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Suppressive Effect of Hyuga-touki Leaf on Postprandial Blood Glucose Level in Healthy Japanese:

A Randomized, Double-blind, Placebo-controlled Crossover 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 suppressing an increase in postprandial blood glucose level.
Methods: A single dose cross-over trial was conducted. We administered oral glucose tolerance test (OGTT) and appraised the changes of postprandial blood glucose levels.
Results: As the result of intergroup analysis, 0.5h-OGTT and three point of AUC (0-0.5h, 0-1h, and 0-1.5h) showed significant differences. Moreover no adverse effects were observed by the ingestion of the test food.
Conclusion: We found out that the ingestion of Hyuga-touki leaf containing YN-1 and isopteryxin could help moderate the elevation of postprandial blood glucose level. Additionally, no safety-related matter occurred during the study.

Key Words: Hyuga-touki, *Angelica Furcijuga* Kitagawa, YN-1, isoepoxypteryxin, isopteryxin, postprandial blood glucose level, OGTT, AUC

INTRODUCTION

According to "National Health and Nutrition Survey 2016" conducted by Ministry of Health, Labour and Welfare, the number of people suffering from diabetes has exceeded 10 million, increased by 500 thousand from the survey in 2012, and it was estimated that prediabetics were to be 10 million[1)]. Diabetes will not get cured once it develops, and if not treated, diabetes can cause complicated disorders such as retinopathy, nephropathy, and neuropathy, and may require blindness and dialysis treatment. Since diabetes promotes the pathogenesis or development of cardiovascular conditions such as cerebrovascular and ischemic cardiac disease, it not only reduces the patient's QOL seriously but also places a burden on society heavily in terms of medical economics[2)]. The residual glucose in the blood which is not consumed as energy is transported to the fat tissues and stored as fat, accordingly this can cause obesity[3)]. Further, the condition of postprandial hyperglycemia is reported to rise in risk for dementia[4)], therefore the increase in the postprandial plasma glucose level can bring on various diseases.

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[5)], prevent inflammation and allergy[6)]. For modern Japanese, who tend to lead an irregular lifestyle and intake an excess of 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 glucose and the safety by a randomized, double-blind, placebo-controlled, crossover study.

MATERIALS AND METHODS

1. Test material

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

2. Subjects

The inclusion criteria were set according to Japanese

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

foods for specified health uses (FOSHU)[7]; (1) healthy Japanese aged between 30 and 59 years, (2) 125 mg/dL ≥ FBG (fasting blood glucose levels) or 199 mg/dL ≥ 2h-OGTT (postprandial blood glucose levels 120 minutes after glucose tolerance). The following exclusion criteria were applied; (1) dyslipidemia, (2) food allergies, (3) pregnant or lactating, (4) medicinal products or functional food intake that could affect the outcome of the study, and (5) judged as unsuitable for the study by the principle investigator. Written informed consent before the study begun was obtained from all subjects.

3. Ethics review board

This study was conducted in accordance with the ethical principles of the declaration of Helsinki (1975, as revised in 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (2014, as partially revised in 2017). The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). This trial was registered at UMIN Clinical Trial Registry (Trial ID: UMIN000027204).

4. Test procedure

These trials were conducted with the aid of a fund from Meigen Inc. (Kagoshima) at two centers (KUROSU HOSPITAL, Tokyo and Japan Clinical Trial Association; JACTA, Tokyo). All of the subjects in this study were public volunteers who had enrolled in the monitor bank of HUMA CORP (Tokyo). 20 candidates were screened using a pretest comprising a physician's interview. 2 subjects were eliminated due to inclusion/ exclusion criteria, thus 18 subjects were finally selected for this study. A randomized, placebo- controlled, double-blind cross-over trial was conducted in May 2018. The study consisted of 2 days separated by a washout, the 1st and 2nd intake were single dose each and the washout period was 7days. On each test day, the subjects visited at the same time without eating or drinking (except water) 12 hours prior to the visit. The subjects took a 15 minute rest before proceeding with the first blood extraction to measure FBG, then the subjects consumed 6 tablets of test food and drank 75 g of glucose solution, and had their blood drawn 30, 60, and 90 minutes after glucose tolerance to measure postprandial glucose levels. During wash out period, subjects were instructed 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, and to avoid excessive intake of foods which may influence blood glucose level.

5. Randomization

A person in charge of allocation, who was not otherwise involved in the study, verified the indistinguishability of the Active and the Placebo, and performed allocation using a random number table. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

Table 1 Participant's characteristics

Item	Mean ± SD
Sex (number)	men 11 / women 7
Age (year)	45.3 ± 8.1
Body weight (kg)	57.6 ± 7.8

(n = 18)

6. Data analysis

All statistics were expressed as mean ± standard deviation (SD). Student's t-test was used for comparisons for intergroup analysis. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Statcel 4 (Yanai, 2015), and the results were considered significant at a < 5% level in the two-sided test.

RESULTS

The subject's characteristics (11 men, 7 women) are shown **Table 1**. The mean age45.3 ± 8.1 y.o. The blood glucose levels measured over time are shown **Figure 1**, and incremental postprandial areas under the curve (AUC) are shown **Figure 2**. At 30 min after glucose tolerance, the blood glucose level of Active remained lower than that of Placebo significantly ($p = 0.004$), and 60-min of Active indicate significant tendency to suppress the blood glucose level compared to the Placebo ($p = 0.067$). As for AUC, all time points of 30, 60, and 90-min depicted significant differences between Active and Placebo ($p = 0.005$, $p = 0.003$, $p = 0.005$, respectively). Moreover, no adverse events associated with the test product were observed in the course of the reporting.

DISCUSSION

This study examined the efficacy of ingesting Hyuga-touki leaf containing YN-1 and isopteryxin, a type of coumarin compound. The pre-meal intake of Hyuga-touki leaf inhibited the rise in postprandial blood glucose level significantly. 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

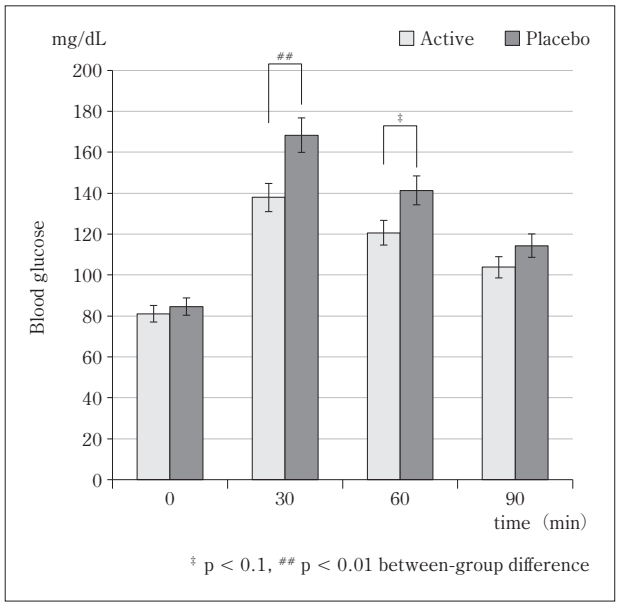

Figure 1　Plasma glucose concentration

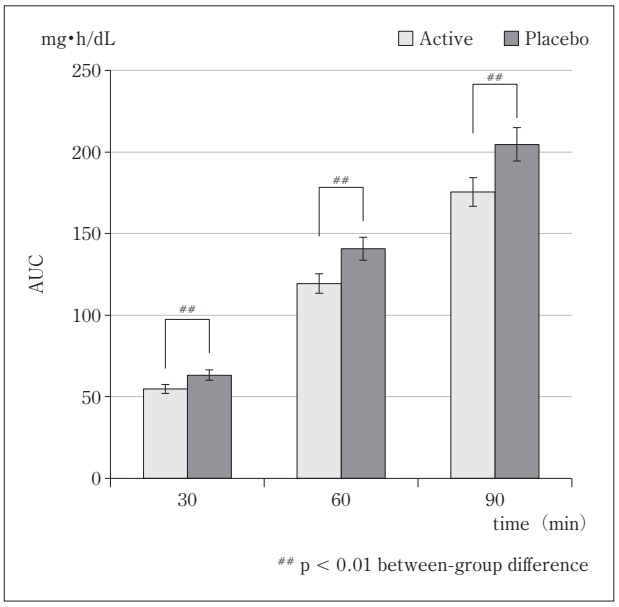

Figure 2　AUC of blood glucose

begun since the 1980s. The Hyuga-touki contains YN-1 (isoepoxypteryxin) and isopteryxin, a type of coumarin-based compound, and coumarin is said to have antibacterial effects [8], anticoagulant action [9], and alleviates swelling [10]. In addition, many anti-hyperglycemic effects of coumarin contained in plants have also been reported [11)-13)].

"Postprandial hyperglycemia" is regarded as an important index to diabetes or pre-diabetes [14]. It is thought the fact that the postprandial blood glucose level is high means the state of "impaired glucose tolerance (IGT)". This means that the amount or function of insulin is decreased, glucose is not able to be metabolized sufficiently inside of the body, and the function to return the blood glucose level to normal is very weak. As IGT promotes arteriosclerosis and causes coronary heart disease and others [15], it is important for us to suppress postprandial blood glucose level in order to extend healthy life expectancy and to maintain and improve QOL. Ninomiya (2011) reported that Hyuga-touki root extract enhanced glucose consumption in hepatocytes. Moreover, the khellactone type coumarin hyuganin A, the coumarin glycoside apiosylskimmin, and isopteriyxin, which are contained in the extract, also showed evidence of promoting glucose consumption [16]. Many studies have also reported that coumarin inhibits α-glucosidase and reduces oxidative stress or inflammation [17)-19)]. Based on these, we could consider that the coumarin contained in Hyuga-touki suppressed the increase in postprandial blood glucose level.

Hyuga-touki root was acknowledged as medicine by the Ministry of Health, Labour and Welfare in 2002 [20]. 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 [21]. The test food used as an intervention in this trial is a tablet made from drying leaves of Hyuga-touki into powder, so it was thought that the similar effect to the root was obtained.

Through the ingestion of the test food, OGTT, and blood drawing, we observed no harmful incidences to the subjects, and this result indicated the safety of the ingestion of the test product. In this study we conducted single intake trials targeted for normal blood sugar level, or "FBG or OGTT is boundary type" (FBG; 110-125 mg/dL, 2h-OGTT; 140-199 mg/dL) which standards regulated by the Consumer Affairs Agency [7]. Future study such as tests on subjects with IGT (impaired glucose tolerance) and long-term intake tests would be expected.

CONCLUSION

In conclusion, we found out that the ingestion of "NIHON-YAMANINJIN tablet" containing YN-1 (isoepoxypteryxin) and isopteryxin suppressed the increase in postprandial blood glucose level of healthy Japanese. In addition, no safety-related matter occurred during the study.

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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