



Evaluation of shoseiryuto for seasonal allergic rhinitis, using an environmental challenge chamber

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ABSTRACT

Background: Complementary and alternative medicine, including Japanese traditional medicine (JTM), has been used for various allergic diseases, but the evidence is limited. Shoseiryuto (Xiao-Qing-Long-Tang), one of the representative JTM drugs, is frequently used to treat allergic rhinitis (AR). However, its efficacy for seasonal AR has not been fully established. Using an Environmental challenge chamber (ECC), we evaluated the therapeutic effects of shoseiryuto on AR induced by Japanese cedar pollen (JCP).

Methods: A placebo-controlled double-blind crossover study with shoseiryuto was conducted using the ECC. The shoseiryuto or placebo was orally administered from 2 weeks before the exposure test. The pollen exposure test was conducted for 3 h, and the pollen concentration was set at 8000 pollen/m³. The primary endpoint of the study was the total nasal symptom score (TNSS) during pollen exposure. A physician certified by the Japanese Society of Oriental Medicine as a specialist checked each participant's "pattern", a comprehensive expression of signs obtained from individual patients' subjective symptoms and other personal findings. Blood samples collected just before the first pollen exposure were stimulated with cedar antigens and used for immunological studies.

Results: The results of the 46 participants were analyzed, and no significant side effects were detected. There was no significant difference in TNSS during pollen exposure for 3 h in the ECC between the shoseiryuto and placebo groups. However, some symptoms were improved in the shoseiryuto group after leaving the ECC. There was no significant correlation between the "fluid retention pattern" and TNSS. In immunological studies, shoseiryuto did not inhibit Th2-type cytokine production and mRNA expression.

Conclusions: Oral administration of shoseiryuto from 2 weeks before pollen exposure did not prevent or inhibit immediate symptoms of AR induced by JCP in the ECC. Further study is needed to reevaluate the shoseiryuto specific "pattern" in JTM.

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INTRODUCTION

Allergic rhinitis (AR) is an IgE-mediated type I allergic disease of the nasal mucosa, the prevalence of which continues to increase worldwide.¹⁻⁷ In Japan, Japanese cedar pollen (*Cryptomeria japonica*, JCP) is the most common causal allergen of AR induced by pollen, which is prevalent at a rate exceeding 26% of the population.⁷

Complementary and alternative medicine (CAM) is a varied group of medical and health care systems, practices, and products that are not generally considered conventional medicine.⁸ A certain prevalence of CAM use was reported for allergic diseases, including AR.⁹ A report from the United States shows that 38% of adults and 12% of children used CAM,¹⁰ and a report from Italy shows that 20% of patients with moderate to severe AR used CAM.¹¹ In our study, 19.2% of adults and 7.1% of children with AR in Japan were also using CAM.¹² Thus, although CAM is used worldwide in many patients with AR, there is insufficient evidence for its therapeutic effects. Evidence has not been established even for homeopathy and herbal therapies, for which numerous papers have been reported.^{13,14} The accumulation of objective and convincing clinical trials is desired.^{11,12,15-17}

Traditional Chinese medicine (TCM), a representative of CAM, has a history of more than 2000 years¹⁸ and has spread throughout Asia, especially in Taiwan, Korea, and Japan. In Japan, TCM was introduced from China in the 6th century and has developed as Japanese traditional (Kampo) medicine (JTM).^{19,20} Shoseiryuto (Xiao-Qing-Long-Tang) is one of the JTM drugs used for treating AR and is covered by the Japanese national health insurance system. However, its efficacy in treating AR has not been fully established. In JTM, the optimal treatment method differs depending on the patient's condition and progress, even if they have the same disease.

JTM physicians diagnose patients by examining their condition and constitution. The diagnosis is determined based on the 3 dualisms (Yin-Yang; positive-negative, Kyo-Jitsu; deficiency-excess, and Netsu-Kan; heat-cold) and the 3 material concepts (Qi; spirit, Ketsu; blood, and Sui; body fluid). The patient's condition concluded through these processes is called a "pattern." It is indispensable to diagnose the "pattern" in JTM, and once the pattern is decided, the corresponding medication is naturally determined.²⁰⁻²² According to this concept of "pattern," patients with AR most frequently show a "fluid retention" pattern, which describes an uneven distribution of water into the nose.²³ Shoseiryuto is likely to have a large effect on patients with "fluid retention" pattern;²⁴ however, there is limited evidence for this effect.

Field tests of seasonal AR are generally conducted during the pollen scattering periods. This limits the evaluation and the results, as it is necessary to consider the changes in weather conditions, pollen-scattering volume, and differences in subjects' conditions. Instead, a pollen-scattering room is useful for scientifically assessing treatment because constant pollen levels can be maintained under the same conditions, allowing clinical tests to be performed with high reproducibility.²⁵

In this study, we conducted a randomized, double-blind, placebo-controlled, crossover study using an environmental challenge chamber (ECC) to confirm the effect of shoseiryuto on AR induced by JCP after a specialist in JTM assessed the "fluid retention pattern" of subjects.

MATERIALS & METHODS

Subjects

Volunteers aged 20-65 years old with AR induced by JCP were included in this study. The diagnosis of AR induced by JCP was based on the presence of seasonal symptoms in the last 2 years

or more and a serum cedar pollen-specific IgE antibody titer of ImmunoCAP (SRL Inc., Tokyo, Japan) class 2 or higher. Class levels were as follows: 6, ≥ 100 UA/ml; 5, 50.0–99.9 UA/ml; 4, 17.5–49.9 UA/ml; 3, 3.50–17.4 UA/ml; 2, 0.70–3.49 UA/ml; 1, 0.35–0.69 UA/ml; and 0, ≤ 0.34 UA/ml. Cases with no nasal symptoms during the non-cedar pollen season and those who developed moderate or more severe symptoms upon screening exposure in the ECC were included. Moderate symptoms were defined as a mean total nasal symptom score of 3 or more at 120, 150, and 180 min after the onset of pollen exposure, using the assessment method described in Section **Materials & methods** (4) below.

Those who had allergic side effects to shoseiryuto and those with aldosteronism, myopathy, and hypokalemia included in the contraindications for shoseiryuto, were excluded. Other exclusion criteria were as follows: patients with AR symptoms that required treatment during the cedar pollen-free season; patients with nasal diseases that could affect the evaluation of the study; patients with serious complications such as respiratory, cardiac, hepatic, renal, or autoimmune diseases currently under treatment; those pregnant, possibly pregnant, or lactating; or those receiving antihistamines, anti-leukotrienes, nasal spray steroids, oral steroids, herbal medicines, or immunotherapy within 2 weeks of the start of the study; or those deemed unsuitable by physicians to safely participate in the study.

Based on the previous studies conducted at our institution, a mean score of 5 with a standard deviation (SD) of about 1.5 was assumed in the placebo group.^{26,27} When comparing the placebo group with the shoseiryuto group, a mean score difference of about 1 between the groups was considered to indicate greater effectiveness than in the placebo group. Assuming a crossover design, a group difference of 1, an SD of 1.5, a two-sided significance level of 5%, and power of 90%, the required number of eligible patients would be 50, even if the correlation coefficient was conservatively set to 0. Because the correlation coefficient is expected to be greater than 0 in practice, we considered some dropouts and set the target number of cases for the study at 50. All participants received an explanation of the study orally and in writing, and written consent was

obtained. The study was approved by the Clinical Research Ethics Review Board of Chiba University Hospital (approval number: G30008) on July 20, 2018, and was conducted following the ethical guidelines for medical research involving human subjects and the Declaration of Helsinki. This clinical trial was registered in the UMIN-CTR (A study of the effect of shoseiryuto on Japanese cedar pollinosis, UMIN000033286) on July 5, 2018.

Study design

Study schedule and drug allocation

The study was a randomized, placebo-controlled, crossover, double-blind study conducted during the non-cedar pollen season from June 2018 to December 2018. The start of cedar pollen dispersal in Chiba City in 2019 was confirmed to be February 13. Questionnaires, blood samples, and a screening pollen exposure test were conducted to select eligible cases. The “fluid retention pattern” was also assessed during screening. The test items (shoseiryuto and placebo) were orally administered before every meal from 2 weeks before the exposure test until the night of the test. Following a 5-week washout period after the first exposure test, another test for each subject was performed using the other test item. Allocations were made by a central registry in the Data Management Office, Clinical Trials Department, Chiba University Hospital. The allocation adjustment factors included a total nasal symptom rating of moderate (symptom score: 3–5 points) or severe (symptom score: 6–12 points) at screening exposure and cedar pollen-specific IgE (class 2 or higher).

Study drug

Six grams of shoseiryuto (Kracie Shoseiryuto Extract Fine Granules®; Kracie Holdings, Inc.) or lactose (lactose hydrate [Sioe®]; Sioe Pharmaceutical Co.) as a placebo was placed in drug inclusion capsules (DBcaps®; size B). They were made to maintain the double-blind study design regarding their color, shape, odor, and weight. Drug encapsulation was performed by a pharmacist not directly related to the study. The study drug was administered at 6 g per day, 3 times per day before each meal, starting 2 weeks before the exposure test and continuing until the test night. Kracie’s shoseiryuto used in this clinical trial is

manufactured under GMP grade in China and Japan. The manufacture also uses three-dimensional PDA (Photo Diode Array) pattern analysis and mass spectrometry to ensure stable quality. It is thought that there is little variation from lot to lot.

Assessment of drug adherence

Participants recorded their intake of the study drugs in a diary. In addition, the empty package of study drugs and unused study drugs were collected and checked for consistency with the diary records. Adherence was determined as the ratio of actual doses taken to total amounts during the study period in all participants.

Exposure examination in the ECC

The examination was conducted in the ECC at Chiba University Hospital. Based on previous performance tests, the standard concentration of cedar pollen dispersal was 8,000 grains/m³,²⁵ and it was confirmed that the symptoms peaked at about 2–3 h. Pollen exposure examinations were conducted from 9:00 a.m. to 12:00 p.m. for 3 hours to evaluate symptoms. Fifty-six pollen concentration measuring instruments (Shinyei Co., Kobe, Japan) were installed in the ECC to monitor the pollen concentration every 5 min. The use of a rescue drug before 9:00 p.m. on the day of symptom assessment was prohibited, while using it after 9:00 p.m. on the day was permitted. The rescue drug was 60 mg of fexofenadine (Allegra®). The use of medications with antiallergic effects, including antihistamines, were generally prohibited during the rest of the study.

Evaluation of symptoms

A mobile terminal (Willcom Co., Tokyo, Japan) was used in the ECC, and symptoms were recorded in an electronic database. Symptoms of sneezing, rhinorrhea, nasal congestion, itchy nose, itchy eyes, and watery eyes were evaluated every 30 min in the ECC and graded as follows: 0, none; 1, mild; 2, moderate; and 3, severe. Symptoms at 3:00, 6:00, and 9:00 p.m. were recorded in the diary: the total score of the 4 nasal symptoms was used as the total nasal symptom score (TNSS; 0–12). The total score of the 2 ocular symptoms was used as the total ocular symptom score (TOSS; 0–6), and the sum of the total nasal and total ocular symptom scores was the total nasal-ocular

symptom score (TNOSS; 0–18). The frequency of sneezing and nose blowing were also recorded. In particular, the mean total nasal symptom scores at 120, 150, and 180 min in the ECC were the primary endpoints, while the mean nasal symptom scores at 3:00, 6:00, and 9:00 p.m. were set as the secondary endpoints.

Definitions of good and poor responders

Subjects with and without differences in mean scores for whole nasal symptoms of 1 or more points at each assessment time between shoseiryuto and placebo treatments were defined as good and poor responders, respectively.

Evaluation of “fluid retention pattern” (Terasawa’s fluid retention score)

A specialist in JTM examined all participants during screening and assessed the “pattern,” and scored 17 items in Table 1 (highest score: 84) according to the criteria developed by Terasawa.²⁷ A score of 13 or higher was judged to be the pattern of “fluid retention”.

Serum eosinophil cationic protein (ECP)

Blood was collected immediately before and after room entry for each evaluation exposure to measure serum ECP (SRL Inc., Tokyo, Japan).

Examination of immunological changes in peripheral blood mononuclear cells (PBMCs) after oral administration of shoseiryuto

Blood sampling and cell culture

Before entering the ECC at each assessment, blood was collected, and PBMCs were extracted using the Ficoll-Hypaque technique, as previously described,²⁸ and stored in a freezer at –80 °C with CELLBANKER-1® cell freezing medium until the time of use. RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with fetal calf serum (10%), L-glutamine (2 µmol/L), penicillin (100 U/mL), streptomycin (100 µg/mL), HEPES (10 mmol/L), 2-mercaptoethanol (55 µmol/L), nonessential amino acids (1%), and sodium pyruvate (1 mmol/L) (all from Gibco BRL, Grand Island, NY, USA) was used as the culture medium. PBMCs were labeled using the CellTrace™ Violet Cell Proliferation Kit (CTV) (Life Technologies, Carlsbad, CA, USA), Purified Sugi (Japanese cedar) Basic Protein (2 µg/ml; Funakoshi, Tokyo, Japan),

Item	Score
Feeling of fatigue	0-3
Throbbing headache	0-4
Dull headache	0-3
Getting car sick easily	0-5
Vertigo	0-5
Dizziness	0-5
Watery nose	0-3
Hypersalivation	0-3
Foamy sputum	0-3
Nausea and vomiting	0-3
Enhancement of bowel peristalsis	0-3
Morning stiffness	0-7
Edema and splashing sound epigastric region	0-15
Brisk pulsation in the supra-umbilical region	0-5
watery diarrhea	0-5
Decreased urine volume	0-7
Increased urine volume	0-5

Table 1. Fluid retention score. A score of 13 or higher is judged to be the pattern of fluid retention

and recombinant IL-2 (5 U/ml) for 3 days for real-time PCR or 8 days for flow cytometry and cytokine measurement in the supernatant at 37 °C.

Cytokine measurement in the supernatant

IFN- γ , IL-4, IL-5, and IL-13 were measured in PBMC culture supernatants using CBA (BD, Franklin Lakes, NJ, USA), as per the manufacturer's protocol. The amount of each cytokine was compared between the shoseiryuto group and the placebo group.

Real-time PCR

We measured the mRNA expression of *IFNG*, *IL4*, *IL5*, and *IL13* in cultured PBMCs and compared it between the shoseiryuto group and the placebo group. RNA was extracted from cultured PBMCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized

using SuperScript®VILO™ MasterMix (Invitrogen). Semi-quantitative real-time PCR was performed by the TaqMan™ method using the Applied Biosystems Step One Plus Real-time PCR System, and relative gene expression was measured by the comparative CT method. *ACTB* was used for normalization as a housekeeping gene. Primers and probes were used with TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA), as follows: *IL4*, Hs00174122-m1; *IL5*, Hs01548712-g1; *IL13*, Hs00174379-m1; *IFNG*, Hs00174379-m1; and *ACTB*, Hs99999903-m1.

Flow cytometry

PBMCs were stained with anti-human antibodies after culture, and the numbers of IL-4-, IL-5-, IL-13-, and IL-31-producing cells were measured using FACS Canto II (BD). The proportions of each cytokine-producing cell type among the cedar pollen-specific CD4⁺ cells were compared between the shoseiryuto and placebo groups. Intracellular staining was performed as per the manufacturer's protocol using Intracellular Fixation and Permeabilization Buffer Set (eBioscience, San Diego, CA, USA). Cells were pre-cultured in FcR Blocking Reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) to prevent non-specific binding to antibodies. Cells with reduced CTV levels after cedar stimulation (CTV_{dim} cells) were defined as cedar antigen (JCP)-specific cells, and the proportion of cytokine-producing cells among them was analyzed. Data were analyzed using the FlowJo software program (Tree Star Inc., Ashland, OR, USA). The antibodies used were as follows: PE-Cy7-anti-IL-4 (Invitrogen), PerCP-Cy5.5-anti-CD4, APC-anti-IL-5, PE-anti-IL-13 (BD), and Alexa Fluor 488-anti-IL-31 (Biolegend, San Diego, CA, USA). Dead cells were stained with Fixable Viability Dye 780 (eBioscience).

Adverse events

A diary was provided to all participants during the study period, in which they recorded adverse events and associated treatments.

Statistical analysis

All subjects who were enrolled in the study took the study drug at least once after randomization, visited the hospital at least once during the intervention period, and had the data of pollen

exposure symptoms were included full analysis set (FAS).

Regarding the subjects' background, the frequency and proportion of categories are presented for nominal and ordinal variables. For continuous variables, means and SD (or medians and ranges) are shown. An analysis of the variance model with treatment and timing as fixed effects was used to mean nasal eye symptom scores. The group differences in means and their 95% confidence intervals were calculated. A two-sample *t*-test was used to compare the frequency of sneezing and nose blowing and the groups' ECP values. Two-sample *t*-test, Fisher's exact test, Fisher's exact test, and Wilcoxon's rank-sum test were used to compare drug efficacy groups, as appropriate. An unpaired *t*-test was used to compare the presence of "fluid retention" with drug efficacy. The frequency and rate of adverse events were tabulated for each group to assess safety. A two-sample *t*-test was used for the analysis of the cell culture experiments.

The level of significance for these statistical analyses was set at 5% for both sides. The study's data management was performed at the Clinical Research Data Center, Chiba University Hospital, and statistical analysis was performed at the Biostatistics Office, Clinical Trials Division. This analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Participants' background

Seventy-one patients participated in the screening. A total of 22 had a mean total nasal symptom score of less than 3 at 120, 150, and 180 min, and 2 withdrew from the study for personal reasons. Forty-seven participants underwent drug allocation, but 1 participant withdrew from the study before exposure assessment. Finally, 46 subjects were analyzed (Fig. 1). The participants included 27 females (58.7%) and 19 males (41.3%), with a mean age of 46.5 years and a mean age of onset of AR induced by JCP of 20.8 years. The mean JCP-specific IgE was 45.2 UA/mL. Two patients missed 1 evaluation exposure due to ill health. The study medication adherence was 97.9% for both the shoseiryuto and placebo groups, showing good compliance (Table 2).

Changes in symptom score over time

Each nasal symptom score

Across all symptoms, the symptom scores increased after the start of pollen exposure and persisted after leaving the ECC. There was no significant difference in the scores of all symptoms in the ECC (Fig. 2).

For sneezing, the scores were significantly lower at 3:00 p.m. ($p < 0.05$), 6:00 p.m. ($p < 0.05$), and 9:00 p.m. ($p < 0.01$) in the shoseiryuto group than in the placebo group (Fig. 2A). For rhinorrhea, no significant differences occurred (Fig. 2B). For nasal congestion, significantly lower values were found at 3:00 p.m., 6:00 p.m., and 9:00 p.m. (all $p < 0.05$) in the shoseiryuto group than in the placebo group (Fig. 2C). For nasal itching, significantly lower values were found at 9:00 p.m. ($p < 0.01$) in the shoseiryuto group than in the placebo group ($p < 0.01$) (Fig. 2D).

Ocular symptom score

For itchy eyes, there were no significant differences between the shoseiryuto group and the placebo group at any point (Fig. 2E). For watery eyes, the scores were significantly lower at 6:00 p.m. ($p < 0.05$) in the shoseiryuto group than in the placebo group (Fig. 2F).

Total symptom score

For TNSS, the values in the shoseiryuto group were significantly lower at 6:00 p.m. ($p < 0.05$) and 9:00 p.m. ($p < 0.01$) than in the placebo group (Fig. 2G). TOSS was significantly lower at 6:00 p.m. ($p < 0.05$) in the shoseiryuto group than in the placebo group (Fig. 2H). TNOSS was significantly lower at 3:00 p.m. ($p < 0.05$), 6:00 p.m. ($p < 0.01$), and 9:00 p.m. ($p < 0.01$) in the shoseiryuto group than in the placebo group (Fig. 2I).

Comparison of symptom scores between 120 and 180 min in the ECC and 3, 6, and 9 p.m.

Table 3 shows the mean symptom scores at 120, 150, and 180 min in the ECC and 3:00, 6:00, and 9:00 p.m. There were no significant differences between the shoseiryuto and placebo groups in the 120–180 min exposure period, including the primary endpoint of TNSS. At 3:00–9:00 p.m., the shoseiryuto group had lower scores than the

Flow of Participants through the trial

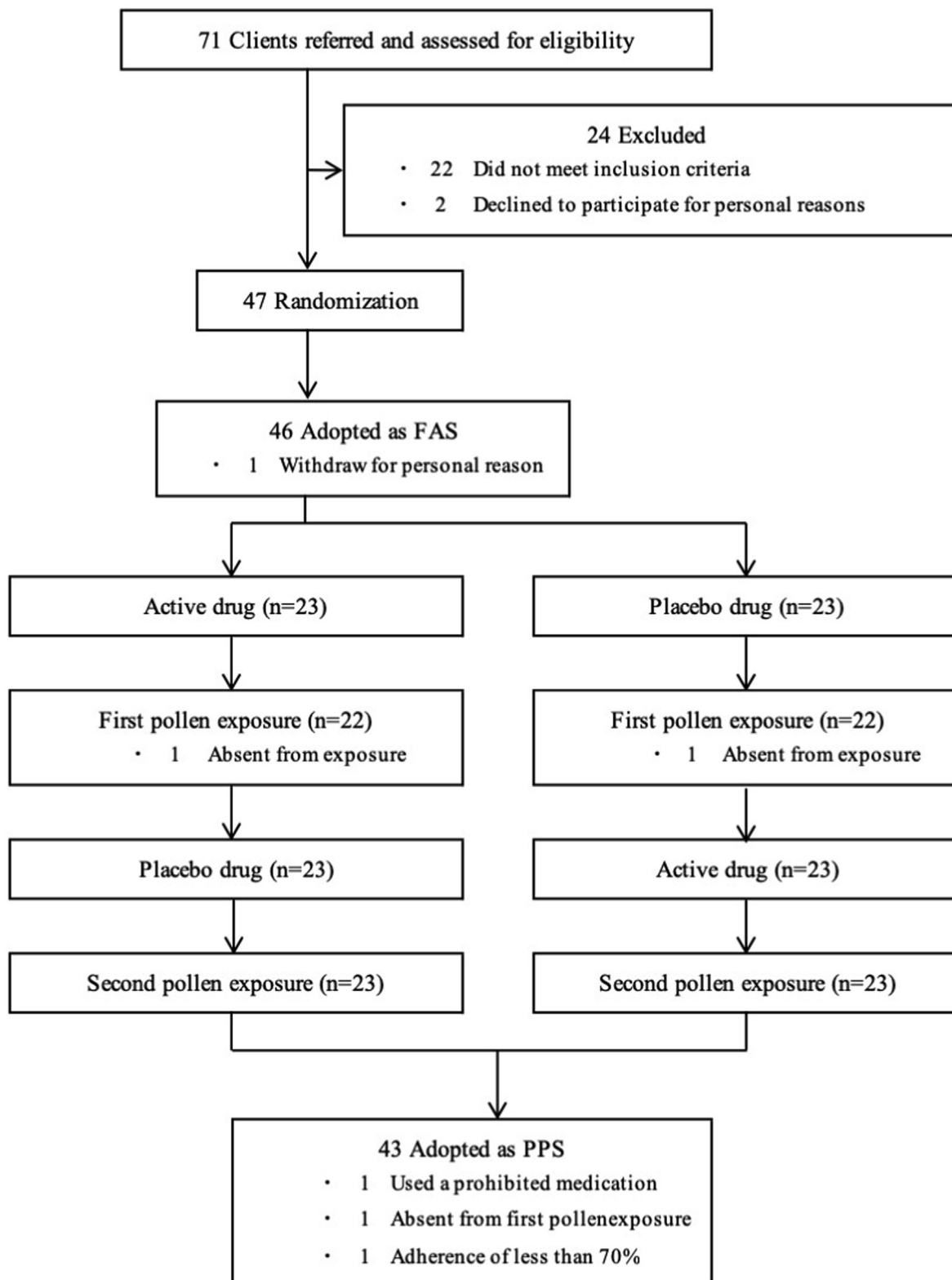


Fig. 1 Participant flow Abbreviation: FAS, Full Analysis Set; PPS, Per Protocol Set

placebo group in all categories except for rhinorrhea.

The frequency of sneezing and nose blowing

A comparison of the total frequency of sneezing and nose blowing is shown in Fig. 2J and K. There was no significant difference between the shoseiryuto and the placebo groups either in the ECC (at 9:00 a.m.–12:00 p.m.) or after leaving the ECC (at 3:00–9:00 p.m.).

Comparison of eosinophil cationic protein (ECP)

ECP values before and after exposure in each group are shown in Supplemental Fig. E1A. When

analyzing the ECP differences between before and after exposure, there was no significant difference between the shoseiryuto and placebo groups.

Association between presence of “fluid retention pattern” and treatment effect

We analyzed whether there was a difference in treatment effect after leaving the ECC (at 3:00–9:00 p.m.) between those judged with fluid retention pattern and those who were not. There was no significant difference between the 2 groups in TNSS. (Supplemental Fig. E1B).

Backgrounds		Total (n = 46)
Gender		
Female	n (%)	27 (58.7)
Male	n (%)	19 (41.3)
Age, years	Mean (SD)	46.5 (9.6)
Age of onset of JCP-induced AR, years	Mean (SD)	20.8 (8.4)
Severity of JCP-induced AR		
Very severe	n (%)	3 (6.5)
Severe	n (%)	27 (58.7)
Moderate	n (%)	16 (34.8)
Medial history of allergic disease		
Bronchial asthma		
Past	n (%)	2 (4.3)
Current	n (%)	0 (0)
Atopic dermatitis		
Past	n (%)	3 (6.5)
Current	n (%)	1 (2.2)
Food allergy		
Past	n (%)	0 (0.0)
Current	n (%)	3 (6.5)
Perennial AR		
Past	n (%)	0 (0.0)
Current	n (%)	7 (15.2)
Total IgE, IU/mL	Mean (SD)	232.4 (347.2)
JCP-specific IgE, UA/mL	Mean (SD)	45.2 (70.9)
HDM-specific IgE, UA/mL	Mean (SD)	7.1 (26.0)
Fluid retention score	Mean (SD)	8.7 (4.8)

Table 2. Background characteristics of subjects. Abbreviations: AR, Allergic rhinitis; HDM, House dust mite; IgE, immunoglobulin E; JCP, Japanese cedar pollen; SD, standard deviation

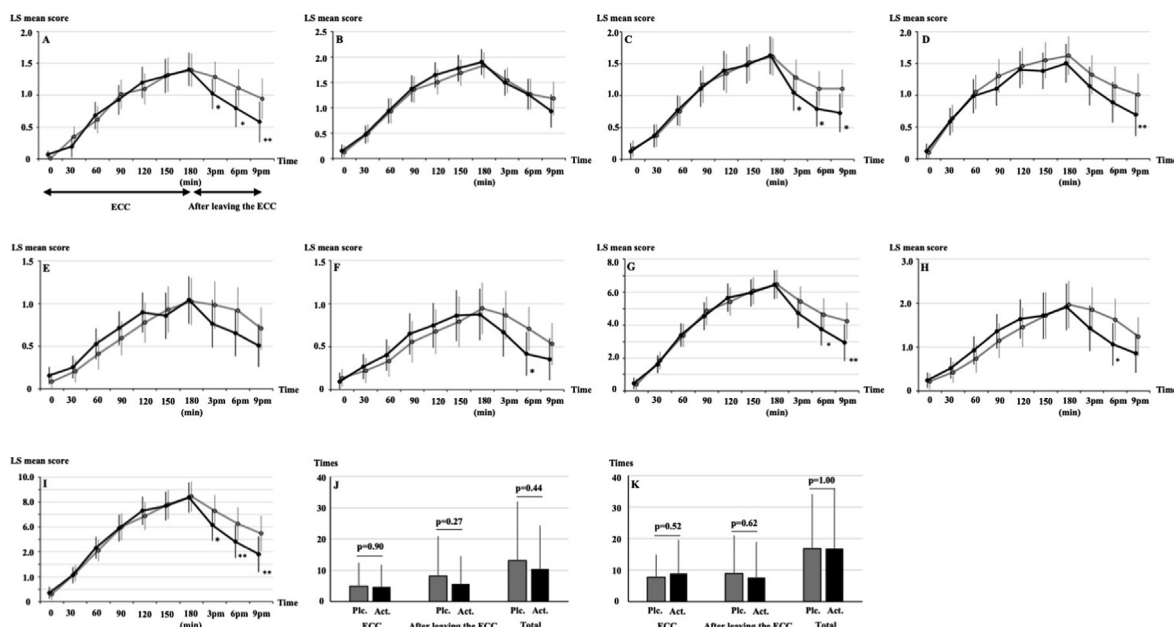


Fig. 2 Change of symptom scores, and frequency of sneezing and nose blowing values represent means \pm 95% confidence intervals. A: sneezing, B: rhinorrhea, C: nasal congestion, D: nasal itching, E: eye itching, F: watery eye, G: total nasal symptoms, H: total eye symptoms, I: total nasal-eye symptoms, J: frequency of sneezing, K: frequency of nose blowing. \circ —: Placebo, \bullet —Active treatment. *: $p < 0.05$, **: $p < 0.01$.

Comparison of background factors between good and poor responders

We compared the background factors of good and poor responders regarding TNSS at 3:00-9:00 p.m. after leaving the ECC. There were no significant differences in background factors between these 2 groups (Table 4). There was also no significant relation between “fluid retention pattern” and efficacy. There was a tendency for the ECP to be lower in the good responders than in the poor responders, but the difference was not significant.

Analysis of cytokine protein in supernatants and mRNA expression in PBMCs

There were no significant differences in IFN- γ , IL-4, IL-5, and IL-13 proteins in the PBMC culture supernatants between the shoseiryuto and placebo groups in blood samples obtained before the first pollen exposure (Supplemental Fig. E2). The mRNA expression of *IFNG*, *IL4*, *IL5*, and *IL13* in post-culture PBMCs also did not differ between the shoseiryuto and placebo groups (Supplemental Fig. E3).

Examination of JCP-specific cytokine-producing cells

A comparison of IL-4-, IL-5-, IL-13-, and IL-31-producing cells after the stimulation of PBMCs with JCP antigen is shown in Supplemental Fig. E4. There were no significant differences between the shoseiryuto and placebo groups in the 2 blood samples obtained before the first pollen exposures.

Adverse events

The numbers of patients with adverse events were 4 (8.5%) and 13 (27.7%) in the placebo and shoseiryuto groups, respectively (Table 5). None of the events required discontinuation of the study drug and were not related to the study items. All events were transient and slight, with no serious adverse events being observed.

DISCUSSION

Eight herbs are used in shoseiryuto: Ephedra herb, peony root, processed ginger, Glycyrrhiza, cinnamon bark, Asiasarum root, Schisandra fruit, and Pinellia tuber. For example, ephedrine or

pseudoephedrine in the Ephedra herb is a sympathomimetic amine that could be expected to be effective in treating AR symptoms associated with nasal congestion. From *in vitro* or *in vivo* animal studies, it has been reported that shoseiryuto inhibits histamine release from mast cells,²⁹ basophil differentiation,³⁰ Th2-type allergic reactions,^{31,32} the production of tumor necrosis factor-alpha in peripheral blood mononuclear cells,³³ acetylcholine stimulation of nasal gland cells,³⁴ infiltration of mast cells and eosinophils into the nasal mucosa,^{35,36} and

increases in the levels of IL-4 and leukotrienes in the blood.³⁵

However, JTM drugs are composed of multiple crudes that exhibit complex pharmacological action, and their efficacy depends on the patient's "pattern". This concept is markedly different from that of Western medicine. It is not easy to evaluate the effectiveness of JTM drugs in animal experiments. A few clinical studies on shoseiryuto have been reported. Only 2 shoseiryuto-related studies

Symptoms	LS mean (Active vs Placebo)	LS mean difference	Adjusted LS mean two-sided 95%CI	Adjusted p-value
From 120 to 180 min in ECC				
Sneezing	1.30 vs 1.27	0.03	-0.20 to 0.27	0.79
Rhinorrhea	1.78 vs 1.67	0.11	-0.20 to 0.41	0.49
Nasal congestion	1.50 vs 1.50	0.01	-0.22 to 0.23	0.96
Nasal itching	1.43 vs 1.55	-0.12	-0.32 to 0.09	0.27
Eye itching	0.92 vs 0.91	0.01	-0.21 to 0.24	0.90
Watery eye	0.82 vs 0.80	0.02	-0.20 to 0.24	0.84
TNSS	6.01 vs 5.99	0.02	-0.79 to -0.82	0.97
TOSS	1.75 vs 1.71	0.04	-0.37 to 0.46	0.83
TNOSS	7.76 vs 7.71	0.05	-1.04 to 1.15	0.92
From 3 to 9 h after leaving the ECC				
Sneezing	0.80 vs 1.11	-0.31	-0.51 to -0.11	<0.01
Rhinorrhea	1.23 vs 1.33	-0.10	-0.33 to 0.14	0.41
Nasal congestion	0.86 vs 1.17	-0.31	-0.54 to -0.08	<0.01
Nasal itching	0.91 vs 1.16	-0.25	-0.47 to -0.03	<0.05
Eye itching	0.64 vs 0.87	-0.23	-0.46 to -0.01	<0.05
Watery eye	0.48 vs 0.70	-0.22	-0.44 to -0.01	<0.05
TNSS	3.80 vs 4.77	-0.97	-1.68 to -0.25	<0.01
TOSS	1.12 vs 1.57	-0.45	-0.86 to -0.05	<0.05
TNOSS	4.91 vs 6.34	-1.42	-2.40 to -0.45	<0.01

Table 3. Comparison of mean symptom scores. Abbreviations: ECC, environmental challenge chamber; LS mean, least square mean; CI, confidence interval; S, Shoseiryuto; P, Placebo; JC, Japanese cedar; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TNOSS, total nasal-ocular symptom score

Backgrounds		Good responders (n = 17)	Poor responders (n = 27)	p-value
Gender				
Female	n (%)	11 (42.3)	15 (57.7)	0.75
Male	n (%)	6 (33.3)	12 (66.7)	
Age, years	Mean (SD)	47.9 (7.2)	46.0 (10.6)	0.50
Age of onset of JCP-induced AR, years	Mean (SD)	21.8 (6.7)	20.4 (9.0)	0.59
Severity of JCP-induced AR				
Very severe	n (%)	0 (0.0)	3 (100)	0.30
Severe	n (%)	9 (36.0)	16 (64.0)	
Moderate	n (%)	8 (50.0)	8 (50.0)	
Medial history of allergic disease				
Bronchial asthma				
None	n (%)	17 (39.5)	26 (60.5)	1.00
Past	n (%)	0 (0.0)	1 (100.0)	
Atopic dermatitis				
None	n (%)	17 (41.5)	24 (58.5)	0.27
Past	n (%)	0 (0.0)	3 (100)	
Food allergy				
None	n (%)	15 (36.6)	26 (63.4)	0.55
Current	n (%)	2 (66.7)	1 (33.3)	
Perennial AR				
None	n (%)	16 (43.2)	21 (26.8)	0.22
Current	n (%)	1 (14.3)	6 (85.7)	
Total IgE, IU/mL	Mean (SD)	309.4 (386.1)	194.9 (331.1)	0.30
JCP-specific IgE UA/mL	Mean (SD)	60.2 (94.1)	37.9 (54.6)	0.32
HDM-specific IgE UA/mL	Mean (SD)	12.6 (40.1)	2.9 (4.6)	0.33
Difference of ECP ^a	Mean (SD)	-1.88 (5.45)	0.56 (3.17)	0.067
Fluid retention pattern	n (%)	5 (45.5)	6 (54.5)	0.72

Table 4. Comparison of backgrounds between good and poor responders. Abbreviations: AR, Allergic rhinitis; ECC, environmental challenge chamber; ECP, eosinophil cationic protein; HDM, House dust mite; IgE, immunoglobulin E; JCP, Japanese cedar pollen; SD, standard deviation; TNSS, total nasal symptom score ^aDifference of ECP measured after leaving the ECC (active - placebo).

on AR induced by house dust mite (HDM) have been conducted, in which shoseiryuto showed a slow and mild effect in both studies.^{37,38} However, no clinical trials investigating the effects on AR induced by pollen have been reported. Symptoms of AR induced by pollen immediately

appear at the start of pollen dispersal and worsen quickly when much pollen is dispersed. Therefore, AR induced by pollen is an acute allergic-inflammatory disease and differs from HDM-induced AR, which is a chronic disease. Treatment of AR induced by pollen should focus

Adverse events	Placebo, n (%)	Active, n (%)
Otorrhea	0 (0)	1 (2.1)
Abdominal pain	0 (0)	1 (2.1)
Diarrhea	0 (0)	1 (2.1)
Pain of limbs	0 (0)	1 (2.1)
Headache	2 (4.2)	4 (8.5)
Coughing	0 (0)	1 (2.1)
Nasopharyngitis	2 (4.2)	5 (10.6)
Tooth extraction	1 (2.1)	0 (0)
Total	4 (8.5)	13 (27.7)

Table 5. Adverse events.

on the rapid improvement of symptoms and preventive medicine to avoid the onset of severe symptoms.

In this study, using the ECC, which is considered reproducible and objective in assessing symptoms, we conducted a double-blind, placebo-controlled study to determine whether treatment with shoseiryuto from 2 weeks before pollen exposure prevents the onset of symptoms or ameliorates the symptoms. Furthermore, this is the first study conducted in collaboration with a physician specializing in JTM treatment to examine the relationship between the JTM drug's effect and the "fluid retention pattern".

The results showed that 2 weeks of oral shoseiryuto had no effect on the onset of symptoms at 3 hours of pollen exposure, and its preventive effect was not apparent. However, it had some effect on the improvement of the symptoms after exposure. Even if clothes are changed, and faces and noses are washed when leaving the ECC to avoid bringing pollen to the outside of the chamber, delayed symptoms usually appear after leaving the chamber. We previously reported that increases in histamine, leukotriene, substance P, IL-5, and IL-31 were detected in nasal lavage fluid after leaving the ECC. These mediators and cytokines were considered to be associated with the development of the delayed symptoms.³⁹ The mechanism of the effect of shoseiryuto in this study is unknown;

however, the absence of effects on blood cytokines and JCP-specific Th2 cells suggested that shoseiryuto does not affect allergy pathogenesis.

Placebo is well known to have a very strong effect on AR.^{40,41} In this study, shoseiryuto did not cause any problematic side effects and effectively treated the delayed symptoms. However, in a previous study using the ECC, a single dose of non-sedating antihistamine administered the day before exposure suppressed both the immediate symptoms in the ECC and the delayed symptoms after leaving the ECC.^{27,42} In the ARIA (Allergic Rhinitis and its Impact on Asthma) report, allergen exposure chambers were considered to be particularly useful for studying the timing of the onset of medicines' effects.⁴³ In any case, shoseiryuto needs a certain amount of time to exert its effects. The significance of shoseiryuto in the treatment of AR induced by pollen also needs to be considered cost-effectiveness.

Limitation of the study

One of the main characteristics of JTM involves identification of the "pattern" of the patients. This "pattern" has been considered to be essential in predicting efficacy, and has been used as an indicator for JTM formulation. However, the scientific evidence for this pattern is insufficient, and herein lies the limitation of this study. The main aim of this study is to determine the efficacy of shoseiryuto and the validity of the "pattern" by scientific analysis of JTM. The pattern classification that we used in this study is the one most widely used in JTM. However, the results of the study failed to show the "pattern" for prediction of efficacy. The ambiguity of the pattern in JTM examination is one of the factors preventing the accumulation of evidence for JTM, and further studies are needed to develop an objective method for evaluating JTM.

CONCLUSION

This study showed that the oral administration of shoseiryuto for 2 weeks before pollen exposure did not prevent or inhibit the immediate symptoms. The relationship between the "fluid retention score" and the therapeutic effect was not clear.

Further study is needed to reevaluate the “pattern” in JTM treatment.

Abbreviations

AR, Allergic rhinitis; CI, Confidence interval; ECC, Environmental challenge chamber; ECP, Eosinophil cationic protein; HDM, House dust mite; IgE, Immunoglobulin E; JCP, Japanese cedar pollen; JTM, Japanese traditional medicine; RCT, Randomized controlled trial; SD, standard deviation; TNOSS, Total nasal-ocular symptom score; TNSS, Total nasal symptom score; TOSS, Total ocular symptom score.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, Yoshitaka Okamoto, upon reasonable request.

Authorship contribution

Conception and design of study: Syuji Yonekura, Toyoyuki Hanazawa, Takao Namiki, Yoshitaka Okamoto. Acquisition of data and analysis of data: Junya Kurita, Tomohisa Iinuma, Riyo Yoneda, Sakiko Imamoto, Yohei Kawasaki. Drafting the manuscript: Junya Kurita. Revising the manuscript critically: Syuji Yonekura, Takao Namiki, Yoshitaka Okamoto.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Review Board of Chiba University Hospital (approval number: G30008) on July 20, 2018 and was conducted following the ethical guidelines for medical research involving human subjects and the Declaration of Helsinki. This clinical trial was registered in the UMIN-CTR (A study of the effect of shoseiryuto on Japanese cedar pollinosis, UMIN000033286) on July 5, 2018. All participants received an explanation of the study orally and in writing, and written consent was obtained.

Submission declaration

The work described in this paper has never been published before and is not under consideration for publication elsewhere.

Declaration of competing interest

All authors have no conflicts of interest directly relevant to the content of this article.

Consent for Publication

All authors have consented to the publication of their work and have reviewed and agreed to the publication’s ethical statement and editorial policies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.waojou.2022.100636>.

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