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Efficacy and Safety of Long-term Ingestion of the Hyuga-touki Leaf on Blood Pressure in Healthy Japanese:

A Randomized, Double-blind, Placebo-controlled Study

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● **Abstract**

Objectives: The objective of this study is to examine how the ingestion of Hyuga-touki leaf containing YN-1 (isoepoxypteryxin) and isopteryxin contributes to the reduction of blood pressure.
Methods: A randomized, double-blind, placebo-controlled study was conducted to elucidate an effect of food ingestion. In this study we measured systolic blood pressure (SBP) and diastolic blood pressure (DBP). We also evaluated subjective reporting and biochemical analysis of blood and urine for safety as the secondary outcome, and adverse events were collected by means of a written questionnaire during the study.
Results: 59 subjects were randomly assigned to two intervention groups, active and placebo, and made a start with ingestion. 18 subjects discontinued due to personal reasons, and the remaining 41 subjects completed the study. 5 were eliminated because of an efficacy eligibility (insufficient intake of the food less than 80% of the expected dose), thus data obtained with 36 subjects (Active; 16, Placebo; 20) was used for the analysis. In addition, stratified analysis was applied with 31 subjects excluding 5 subjects with I degree high blood pressure (SBP ≥ 140 or DBP ≥ 90). As the result of an analysis of normotensive and high-normotensive healthy person, the intergroup analysis showed a significant difference in SBP. Regarding subjective reporting, there was no significant difference between the groups in all items. No adverse effects were observed after the ingestion of the test food.
Conclusion: We found out that the ingestion of Hyuga-touki leaf containing YN-1 and isopteryxin for 12 weeks improved SBP. Additionally, no safety-related matter occurred during the 12-week of test period.

Key Words: Hyuga-touki, *Angelica Furcijuga* Kitagawa, YN-1, isoepoxypteryxin, isopteryxin, blood pressure, SBP, high-normal, tablet

1. INTRODUCTION

Although the blood pressure of Japanese has declined significantly in both males and females even in the past ten years, the proportion of hypertension with systolic blood pressure (SBP) more than 140 mmHg including those taking antihypertensive medicine is still high as 38.6% in males and 28.7% in females over 40 years old (National Health and Nutrition Survey, 2016)[1)]. Hypertension is one of the most frequent diseases in Japan. The government declared Healthy Japan 21 (the second) in 2012 which also aims to improve hypertension and to decrease dyslipidemia (risks manifesting cerebrovascular disease and ischemic heart disease). By 2022, the government hopes to set an SBP goal of 134 in males and 129 in females[2)]. In order to prevent and improve hypertension and arteriosclerosis, it is said to be important to improve lifestyle mainly with diet therapy such as reducing salt and alcohol drinking and with physical exercise.

Meanwhile, the traditional medicinal plant in northern Miyazaki Prefecture named Hyuga-touki is called Nihon-yamaninjin and has been eaten as it suppresses blood pressure and blood glucose levels[3)], prevent inflammation and allergy[4)]. For modern Japanese, who tend to lead an irregular lifestyle and intake excessive calories, we think that it is beneficial to be able to prevent lifestyle diseases by easily ingesting food as a supplement. In this study, we examined the effect of Hyuga-touki leaf for blood pressure and the safety by a randomized, double-blind, placebo-controlled study.

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from Meigen Inc.

1) JACTA (Japan Clinical Trial Association)
2) Nihonbashi M's Clinic
3) Meigen Inc.

Table 1　Schedule for the study

Item \ Term	Screening	Pretrial test	Test period	
			6 w	12 w
Informed consent	●			
Selection and/or allocation	●			
SBP/ DBP	●	●	●	●
Subjective reporting		●	●	●
Blood and urine test		●	●	●
Ingestion of test foods			↔	↔
Log			↔	↔

● : Implementation
↔ : Daily practice during the test period

(Kagoshima) at two centers (KUROSU HOSPITAL, Tokyo and Japan Clinical Trial Association; JACTA, Tokyo). The study period was 12 weeks, from September 2017 to January 2018, and divided into two groups with different testing periods. This study was conducted in accordance with the ethical principles of the declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all Subjects. This trial was registered at UMIN Clinical Trial Registry (Trial ID: UMIN000027204). The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of TRIBELATE CORPORATION (Tokyo), recruited from August through October, 2017.

2.2.1. Inclusion criteria

(1) Healthy Japanese aged between 30 and 59 years;
(2) Subjects with normotensive, high-normotensive, and 1 degree hypertension.

2.2.2. Exclusion criteria

(1) Subjects with chronic hypertension;
(2) Subjects with food allergies;
(3) Subjects who are pregnant or lactating;
(4) Subjects who consume medicinal products which may influence the outcome of the study;
(5) Subjects who consume foods which may influence the outcome of the study;
(6) Subjects who are judged as unsuitable for the study by the principle investigator.

2.3. Randomization

From all 88 applicants, 29 were eliminated due to systolic blood pressure (SBP) or diastolic blood pressure (DBP). The inclusion/ exclusion criteria were judged by the principle investigator. All subjects were sequentially allocated to Group A (n＝30, M; 14, F; 16) and Group B (n=29, M; 13, F; 16) using a random number table. In the process of subject assignment, background factors such as gender, age, SBP, and DBP were taken into consideration to avoid biased distribution. Subjects in Group A ingested the test sample of *Angelica furcijuga* leaf and subjects in Group B ingested placebo for 12 weeks.

2.4. Description of test foods and blinding

The test product was “NIHON-YAMANINJIN tablet” (also called Hyuga-touki, hereinafter called “Active”) containing YN-1 (isoepoxypteryxin) and isopteryxin, prepared by Meigen Inc. The amount of daily intake was 6 tablets (1 tablet weighs 240 mg), which include 8.0 mg of YN-1 and 1.4mg of isopteryxin per day. The placebo (“Placebo”) does not include Hyuga-touki, YN-1, nor isopteryxin. Both tablets were indistinguishable in shape, color, smell, and taste, and were managed by an identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 6 tablets of the supplement with hot or cold water before every supper for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid taking other supplements; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to avoid excessive intake of foods which may influence blood pressure; to maintain a daily record of every meal eaten and the number of steps measured by a pedometer during the test period; and to send the diary to the study coordinator.

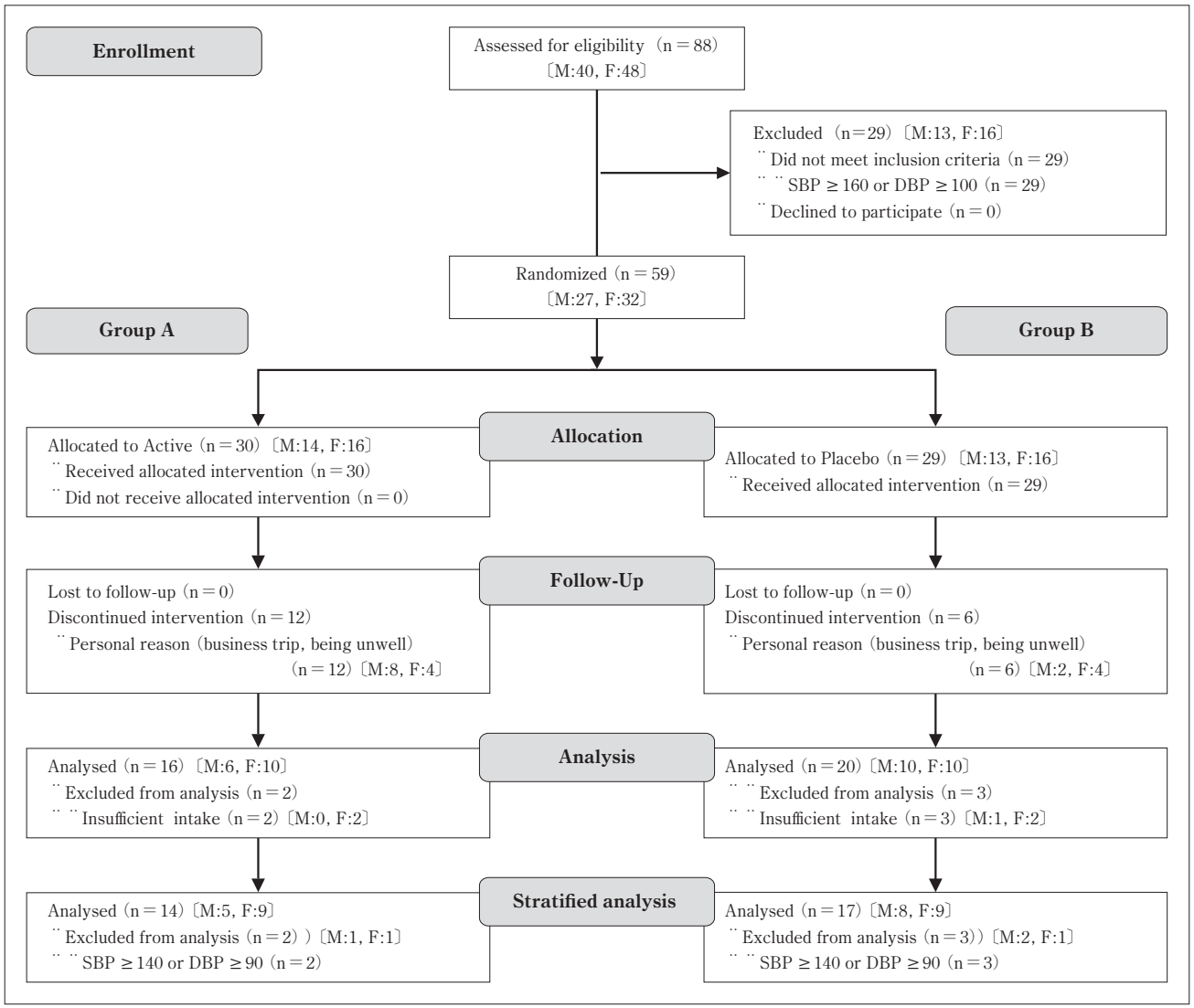

Figure 1 Flow diagram of subject disposition

2.5.2. Outcome

The objective of this study was to elucidate the effect on lowering blood pressure by consuming Hyuga-touki leaf containing YN-1 and isopteryxin. To evaluate this objective, the blood pressure (SBP and DBP) was measured as the primary outcome. As the secondary outcome, subjective reporting about frequency of urination, thirst, fatigue, headache, feeling heavy headed, and dizziness were observed by a questionnaire. Responses to each question were rated on an ordinal scale of 1 to 9, with higher scores indicating a better result. Furthermore, blood and urine biochemical parameters were recorded to evaluate the safety of Hyuga-touki, and adverse events were collected by means of a written questionnaire during the study. According to the schedule shown in **Table 1**, parameters on efficacy and safety were measured. These assessments were conducted upon pre- and post- intervention.

At every clinical visit, the subjects were not allowed to eat or drink (except water) 12 hours prior to the visit and were instructed to visit all at the same time. After taking a 15 minute rest, the subjects had their blood pressure measured. SBP and DBP were tested once via the right arm of subjects by a clinical nurse using TAMANO mercury sphygmomanometer (Sanden Medical Industry Co., Ltd., Tokyo). Next, the subjects had their blood and urine taken, and evaluated the subject reporting respectively.

2.6. Data analysis

A per protocol set (PPS) was adopted in the study and no sample size design was used. All statistics were expressed as mean ± SD. For SBP and DBP, the main effects and interactions of the food and duration of ingestion were analyzed by two-way repeated measures analysis of variance (ANOVA) and paired t-test was used for intragroup analysis. For subjective reporting and biochemical parameters, Student's t-test was used for intergroup comparisons, and paired t-test was used for intragroup analysis. The Chi-square test and Student's

Table 2　Subject demographics

Item	Unit	Active	Placebo
Subjects	numbers	16	20
Male:Female *	numbers	6:10	10:10
Age *	years	45.9 ± 6.9	44.1 ± 8.9
SBP *	mmHg	118.1 ± 17.8	116.8 ± 14.0
DBP *	mmHg	67.7 ± 10.6	69.0 ± 7.4

mean ± SD

* No significant difference

Table 3　Results of blood pressure

Item	Unit	Time points	Values		P-value (ANOVA)
			Active (n = 16)	Placebo (n = 20)	
SBP	mmHg	0-week	118.1 ± 17.8	116.8 ± 14.0	
		6-week	116.1 ± 16.4	114.1 ± 12.3	
		⊿ 0-6 w	− 1.9 ± 16.3	− 2.7 ± 10.1	
		12-week	111.1 ± 10.0	116.5 ± 12.8	
		⊿ 0-12 w	− 6.9 ± 12.3 *	− 0.3 ± 8.1	0.108
DBP	mmHg	0-week	67.7 ± 10.6	69.0 ± 7.4	
		6-week	66.1 ± 6.9	66.2 ± 7.4	
		⊿ 0-6 w	− 1.6 ± 8.4	− 2.8 ± 5.9 *	
		12-week	67.6 ± 8.3	69.0 ± 7.6	
		⊿ 0-12 w	− 0.1 ± 6.9	0.1 ± 4.9	0.788

Values are expressed as the mean ± SD.

* $p < 0.05$ against 0-week.

t-test were used to compare subject's backgrounds between groups. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. The subjects who came under the following criteria of exclusion were eliminated before the allocation list was opened, 1; consumed less than 80% of the expected dose, 2; without adequate records, 3; fell under the exclusion criteria after enrollment or had justifiable reason for exclusion.

Statistical analyses were performed using Statcel 4 (Yanai, 2015) and Excel Tokei 2015 (SSRI). The results were considered significant at a < 5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 59 subjects were randomly assigned to an intervention group and made a start with ingestion. 18 subjects were withdrawn due to personal reasons (business trip or being unwell), and the remaining 41 subjects completed the study. Due to an efficacy eligibility (insufficient intake of the food less than 80% of the expected dose), 5 (Active; 2, Placebo; 3) were eliminated, thus data obtained with 36 subjects (Active; 16 [M 6, F 10], Placebo; 20 [M 10, F 10]) was used for the analysis of efficacy (**Figure 2**). The subject's age range was 31-58 years of age (mean age 44.9 ± 8.0 y.o.). There was no significant difference in gender, age, SBP, and DBP between groups (**Table 2**).

3.2. SBP and DBP

Table 3 depicts the results of blood pressure. As for SBP, there was no interaction of the food and duration of ingestion, though the Active showed a significant decrease at week 12 in intragroup analysis. In regard to DBP, a significant difference was observed at week 6 of Placebo.

3.3. Stratified analysis

According to the classification of hypertension, 5 subjects (Active; 2, Placebo; 3) were excluded with I degree high blood pressure (**Figure 2**). 5 had exceeded 139 of SBP and 1 had exceeded 89 of DBP (1 had exceeded both SBP and DBP), so we applied stratified analysis of the remaining 31 subjects. The subject's age range was 31-58 years of age (mean age 45.0 ± 8.1 y.o.). There was no significant difference in gender, age, SBP, and DBP

Table 4 Subject demographics (stratified analysis)

Item	Unit	Active	Placebo
Subjects	numbers	14	17
Male:Female *	numbers	5:9	8:9
Age *	years	45.1 ± 7.0	44.9 ± 9.1
SBP *	mmHg	113.1 ± 12.3	112.4 ± 9.5
DBP *	mmHg	65.6 ± 9.2	67.1 ± 6.3

mean ± SD

* No significant difference

Table 5 Stratified analysis

Item	Unit	Time points	Values [1)]		P-value [2)] (ANOVA)
			Active (n = 14)	Placebo (n = 17)	
SBP	mmHg	0-week	113.1 ± 12.3	112.4 ± 9.5	
		6-week	115.1 ± 13.8	111.4 ± 9.3	
		⊿ 0-6 w	2.1 ± 9.0	− 0.9 ± 8.0	
		12-week	109.7 ± 9.9	113.8 ± 11.0	
		⊿ 0-12 w	− 3.4 ± 7.6	1.4 ± 6.7	0.023 #
DBP	mmHg	0-week	65.6 ± 9.2	67.1 ± 6.3	
		6-week	65.6 ± 6.1	64.7 ± 6.2 †	
		⊿ 0-6 w	− 0.1 ± 7.9	− 2.4 ± 4.8	
		12-week	66.1 ± 7.7	67.2 ± 6.6	
		⊿ 0-12 w	0.5 ± 6.8	0.1 ± 5.0	0.495

Values are expressed as the mean ± SD.

1) † $p < 0.1$ against 0-week.

2) # $p < 0.05$ between-group difference

between groups (**Table 4**).

Table 5 shows the results of the stratified analysis of blood pressures. Significant difference was observed between two groups in SBP, while DBP yielded no significant changes.

3.4. Subjective reporting

Table 6 shows the results of subjective reporting. After 12-weeks of ingestion, there was no significant difference of change between two groups. As for intragroup analysis, about "Fatigue" of Active and Placebo (at week 6), "Headache" of Active (at week 12), and "Feeling heavy headed" of Placebo (at week 12) depicted significant differences from baseline. Other questions did not show any significant difference.

3.5. Biochemical blood and urine test

Table 7 shows blood and urine biochemical parameters (data about week 6 were not shown). With respect to the blood test, significant differences were observed in Potassium of Active and Creatinine (male) of Placebo after 12-weeks ingestion, since there was no significant difference of change between two groups. As for urine, no significant difference was observed in pH, and no problematic findings were found in all items of uric protein, sugar, urobilinogen, ketone bodies, occult blood, and bilirubin (data on urine qualitative analysis is not shown). The investigator judged differences as minor and within the range of physiological variation (or clinically safe).

3.6. Adverse Event

No adverse events associated with the test product were observed in the course of the reporting.

4. DISCUSSION

We conducted a randomized, double-blind, placebo-controlled study for examining the effect of Hyuga-touki leaf containing YN-1 and isopteryxin on blood pressure. As the result of stratified analysis of normotensive and high-normotensive healthy subjects excluding 1 degree high blood pressure, Active showed a significant decreasing in SBP compared to the Placebo after 12-weeks of ingestion. As for the secondary outcome, there was no significant difference between the groups with regard to the subjective reporting, though it proved that no abnormal change was triggered by the ingestion

Table 6　Results of subjective reporting

Item	Time point	Active（n = 16）	Placebo（n = 20）
1　Frequency of urination	0-week	4.9 ± 0.3	4.8 ± 0.6
	6-week	5.0 ± 1.0	4.5 ± 0.9
	⊿ 0-6 w	0.1 ± 0.8	− 0.4 ± 0.9
	12-week	4.9 ± 1.0	5.0 ± 0.6
	⊿ 0-12 w	− 0.1 ± 0.9	0.2 ± 0.7
2　Thirst	0-week	4.9 ± 0.3	5.0 ± 0.0
	6-week	5.1 ± 0.8	5.0 ± 0.6
	⊿ 0-6 w	0.2 ± 0.9	− 0.1 ± 0.6
	12-week	4.8 ± 0.7	4.8 ± 0.7
	⊿ 0-12 w	− 0.1 ± 0.7	− 0.2 ± 0.7
3　Fatigue	0-week	4.9 ± 0.3	4.9 ± 0.3
	6-week	5.4 ± 0.8 *	5.1 ± 0.3 *
	⊿ 0-6 w	0.5 ± 0.9	0.2 ± 0.4
	12-week	5.4 ± 1.2 †	5.2 ± 0.5 †
	⊿ 0-12 w	0.6 ± 1.3	0.3 ± 0.6
4　Headache	0-week	4.9 ± 0.3	5.1 ± 0.4
	6-week	5.3 ± 0.5 *	5.4 ± 1.3
	⊿ 0-6 w	0.4 ± 0.6	0.3 ± 1.1
	12-week	5.3 ± 1.1	5.6 ± 1.3 †
	⊿ 0-12 w	0.4 ± 1.1	0.5 ± 1.1
5　Feeling heavy headed	0-week	4.9 ± 0.3	5.0 ± 0.0
	6-week	5.4 ± 1.0	5.2 ± 0.7
	⊿ 0-6 w	0.4 ± 1.0	0.2 ± 0.7
	12-week	5.4 ± 1.0	5.5 ± 0.9 *
	⊿ 0-12 w	0.4 ± 1.0	0.5 ± 0.9
6　Dizziness	0-week	4.9 ± 0.3	5.2 ± 0.9
	6-week	5.2 ± 1.1	5.2 ± 0.9
	⊿ 0-6 w	0.3 ± 1.1	0.0 ± 0.0
	12-week	5.3 ± 1.1	5.4 ± 1.1
	⊿ 0-12 w	0.3 ± 1.1	0.2 ± 0.7

Unit; score, Scores are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$ against 0-week.

of the test food.

The Hyuga-touki used in this study is one of the Apiaceae plants, which grows naturally from the northern part of Miyazaki prefecture to the mountainous area of Kagoshima prefecture, and has been used as an indigenous drug such as cordial medicine called the "grass of God" since ancient times. In 1971, it was announced as a new species, named "*Angelica Furcijuga* Kitagawa", and named "Hyuga-touki" as the Japanese name. The Hyuga-toki is also called Nihon-yamaninjin, and it is said that full-fledged cultivation spreading activity had begun since the 1980s. The Hyuga-touki contains YN-1 and isopteryxin, a type of coumarin-based compound, and coumarin is said to have antibacterial effects[5], anticoagulant action[6], and alleviating swelling[7]. Moreover, there have been many reported effects of coumarin on antihypertensive[8)9)]. In particular, it has been shown that plant-derived coumarin compounds suppress blood pressure increase by inhibiting angiotensin II[10]. Pickering in Britain (1955) described, "essential hypertension is one in which both DBP and SBP were rising"[11]. It is well known that hypertension causes complicated diseases such as cardiac dysfunction, cerebrovascular disorder, kidney impairment, and eye lesion. In actuality, according to the 18-year follow-up study of Framingham Heart Study (FHS), it was reported that the hypertensive group had incidence 7 times as cerebral infarction, 3 times as coronary artery disease,

Table 7-1 Results of biochemical blood and urine test (1)

Item	Unit	Std. Value	Gender	Time points	Values	
					Active (n = 16) 〔M 6, F 10〕	Placebo (n = 20) 〔M 10, F 10〕
Total Bilirubin	mg/dL	0.2-1.2	M/F	0-weeke	0.76 ± 0.23	0.76 ± 0.26
				12-week	0.76 ± 0.31	0.82 ± 0.24
				⊿ 0-12 w	0.00 ± 0.32	0.07 ± 0.23
Total Protein	g/dL	6.7-8.3	M/F	0-weeke	7.4 ± 0.4	7.2 ± 0.3
				12-week	7.3 ± 0.4	7.1 ± 0.3
				⊿ 0-12 w	− 0.1 ± 0.3	− 0.1 ± 0.3
Albumen	g/dL	3.8-5.2	M/F	0-weeke	4.5 ± 0.2	4.5 ± 0.3
				12-week	4.4 ± 0.2	4.4 ± 0.3
				⊿ 0-12 w	− 0.1 ± 0.2	− 0.1 ± 0.2
AST (GOT)	U/L	10-40	M/F	0-weeke	22.1 ± 7.1	20.8 ± 4.1
				12-week	23.8 ± 8.8	21.4 ± 7.4
				⊿ 0-12 w	1.6 ± 3.8	0.6 ± 5.8
ALT (GPT)	U/L	5-45	M/F	0-weeke	19.1 ± 9.0	19.1 ± 11.6
				12-week	20.6 ± 11.4	19.3 ± 12.5
				⊿ 0-12 w	1.4 ± 7.4	− 0.4 ± 5.8
ALP	U/L	100-325	M/F	0-weeke	200.4 ± 47.5	190.5 ± 45.4
				12-week	210.0 ± 51.2	187.4 ± 49.1
				⊿ 0-12 w	9.6 ± 20.0	− 3.1 ± 20.8
LD (LDH)	U/L	120-240	M/F	0-weeke	183.7 ± 22.6	185.2 ± 30.3
				12-week	191.1 ± 28.4	196.5 ± 43.4
				⊿ 0-12 w	7.4 ± 24.5	11.3 ± 41.4
γ-GT (γGTP)	U/L	80 and under	M	0-weeke	49.8 ± 48.4	35.7 ± 16.0
				12-week	55.7 ± 49.2	34.8 ± 14.1
				⊿ 0-12 w	5.8 ± 20.0	− 0.9 ± 6.9
		30 and under	F	0-weeke	17.8 ± 4.1	21.1 ± 9.5
				12-week	19.6 ± 7.1	18.8 ± 9.8
				⊿ 0-12 w	1.8 ± 4.1	− 2.3 ± 10.7
CK (CPK)	mg/dL	60-270	M	0-weeke	126.2 ± 30.4	133.8 ± 39.1
				12-week	160.8 ± 94.9	130.8 ± 34.6
				⊿ 0-12 w	34.7 ± 87.1	− 3.0 ± 17.7
		40-150	F	0-weeke	85.1 ± 21.1	84.9 ± 48.0
				12-week	93.7 ± 35.2	81.6 ± 33.7
				⊿ 0-12 w	8.6 ± 24.8	− 3.3 ± 30.7
Total Cholesterol	mg/dL	120-219	M/F	0-weeke	206.3 ± 36.9	207.5 ± 27.6
				12-week	212.7 ± 34.7	204.3 ± 36.5
				⊿ 0-12 w	6.4 ± 16.9	− 3.2 ± 22.2
Neutral Fat (TG)	mg/dL	30-149	M/F	0-weeke	81.1 ± 42.5	94.0 ± 45.5
				12-week	84.6 ± 94.3	110.3 ± 145.5
				⊿ 0-12 w	3.5 ± 78.2	16.3 ± 141.8
Sodium	mEq/L	137-147	M/F	0-weeke	142.3 ± 1.9	141.6 ± 1.6
				12-week	141.9 ± 2.3	141.3 ± 1.8
				⊿ 0-12 w	− 0.4 ± 1.4	− 0.3 ± 1.7

Values are expressed as the mean ± SD.

Table 7-2　Results of biochemical blood and urine test （2）

Item	Unit	Std. Value	Gender	Time points	Values	
					Active （n = 16） 〔M 6, F 10〕	Placebo （n = 20） 〔M 10, F 10〕
Chloride	mEq/L	98-108	M/F	0-weeke	102.9 ± 2.4	102.9 ± 2.9
				12-week	103.7 ± 2.2	103.1 ± 2.4
				⊿ 0-12 w	0.8 ± 4.0	0.2 ± 3.9
Potassium	mEq/L	3.5-5.0	M/F	0-weeke	4.2 ± 0.3	4.2 ± 0.3
				12-week	4.5 ± 0.4 *	4.4 ± 0.5
				⊿ 0-12 w	0.3 ± 0.5	0.3 ± 0.6
Calcium	mg/dL	8.4-10.4	M/F	0-weeke	9.4 ± 0.3	9.3 ± 0.3
				12-week	9.3 ± 0.3	9.1 ± 0.4
				⊿ 0-12 w	− 0.2 ± 0.4	− 0.2 ± 0.4
Inorganic Phosphorus	mg/dL	2.5-4.5	M/F	0-weeke	3.3 ± 0.4	3.3 ± 0.4
				12-week	3.5 ± 0.4	3.3 ± 0.5
				⊿ 0-12 w	0.2 ± 0.5	0.1 ± 0.5
Urea Nitrogen	mg/dL	8.0-20.0	M/F	0-weeke	12.3 ± 2.8	12.2 ± 2.1
				12-week	13.9 ± 3.1	13.3 ± 4.3
				⊿ 0-12 w	1.6 ± 3.7	1.1 ± 3.4
Creatinine	mg/dL	0.61-1.04	M	0-weeke	0.77 ± 0.10	0.87 ± 0.08
				12-week	0.76 ± 0.07	0.81 ± 0.09 *
				⊿ 0-12 w	− 0.00 ± 0.11	− 0.06 ± 0.07
		0.47-0.79	F	0-weeke	0.58 ± 0.05	0.63 ± 0.08
				12-week	0.59 ± 0.06	0.60 ± 0.07
				⊿ 0-12 w	0.01 ± 0.04	− 0.03 ± 0.05
Blood Sugar （serum）	mg/dL	70-109	M/F	0-weeke	83.1 ± 8.9	87.3 ± 8.9
				12-week	83.4 ± 8.3	85.5 ± 9.8
				⊿ 0-12 w	0.3 ± 8.0	− 1.8 ± 6.9
pH （urine）	−	5.0-7.5	M/F	0-weeke	6.5 ± 0.7	6.3 ± 0.8
				12-week	6.6 ± 0.8	6.4 ± 0.8
				⊿ 0-12 w	0.1 ± 0.5	0.1 ± 1.0

Values are expressed as the mean ± SD.
* $p < 0.05$ against baseline.

and 4 times as congestive heart failure, compared to the normotensive group[12]. It is important for us to suppress hypertension in order to extend healthy life expectancy and to maintain and improve QOL. Hyuga-touki has been used as an indigenous medicine so far, while its part of the root was acknowledged as medicine by the Ministry of Health, Labour and Welfare in 2002[13]. On the other hand, there are reports that the leaves and stems of Hyuga-touki also contain YN-1 and isopteryxin similar to the root[14]. The test food used as an intervention in this trial is a tablet made from dried leaves of Hyuga-touki into powder, so it was thought that the similar effect to root was obtained.

As the secondary outcome, we evaluated the subjective effects of the Hyuga-touki leaf in this study, though there was no significant difference between the groups in all items. In this study, the screening was performed to choose subjects with normal level of blood pressure or slight hypertensive (normotensive, high-normotensive, and I degree high blood pressure). Actually, the majority of subjects analyzed were normal or high-normal, so it is considered that the subjective effect did not appear. Due to these conditions, the effect of Hyuga-touki leaf observed in this study would have been limited, and different study designs in the future would be expected. In addition, we examined the safety of the test food by the biochemical analysis of blood and urine. A significant difference was observed in Potassium of Active and Creatinine (Male) of Placebo after 12-weeks of ingestion, while there was no significant difference of change between two groups. The principle investigator judged the differences as minor and within the range of

physiological variation (or clinically safe). During the test period 18 subjects discontinued the test. The reasons for discontinuance were personal circumstances such a business trip or being unwell. 5 subjects were withdrawn from analysis due to insufficient consumption of the test food and had nothing to do with the ingestion of the test product. Therefore, based upon the blood and urine test, and the diaries of the subjects, we observed no harmful influence against biochemical and/or physiological matters of the subjects, and this result indicated the safety of ingestiing of the test product for 12 weeks of the test period.

In recent years, hypertension has been found to affect the occurrence or progression of dementia. It is supposed that hypertension leads to atherosclerosis, arteriosclerosis, and cerebrovascular lesion. In addition, the presence of cerebrovascular lesion lowers the threshold of cognitive dysfunction, and amyloid β-protein is advanced in the brain from disorders of ischemic and endothelial, or increased vascular permeability. Thus Alzheimer's lesion accelerates with obstruction of clearance [15]. Conversely, there is also a report that YN-1 and isopteryxin contained in *Angelica shikokiana* reduces amyloid β-proteins [16], therefore, future studies are expected on this.

5. CONCLUSION

In conclusion, we found out that the ingestion of "NIHON-YAMANINJIN tablet" containing YN-1 (isoepoxypteryxin) and isopteryxin for 12 weeks decreased SBP in normotensive and high-normotensive healthy subjects. In addition, no safety-related matter occurred during the test period.

CONFLICT OF INTEREST

All parts of this study were funded by Meigen Inc. Masahiro Yamamoto is the principal. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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