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ORIGINAL RESEARCH ARTICLE

Daily intake of *Citrus jabara* fruit peel powder (Japanese Patent No. 5,323,127) improves allergy-like symptoms: A randomized double-blind parallel-group comparative study

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ABSTRACT

Citrus jabara (CJ) is a rare citrus fruit that used to grow naturally only in the southern part of the Kii Peninsula in Japan. Human intervention studies with oral intake of CJ fruit have shown its anti-allergic effects, but the testing method was a pre-post comparison study. In this study, we conducted a randomized, double-blind, parallel-group interventional study to evaluate the volume-dependent effects of oral intake of CJ fruit peel powder (Japanese Patent No. 5,323,127) on nasal and eye allergy-like symptoms. Ninety healthy adults were allocated to three groups and given test foods containing 1,000, 500, and 0 mg of CJ peel powder, with one packet per day for 4 weeks. After excluding those who dropped out or deviated from the study protocol, 73 were included in the efficacy analysis and 86 in the safety analysis. The high-dose group (1,000 mg/day) was significantly lower than the placebo group in the scores of "nasal and eye symptoms" at week 4, and "blocked nose" at weeks 2 and 4 in the evaluation of question I of Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No. 1). The changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire showed a dose-dependent reduction in rhinorrhea. In the safety evaluation, there were no significant differences in examinations of physiology, hematology, and blood biochemistry between the groups, and no adverse events attributable to the test foods were observed. These results suggest that intake of CJ peel powder can alleviate allergy-like symptoms.

Keywords: *Citrus jabara* Peel Powder; Allergy-like Symptoms; Randomized-double-blind-parallel-group Interventional Study; Foods with Function Claims

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1. Introduction

In the epidemiological Survey of Allergic Rhinitis in Japan 2019, the prevalence of allergic rhinitis in our country was 49.2%, pollinosis (including Japanese cedar and other pollinosis) was 38.8%, and perennial allergic rhinitis was 24.5%, showing an increase in prevalence compared to past surveys[1]. Meanwhile, in an effort to curb medical costs, the Federation of Health Insurance Associations has proposed that medical hay fever treatments similar to over-the-counter (OTC) drugs be excluded from insurance coverage.

In addition, attention to “ME-BYO” and “preventive medicine” that focuses on prevention of disease and serious illness is increasing for reasons of both cost and quality of life (QOL). Reflecting this situation, “allergies” were added to the health claims of “Foods with Function Claims” under the jurisdiction of the Consumer Affairs Agency in spring 2019.

Citrus jabara (CJ) is a rare citrus fruit that used to grow naturally only in the southern part of the Kii Peninsula in Japan, and its fruits been reported to have anti-allergic and anti-inflammatory effects[2-7]. Some human intervention studies with oral intake of CJ fruits have evaluated the anti-allergic effects of fruit juice[6] and fermented product[7], but the test methods were before-and-after comparison studies.

These anti-allergic and anti-inflammatory effects are attributed, at least in part, to the flavonoid narirutin, which is abundantly contained in CJ fruit. Narirutin has been reported to inhibit increases in eosinophils and blood immunoglobulin (Ig) E in mouse models of asthma[8] and to suppress inflammation[9].

We focused on the fact that narirutin is unevenly distributed in CJ fruit, with the vast majority (about 90%) in the peel rather than in the juice, which has been conventionally used. Furthermore, the safety of CJ fruit peels has improved, with the development of a CJ fruit peel powder with high narirutin content and proven safety[2,10].

In the present study, we report the results of a randomized, double-blind, parallel-group, comparative interventional study on the volume-dependent effects of oral intake of CJ peel powder[10] on nasal and eye allergy-like symptoms.

2. Experimental

2.1 Participants

The participants were healthy Japanese men and women between the ages of 20 and 65 with subjective symptoms of eye and nose discomfort (sneezing, runny nose, nasal congestion, itchy eyes, etc.) in daily life. Ninety participants were selected based on the results of blood tests and medical interviews.

The exclusion criteria for the selection of participants were as follows: (1) those with severe or worse allergic rhinitis symptoms, (2) those with acute rhinitis, sinusitis, nasal polyps, hypertrophic rhinitis, or deviated nasal septum, (3) those with bronchial asthma, (4) those with serious liver, heart, kidney, respiratory, endocrine, or metabolic diseases, (5) those who were undergoing or had undergone specific desensitization therapy, (6) those who were receiving any medication for treatment, (7) those who had current or previous drug allergies or food allergies, (8) those who had a history of discomfort or problems with physical symptoms after eating citrus fruits, (9) those who routinely consumed “Foods for specific health uses” or “Foods with Function Claims” (however, this did not apply to those who are able to suspend their intake during the study period at the time of obtaining consent), (10) those who were pregnant, lactating, or who wished to become pregnant during the study, (11) those who had experienced sickness or deterioration of physical condition due to blood collection in the past, or those who had been told that their blood vessels are too small to facilitate blood collection, (12) those who had participated or were currently participating in other clinical trials within one month prior to obtaining consent, or those who planned to thus participate during the study, (13) those who might change their lifestyle during the study, such as taking a long trip, (14) heavy alcohol drinkers (60 g/day in alcohol equivalent), (15) those with extremely irregular dietary habits and irregular life rhythms, such as those who work in shifts or late at night, and (16) others who were judged by the responsible medical doctor to be unsuitable as subjects for this study.

This study was conducted under the ethical review and approval of the Clinical Trial Review Committee of Hakusui-Kai Suda Clinic Medical Corporation (approved on January 26, 2021, approval number: 2021-004), in compliance with the “Helsinki Declaration” and the “Ethical Guidelines for Medical Research Involving Human Subjects” (Ministry of Education, Culture,

Sports, Science and Technology (MEXT), and the Ministry of Health, Labor and Welfare (MHLW)). The study was conducted under the supervision of a physician and with the cooperation of a third-party CRO to ensure the human rights and safety of the participants and the reliability of the study data. The study protocol for this study was registered in advance with the University Hospital Medical Information Network (UMIN) (UMIN000043224).

2.2 Test foods

The CJ peel powder[10] used in this study was produced by Jabara Laboratory Co., Ltd. This powder was standardized to contain at least 75 mg/g of narirutin and 70 μg/g of chlorophyll a and b. Maltodextrin, which does not affect eye and nasal health functions, was used for the placebo food. The test foods containing CJ peel powder, i.e., the high-dose test food consisting of 1,000 mg of CJ peel powder and 1,000 mg of reduced maltose, the low-dose test food consisting of 500 mg of CJ peel powder and 1,500 mg of reduced maltose, and the placebo food consisting of 1,000 mg of maltodextrin and 1,000 mg of reduced maltose, were manufactured by Asunaro Institute Chemical Co., Ltd. These foods were packaged in aluminum pouches and were visually indistinguishable.

2.3 Study design

The study was conducted in a randomized, double-blind, parallel-group trial under the supervision of a physician. Participants were given a full explanation of the study, and written consent was obtained from all participants. Randomization was performed by Contract Research Organization (CRO)-affiliated personnel not directly involved in the study, using Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) question I, nasal remarks scores, and scores on the Nasal and Eye Symptom Questionnaire as adjustment factors. All groups were asked to take one packet a day with water or lukewarm water every day before breakfast for four weeks, and to record whether or not they took the packet in an electronic diary. The intake period of the test and placebo foods was conducted from May 2021 to June 2021.

During the study, the intake of health foods and supplements as well as foods with anti-allergic effects, such as Tencha (sugar beet), was prohibited. Except in emergencies, drugs were to be used only with the permission of the investigator, and when used, the reason for use, name of drug used, amount used, duration of use, etc. were to be entered in the electronic diary and the investigator was to be notified.

During the study, the participants were expected to lead the same lifestyle as before participating in the study. In particular, binge drinking, excessive dietary restrictions, changes in eating habits due to overseas travel, changes in exercise habits, lack of sleep due to excessive late nights, or changes in drinking habits were not allowed. Participants who significantly violated these compliance requirements were excluded from the study. In addition, participants who skipped the test foods more than 3 days during the test periods were also excluded.

2.4 Endpoint

2.4.1 Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No. 1)

The scores of nasal and eye symptoms (runny nose, sneezing, blocked nose, itchy nose, itchy eyes, watery eyes) in the JRQLQ No. 1 were evaluated as the primary endpoint, and the total score of the JRQLQ No. 1 was evaluated as the secondary endpoint. The participants were asked to record the results in a questionnaire at screening and at the end of weeks 2 and 4 of the study.

2.4.2 Evaluation of Nasal Remarks Score

At the time of screening and the end of the study (at week 4), following 4 endpoints such as “swelling of concha nasalis inferior mucosa”, “color of concha nasalis inferior mucosa”, “aqueous secretion”, and “character of nasal mucus” were evaluated by an otolaryngologist on a 4-point scale, according to the practical guideline for the management of allergic rhinitis in Japan[11].

2.4.3 Nasal and Eye Symptom Questionnaire

At the end of each day during the study, the participants were asked to evaluate and record scores for "paroxysmal sneezing", "rhinorrhea", "nasal blockage" "itchy eyes", and "watery eyes" on a 5-point scale[12]. The scores for each endpoint in the diary questionnaire were summed and compared for the two weeks of the pre-observation period, the first half, and the second half of the study.

2.5 Physical examination and blood tests

In the physical examination, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate (P) were measured. Hematological tests included white blood cell count, red blood cell count (WBC), platelet count (RBC), hemoglobin (Hb), and hematocrit (HCT). Biochemical blood analysis included values for total protein (TP), total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), total bilirubin (T-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP), creatine phosphokinase (CPK), fasting blood glucose (GLU), and hemoglobin A1c (HbA1c). Serum immunoglobulin E (IgE) specific for house dust, *Dermatophagoides pteronyssinus*, Japanese cedar pollen, and Japanese cypress pollen were measured. Of the above endpoints, height, HbA1c, and specific IgE were measured only at the screening, while all other clinical test items were measured at the screening and at the end of the study (week 4).

2.6 Statistical analysis

Statistical analysis was performed using the computer software "IBM SPSS Statistics Subscription". Each endpoint was presented as a mean ± standard deviation (SD). A two-tailed test was used to determine the significance probability, with "significant difference" determined when the significance level was less than 5%, and "trend" when the significance level was between 5% and 10%.

For the comparison before and after the intake of the test or placebo foods, normality was first tested by the Shapiro-Wilk test. The paired *t*-test (PTT) was used when normality could be assumed, and the Wilcoxon signed-rank test (PWT) was used when normality could not be assumed. For between-group comparisons of the low-dose, high-dose, and placebo groups, the Tukey-Kramer test (TK) was performed when normality and homoscedasticity could be assumed, and the Kruskal-Wallis test followed by Bonferroni correction (KWB) was performed when normality could not be assumed. The same was done for the comparison of the amount of change.

The analysis for efficacy was based on the participants who completed the study, excluding those who met the exclusion criteria. For the safety analysis, all participants were included in the study.

3. Results

3.1 Participants

The flow of participant selection is shown in **Figure 1**. In this study, 182 candidates were recruited, 90 participants started taking the test foods, and 86 completed the prescribed schedule. The reasons for the dropout of the other 4 participants were confirmed to be unrelated to the study procedures or their effects. Of the 86 participants, 13 deviated from the study protocol, leaving 73 participants in the efficacy analysis and 86 in the safety analysis. Reasons for dropout or exclusion from the analysis are shown in **Table 1**, and participant background is shown in **Table 2**. There were no significant differences between groups in gender, age, height, weight, BMI, SBP, DBP, P, Hb1Ac, and "nasal and nose symptoms" in JRQLQ No. 1. The number of participants using allergy medication in this study was 10 out of 90 at the pre-observation period, 1 out of 90 at week 2, and 1 out of 86 at week 4. The drug scores of the allergy medi-

cation users were in the mild range. These results indicate that more than half of participants were in the healthy range.

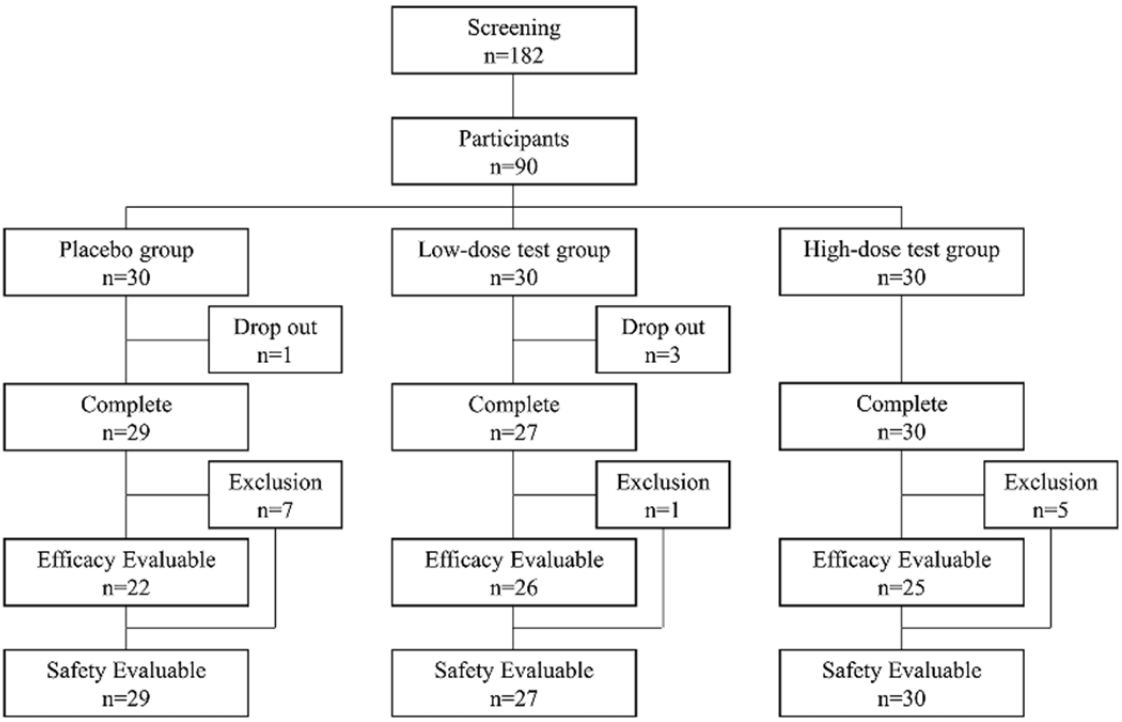

Figure 1. The flow of participant selection and evaluation.

Table 1. Reason for exclusion for participants

ID	Reason for exclusion
8	Noncompliance with restrictions
14	Withdrawal of consent
16	Noncompliance with restrictions
23	Noncompliance with restrictions
37	Noncompliance with restrictions
39	Less than 90% intake of test foods
40	Noncompliance with restrictions
41	Noncompliance with restrictions
45	Noncompliance with restrictions
54	Noncompliance with restrictions
66	Noncompliance with restrictions
67	Withdrawal of consent
71	Noncompliance with restrictions
82	Noncompliance with restrictions
83	Withdrawal of consent
85	Noncompliance with restrictions
89	Withdrawal of consent

Table 2. Participant background

	Placebo	Low-dose	High-dose
Number	30 (M: 13, F: 17)	30 (M: 14, F: 16)	30 (M: 14, F 16)
Age (years)	44.4 ± 12.0	44.9 ± 12.7	44.7 ± 11.4
Height (cm)	162.8 ± 8.1	164.4 ± 7.3	163.5 ± 8.6
Weight (kg)	59.2 ± 10.1	60.2 ± 11.0	58.6 ± 10.3
BMI	22.2 ± 3.0	22.2 ± 3.1	21.8 ± 2.5
SBP (mmHg)	112.8 ± 13.2	112.9 ± 14.2	115.1 ± 12.8
DPB (mmHg)	75.3 ± 8.7	74.5 ± 10.9	77.6 ± 8.2
P (pbm)	72.2 ± 9.1	72.0 ± 8.7	72.8 ± 12.5
HbA1c	5.34 ± 0.29	5.27 ± 0.33	5.23 ± 0.28
Nasal and eye symptom score in the question I of JRQLQ No. 1	12.1 ± 3.6	12.4 ± 3.8	12.3 ± 3.9

Values are shown as means ± SDs.

3.2 JRQLQ No. 1

The results of Question I of the JRQLQ No. 1 are shown in **Table 3**. There were no significant differences among the low-dose, high-dose, and placebo groups in scores for each endpoint or "nasal and eye symptoms" in the preliminary screening. In the between-group comparison at week 2, the high-dose group was significantly lower than the placebo group in the score for "blocked nose". At week 4, the high-dose group had significantly lower scores than the placebo group for "blocked nose" and "nasal and eye symptoms", and the low-dose group tended to have lower scores than the placebo group for "sneezing". There were no significant differences in comparisons of other endpoints and in the amount of change in scores. In the before-and-after comparison, scores at weeks 2 and 4 were significantly lower than those at screening for all endpoints in the low-dose, high-dose, and placebo groups.

The total score of JRQLQ No. 1 tended to be lower in the high-dose group than in the placebo group at week 2. There were no significant differences between groups at other time points or in comparisons of the amount of change in scores. In the before-and-after comparison, scores at weeks 2 and 4 were significantly lower than at screening for all endpoints in the low-dose, high-dose, and placebo groups.

Table 3. Results of JRQLQ No. 1

Parameter	Group	Score			Amount of change in score	
		Screening	Week 2	Week 4	Week 2 – SCR	Week 4 – SCR
Nasal and eye symptoms	Placebo	12.1 ± 3.7	5.8 ± 2.8***	5.1 ± 2.9***	–6.3 ± 3.6	–7.0 ± 4.0
	Low-dose	12.1 ± 3.4	4.7 ± 2.5***	3.8 ± 1.9***	–7.4 ± 3.2	–8.3 ± 3.2
	High-dose	11.6 ± 3.5	4.2 ± 2.0***	3.2 ± 1.9$^{***\#\#}$	–7.4 ± 4.2	–8.4 ± 3.9
Runny nose	Placebo	2.3 ± 0.9	1.0 ± 0.7***	0.8 ± 0.9***	–1.4 ± 0.7	–1.5 ± 1.0
	Low-dose	2.2 ± 1.0	0.8 ± 0.5***	0.7 ± 0.5***	–1.5 ± 1.0	–1.5 ± 1.0
	High-dose	2.1 ± 0.9	0.8 ± 0.5***	0.6 ± 0.6***	–1.4 ± 1.0	–1.5 ± 1.0
Sneezing	Placebo	2.1 ± 0.9	1.2 ± 0.8***	1.3 ± 0.9***	–0.9 ± 1.0	–0.8 ± 1.0
	Low-dose	2.0 ± 0.9	1.0 ± 0.5***	0.8 ± 0.4$^{***\#}$	–1.0 ± 1.0	–1.1 ± 0.9
	High-dose	1.9 ± 0.8	1.0 ± 0.4***	1.0 ± 0.4***	–0.8 ± 0.8	–0.9 ± 0.9
Blocked nose	Placebo	2.0 ± 1.2	1.1 ± 0.9***	0.9 ± 0.6***	–1.0 ± 1.0	–1.2 ± 1.0
	Low-dose	1.9 ± 0.9	0.8 ± 0.6***	0.7 ± 0.6***	–1.1 ± 0.8	–1.2 ± 0.9
	High-dose	2.0 ± 0.8	0.6 ± 0.6$^{***\#\#}$	0.4 ± 0.6$^{***\#\#}$	–1.4 ± 1.1	–1.5 ± 1.0
Itchy nose	Placebo	1.8 ± 0.9	0.7 ± 0.6***	0.7 ± 0.8***	–1.1 ± 0.9	–1.1 ± 0.8
	Low-dose	1.7 ± 1.0	0.7 ± 0.8***	0.5 ± 0.6***	–0.9 ± 1.1	–1.1 ± 1.0
	High-dose	1.6 ± 0.7	0.4 ± 0.5***	0.3 ± 0.5***	–1.2 ± 0.8	–1.3 ± 0.9
Itchy eyes	Placebo	2.5 ± 0.9	1.1 ± 0.7***	0.9 ± 0.6***	–1.4 ± 1.0	–1.6 ± 1.0
	Low-dose	2.6 ± 0.9	0.8 ± 0.7***	0.7 ± 0.7***	–1.8 ± 1.0	–1.9 ± 0.9
	High-dose	2.5 ± 0.8	0.8 ± 0.5***	0.5 ± 0.5***	–1.7 ± 1.0	–2.0 ± 0.9
Watery eyes	Placebo	1.5 ± 1.1	0.8 ± 0.8**	0.6 ± 0.6***	–0.7 ± 1.4	–0.8 ± 1.2
	Low-dose	1.7 ± 0.9	0.6 ± 0.5***	0.3 ± 0.5***	–1.2 ± 0.9	–1.4 ± 0.9
	High-dose	1.5 ± 0.9	0.6 ± 0.6***	0.4 ± 0.5***	–1.0 ± 1.0	–1.1 ± 0.9
Total score of the JRQLQ No. 1	Placebo	40.2 ± 18.7	14.9 ± 10.4***	12.3 ± 11.6***	–25.3 ± 16.9	–27.9 ± 20.0
	Low-dose	37.3 ± 13.8	12.0 ± 8.7***	9.0 ± 6.4***	–25.4 ± 14.4	–28.3 ± 13.9
	High-dose	38.8 ± 16.9	9.7 ± 7.9$^{***\#}$	7.6 ± 6.4***	–29.1 ± 18.6	–31.2 ± 18.0

Differences in individual scores at screening and after intake were evaluated within groups by PTT or PWT, and between groups by TK or KWB. **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#}$, significant trend to placebo (p <0.1); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as means ± SDs.

3.3 Evaluation of Nasal Remarks Score

The results of the evaluation of nasal signs are shown in **Table 4**. In the comparison between groups, "swelling of concha nasalis inferior mucosa" at week 4 was significantly lower in the low-dose group than in the placebo group, and also tended to be lower in the high-dose group.

Table 4. Evaluation of nasal remarks score

Parameter	Group	Score		Amount of change in score
		Screening	Week 4	Week 4 - SCR
Swelling of concha nasalis inferior mucosa	Placebo	2.6 ± 1.0	2.6 ± 0.8	0.0 ± 0.7
	Low-dose	2.2 ± 0.9	2.0 ± 0.5$^{*\#\#}$	–0.3 ± 0.8
	High-dose	2.7 ± 1.0	2.4 ± 0.9**	–0.3 ± 0.6
Color of concha nasalis inferior mucosa	Placebo	2.4 ± 1.1	2.0 ± 0.7	–0.3 ± 1.1
	Low-dose	2.2 ± 0.8	1.8 ± 0.4***	–0.4 ± 0.7
	High-dose	2.5 ± 0.9	1.9 ± 0.4***	–0.6 ± 1.0
Watery secretions	Placebo	2.2 ± 0.8	1.8 ± 0.5**	–0.4 ± 0.7
	Low-dose	1.9 ± 0.7	1.7 ± 0.5	–0.2 ± 0.8
	High-dose	2.1 ± 0.9	1.6 ± 0.5**	–0.5 ± 0.8
Character of nasal mucus	Placebo	3.2 ± 1.1	2.8 ± 1.1	–0.4 ± 1.3
	Low-dose	2.6 ± 1.2	2.3 ± 1.0	–0.2 ± 1.5
	High-dose	2.8 ± 1.3	2.3 ± 1.1**	–0.6 ± 1.2

Differences in individual scores at screening and after intake were evaluated within groups by PWT, and between groups by KWB. *, tend to screening (p <0.1); **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as mean ± SD.

On the other hand, there was no significant difference in "color of concha nasalis inferior mucosa", "aqueous secretion", or "character of nasal mucus" among the low-dose, high-dose, and placebo groups.

In the comparison of the changes in scores (differences from screening), there were no significant differences among the groups for changes in "swelling of concha nasalis inferior mucosa", "color of concha nasalis inferior mucosa", "aqueous secretion", and "character of nasal mucus".

In the before-and-after comparison, "swelling of concha nasalis inferior mucosa" showed a decreasing trend in the low-dose group and a significant decrease in the high-dose group at week 4 compared to the screening. The "color of concha nasalis inferior mucosa" score was significantly lower in the low-dose and high-dose groups at week 4 compared to the screening. For "aqueous secretion", the high-dose and placebo groups had significantly lower scores at week 4 than at the screening, with no significant change in the low-dose group. In character of nasal mucus, the high-dose group showed a significantly decreased score at week 4 compared to the screening, while the low-dose and placebo groups showed no significant change.

3.4 Nasal and Eye Symptoms Questionnaire

The results of the Nasal and Eye Symptoms Questionnaire are shown in **Table 5**. In the comparison of the scores of each endpoint on the nasal and eyes symptom questionnaire, there was no significant difference among the low-dose, high-dose and placebo groups for any of the following two weeks: pre-observation period, and the first and the second half of the study period.

In the comparison of changes in scores among the 3 groups (difference from the pre-observation period), the reduction in "paroxysmal sneezing" was significantly greater in the low-dose group than in the placebo group, for both the first half and second half of the study period. The reduction in "rhinorrhea" was significantly greater in the high-dose than the placebo group in both the first and second half of the study period; in addition, it was significantly greater in the low-dose than the placebo group in the first half of the study period and tended to be greater in the second half of the study period. The reduction in "itchy eyes" tended to be greater in the low-dose group than in the placebo group in the first half of the study period.

Table 5. Nasal and Eye Symptoms Questionnaire

Parameter	Group	Score			Amount of change in score	
		Pre observation	First half	Second half	First half - pre	Second half - pre
Paroxysmal sneezing	Placebo	19.2 ± 8.9	12.6 ± 9.0***	12.4 ± 8.8***	–6.5 ± 7.0	–6.8 ± 8.8
	Low-dose	21.6 ± 7.9	9.3 ± 6.8***	8.4 ± 6.8***	–12.3 ± 7.6$^{\#\#}$	–13.2 ± 9.0$^{\#\#}$
	High-dose	23.0 ± 8.3	11.8 ± 6.0***	11.0 ± 5.2***	–11.2 ± 8.8	–12.0 ± 8.5
Rhinorrhea	Placebo	22.6 ± 11.0	15.4 ± 8.9***	14.4 ± 8.8***	–7.2 ± 8.3	–8.2 ± 9.3
	Low-dose	23.9 ± 9.9	11.3 ± 8.7***	10.3 ± 8.2***	–12.7 ± 6.2$^{\#\#}$	–13.6 ± 7.0$^{\#}$
	High-dose	24.3 ± 9.5	11.6 ± 6.6***	9.8 ± 6.5***	–12.7 ± 8.2$^{\#\#}$	–14.4 ± 8.4$^{\#\#}$
Nasal blockage	Placebo	17.6 ± 11.1	9.5 ± 7.6***	7.3 ± 6.8***	–8.2 ± 7.4	–10.4 ± 9.1
	Low-dose	19.6 ± 9.2	7.2 ± 8.1***	5.5 ± 7.2***	–12.4 ± 8.3	–14.1 ± 8.7
	High-dose	17.3 ± 8.1	5.6 ± 6.7***	4.2 ± 5.6***	–11.7 ± 8.0	–13.1 ± 7.9
Itchy eyes	Placebo	22.0 ± 9.6	12.6 ± 10.2***	9.2 ± 9.5***	–9.5 ± 8.3	–12.8 ± 9.2
	Low-dose	24.8 ± 9.9	8.6 ± 8.6***	8.0 ± 9.4***	–16.2 ± 8.9$^{\#}$	–16.7 ± 9.1
	High-dose	23.0 ± 10.7	8.6 ± 8.3***	5.8 ± 6.2***	–14.4 ± 10.9	–17.2 ± 10.6
Watery eyes	Placebo	14.8 ± 9.3	7.8 ± 8.4***	4.4 ± 6.2***	–7.0 ± 7.0	–10.4 ± 7.5
	Low-dose	17.2 ± 11.6	4.3 ± 5.0***	3.6 ± 5.6***	–12.9 ± 10.8	–13.6 ± 10.5
	High-dose	15.7 ± 10.4	4.4 ± 6.0***	2.9 ± 4.2***	–11.4 ± 8.6	–12.8 ± 10.0

Differences in individual scores at screening and after intake were evaluated within groups by PTT or PWT, and between groups by TK or KWB. *, significant trend to screening (p <0.1); **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#}$, significant trend to placebo (p <0.1); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as means ± SDs.

3.5 Safety evaluation

There were no significant differences in examinations of physiology, hematology, and blood biochemistry between the screening and

end of the study (week 4) for either the low-dose, high-dose or placebo groups; fluctuations in values before and after the test were within physiological variations (**Tables 6-8**). In addition, no adverse events attributable to the test foods were observed in this study.

Table 6. Physical analysis

Parameter	Standard value	Group	Screening	Week 4
Weight (kg)	—	Placebo	59.6 ± 9.9	58.7 ± 10.1
		Low-dose	60.0 ± 10.1	59.3 ± 9.4
		High-dose	58.6 ± 10.3	57.5 ± 9.9
BMI (kg/m^2)	16.0-30.0	Placebo	22.4 ± 2.9	22.0 ± 3.1
		Low-dose	22.1 ± 3.1	21.9 ± 2.8
		High-dose	21.8 ± 2.5	21.4 ± 2.6
SBP (mmHg)	<150	Placebo	113.5 ± 12.8	111.5 ± 10.8
		Low-dose	112.7 ± 14.2	116.7 ± 13.8
		High-dose	115.1 ± 12.8	118.4 ± 11.0
DPB (mmHg)	<100	Placebo	75.9 ± 8.1	74.0 ± 7.0
		Low-dose	75.1 ± 11.1	77.1 ± 11.8
		High-dose	77.6 ± 8.2	78.7 ± 7.6
P (bpm)	50-100	Placebo	72.2 ± 9.2	72.6 ± 9.7
		Low-dose	72.0 ± 9.1	76.1 ± 10.5
		High-dose	72.8 ± 12.5	75.9 ± 12.0

Values are shown as means ± SD.

Table 7. Hematological analyses

Parameter	Standard value		Group	Screening	Week 4
	Male	Female			
WBC ($x10^3/\mu L$)	3.5-9.7		Placebo	6.04 ± 1.19	6.67 ± 1.40
			Low-dose	5.46 ± 1.08	6.54 ± 1.69
			High-dose	5.79 ± 1.45	6.26 ± 1.49
RWC ($x10^4/\mu L$)	438-577	376-516	Placebo	469 ± 35.9	464 ± 32.4
			Low-dose	463 ± 35.0	462 ± 43.8
			High-dose	467 ± 46.8	46.2 ± 51.8
Hb (g/dL)	13.6-18.3	11.2-15.2	Placebo	14.1 ± 1.2	13.9 ± 1.0
			Low-dose	14.1 ± 1.2	14.1 ± 1.3
			High-dose	14.2 ± 1.3	13.9 ± 1.4
HCT (%)	40.4-51.9	34.3-45.2	Placebo	44.0 + 3.5	43.0 ± 2.8
			Low-dose	44.4 ± 3.3	43.5 ± 3.5
			High-dose	44.2 ± 3.8	43.1 ± 3.8
PLT ($x10^4/\mu L$)	14.0-37.9		Placebo	29.6 ± 6.0	29.6 ± 5.9
			Low-dose	26.4 ± 4.3	26.9 ± 4.1
			High-dose	27.8 ± 4.8	27.1 ± 5.1

Values are shown as means ± SD.

4. Discussion

In this study, a 4-week, double-blind, parallel-group study was conducted to evaluate the effects of oral consumption of CJ peel powder[10] on allergy-like symptoms in healthy Japanese men and women with subjective symptoms of eye and nose discomfort in daily life.

In the comparison of responses to JRQLQ No. 1 (**Table 3**) between the high-dose group and placebo group, the high-dose group was significantly lower than the placebo group in the scores of "nasal and eye symptoms" at week 4, and "blocked nose" at weeks 2 and 4. In the comparison of the changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire, the decrease in "rhinorrhea" in the high-dose group was significantly greater than that in the placebo group in both the first and second half of the study period (**Table 5**). These results indicate that intake of high doses (1,000 mg/day) of CJ peel powder significantly reduced eye and nose discomfort, nasal congestion, and rhinorrhea.

In the comparison between the low-dose group and the placebo group regarding JRQLQ No. 1, the mean value of the low-dose group was lower than that of the placebo group for all endpoints although none of the differences were significant (**Table 3**). The changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire showed that the decrease in "paroxysmal sneezing" in the low-dose group was significantly greater than that in the placebo group in both the first and second half of the study period (**Table 5**). The decrease in "rhinorrhea" was significantly greater in the low-dose group than in the placebo group

in the first half of the study period and tended to be greater in the second half. Compared to the results of the high-dose group, the results for "paroxysmal sneezing" showed no dose-dependent effect, so an accidental effect cannot be ruled out, but the results for "rhinorrhea" showed a dose-dependent effect, indicating that the low-dose group also had a relieving effect on "rhinorrhea".

Table 8. Biochemical blood analyses

Parameter	Standard value		Group	Screening	Week 4
	Male	Female			
TP (g/dL)	6.5-8.2		Placebo	7.3 ± 0.3	7.3 ± 0.3
			Low-dose	7.2 ± 0.3	7.3 ± 0.3
			High-dose	7.2 ± 0.3	7.2 ± 0.4
TC (mg/dL)	150-219		Placebo	214 ± 36.7	205 ± 34.5
			Low-dose	209 ± 31.7	207 ± 32.1
			High-dose	204 ± 31.8	201 ± 33.8
LDL-C (mg/dL)	70-139		Placebo	122 ± 29.6	117 ± 30.9
			Low-dose	124 ± 22.7	125 ± 27.4
			High-dose	114 ± 27.0	114 ± 34.5
HDL-C (mg/dL)	40-80	40-90	Placebo	72.6 ± 14.5	68.5 ± 14.0
			Low-dose	67.5 ± 12.3	65.1 ± 11.4
			High-dose	71.1 ± 22.8	67.9 ± 24.5
TG (mg/dL)	50-149		Placebo	75.5 ± 41.0	75.7 ± 40.0
			Low-dose	84.0 ± 41.5	83.0 ± 37.3
			High-dose	79.0 ± 40.3	85.1 ± 51.6
BUN (mg/dL)	8.0-20.0		Placebo	13.1 ± 3.5	13.1 ± 4.9
			Low-dose	13.0 ± 2.2	14.1 ± 3.6
			High-dose	12.6 ± 4.5	13.3 ± 3.6
CRE (mg/dL)	0.65-1.09	0.46-0.82	Placebo	0.7 ± 0.1	0.7 ± 0.1
			Low-dose	0.7 ± 0.2	0.8 ± 0.2
			High-dose	0.7 ± 0.2	0.7 ± 0.2
UA (mg/dL)	3.6-7.0	2.7-7.0	Placebo	5.0 ± 1.3	5.0 ± 1.3
			Low-dose	5.2 ± 1.2	5.3 ± 1.2
			High-dose	5.3 ± 1.2	5.6 ± 1.2
T-Bil (mg/dL)	0.3-1.2		Placebo	0.7 ± 0.2	0.8 ± 0.2
			Low-dose	0.7 ± 0.3	0.8 ± 0.4
			High-dose	0.8 ± 0.3	0.9 ± 0.4
AST (U/L)	10-40		Placebo	22.8 ± 6.2	21.4 ± 5.5
			Low-dose	22.3 ± 6.1	22.3 ± 8.6
			High-dose	21.8 ± 5.8	23.1 ± 12.0
ALT (U/L)	5-45		Placebo	22.2 ±14.2	20.0 ± 11.3
			Low-dose	20.9 ± 10.8	20.2 ± 11.9
			High-dose	19.7 ± 11.1	20.2 ± 15.4
ALP (U/L)	38-113		Placebo	65.7 ± 20.4	66.7 ± 18.9
			Low-dose	57.6 ± 14.3	62.1 ± 15.7
			High-dose	57.9 ± 17.9	60.4 ± 17.5
LDH (U/L)	120-245		Placebo	174 ± 30.3	171 ± 27.8
			Low-dose	168 ± 22.6	173 ± 29.6
			High-dose	166 ± 27.8	167 ± 25.3
γ-GPT (U/L)	<79	<48	Placebo	24.5 ± 14.8	24.1 ± 20.7
			Low-dose	26.2 ± 11.6	24.8 ± 10.6
			High-dose	25.2 ± 15.9	23.3 ± 12.6
CPK (U/L)	50-230	50-210	Placebo	118 ± 87.7	114 ± 78.9
			Low-dose	112 ± 41.9	125 ± 66.8
			High-dose	110 ± 80.4	100 ± 51.4
GLU(mg/dL)	70-109		Placebo	91.8 ± 7.6	88.8 ± 5.3
			Low-dose	91.3 ± 8.3	90.9 ± 9.4
			High-dose	89.7 ± 7.5	94.5 ± 21.3

Values are shown as means ± SDs.

In this study, there was an improvement in symptoms in the high-dose, low-dose, and placebo groups compared to the pre-observation period, which may be attributed to decrease in pollen levels during the study period compared to the time of pre-observation period[12]. In addition,

the period of this study coincided with the time when people were required to refrain from going out and to wear masks when going out and in the office as countermeasures against COVID-19, and these factors may have impacted the allergen exposure among the study participants. These circumstances suggest that during this study, it was more difficult than usual to find a significant difference from the placebo group. Furthermore, the declaration of a state of emergency due to the expansion of COVID-19 during the study may have disrupted the lives of some participants, resulting in more cases of exclusion.

In the safety evaluation of the test food, there were no significant differences in examination of physiology (**Table 6**), hematology (**Table 7**), and blood chemistry (**Table 8**) among the low-dose, high-dose, and placebo groups, and the pre- and post-test variations were within physiological levels. In addition, there were no adverse events caused by the test foods in this study. These results indicate that the CJ fruit peel food in this study is safe at both low and high doses.

Comparing the results of this study with those of previous reports, the anti-inflammatory and anti-allergic effects of CJ fruits and narirutin, which is abundantly contained in the fruit, have been recognized in cell[6,7,9] and animal[3,8] experiments, as well as in oral intake intervention studies using before-and-after comparison[6,7] tests. In addition, the CJ peel powder[10] used in this study has been shown to be safe in non-clinical studies, healthy volunteers, and patients with atopic dermatitis (AD), and to be useful for patients with AD[2]. The results of this study are consistent with the anti-allergic effects reported above. To our knowledge, this is the first double-blind, parallel-group study showing the effects of oral consumption of any part of CJ fruit.

5. Conclusion

A randomized, parallel-group human food study of CJ peel powder[10] using a high-dose group (1,000 mg/day), a low-dose group (500 mg/day), and a placebo group showed that high-dose CJ peel powder improved the “eye and nasal symptoms” of allergic rhinitis symptoms, while low-dose CJ peel powder improved “nasal symptoms”. In addition, the safety of 4-week continuous intake of CJ peel powder was demonstrated.

Ethics statement

The Clinical Trial Review Committee of Hakusui-Kai Suda Clinic Medical Corporation approved this study (Approval number: 2021-004). All study participants provided informed consent. The study protocol for this study was registered in advance with the University Hospital Medical Information Network (UMIN) (UMIN000043224).

Conflict of interest

This study was funded by Jabara Laboratory Co., Ltd., but the study was conducted by a third-party organization. Yoshinobu Murakami belongs to the Department of Aesthetics and Health Sciences, Wakayama Medical University, which is funded by Jabara Laboratory Co., Ltd. Kiyoshi Nakamura was involved in this study as the investigator, but no research expenses or honoraria were paid to him by Jabara Laboratory Co., Ltd.

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