormones has highlighted the need for the discovery of substances that do not affect female hormones, while still relieving menstrual symptoms. In this clinical study, neither changes in female hormone levels nor medical problems were observed after ingestion of VENETRON®. Therefore, VENETRON® is considered to be a safer option for improving complaints related to menstruation.

CONCLUSION

In this study, a randomized, double-blind, placebo-controlled parallel-group comparison study was performed in healthy female subjects, in which tablets containing 50 mg of *Apocynum venetum* leaf extract (VENETRON®) per day were ingested for one menstrual cycle. Results indicated that the test food significantly reduced total scores in MDQ before menstruation and during menstruation than the placebo. The scores of Autonomic Reactions, Negative Affect, Concentration, and Behavioral Change before menstruation and scores of Pain, Concentration, and Control during menstruation were significantly decreased in the test food group after ingestion of VENETRON® than in the placebo group. In OSA-MA, the results showed significant improvement in 'Initiation and maintenance of sleep' before menstruation in the test food group than in the placebo group. These results may be due to the serotonin-increasing effect of VENETRON® and the accompanying increase in melatonin. The serotonin-increasing effect has been confirmed in animal studies[5], but we did not measure serotonin level in the present study, therefore it is still uncertain whether VENETRON® increases serotonin level in women with unidentified complaints relating menstruation. This is a limitation of this study, and further research is needed to elucidate the mechanisms of improvement of unidentified complaints relating

menstruation and sleep quality before menstruation. On the other hand, the fact that neither female hormonal changes nor medically problematic changes occurred with the ingestion of VENETRON® indicates the safety of VENETRON®. In conclusion, VENETRON® can be anticipated to improve the unidentified complaints relating menstruation in healthy women and the quality of sleep, and contributes to the improvement of women's quality of life.

【Conflict of interest】 This trial was conducted by ORTHOMEDICO Inc., of which N. S. is employee. Tokiwa Phytochemical Co., Ltd., of which T. K., Y. K., and J. Y. are employees, provided the financial support and test products for this trial. T. T.（at Medical Corporation Seishinkai, Takara Clinic）declare that there is no conflict of interest.

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