Effects and safety of daily ingestion of plum extract on blood pressure: randomized, double-blinded, placebo-controlled parallel group comparison study

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ABSTRACT

Background: Hypertension is an increasing health issue in Japan. Plums are widely consumed in Japan and are reported to have various health benefits, including improvements to blood flow. However, clinical trials investigating the effects of plum extract on blood pressure have not yet been conducted. Therefore, we evaluated the effects and safety of plum extract on blood pressure in this randomized, double-blinded, and placebo-controlled parallel group comparison study.

Methods: Seventy-four healthy Japanese subjects with systolic blood pressure (SBP) ≥130 and <160 mmHg were randomly divided into test and placebo groups. Subjects were given either plum extract-processed food (3.0 g of plum extract, containing 30 mg of mumefural and 1.119 g of citric acid) or placebo food daily for 12 weeks. Physical examinations, blood measurements, and medical interviews were performed at weeks 0, 4, 8, and 12 and at 2 weeks after the intake period.

Results: SBP and diastolic blood pressure (DBP) did not significantly differ between the groups. However, in subjects with grade I hypertension, DBP was significantly lower in the active test food group than in the placebo food group at week 12 and at 2 weeks after the intake period. An exploratory subgroup analysis revealed that plum extract improved DBP in subjects with normal to high obesity/class I obesity at week 12. Moreover, plum extract had positive effects on fatigue and bowel movements as determined by visual analog scale questionnaire evaluation. No abnormal changes or severe adverse events were observed in the physical examinations, blood measurements, or medical interviews in this trial.

Conclusion: These results suggest that plum extract is safe for long-term intake and improves DBP in subjects with grade I hypertension.

Keywords: blood pressure, clinical trial, mild hypertension, plum extract

Clinical trial registration: UMIN000023184

BACKGROUND:

It is estimated that there are 43 million people with treated or untreated hypertension in Japan [1]. In recent years, hypertension has increasingly been shown to be accompanied by lifestyle diseases such as obesity, dyslipidemia, and diabetes [2-3]. Research has shown that hypertension is related to the cause of cerebrovascular disease, cardiovascular disease, and dementia [4-5]. The prevalence of hypertension increases with age; accordingly, there is concern that the number of patients with hypertension will increase with Japan's aging society [6]. Although various antihypertensive drugs with different mechanisms have been developed, multiple drug combinations are used in current standard therapy. Therefore, lifestyle improvement, such as diet modification, has become extremely important in addition to medical therapy; various functional foods for prevention of hypertension were also researched [7-8].

Plums (*Prunus mume* Sieb. et Zucc) have been consumed for centuries in Japan and processed into various foods, such as "Umeboshi," plum wine or plum extract [9]. Approximately 200 varieties are cultivated as edible fruits, and the fruit has been used in folk medicine to alleviate fever, cough, and intestinal disorders [10] and has been consumed for many years in Wakayama, Japan. It is believed to have antibacterial effects and cure typhus fever, diarrhea caused by dysentery/diarrhea, and food poisoning [9]. Moreover, recent research has suggested that plum extract has various other effects, such as antigastric ulcer, antioxidant effects, and antivascular

hypertrophy [9]. Plum extract is produced by concentrating plum to a moisture content of around 20%. It contains a high amount of citric acid [10], an α-hydroxy acid abundant in citrus fruits. It plays a central role in energy metabolism and has antifatigue effects [11]. Citric acid has also shown to promote antiplatelet aggregation [12] and has antiblood coagulation effects [13].

Mumefural (1-[5-(2-formylfuryl)methyl] dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate) was identified in plum extract as a functional component and is produced by heating sugar and the citric acid contained in unripe plums [14]. It was revealed that mumefural promotes erythrocyte deformability, inhibits platelet aggregation, and improves blood flow [9]. Previous research identified mumefural and its analogs in plum extract and intake of plum extract improved blood flow in healthy subjects [14]. Furthermore, mumefural was gradually degraded to citric acid and 5-hydroxymethyl-2-furfural in blood. Therefore, mumefural may contribute to sustaining the concentration of citric acid. These findings suggest that improved blood flow by plum extract was not only due to citric acid but also mumefural. However, clinical trials investigating the effects of plum extract on blood pressure have not yet been conducted.

We assessed the efficacy of plum extract on blood pressure in 80 healthy subjects (aged 30–69 years) with hospital systolic blood pressure (SBP) ≥130 and <160 mmHg in a 12-week randomized, double-blinded, placebo-controlled parallel group comparison study.

METHODS:

Study Subjects

We recruited 161 volunteers with mild elevated blood pressure. All volunteers provided written informed consent to participate in this clinical study. Finally, through screening tests, we selected 80 healthy Japanese subjects (aged 30–69 years). These included individuals with an SBP ≥130 and <160 mmHg and who were judged by a physician as not needing hospital treatment for hypertension. Inclusion and exclusion criteria is shown in Table 1.

The 80 eligible subjects were randomly assigned to active test food or placebo food groups, with adjustments for sex, age, and level of SBP. A randomization sequence was created using a permuted block randomization design stratified by sex, age, and level of SBP. To ensure a balanced design, each subject was assigned to a relevant group by a third-party data center according to the randomization. The third-party data center concealed the assignment information, including the subjects' personal data, keeping it secure for the duration of the trial so that all staff (doctors, nurses, clinical research coordinators, and statistical analyzers) had no knowledge of the participating subjects. This information was only disclosed after the laboratory and analysis data were fixed, and the method of statistical analysis was finalized.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	1. SBP ≥130 and <160 mmHg
	2. Judged by a physician as not needing hospital treatment for hypertension
Exclusion criteria	1. Receiving medication for hypertension
	2. Serious cerebrovascular, cardiac, hepatic, renal, or gastrointestinal disease
	3. Had a major surgical history related to the digestive system
	4. Unusually abnormal hematological data or serious anemia
	5. Regularly taking medicine, functional foods, and/or supplements that would improve hypertension
	6. Heavy smokers, alcohol addicts, or subjects with an irregular lifestyle
	7. A severe allergic reaction to foods, particularly plum, fruits of the family Rosacea, and/or white birch pollen
	8. Other medical reasons, as judged by the principal investigator

Study Design

The clinical study was conducted as a randomized, double-blinded, placebo-controlled parallel group comparison study and was carried out in the Hokkaido Information University, Health Information Science Research Center (Ebetsu city, Hokkaido, Japan). The examination schedule for this study is shown in Table 2. We performed vital signs measurements and visual analog scale (VAS) questionnaires at weeks 0 (baseline), 4, 8, and 12 after the start of test food ingestion and 2 weeks after the end of test food ingestion (week 14). At each time point, a medical interview was conducted by a physician. Physical, hematological, and biological examinations were performed at weeks 0, 4, 8, and 12. We asked the subject groups to consume 10 g of test food three times a day (total 30.0 g/day) before each meal, with either active test food containing 1.0 g of plum extract (total 3.0 g/day) or placebo food. The volume of plum extract was determined using primary clinical trial. During this study, subjects were asked to not change their daily activities, including food consumption, medication, and exercise, and keep a life diary during the study period. The primary outcome was hospital blood pressure, and the secondary outcome was the VAS questionnaire score.

Table 2. Examination schedule

| | Guidance and agreement | Screening | Randomization | Test food intake period | | | | |
				Week 0	Week 4	Week 8	Week 12	Week 14
Date	Jul 20－23, 2016	Jul 20－23, 2016	Aug 1, 2016	Sep 1－3	Sep 29－Oct 1	Oct 27－29	Nov 24－26	Dec 8－10
Medical interview	—	○	—	○	○	○	○	○
Vital signs measurement	—	○	—	○	○	○	○	○
Physical measurement	—	○	—	○	○	○	○	—
Blood sampling	—	○	—	○	○	○	○	—
VAS questionnaire	—	—	—	○	○	○	○	○
Diary record	—	—	—	←				→

Preparation of Test Food

The plum used in the active test food in this trial was cultivated in Wakayama, Japan. The manufacturing process for active test food containing 3.0 g plum extract was the following: materials (plum extract, erythritol, xanthan gum, fragrance, and sweetener) were mixed, followed by hot filling at 90°C. The manufacturing process for the placebo food was the same except for the materials used (caramel, erythritol, L-tartaric acid, xanthan gum, fragrance, and sweetener). Analytical results of the nutrient composition for active test food and placebo food used in this study are provided in Table 3. Analysis of the nutrient composition was performed by the Japan Food Research Laboratories (Chitose, Japan). Citric acid was measured using high-performance liquid chromatography (HPLC) at the Japan Food Research Laboratories. Mumefural was measured using HPLC at NAKANO BC, Co Ltd. The active test food and placebo food were identical in appearance. The test foods were manufactured under strict quality control at the NAKANO BC Co. Ltd. with ISO9001 certification.

Table 3. Nutrient composition of test foods (per 30 g)

	Placebo food	Active test food
Calories (kcal)	14.4	15.3
Water (g)	26.31	25.71
Proteins (g)	—	0.12
Lipids (g)	—	—
Carbohydrates (g)	3.63	4.02
Ash (g)	0.06	0.15
Sodium (mg)	37.5	7.32
Mumefural (mg)	—	30
Citric acid (g)	0.021	1.119

Hospital Blood Pressure Measurements

Blood pressure (BP) was measured by a doctor or nurse using an Automatic Blood Pressure Monitor HEM-7080IC (Omron Colin Co., Ltd., Tokyo, Japan) using the upper arm on the nondominant arm after >10-min rest. Three sequential measurements were performed, and the median of the measurements was taken at each evaluation point.

VAS Questionnaire

To evaluate the effects of plum extract on fatigue, chillness, and bowel movements, the subjects completed a VAS questionnaire consisting of eight questions assessing (1) fatigue, (2) stiffness of the neck and shoulders, (3) stiffness of the waist, (4) chillness in hands and feet, (5) defecation regularity, (6) defecation difficulty, (7) defecation satisfaction, and (8) fecal odor. Subjects were instructed to mark a "cross" on a 100-mm line to answer each question based on their current health condition. The left end of the line (0 mm) was defined as the worst condition and the right end (100 mm) as the best condition. The questionnaire results were assessed by evaluating the length from the start of the line on the left to the cross. An increase in VAS score indicated an improvement for each symptom.

Physical, Hematological, and Biological Measurements

Blood was collected after a 12-h fast. General blood tests were performed, including measurement of complete blood counts (CBCs; WBCs, RBCs, Hb, Ht, and Plt), liver function (AST, ALT, γ-GTP, ALP, and LDH), kidney function (BUN, CRE, and UA). Blood tests were performed by the Sapporo Clinical Laboratory, Inc. (Sapporo, Japan). Each subject's body composition was measured using a Body Composition Analyzer DC-320 (Tanita Corp, Tokyo, Japan).

Ethics Committee

All subjects provided written informed consent prior to undergoing any of the tests related to this study. The Ethics Committee of Hokkaido Information University approved the study protocol conforming to the Helsinki Declaration and Ethical Guidelines for Medical and Health Research Involving Human Subjects (approval date, Jul 21, 2016; approval number 2016-02). This study was registered with UMIN Clinical Trials Registry (approval number UMIN000023184).

Sample Size

The sample size was statistically determined to obtain a power of 80% with an alpha of 0.05. To demonstrate SBP at week 12, which was postulated to have an intergroup difference of 7 mmHg with a standard deviation (SD) of 10 mmHg, a sample size of 72 (36 in each group) was required. Assuming a 10% loss in the follow-up rate, 80 subjects were selected.

Statistical Analysis

Values are presented in the tables as means ± SD. Changes in subject values were analyzed using repeated measures ANOVA between the groups for evaluation of BP. Moreover, changes in subject values were analyzed using Student's *t* test for evaluations of physical, hematological, and biological parameters between active test food and placebo food at each evaluation point. *p* value <0.05 was considered to be significant. Statistical analyses were performed using SPSS Statistics 19 (IBM, Armonk, NY, USA).

RESULTS

Subject Dropouts, Exclusions, and Characteristics

During the trial, five subjects withdrew (started treatment, *n* = 3; irregular lifestyle, *n* = 1; personal reasons, *n* = 1). Consequently, 75 subjects completed this trial (placebo food group, *n* = 38; active test food group, *n* = 37). One subject was excluded from the analysis of the "per-protocol set" because of an irregular lifestyle (placebo food group, *n* = 1). We included 74 subjects in the PPS analysis for efficacy analysis (placebo food group, *n* = 37; active test food group, *n* = 37), and 80 subjects in ITT analysis for safety analysis (placebo food group, *n* = 40; active test food group, *n* = 40). The study flow diagram is shown in Fig. 1.
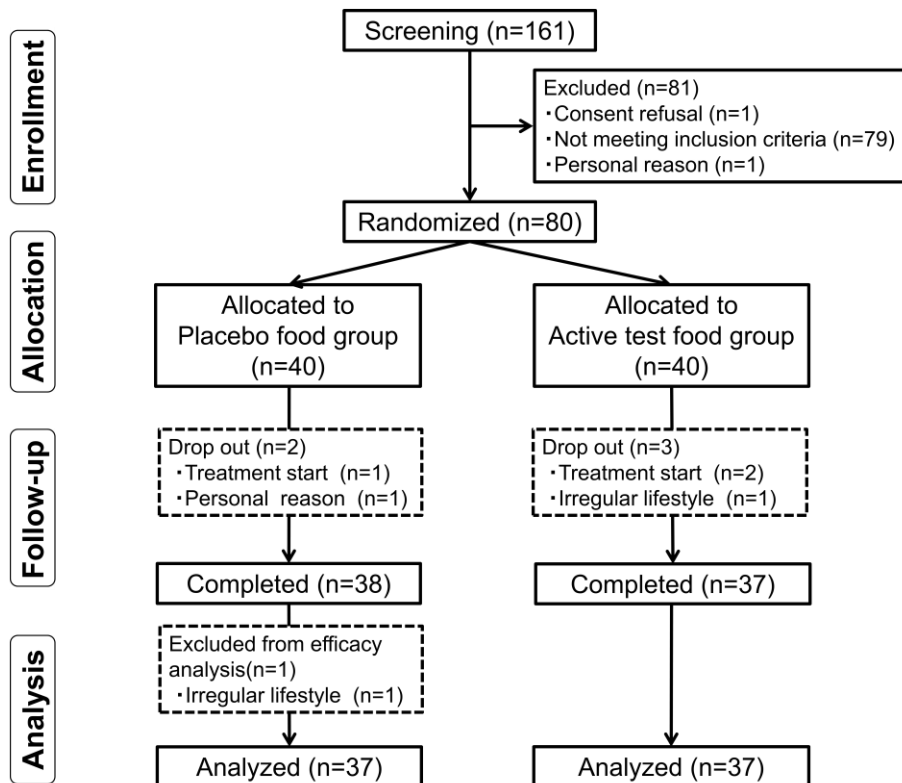

Figure 1. Flow diagram of the trial.

The mean age, height, body weight, body mass index, SBP, and DBP for each group are presented in Table 4. These data did not differ significantly between the active test food and placebo food groups, confirming the appropriate allocation of subjects into the two groups. Moreover, there were no differences between the active test food and placebo food groups regarding intake rate (Table 4).

Table 4. Characteristics of the subjects in the active test food and placebo groups

Characteristic	Placebo	Active	*p*
Subjects, *n*	37	37	–
Male, *n*	10	12	0.80
Age, years	57.78 ± 8.78	57.49 ± 9.21	0.89
Height, cm	157.34 ± 7.02	160.56 ± 8.58	0.08
Body weight, kg	55.91 ± 11.03	58.12 ± 8.66	0.34
Body mass index, kg/m^2	22.44 ± 3.17	22.49 ± 2.36	0.95
Systolic blood pressure, mmHg	139.11 ± 11.03	141.03 ± 12.40	0.48
Diastolic blood pressure, mmHg	81.51 ± 9.05	84.41 ± 8.79	0.17
Intake rate, %	98.39 ± 3.01	99.04 ± 1.61	0.26

Values are shown as the mean ± standard deviation. Analyses were performed using Student's *t* test for age, height, body weight, body mass index, systolic blood pressure, and diastolic blood pressure, and chi-square test was used for gender. Mann–Whitney's *U* test was used to analyze the intake rate. *n*, number of subjects.

Effects of Plum Extract on Blood Pressure

We evaluated the effects of plum extract on BP (Table 5). There were no differences between the active test food and placebo food groups regarding changes in SBP and DBP. However, in subjects with grade I hypertension (SBP ≥140 and <169 mmHg or DBP ≥90 and <100 mmHg), DBP was significantly lower in the active test food group than the placebo food group at weeks 12 and 14 (change from baseline to week 12: placebo, 4.21±4.92 mmHg; active, −1.53±7.28 mmHg, *p* = 0.019 and change from baseline to week 14: placebo, 3.86 ± 4.07 mmHg; active, −2.80±7.08 mmHg, *p* = 0.005). In an exploratory subgroup analysis, DBP significantly decreased at week 12 (change from baseline to week 12: placebo, 4.50 ± 7.68 mmHg; active, −1.64 ± 7.28 mmHg, *p* = 0.047) and the time–group interaction of change value of DBP was significant (*p* = 0.009) following ingestion of the active test food in subjects with normal to high obesity or class I obesity. Moreover, SBP tended to decrease at week 12 (change from baseline to week 12: placebo, 3.58 ± 12.84 mmHg; active, −4.36 ± 13.70 mmHg, *p* = 0.14) and the time–group interaction of change value of SBP was significant in the same subject group (*p* = 0.008).

Table 5. Measurement of blood pressure

		Week 0	△week 4	△week 8	△week 12	△week 14	p^b
SBP in all subjects (mmHg)	Placebo	132.16±10.60	1.24±10.59	2.41±9.69	-0.22±11.41	-1.92±11.86	
	Active	132.81±12.34	3.68±10.88	1.97±13.87	1.62±12.84	-2.19±12.03	0.59
	p^a	0.81	0.33	0.88	0.52	0.92	
DBP in all subjects (mmHg)	Placebo	81.03±8.14	1.73±5.72	2.32±4.88	2.03±5.71	0.43±5.62	
	Active	81.92±7.91	1.19±5.98	1.17±7.55	0.81±7.21	-0.51±7.45	0.91
	p^a	0.63	0.69	0.44	0.43	0.54	
SBP in subjects with high-normal blood pressure (mmHg)	Placebo	128.30±9.56	0.70±12.00	3.55±9.54	-0.47±11.25	-1.60±12.60	
	Active	128.21±11.89	3.26±11.49	3.56±13.10	2.53±13.06	-0.11±11.51	0.67
	p^a	0.98	0.50	1.00	0.45	0.70	
DBP in subjects with high-normal blood pressure (mmHg)	Placebo	80.35±7.87	0.90±5.68	1.95±5.31	1.21±5.57	-1.20±5.60	
	Active	80.05±7.42	1.63±5.69	2.06±6.47	2.26±7.36	1.47±7.93	0.25
	p^a	0.90	0.69	0.96	0.62	0.23	
SBP in subjects with grade I hypertension (mmHg)	Placebo	134.93±10.20	2.29±7.62	2.14±10.47	0.50±12.94	-1.29±11.93	
	Active	136.33±11.71	3.00±11.04	-1.60±15.44	-0.07±14.09	-5.07±12.44	0.57
	p^a	0.73	0.84	0.46	0.91	0.41	
DBP in subjects with grade I hypertension (mmHg)	Placebo	80.07±8.29	3.71±5.24	3.57±4.24	4.21±4.92	3.86±4.07	
	Active	84.27±8.53	1.07±6.63	-0.53±8.98	-1.53±7.28	-2.80±7.08	0.23
	p^a	0.19	0.24	0.13	0.019*	0.005**	
SBP in subjects with normal weight (mmHg)	Placebo	132.25±11.62	2.13±11.04	1.00±9.91	-2.13±10.39	-3.42±12.30	
	Active	131.48±12.64	3.30±9.95	3.36±12.32	5.26±11.06	-1.83±9.67	0.17
	p^a	0.83	0.70	0.48	0.023*	0.63	
DBP in subjects with normal weight (mmHg)	Placebo	80.88±8.59	1.79±5.39	1.71±4.82	0.79±4.09	-0.83±4.72	
	Active	80.30±8.41	0.48±5.81	1.86±7.50	2.30±6.90	-0.43±7.52	0.30
	p^a	0.82	0.43	0.93	0.36	0.83	
SBP in subjects with normal-high / class I obesity (mmHg)	Placebo	132.00±8.84	-0.38±9.91	5.00±9.08	3.58±12.84	0.85±10.92	
	Active	135.00±11.97	4.29±12.63	-0.21±16.25	-4.36±13.70	-2.79±15.56	0.008**
	p^a	0.47	0.30	0.32	0.14	0.49	
DBP in subjects with normal-high obesity/ class I obesity(mmHg)	Placebo	81.31±7.55	1.62±6.53	3.46±4.98	4.50±7.68	2.77±6.55	
	Active	84.57±6.45	2.36±6.30	0.07±7.79	-1.64±7.28	-0.64±7.62	0.009**
	p^a	0.24	0.77	0.19	0.047*	0.23	

SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ week 4, change from baseline to week 4; Δ week 8, change from baseline to week 8; Δ week 12, change from baseline to week 12; Δ week 14, change from baseline to week 14. Values are shown as the mean ± standard deviation. Changes in subject values were analyzed using repeated measures ANOVA between the groups (p^b). Changes in subject values were analyzed using Student's *t* test to compare the mean of the test food group and the placebo food group at each evaluation point (p^a). Statistically significant, *p < 0.05,**p < 0.01.

Effects of Plum Extract on VAS Scores: To confirm the effects of plum extract on fatigue, chillness, and bowel movements, we evaluated changes in VAS based on a questionnaire rating eight factors (Table 6). "Fatigue" was improved after ingestion of the active test food compared with the placebo food group (change from baseline to week 4: placebo, −8.62 ± 21.69 mm; active, 5.05 ± 16.81 mm, $p = 0.002$; change from baseline to week 12: placebo, −1.64 ± 19.33 mm; active, 11.22 ± 19.26 mm, $p = 0.007$; and change from baseline to week 14: placebo, 0.51 ± 21.96 mm; active, 9.76 ± 14.53 mm, $p = 0.021$). Furthermore, ingestion of the active test food improved "defecation difficulty" at week 12 compared with the placebo food group (change from baseline to week 12: placebo, −4.78 ± 26.16 mm; active, −0.41 ± 20.70 mm, $p = 0.045$).

Table 6. Scores of VAS questionnaire

		Week 0	Δ Week 4	Δ Week 8	Δ Week 12	Δ Week 14
Fatigue (mm)	Placebo	72.11 ± 21.31	−8.62 ± 21.69	1.05 ± 19.47	−1.64 ± 19.33	0.51 ± 21.96
	Active	57.54 ± 23.45	5.05 ± 16.81	6.47 ± 17.71	11.22 ± 19.26	9.76 ± 14.53
	p	0.007**	0.002**	0.09	0.007**	0.021*
Stiffness of the neck and shoulder (mm)	Placebo	61.35 ± 30.50	1.97 ± 17.73	6.76 ± 19.93	4.14 ± 22.68	3.05 ± 22.84
	Active	56.08 ± 32.72	1.81 ± 22.80	3.81 ± 27.18	−0.38 ± 19.15	2.03 ± 22.04
	p	0.56	0.97	0.46	0.35	0.70
Stiffness of waist (mm)	Placebo	72.43 ± 26.68	3.03 ± 19.71	2.46 ± 23.88	5.06 ± 30.32	3.24 ± 26.64
	Active	75.95 ± 23.55	−3.68 ± 16.95	−7.06 ± 23.80	−6.78 ± 22.70	−4.14 ± 18.89
	p	0.88	0.64	0.21	0.23	0.48
Chillness in hands and feet (mm)	Placebo	84.51 ± 22.34	−2.16 ± 20.11	−21.78 ± 29.49	−16.64 ± 35.56	−12.62 ± 33.02
	Active	81.84 ± 19.92	−4.51 ± 18.53	−19.86 ± 25.25	−15.84 ± 28.67	−12.89 ± 20.26
	p	0.20	0.59	0.92	1.00	0.69
Defecation regularity (mm)	Placebo	66.08 ± 34.14	2.57 ± 18.06	3.57 ± 21.30	5.31 ± 18.00	3.59 ± 19.33
	Active	62.08 ± 27.99	−1.62 ± 23.91	1.17 ± 21.39	2.76 ± 23.23	0.73 ± 26.98
	p	0.42	0.60	0.87	0.60	0.96
Defecation difficulty (mm)	Placebo	78.38 ± 22.84	5.03 ± 21.18	−1.49 ± 18.52	−4.78 ± 26.16	−3.86 ± 28.59
	Active	69.14 ± 26.25	2.11 ± 16.34	2.06 ± 22.00	−0.41 ± 20.70	−0.51 ± 20.94
	p	0.14	0.38	0.14	0.045*	0.46
Defecation satisfaction (mm)	Placebo	74.62 ± 23.72	−0.05 ± 22.08	0.84 ± 21.35	0.19 ± 21.03	0.89 ± 26.73
	Active	73.19 ± 26.42	0.84 ± 19.78	−1.00 ± 23.24	−0.22 ± 22.23	−3.32 ± 24.92
	p	0.95	0.60	0.85	0.89	0.57
Fecal odor (mm)	Placebo	65.76 ± 25.67	7.16 ± 22.90	2.43 ± 24.25	1.86 ± 25.11	5.89 ± 28.08
	Active	63.89 ± 22.53	2.68 ± 17.02	3.14 ± 12.51	3.16 ± 15.37	4.92 ± 15.83
	p	0.75	0.54	0.87	0.72	0.63

Δ week 4, change from baseline to week 4; Δ week 8, change from baseline to week 8; Δ week 12, change from baseline to week 12. Values are shown as the mean ± standard deviation. Changes in subject values were analyzed using Mann–Whitney's *U* test to compare the mean of the test food group and the placebo food group at each evaluation point. *Statistically significant, $p < 0.05$.

***Safety*:** We evaluated body composition, CBCs, and liver and renal function after ingestion of the test foods (Table 7). Although minimal changes were observed, these changes were within the normal range. Moreover, few subjects exhibited adverse effects. However, these subjects presented only mild symptoms and recovered within a few days. Therefore, the principal investigator judged that were no adverse events related to the ingestion of the test food. There were also no abnormal changes or severe adverse events observed in the physical, hematological, and biological examinations, and the medical interview in this trial and long-term intake of 3.0 g of plum extract was considered safe.

Table 7. Body composition, CBC parameters, liver, and renal function

		Week 0	Week 4	Week 8	Week 12
BW (kg)	Placebo	56.29 ± 11.27	56.76 ± 11.03	56.35 ± 10.94	55.88 ± 11.09
	Active	57.88 ± 8.51	58.04 ± 8.47	58.42 ± 8.94	58.24 ± 9.01
BFP (%)	Placebo	27.16 ± 6.36	28.46 ± 5.98	28.12 ± 7.26	29.11 ± 6.14
	Active	27.26 ± 7.48	27.56 ± 7.27	27.90 ± 7.41	27.91 ± 7.11
BMI (kg/m^2)	Placebo	22.40 ± 3.14	22.59 ± 3.06	22.55 ± 3.15	22.48 ± 3.24
	Active	22.49 ± 2.43	22.49 ± 2.33	22.58 ± 2.41	22.53 ± 2.42
WBCs (×10^3/μl)	Placebo	5.39 ± 1.37	5.39 ± 1.17	5.61 ± 1.51	5.56 ± 1.26
	Active	5.62 ± 2.03	5.14 ± 1.43	4.97 ± 1.40	5.38 ± 1.72
RBCs (×10^4/μl)	Placebo	467.08 ± 56.25	472.18 ± 53.31	475.28 ± 52.80	478.97 ± 51.92
	Active	463.08 ± 44.08	461.62 ± 49.07	470.25 ± 49.08	471.41 ± 51.75
Hb (g/dl)	Placebo	13.60 ± 1.18	13.81 ± 1.24	13.88 ± 1.34	13.80 ± 1.29
	Active	13.67 ± 1.49	13.69 ± 1.69	13.85 ± 1.67	13.91 ± 1.71
Ht (%)	Placebo	41.40 ± 3.55	42.17 ± 3.61	42.48 ± 3.80	42.53 ± 3.45
	Active	41.57 ± 3.90	41.82 ± 4.72	42.55 ± 4.36	42.65 ± 4.40
Plt (×10^4/μl)	Placebo	23.27 ± 4.37	23.67 ± 4.92	25.05 ± 5.32	25.78 ± 5.05
	Active	23.74 ± 6.03	23.30 ± 5.84	23.41 ± 6.11	23.88 ± 5.75
AST (U/l)	Placebo	22.33 ± 5.63	23.45 ± 7.59	22.80 ± 5.98	22.30 ± 6.05
	Active	22.60 ± 5.72	22.15 ± 4.20	22.25 ± 5.02	23.03 ± 7.11
ALT (U/l)	Placebo	20.31 ± 12.12	20.90 ± 12.93	20.68 ± 12.54	19.65 ± 9.75
	Active	21.13 ± 10.30	20.18 ± 7.37	20.83 ± 8.79	20.35 ± 9.42
γ-GTP (U/l)	Placebo	30.00 ± 28.10	31.55 ± 34.31	30.95 ± 32.89	30.32 ± 27.07
	Active	28.43 ± 20.25	28.49 ± 19.74	27.78 ± 20.58	28.89 ± 20.51
ALP (U/l)	Placebo	213.08 ± 42.70	222.43 ± 56.68	227.28 ± 66.38	228.03 ± 41.16
	Active	211.15 ± 57.76	209.74 ± 56.88	206.92 ± 53.15	209.70 ± 52.61
LDH (U/l)	Placebo	200.28 ± 32.02	198.58 ± 37.09	191.20 ± 30.40	199.92 ± 34.55
	Active	200.53 ± 34.53	193.82 ± 30.79	189.31 ± 32.15	197.35 ± 34.74
BUN (mg/dl)	Placebo	14.13 ± 3.28	14.31 ± 3.75	14.43 ± 4.15	13.75 ± 3.40
	Active	14.17 ± 3.36	14.32 ± 3.19	14.47 ± 3.29	14.11 ± 2.88
CRE (mg/dl)	Placebo	0.77 ± 0.12	0.75 ± 0.11	0.76 ± 0.13	0.74 ± 0.11
	Active	0.77 ± 0.14	0.76 ± 0.13	0.77 ± 0.14	0.76 ± 0.12
UA (mg/dl)	Placebo	5.34 ± 1.23	5.21 ± 1.12	5.12 ± 1.13	4.98 ± 1.11
	Active	5.05 ± 1.34	5.04 ± 1.29	4.98 ± 1.39	4.90 ± 1.16

Values are shown as the mean ± standard deviation.

DISCUSSION

This clinical trial showed that plum extract-processed food displayed more beneficial effects on BP in subjects with SBP ≥130 and <160 mmHg. There were no significant differences between the active test food and placebo food groups regarding changes in the level of SBP and DBP. However, in subjects with grade I hypertension or normal to high obesity/class I obesity, DBP significantly decreased in the active test food group compared with the placebo food group at week 12, results suggesting improvement.

The evaluation of DBP was influenced by endothelial function, such as vascular stiffness. It was reported that citric acid and mumefural improved blood flow via inhibition of platelet aggregation and promotion of erythrocyte deformability [9]. In addition, previous research has been revealed that the active center of mumefural was the residue derived from citric acid [15]. Endothelial function demonstrated a change in morphology, gene, and protein expression of nitric oxide and adhesion molecules and signal transduction of calcium and potassium due to mechanical actions such as shear stress and extension by blood flow [15]. These findings suggested that plum extract inhibited the increase of DBP by improving blood flow via citric acid and mumefural contained in the plum extract. However, the VAS question relating to chillness (improvement of blood flow) was not changed by ingestion of active test food. The biological mechanism of these results needs to be elucidated to fully understand the effects of plum extract. Additionally, DBP was significantly decreased in the active test food group at 2 weeks after the end of test food ingestion. This suggests that the effect of the plum extract persisted after the intake period.

Moreover, as an exploratory subgroup analysis, DBP significantly decreased at week 12 and time–group interaction of change value of DBP was significant following ingestion of the active test food in subject with normal to high obesity or class I obesity. Furthermore, SBP tended to decrease at week 12, and the time–group interaction of change value of SBP was significant in the same subject group. Previous research reported a correlation between obesity and hypertension [16]. Moreover, cytokines and adipokines secreted from increased adipose tissue induced endothelial dysfunction and atherosclerosis [17]. It is suspected that endothelial function of subjects with normal to high obesity/class I obesity is impaired compared with normal weight subjects. These findings suggest that plum extract was more effective in subjects with normal to high obesity/class I obesity.

The VAS questionnaire indicated that fatigue was improved by ingestion of active test food at weeks 4 and 12 and at week 2 after the intake period. Active test food contained 1.119 g of citric acid. A previous clinical study reported that ingestion of drink containing lemon citric acid (2,700

mg) attenuated the fatigue feeling in VAS for people frequently feeling fatigue by continuing intake for 28-week [18]. Other clinical study reported that the continued intake of 2,700 mg/day of citric acid for 8 weeks reduced physiological stress and attenuated physical fatigue [11]. These findings suggest that the citric acid contained in plum extract may improve feelings of fatigue. However, our clinical trial only used an objective evaluation to assess fatigue. Moreover, initial values were significantly different among the two groups. Further research is required to determine the effects of plum extract on fatigue such as evaluating stress markers. Furthermore, the VAS question relating to "defecation difficulty" showed improvement following the ingestion of active test food at week 12. A previous study suggested that consumption of carbohydrate-free plum juice for 11 weeks had the potential to modify fecal microbial ecology in an obese animal model [19]. Further clinical trials into constipation are required to assess the effects of plum extract on intestinal flora.

In our clinical study, no abnormal changes or severe adverse events were observed in the physical and blood examinations or reported in the medical interviews during the trial. These results confirm the safety of 12-week ingestion of 3.0 g of plum extract.

The results of this study reveal that plum extract improved DBP in subjects with grade I hypertension or normal to high obesity/class I obesity. Furthermore, plum extract had positive effects on fatigue and bowel movements.

CONCLUSION

The present study was a 12-week randomized, double-blinded, placebo-controlled parallel group comparison study to determine the effects of consuming plum extract among subjects with normal high blood pressure/grade I hypertension. It was revealed that plum extract improved DBP in subjects with grade I hypertension or normal to high obesity/class I obesity. Moreover, plum extract had positive effects on fatigue and bowel movements. Our results confirmed the safety of 12-week ingestion of 3.0 g of plum extract. Although the biological mechanism of these results needs to be elucidated to fully understand the effects of plum extract, plum extract will be used as a new functional food for mild hypertension in the future.

List of Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BP, blood pressure; BUN, blood urea nitrogen; BFP, body fat percentage; BMI, body mass index; BW, body weight; CBCs, complete blood counts; CRE, creatinine; DBP, diastolic blood pressure; γ-GTP, gamma glutamyl transpeptidase; Ht, hematocrit; Hb, hemoglobin; LDH, lactate dehydrogenase; Plt, platelet counts;

RBCs, red blood cells; SBP, systolic blood pressure; UA, uric acid; VAS, visual analog scale; WBCs, white blood cells.

Authors' Contributions: JN, MN, HK, AK, and NG designed the research. HK, AK, and NG provided test food. JN conducted the research. MN performed statistical analyses. MN and JN wrote the manuscript. JN had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

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