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Seaberry ursolic acid improves daytime frequency and urgency of urination in healthy Japanese subjects: A randomized, placebo-controlled, double-blind trial

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ABSTRACT

Background and objective: The fruit of seaberry (*Hippophae rhamnoides*), which exerts anti-hypertensive, hypoglycemic, anti-obesity, and immunomodulatory effects, contains triterpenoids, namely ursolic acid. We previously reported that seaberry-derived ursolic acid suppressed urination frequency and anxiety related to urination in Japanese subjects; however, some subjects were patients with mild overactive bladder (OAB). Therefore, we conducted a clinical trial on the effects of ursolic acid supplementation on urination frequency and urination scores in healthy Japanese subjects.

Methods: Eighty out of 256 registered volunteers were enrolled in the present study. This was a randomized, placebo-controlled, double-blind study. All subjects were randomly allocated by the stratified block randomization method into the ursolic acid group (n=40) or placebo group (n=40). Tablets containing ursolic acid (0.2 mg/day) or placebo were administered for 8 weeks between January and March 2024. A voiding diary and the core lower urinary tract symptom score (CLSS) were the primary outcomes. The secondary outcomes were the OAB and King's Health Questionnaire (KHQ). A safety analysis measured blood, urine, and other body parameters.

Results: The per-protocol set comprised 35 subjects in the ursolic acid group and 32 in the placebo group. Daytime urination frequency significantly decreased after 3 weeks of ursolic acid supplementation, and a significant correlation between the number of daytime urinations and time was detected in the ursolic acid group. No significant differences were observed in other scores or parameters in the safety analysis.

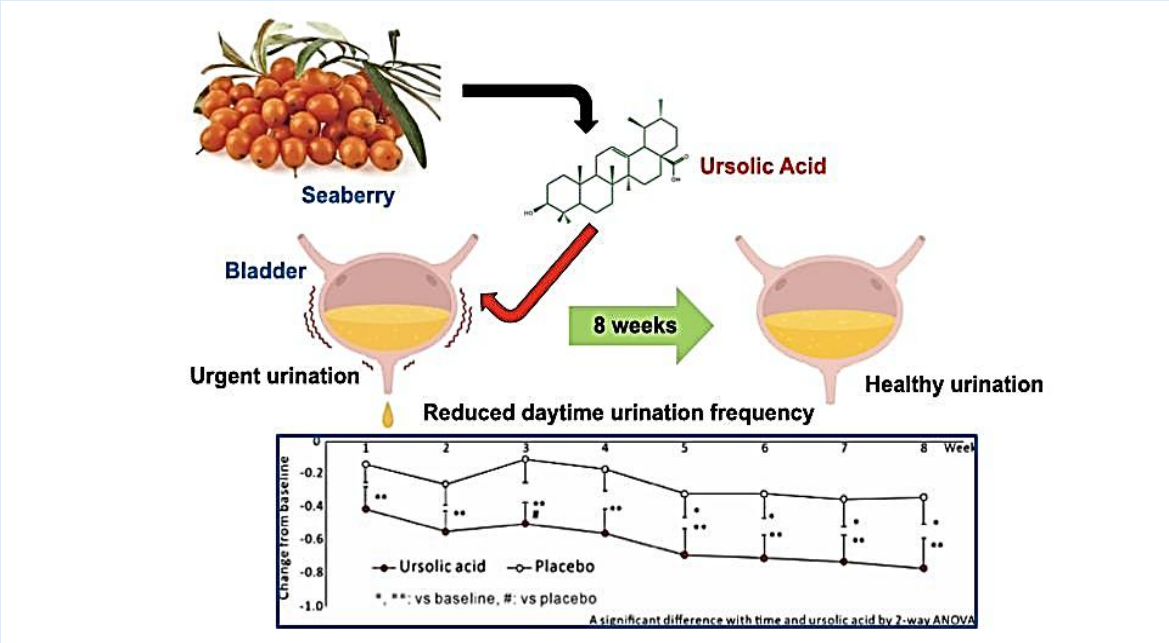
Conclusions: The intake of ursolic acid (0.2 mg/day) reduced daytime urination frequency and urgency. Therefore, seaberry ursolic acid can potentially manage urgent urination in healthy subjects.

Novelty: This study presents the novel finding that a low-dose (0.2 mg/day) ursolic acid supplementation derived from seaberry significantly reduces daytime urination frequency after 3 weeks and the urgency of urination in a randomized, placebo-controlled, double-blind clinical trial conducted explicitly on healthy Japanese adults.

Trial Registration: UMIN-CTR: UMIN000052848

Foundation: Oryza Oil & Fat Chemical Co., Ltd.

Keywords: ursolic acid; seaberry; urination; overactive bladder; core lower urinary tract symptom score; King's health questionnaire; bioactive compounds



Graphical Abstract: Seaberry ursolic acid improves daytime frequency and urgency of urination in healthy Japanese subjects

INTRODUCTION

A sense of urinary urgency characterizes overactive bladder (OAB) and is typically accompanied by frequent urination and nocturia with or without urge incontinence [1]. It is

caused by overactive detrusor muscles (hypercontractility). According to a survey conducted in 2002, OAB affects 8.3 million individuals in Japan [2]; however, very few receive medical treatment due to embarrassment about discussing urinary incontinence in a hospital [3]. A recent study reported that chronic hypercontraction increased the concentration of growth factors that induce fibrosis in bladder cells, resulting in the degeneration of muscle fibers that promote contraction and the expression of stress-induced fibers [4]. In OAB patients, capable urine accumulation is only 40% of the maximum bladder volume and, thus, causes urgent urination [5]. Besides OAB, dysfunctional voiding is a serious issue for women. According to the International Continence Society definition, dysfunctional voiding is characterized by an intermittent and/or fluctuating flow rate due to involuntary intermittent contractions of the periurethral striated or levator muscles during voiding in neurologically normal women [6]. On the other hand, in the case of non-morbid frequent urination, nighttime urination, particularly nocturia, is associated with age-related physical changes. The circadian clock system plays a role in micturition rhythm during sleep [7]. A recent study showed that nocturia was related to sleep-disordered breathing [8]. Therefore, frequent urination during the sleep period suggests a more serious disease status for sleep disturbances and may also be associated with cardiovascular diseases.

To ensure clarity and alignment with established scientific standards, it is imperative to define the terms 'functional food' and 'bioactive compound' clearly according to the Functional Food Center (FFC) guidelines. The FFC defines functional foods as natural or processed food products containing biologically active compounds that, in specified, effective, and non-toxic amounts, provide a clinically proven health benefit for the diagnosis, mitigation, treatment, or prevention of disease, thereby enhancing health and wellness and/or reducing disease risk [9-10]. Bioactive compounds are core components of functional foods. These non-nutritive substances present in foods are recognized for their advantageous physiological effects [11]. Seaberry (Hippophae rhamnoides) fruit is recognized as a rich source of various bioactive compounds, including triterpenoids such as ursolic acid, which are believed to contribute to the diverse health-promoting effects [9, 12]. Ursolic acid is a triterpenoid that is present in seaberries and other fruits. We previously demonstrated that seaberry-derived ursolic acid (0.18 and 0.36 mg/day) improved the scores of several items in King's Health Questionnaire (KHQ) related to urination and the OAB syndrome score (OABSS) for urination urgency [13,14] and frequency [15] in Japanese subjects, including patients with mild OAB. In a previous trial, a stratified analysis excluding subjects with overactive bladder showed that 0.18 mg/day ursolic acid compared to placebo significantly improved the change in urinary frequency at week 8. The quantity of Seaberry Extract-P administered in the present study was consistent with that used in the previous trial. However, the ursolic acid content differed slightly, at 0.2 mg/day in this study compared to 0.18 mg/day in the prior trial. This dosage is approximately equivalent to the ursolic acid content found in five seaberry fruits. The mechanism by which ursolic acid exerts its effects on urination involves suppressing bladder smooth muscle contractile responses [16]. Therefore, ursolic acid has been suggested to mitigate urinary anxiety. This research aims to evaluate the potential of seaberry-derived ursolic acid, a key bioactive compound present in a food source with established functional properties, to improve specific aspects of urinary health in a healthy population. We herein investigated the effects of ursolic acid on urination parameters in healthy Japanese subjects.

MATERIALS AND METHODS

Participants and allocation: The present study was

conducted as a randomized, double-blind, parallel-group controlled method. The protocol (HR-2023-OY05) was approved by the Committee of Ueno Asagao Clinic (Tokyo, Japan) on November 1, 2023. To recruit subjects, 256 volunteers underwent preliminary check-ups, including blood and urine tests, which were applied as day 0 tests (baseline). They also filled in a self-assessment sheet of urination for 7 days, starting the day after baseline, and sent it to TES Holdings, Inc.

According to the following inclusion and exclusion criteria, 80 subjects were selected and provided with a written agreement to participate. Inclusion criteria were as follows:

1) Japanese males and females aged 40 to 64 years old.
2) Healthy, with no history of disease and no current medications
3) Daytime urination frequency of approximately 8 to 10 times.
4) Nighttime urination frequency of 0 or 1 time.

Exclusion criteria were as follows:

1) Currently receiving any medication or regularly using medicines or quasi drugs.
2) Suffering, medicating, or a history of diabetes, kidney or liver failure, heart diseases, thyroid gland diseases, adrenal gland diseases, and other metabolic diseases.
3) A history of heart failure, kidney failure, or type B or C hepatitis.
4) Presence of comorbidities or a history of severe diseases in the digestive tract
5) Prostate-specific antigen (PSA) level ≥4.0 ng/mL.
6) Frequent nighttime urination, benign prostatic hyperplasia (BPH), or OAB.
7) Meet the following criteria for OAB.
   a) Subjects at risk of OAB with ≥2 points in question 3 of the OABSS and a total OABSS score ≥3.
   b) A total OABSS score ≥6.
8) Take medicine to treat diseases within the last month, except PRN, such as for a cold or headache.
9) Body mass index (BMI) ≥30 kg/m^2.
10) Impossible to refrain from alcohol drinking
11) At risk of allergic symptoms
12) Taking or planning to start dietary supplements in the last month.
13) A lifestyle change in the near future, such as taking a long trip during the test period.
14) Pregnant, expecting, or lactating
15) Currently participating in other clinical trials or participated in a trial in the past 3 months.
16) Working in industries producing or selling dietary supplements or cosmetics, including family members.
17) Judged by a physician as not applicable for the intervention.

Subjects were allocated into two groups by the stratified block randomization method based on age, sex, and daytime urination frequency in CLSS [17]. No significant differences were observed between the groups. Allocations were concealed from subjects and study personnel until all outcome data were obtained. Before the initiation of the study, the physician and study staff obtained written informed consent. They thoroughly explained to subjects that they were free to participate or drop out with no risk of disadvantage. All subjects agreed to participate freely and signed the agreement sheet. Subjects were asked to comply with the following:

1) Avoid an irregular lifestyle, including a lack of sleep and excessive eating and drinking.
2) Maintain their usual eating, sleeping, and exercise habits.
3) Refrain from alcohol consumption and taking other dietary supplements.

4) Maintain their usual coffee, tea, and green tea consumption.
5) Avoid energy drinks, tonics, and nourishing beverages.
6) Refrain from excessive exercise.
7) Avoid taking medicines, including quasi drugs, and Kampo medicines.
8) Avoid blood donations.
9) Other behaviors that may affect the results of the study.

In addition, alcohol and food, except water, were prohibited 12 hours before the examination.

Outcomes: The primary outcomes were assessed using indices for urination, including CLSS and a voiding diary [18]. Daytime and nighttime urination frequencies, urgent urination, and the amount of water consumed were recorded in the voiding diary. The OAB questionnaire (OAB-q) [19] and KHQ [20] were selected as the secondary outcomes. The safety analysis measured blood pressure, heart rate, body weight, body fat ratio, and BMI. Blood and urinary parameters were also examined.

Preparation of test tablets: Test supplements (indistinguishable brown tablets containing ursolic acid or placebo) were provided by Oryza Oil & Fat Chemical Co., Ltd. The active tablet contained 0.1 mg of ursolic acid (100 mg of Seaberry Extract-P, seaberry extract powder made by Oryza Oil & Fat Chemical Co., Ltd.). The composition of the active tablet was 100 mg Seaberry Extract-P, 104.5 mg maltitol, 45 mg cellulose, 4.5 mg silicon dioxide, 6 mg calcium stearate, 9 mg caramel, and 31 mg pregelatinized starch. The placebo tablet contained 100 mg maltodextrin instead of Seaberry Extract-P. Maltodextrin was used as the placebo because Seaberry Extract-P comprises seaberry extract and maltodextrin.

Oryza Oil & Fat Chemical Co., Ltd. provided test tablets with identification codes on the packages to TES Holdings Inc. Identification codes were strictly concealed and sent to the allocation controller at TES Holdings Inc. The allocation controller confirmed that the tablets were indistinguishable and changed the identification codes to another sample control number on the sheet as a blind process. These numbers were strictly sealed with a sheet and locked until opened.

Study protocol: This was a placebo-controlled, double-blind, parallel-group study registered with the University Hospital Medical Information Network (UMIN000052848). The present study was performed at Hasegawa Hospital (Medical Corporation Seishukai, Tokyo, Japan) and analyzed by TES Holdings Inc., as well as subjects in the placebo group. The ursolic acid group took two tablets after a meal with water. The daily amount of ursolic acid was 0.2 mg. All subjects took the study tablets for 8 weeks and then completed CLSS, OABq, and KHQ. Subjects also underwent a medical check-up, including a safety analysis with blood and urine parameters.

Ethics, adherence, and compliance: The present study was performed in accordance with the Declaration of Helsinki (2013 revision) and “Ethical guidelines for medical and health research involving human subjects” (Ministry of Education, Culture, Sports and Technology and Ministry of Health and Labor), and was conducted in conformity with the ethical considerations of the Ethics Committee of TES Holdings Inc. Any substantial deviations from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before giving consent to participate.

Laboratory tests: BML Inc. (Tokyo, Japan) performed blood and urine analyses. All items were examined at baseline and after 8 weeks of intake. A venous blood sample was collected from an arm vein, and the following tests were performed for the safety analysis.

The following hematology components were assessed: the red blood cell count, leukocyte count, hemoglobin (Hb), hematocrit, and platelet count. The following biochemical components were examined: protein, albumin, total bilirubin, urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, HbA1c, blood glucose, creatine kinase (CPK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ–glutamyl transferase (γ–GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Na, K, and Cl.

Urine samples were collected to qualitatively evaluate protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood.

Statistical analysis: Each value was reported as the mean and SE. A 2-way ANOVA analyzed urination frequency. Regarding urination frequency, a paired *t*-test and an independent *t*-test were used to compare baseline and post-intervention values and between the groups, respectively. CLSS, OAB-q, and KHQ were analyzed by the Wilcoxon signed-rank test to compare values at baseline and after 8 weeks of intake or by the Mann-Whitney U-test for comparisons among groups. Blood parameters were analyzed by the paired *t*-test for comparisons of values at baseline and after 8 weeks of intake or by an independent *t*-test for comparisons among groups. Urine parameters were analyzed by the Mann-Whitney U test. SAS (SAS9.4, SAS Institute Inc.) and SPSS (Statistics 26, Japan IBM) were used for these analyses.

RESULTS

Study performance: The present study was performed between January and March 2024. During the study period, one subject in the placebo group was unable to continue for personal reasons (Fig. 1). The safety analysis used a full analysis set (FAS) after omitting data from this subject at 8 weeks. Regarding the primary and secondary outcomes, 7 subjects in the placebo group and 5 in the ursolic acid group were excluded from the per protocol analysis (PPS) (Fig. 1). These subjects included 3 who exceeded their daily water intake by 150%, 3 suspected of a nighttime urination disease, and four suspected of OAB. Accordingly, 35 subjects (53.5±1.2 years) were available for analysis in the ursolic acid group and 32 (53.8±1.0 years) in the placebo group (Table 1).

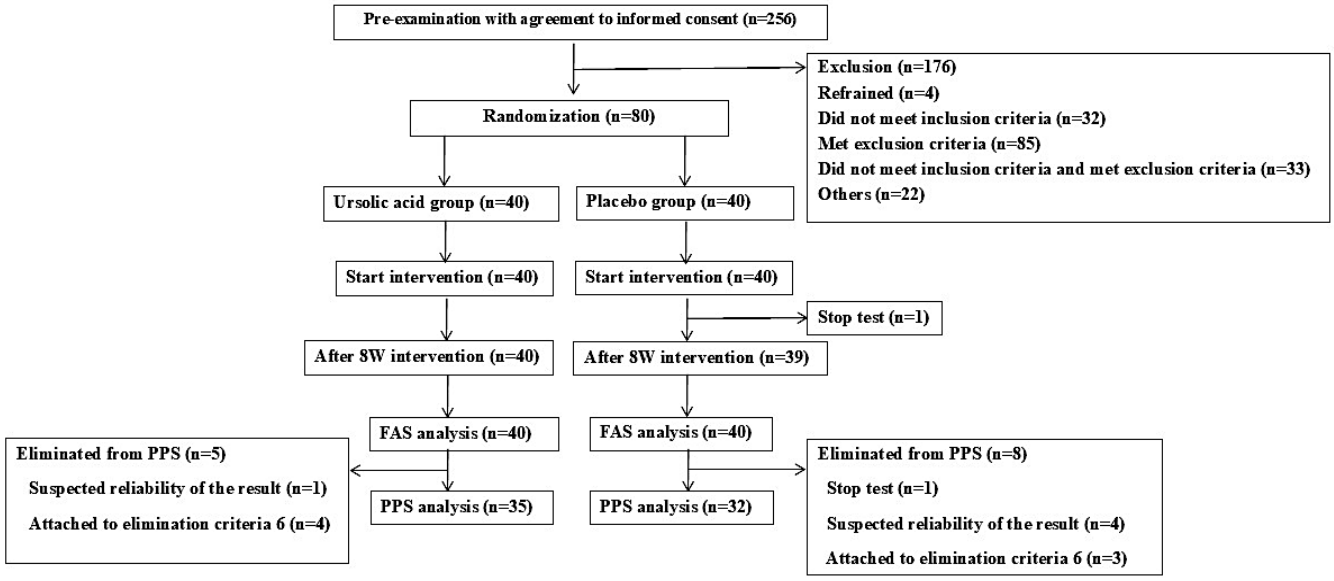


Figure 1. Flow chart of the study.

Table 1. Profiles of subjects

	Ursolic acid	Placebo
Male	10	10
Female	25	22
Age	53.5±1.2	53.8±1.0
Daytime CLS score	1.1±0.1	1.1±0.1
Systolic blood pressure (mmHg)	116.9±2.1	121.8±1.9
Diastolic blood pressure (mmHg)	74.4±1.5	76.9±1.5
Heart rate (beats/min)	73.5±1.8	73.4±1.8

Mean ± SE without significant differences in the groups. Age and daytime CLS scores were examined using PPS (n=35 for ursolic acid, n=32 for placebo). Blood pressure and pulse measured after the 8-week intervention using FAS (n=40). No significant differences were detected by an independent *t*-test among the groups.

CLSS

No significant between-group differences were observed in CLSS parameters. However, nighttime urination frequency, "incontinence when coughing, sneezing, and exercise", and the constant urge to urinate significantly improved from baseline in the ursolic acid group (Table 2).

Table 2. Changes in CLSS by intervention.

		Ursolic acid	Placebo
How many times did you urinate?			
1. Daytime	0 W	1.1 ± 0.1	1.1 ± 0.1
	8 W	0.9 ± 0.1	1.0 ± 0.1
2. Nighttime	0 W	0.8 ± 0.1	0.7 ± 0.1
	8 W	0.5 ± 0.1**	0.6 ± 0.1
3. Impatient to urinate	0 W	1.0 ± 0.1	1.1 ± 0.1
	8 W	0.4 ± 0.1**	0.3 ± 0.1**
4.Incontinence	0 W	0.2 ± 0.1	0.3 ± 0.1
	8 W	0.1 ± 0.0	0.1 ± 0.0*
5. Incontinence when coughing, sneezing, and exercising	0 W	0.4 ± 0.1	0.4 ± 0.1
	8 W	0.2 ± 0.1**	0.3 ± 0.1
6. Slow urination speed	0 W	0.8 ± 0.1	0.5 ± 0.1
	8 W	0.7 ± 0.2	0.5 ± 0.1
7. Require the abdominal muscles for urination	0 W	0.6 ± 0.1	0.4 ± 0.1
	8 W	0.5 ± 0.1	0.4 ± 0.1
8. Constant urge to urinate	0 W	1.0 ± 0.1	0.8 ± 0.1
	8 W	0.7 ± 0.1**	0.6 ± 0.1
9. Feel bladder pain	0 W	0.1 ± 0.1	0.1 ± 0.0
	8 W	0.1 ± 0.0	0.0 ± 0.0
10. Feel urinary tract pain	0 W	0.0 ± 0.0	0.0 ± 0.0
	8 W	0.0 ± 0.0	0.0 ± 0.0
Total CLSS	0 W	6.0 ± 0.4	5.5 ± 0.5
	8 W	4.0 ± 0.4**	3.6 ± 0.4**

Mean ± SE (n=35 for ursolic acid, n=32 for placebo) without significant differences in the groups. Asterisks denote significant differences from the baseline value at *: $p<0.05$, **: $p<0.01$ detected by the Wilcoxon signed-rank test. No significant differences were detected between the groups by the Mann-Whitney U test.

Voiding diary: Daytime urination frequency was significantly lower after 1 to 8 weeks of intake than at baseline in the ursolic acid group (Fig. 2), while a significant decrease was only observed in the placebo group after 5 to 8 weeks of intake. Nighttime urination frequency was significantly lower after 2 to 8 weeks of intake than at baseline in the ursolic acid group and after 1 to 8 weeks of intake in the placebo group.

Notably, for the changed value of daytime urination, a significant reduction was observed in the ursolic acid group by a 2-way factorial analysis of variance. In addition, a significant reduction was observed in week 3.

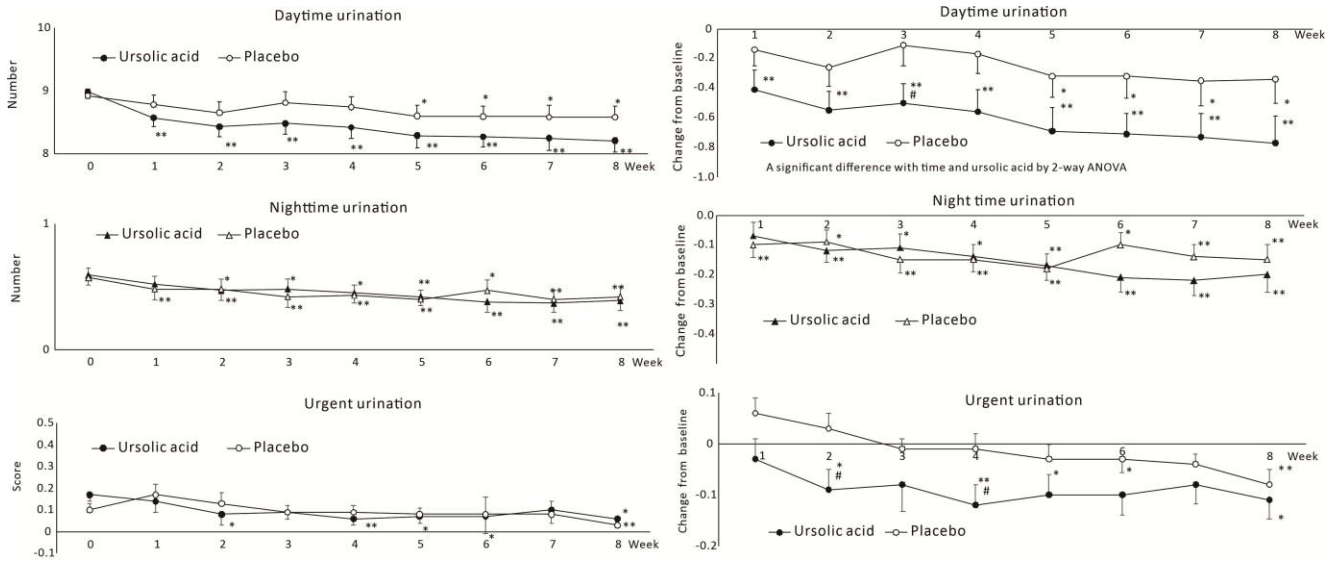


Figure 2. Changes in daytime urination, nighttime urination, and urgent urination.

Each point represents the mean ± SE (n=32 in the ursolic acid group, n=35 in the placebo group). Asterisks denote significant differences from the baseline at *: $p<0.05$, **: $p<0.01$, and ***: $p<0.001$. A significant difference from the placebo at #: $p<0.05$.

Significant differences were detected in time and daytime urination in the ursolic acid group by a 2-way ANOVA.

OAB-q: Table 3 shows the results of OAB-q. No significant differences were observed between the groups. Only "4. Little incontinence unexpectedly" was significantly improved by ursolic acid, but not by the placebo. Scores for "9. Seriously hesitated outing", "11. Tried to stay near bathroom in public space", "14. Felt unhealthy", "15. Could not sleep well", "16. Decrease in opportunity to exercise", "17. Felt tired when I woke up", "21. Tried to stay near bathroom when outing", "23. Frustrated by frequent urination", "28. Decrease in gathering or visiting family", and "32. Planned more carefully for behavior" were increased by ursolic acid, but not by the placebo.

Table 3. Changes in OAB-q by the intervention.

		Ursolic acid	Placebo
1. Frequent daytime urination	0 W	2.9 ± 0.2	2.8 ± 0.2
	8 W	2.0 ± 0.1**	2.1 ± 0.2**
2. Uncomfortable due to urgent urination	0 W	2.6 ± 0.2	2.4 ± 0.2
	8 W	1.8 ± 0.1**	1.8 ± 0.2*
3. Sudden unexpected desire to urinate	0 W	2.0 ± 0.2	2.1 ± 0.2

		Ursolic acid	Placebo
	8 W	1.4 ± 0.1**	1.7 ± 0.2*
4. A little incontinence unexpectedly	0 W	1.7 ± 0.2	1.6 ± 0.1
	8 W	1.3 ± 0.1*	1.4 ± 0.1
5. Nighttime urination	0 W	2.6 ± 0.2	2.3 ± 0.2
	8 W	2.1 ± 0.2	1.9 ± 0.2
6. Wake up for urination during the sleep	0 W	2.5 ± 0.2	2.3 ± 0.2
period	8 W	2.3 ± 0.2	2.0 ± 0.2
7. Impatient for urination	0 W	2.3 ± 0.2	2.3 ± 0.2
	8 W	1.5 ± 0.1**	1.5 ± 0.1**
8.Impatient incontinence	0 W	1.4 ± 0.2	1.4 ± 0.1
	8 W	1.1 ± 0.1	1.1 ± 0.1
9. Seriously, I hesitated outing	0 W	4.5 ± 0.3	5.2 ± 0.2
	8 W	5.1 ± 0.2*	5.3 ± 0.2
10. Felt sleepy in the daytime	0 W	4.3 ± 0.2#	4.9 ± 0.2
	8 W	5.0 ± 0.2**	5.2 ± 0.2*
11. Tried to stay near bathroom in public	0 W	4.1 ± 0.3	4.8 ± 0.2
space	8 W	5.1 ± 0.2**	5.0 ± 0.2
12. Felt painful	0 W	5.0 ± 0.2	5,3 ± 0.2
	8 W	5.7 ± 0.1**	5.7 ± 0.1*
13. Frustrated	0 W	5.4 ± 0.2	5.4 ± 0.2
	8 W	5.7 ± 0.1	5.7 ± 0.1
14. Felt unhealthy	0 W	5.4 ± 0.2	5.4 ± 0.1
	8 W	5.8 ± 0.1**	5.7 ± 0.1
15. Could not sleep well	0 W	4.5 ± 0.3	5.0 ± 0.2
	8 W	4.9 ± 0.2*	5.2 ± 0.2
16. Decrease in opportunity to exercise	0 W	5.4 ± 0.2	5.7 ± 0.1
	8 W	5.8 ± 0.1*	5.8 ± 0.1
17. Felt tired when I woke up	0 W	4.5 ± 0.2	4.7 ± 0.2
	8 W	5.1 ± 0.1**	5.1 ± 0.2
18. Got frustrated with family or friends	0 W	5.8 ± 0.1	5.8 ± 0.1
	8 W	5.9 ± 0.0	5.9 ± 0.0*
19. Got frustrated or worried	0 W	5.5 ± 0.1	5.2 ± 0.2
	8 W	5.5 ± 0.1	5.6 ± 0.1*
20. Increasingly staying home in spite of the desire to go out	0 W	5.7 ± 0.1	5.8 ± 0.1
	8 W	5.8 ± 0.1	5.8 ± 0.1
21. Tried to stay near bathroom when outing	0 W	4.8 ± 0.2	5.1 ± 0.2
	8 W	5.4 ± 0.2**	5.5 ± 0.2
22. Do not go out without a bathroom nearby	0 W	5.3 ± 0.2	5.3 ± 0.2
	8 W	5.5 ± 0.2	5.7 ± 0.1*
23. Frustrated by frequent urination	0 W	4.7 ± 0.2	4.8 ± 0.2
	8 W	5.5 ± 0.1**	5.3 ± 0.1

		Ursolic acid	Placebo
24. Woke up during sleep	0 W	4.2 ± 0.2	4.5 ± 0.2
	8 W	4.5 ± 0.2	4.8 ± 0.2
25. Worried about smell and hygiene	0 W	5.5 ± 0.1	5.4 ± 0.2
	8 W	5.7 ± 0.1*	5.7 ± 0.1*
26. Felt sorry because of the need to go to the bathroom when going out with friends	0 W	4.9 ± 0.2	5.0 ± 0.2
	8 W	5.4 ± 0.2**	5.5 ± 0.1*
27. Affected relationship with family or friends	0 W	5.7 ± 0.1	5.8 ± 0.1
	8 W	5.9 ± 0.1	5.9 ± 0.1
28. Decrease in gathering or visiting family and friends	0 W	5.6 ± 0.1	5.8 ± 0.1
	8 W	5.9 ± 0.1*	5.9 ± 0.1
29. Ashamed	0 W	5.7 ± 0.1	5.8 ± 0.1
	8 W	5.9 ± 0.1	5.9 ± 0.0
30. Could not get enough time to sleep	0 W	5.0 ± 0.3	5.2 ± 0.2
	8 W	5.3 ± 0.2*	5.6 ± 0.1*
31. Causes a problem with a partner	0 W	5.9 ± 0.1	5.9 ± 0.1
	8 W	6.0 ± 0.0	6.0 ± 0.0
32. Planned more carefully for behavior	0 W	5.1 ± 0.2	5.6 ± 0.1
	8 W	5.5 ± 0.2**	5.8 ± 0.1
33. Checked the place of the bathroom immediately when got to the unknown place	0 W	4.6 ± 0.3	4.7 ± 0.2
	8 W	5.3 ± 0.2**	5.4 ± 0.1*

Each value represents the mean ± SE (n=32 for the ursolic acid group, n=35 for the placebo group). The Wilcoxon signed-rank test was performed for comparisons with values at 0 w and significant levels are at *: $p<0.05$, **: $p<0.01$. A significant difference between the groups detected by the Mann-Whitney U test is indicated as #: $p<0.05$.

KHQ: The ursolic acid group showed a significantly higher value for "Q7a. Does your bladder problem affect your sleep?" compared to the placebo group (Table 4). However, the value in the ursolic acid group remained at baseline. Comparisons between values with the intervention and at baseline revealed significant decreases for Q3a, Q4b, and Q6a in the ursolic acid group, but not in the placebo group.

Table 4. Changes in KHQ by the intervention.

		Ursolic acid	Placebo
Q1. How would you describe your health at present?	0 W	1.9 ± 0.1	2.0 ± 0.1
	8 W	2.0 ± 0.1	2.0 ± 0.1
Q2. How much do you think your bladder problem affects your life?	0 W	2.3 ± 0.1	2.1 ± 0.1
	8 W	1.8 ± 0.1**	1.7 ± 0.1**
Q3a. Does your bladder problem affect your household tasks?	0 W	1.4 ± 0.1	1.5 ± 0.1
	8 W	1.2 ± 0.1*	1.3 ± 0.1
Q:3b. Does your bladder problem affect your	0 W	2.0 ± 0.2	1.9 ± 0.1

		Ursolic acid	Placebo
job or your daily activities outside the home?	8 W	1.6 ± 0.1*	1.6 ± 0.1*
Q4a. Does your bladder problem affect your physical activities?	0 W	1.5 ± 0.1	1.7 ± 0.1
	8 W	1.4 ± 0.1	1.4 ± 0.1*
Q4b. Does your bladder problem affect your ability to travel?	0 W	2.0 ± 0.2	2.1 ± 0.2
	8 W	1.7 ± 0.1*	1.8 ± 0.1
Q4c. Does your urination problem affect your public communication?	0 W	1.4 ± 0.1	1.3 ± 0.1
	8 W	1.2 ± 0.1	1.2 ± 0.1
Q4d. Does your urination problem affect your relationship with your friends or when visiting them?	0 W	1.4 ± 0.1	1.4 ± 0.1
	8 W	1.2 ± 0.1	1.2 ± 0.1
Q5a. Does your bladder problem affect your relationship with your partner?	0 W	1.1 ± 0.1	1.1 ± 0.1
	8 W	1.0 ± 0.0#	1.1 ± 0.1
Q5b. Does your bladder problem affect your sex life?	0 W	1.3 ± 0.1#	1.1 ± 0.1
	8 W	1.1 ± 0.1	1.0 ± 0.0
Q5c. Does your bladder problem affect your family life?	0 W	1.2 ± 0.1	1.3 ± 0.1
	8 W	1.1 ± 0.1	1.2 ± 0.1
Q6a. Does your bladder problem make you depressed?	0 W	1.7 ± 0.1	1.6 ± 0.2
	8 W	1.3 ± 0.1*	1.4 ± 0.1
Q6b. Does your bladder problem make you feel anxious or nervous?	0 W	1.9 ± 0.1	2.0 ± 0.2
	8 W	1.8 ± 0.1	1.6 ± 0.1**
Q6c. Does your bladder problem make you feel bad about yourself?	0 W	1.5 ± 0.1	1.8 ± 0.2
	8 W	1.4 ± 0.1	1.3 ± 0.1*
Q7a. Does your bladder problem affect your sleep?	0 W	1.9 ± 0.1	1.8 ± 0.1
	8 W	1.9 ± 0.1#	1.6 ± 0.1*
Q7b. Does your bladder problem make you feel worn out and tired?	0 W	1.6 ± 0.1	1.6 ± 0.1
	8 W	1.5 ± 0.1	1.3 ± 0.1
Q8a. Do you wear pads to stay dry?	0 W	1.3 ± 0.1	1.4 ± 0.1
	8 W	1.2 ± 0.1	1.3 ± 0.1
Q8b. Are you careful about how much fluid you drink?	0 W	2.2 ± 0.2	2.1 ± 0.1
	8 W	1.8 ± 0.1*	1.8 ± 0.1*
Q8c. Do you change your underclothes because they get wet?	0 W	1.2 ± 0.1	1.3 ± 0.1
	8 W	1.1 ± 0.1	1.2 ± 0.1
Q8d. Do you worry in case you smell?	0 W	1.3 ± 0.1	1.4 ± 0.1
	8 W	1.3 ± 0.1	1.3 ± 0.1
Q8e. Do you embarrassed because of your bladder problem?	0 W	1.3 ± 0.1	1.3 ± 0.1
	8 W	1.2 ± 0.1	1.2 ± 0.1

Each value represents the mean ± SE (n=32 for the ursolic acid group, n=35 for the placebo group). The Wilcoxon signed-rank test was performed for comparisons with values at 0 w and significant levels are at *: $p<0.05$, **: $p<0.01$. A significant difference between the groups detected by the Mann-Whitney U test is indicated as #: $p<0.05$.

Laboratory data and adverse effects*:* Table 1 shows blood pressure and heart rate. Tables 5 and 6 show hematology and blood biochemical parameters. No significant differences were observed in hematology parameters

between the groups. LDH was significantly lower among blood biochemical parameters after the ursolic acid intervention. Urinalysis parameters showed no changes in either group (Table 7). The intervention did not cause any adverse effects.

Table 5. Changes in hematology parameters.

	Baseline	After 8 weeks of intake	Standard value
Red blood cells (×10⁴ cells/㎕)			
Ursolic acid	457 ± 7	458 ± 6	M: 438-577
Placebo	458 ± 5	462 ± 5	F: 376-516
Leukocytes (cells/㎕)			
Ursolic acid	5754 ± 209	5007 ±2 17**	3500-9700
Placebo	5646 ± 211	5119 ± 194**	
Hemoglobin (g/dL)			
Ursolic acid	13.9 ± 0.2	13.8 ± 0.2	M: 13.6-18.3
Placebo	13.7 ± 0.2	13.8 ± 0.2	F: 11.2-15.2
Hematocrit (%)			
Ursolic acid	43.1 ± 0.5	41.6 ± 0.5**	M: 40.4-51.9
Placebo	43.1 ± 0.4	41.7 ± 0.4**	F: 34.3-45.2
Platelets (×10⁴ cells/㎕)			
Ursolic acid	28.4 ± 0.9	28.3 ± 1.0	14.0-37.9
Placebo	26.8 ± 0.7	26.7 ± 0.9	

Values are represented as the mean with the SE (n=40). No significant difference was observed between the groups. Asterisks denote significant differences from the baseline value at *: $p<0.05$, **: $p<0.01$ detected by the paired *t*-test.

Table 6. Changes in blood biochemical parameters.

	Baseline	After 8 weeks of intake	Standard value
Total protein (g/dL)			
Ursolic acid	7.3 ± 0.0	7.2 ± 0.1*	6.5-8.2
Placebo	7.4 ± 0.1	7.3 ± 0.1	
Albumin (g/L)			
Ursolic acid	4.5 ± 0.0	4.4 ± 0.0*	3.8-5.2
Placebo	4.5 ± 0.0	4.4 ± 0.0*	
Total bilirubin (mg/dL)			
Ursolic acid	0.8 ± 0.0	0.7 ± 0.0*	0.3-1.2
Placebo	0.7 ± 0.0	0.7 ± 0.0	
Urea N (mg/dL)			
Ursolic acid	12.7 ± 0.6	12.8 ± 0.5	8.0-20.0
Placebo	12.0 ± 0.5	13.1 ± 0.4*	
Creatinine (mg/dL)			

	Baseline	After 8 weeks of intake	Standard value
Ursolic acid	0.69 ± 0.02	0.70 ± 0.02	M:0.65-1.09
Placebo	0.72 ± 0.02	0.74 ± 0.02	F: 0.46-0.82
Uric acid (mg/dL)			
Ursolic acid	4.8 ± 0.2	4.9 ± 0.2	M:3.6-7.0
Placebo	4.9 ± 0.2	5.0 ± 0.2*	F:2.7-7.0
Total cholesterol (mg/dL)			
Ursolic acid	225 ± 6	222 ± 5	150-219
Placebo	221 ± 5	226 ± 6	
LDL-cholesterol (mg/dL)			
Ursolic acid	126 ± 4	126 ± 4	70-139
Placebo	125 ± 4	129 ± 4	
HDL-cholesterol (mg/dL)			
Ursolic acid	79 ± 3	75 ± 3*	M:40-80
Placebo	74 ± 3	74 ± 4	F:40-90
Triglycerides (mg/dL)			
Ursolic acid	84 ± 7	84 ± 7	50-149
Placebo	91 ± 10	96 ± 14	
HbA1c (%)			
Ursolic acid	5.3 ± 0.1	5.4 ± 0.1**	4.6-6.2
Placebo	5.3 ± 0.0	5.4 ± 0.0**	
Blood glucose (mg/dL)			
Ursolic acid	89 ± 1	94 ± 1**	70-109
Placebo	91 ± 1	93 ± 1*	
AST (U/L)			
Ursolic acid	23 ± 1	22 ± 1	10-40
Placebo	23 ± 1	23 ± 1	
ALT (U/L)			
Ursolic acid	21 ± 1	21 ± 1	5-45
Placebo	20 ± 1	20 ± 1	
CPK (U/L)			
Ursolic acid	114 ± 13	105 ± 9	M:50-230
Placebo	117 ± 8	127 ± 12	F: 50-210
□-GTP (U/L)			
Ursolic acid	23 ± 2	25 ± 3	Male: ≤79
Placebo	27 ± 3	28 ± 4	Female: ≤48
ALP (U/L)			

	Baseline	After 8 weeks of intake	Standard value
Ursolic acid	70 ± 3	69 ± 3	38-113
Placebo	72 ± 3	73 ± 3	
LDH (U/L)			
Ursolic acid	178 ± 6	167 ± 5**, #	120-245
Placebo	188 ± 4	181 ± 4*	
Na^+ (mEq/L)			
Ursolic acid	140 ± 0	141 ± 0	135-145
Placebo	141 ± 0	141 ± 0	
K^+ (mEq/L)			
Ursolic acid	4.3 ± 0.1	4.3 ± 0.1	3.5-5.0
Placebo	4.3 ± 0.1	4.3 ± 0.1	
Cl (mEq/L)			
Ursolic acid	102 ± 0	104 ± 0**	98-108
Placebo	102 ± 0	104 ± 0**	

Each value represents the mean ± SE (n=40). The paired t-test was performed for comparisons of 8 w values with values at 0 w, and significant levels are at *: $p<0.05$, **: $p<0.01$. A significant difference from the placebo at #: $p<0.05$ was detected by an independent t-test.

Table 7. Changes in urine parameters.

	Week	Ursolic acid	Placebo
Protein	0	0.01 ± 0.01	0.01 ± 0.01
	8	0.06 ± 0.03	0.03 ± 0.02
Glucose	0	0.0 ± 0.0	0.0 ± 0.0
	8	0.0 ± 0.0	0.0 ± 0.0
Urobilinogen	0	0.0 ± 0.0	0.0 ± 0.0
	8	0.0 ± 0.0	0.0 ± 0.0
Bilirubin	0	0.0 ± 0.0	0.0 ± 0.0
	8	0.0 ± 0.0	0.0 ± 0.0
pH	0	6.38 ± 0.11	6.15 ± 0.12
	8	6.20 ± 0.11	6.31 ± 0.13
Occult blood	0	0.19 ± 0.07	0.04 ± 0.02
	8	0.20 ± 0.07	0.21 ± 0.09
Ketone bodies	0	0.1 ± 0.1	0.1 ± 0.1
	8	0.0 ± 0.0	0.0 ± 0.0
Specific gravity	0	1.0159 ± 0.0012	1.0150 ± 0.0011
	8	1.0160 ± 0.0011	1.0144 ± 0.0012

Data are represented as the mean ± SE (n=40). No significant differences were observed between the groups.

DISCUSSION

The present study investigated whether the continuous intake of seaberry-derived ursolic acid (0.2 mg/day) improved the quality of urination, particularly its frequency. This was a randomized, double-blind, placebo-controlled, parallel-group study performed on healthy men and women aged 40 to 64 years who were dissatisfied with the frequency of urination during the day or night. The primary outcomes were CLSS and a voiding diary; the secondary outcomes were OAB-q and KHQ. The results showed no significant differences in CLSS between the groups. At the same time, daytime urination frequency (averaged over 7 days) in the voiding diary was significantly lower in the ursolic acid group than in the placebo group after 3 weeks of intake. In addition, a significant reduction was detected in the ursolic acid group by a 2-way ANOVA of the entire period. These results imply that the intake of ursolic acid reduced urination frequency. Additionally, daytime urination frequency was lower after 4, 5, 6, and 8 weeks of the ursolic acid intervention. On the other hand, although no significant differences were noted in nighttime urination frequency, it was slightly lower in the ursolic acid group than in the placebo group after 6 weeks of intake. Furthermore, urgent urination (throughout the day) was significantly lower in the ursolic acid group than in the placebo group after 2 and 4 weeks of intake.

Therefore, based on the primary outcomes, no significant difference was observed in CLSS, whereas daytime urination frequency in the voiding diary significantly decreased. This result is consistent with our previous findings showing that ursolic acid (0.18 mg/day) reduced urination frequency after 8 weeks of intake [16]. In this study, subjects presented with a baseline of approximately 8 to 10 daytime urination frequencies and 0 or 1 nighttime urination frequency. Therefore, the observed effects were specific to daytime urinary frequency. Considering the potential mechanism of effect in suppressing overactive bladder symptoms, future studies in subjects with higher nighttime urination frequency may lead to further insights.

OAB-q, as the secondary outcome, did not significantly differ between the groups. We previously reported that the intake of 0.36 mg/day of ursolic acid for 8 weeks improved scores for urination urgency, such as “How often do you have a sudden desire to urinate, which is difficult to defer?” [13-14]. Therefore, the administration of a higher dose of ursolic acid may be required to attenuate anxiety related to urgent urination. Regarding KHQ scores, a significantly higher value was observed for Q7a “Does your bladder problem affect your sleep?” with the ursolic acid intervention. However, the value in the ursolic acid group remained at baseline [13,14]. Therefore, the higher value compared to the placebo was not problematic. On the other hand, significant decreases were observed in scores related to household tasks, travel, and a depressed feeling with the ursolic acid intervention. In our previous study, the 8-week intake of ursolic acid (0.36 mg/day) improved the scores for “impact of incontinence” and “emotion”, including Q6a “Does your bladder problem make you depressed?” and Q6c “Does your bladder problem make you feel bad about yourself?”. Therefore, a higher dose of ursolic acid may be needed to attenuate the depressed feeling related to urination in KHQ and OABSS.

In the safety analysis of ursolic acid, no significant changes were observed in blood pressure, heart rate, hematological, blood biochemical, or urine parameters, or the adverse event assessment by a physician.

The observed reduction in daytime urination frequency and urgency in healthy adults following low-dose seaberry ursolic acid supplementation suggests a potential natural approach for managing these common lower urinary tract symptoms. This finding could lead to the development of dietary supplements or functional food ingredients derived from seaberry to support bladder

health and improve the quality of life for individuals experiencing frequent or urgent urination, even without a diagnosed overactive bladder. While our study utilized seaberry extract, it is reasonable to hypothesize that pure ursolic acid at the exact dosage would exhibit similar effects. However, the practical application of pure ursolic acid in food products is limited due to regulatory constraints under the Japanese Food Sanitation Act, which makes its production for such purposes either unfeasible or extremely costly. In conclusion, the intake of seaberry ursolic acid for 8 weeks reduced daytime urination frequency and urination urgency in healthy Japanese subjects.

Abbreviations: ANOVA, analysis of valance; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; BPH, benign prostatic hyperplasia; CLSS, core lower urinary tract symptom score; CPK, creatinine kinase; GTP, glutamyl transferase; HDL, high-density lipoprotein; Hb, hemoglobin; KHQ, King's Health Questionnaire; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; OAB, overactive bladder; OAB-q, OAB questionnaire; OABSS, urination and the OAB syndrome score; PPS, per protocol analysis; PSA, prostate-specific antigen; SE, standard error; TG, triglyceride

Competing Interests: The authors declare no conflicts of interest in this manuscript.

Authors' Contributions: The authors' contributions to this study are as follows: Shimizu N. was responsible for Conceptualization and Methodology; Yagihashi M. contributed to Data curation and Formal analysis; Hirano M. provided Resources; Shimizu R. contributed to Investigation, Methodology, Project administration, Resources and Visualization; Dr. Shimizu H. was responsible for Investigation and Supervision; Dr. Shimoda H. handled Writing - Original draft preparation; and Dr. Yamada S. contributed to Writing - Review and editing.

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