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Improvement of glucose metabolism and safety of proanthocyanidins derived from acacia bark in healthy Japanese adults: A randomized, double-blind, placebo-controlled, parallel-group comparison trial

Asami Baba[1*] Tomohiro Hoshino[1] Sosuke Ogawa[2] Tsuyoshi Takara[3]**

[1]ORTHOMEDICO Inc. 2F Sumitomo Fudosan Korakuen Bldg., 1-4-1 Koishikawa, Bunkyo-ku, Tokyo, Japan; [2]Acacia-No-Ki Co., Ltd. 4291-1 Miyauchi, Hatsukaichi-shi, Hiroshima, Japan; [3]Medical Corporation Seishinkai, Takara Clinic 9F Taisei Bldg., 2-3-2, Higashi-gotanda, Shinagawa-ku Tokyo, Japan

***Corresponding author:** Asami Baba ORTHOMEDICO Inc. 2F Sumitomo Fudosan Korakuen Bldg., 1-4-1 Koishikawa, Bunkyo-ku, Tokyo, Japan

**** Principal investigator:** Tsuyoshi Takara Medical Corporation Seishinkai, Takara Clinic 9F Taisei Bldg., 2-3-2, Higashi-gotanda, Shinagawa-ku Tokyo, Japan

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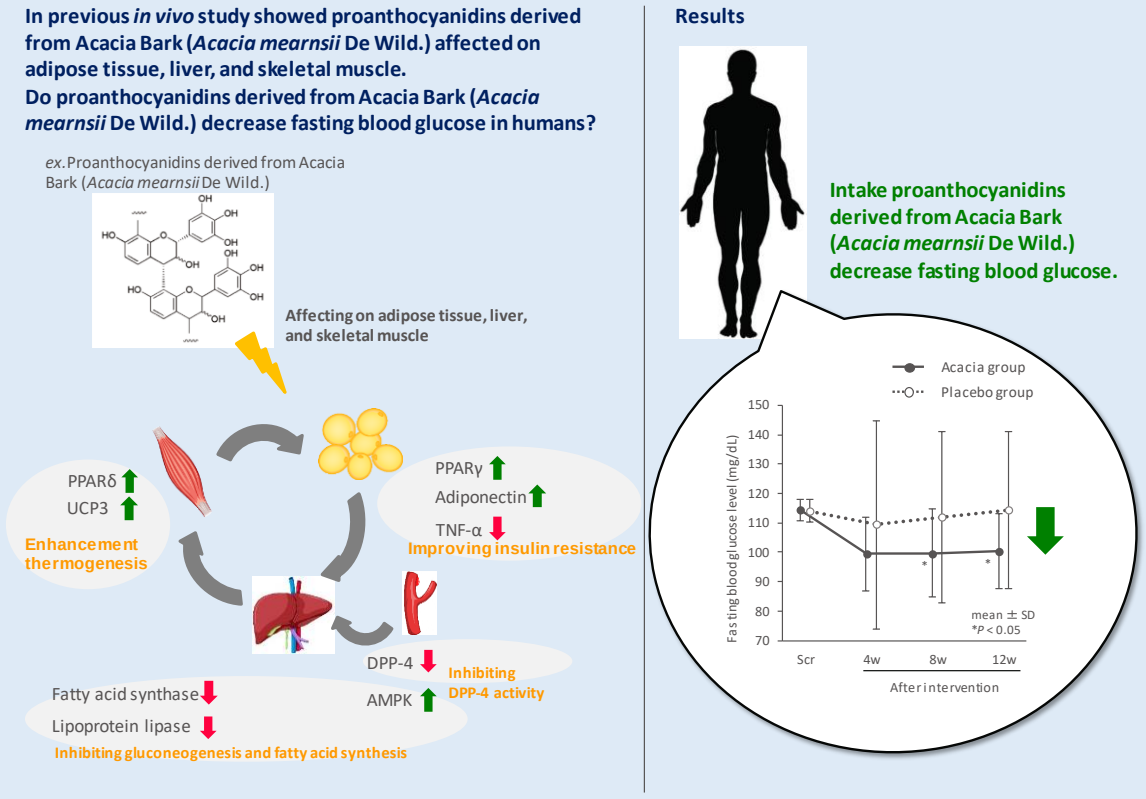
ABSTRACT

Objective: Proanthocyanidins derived from acacia bark exhibit antioxidant, anti-inflammatory, anti-diabetic, hypotensive effects, and improving skin condition, in addition to beneficial effects on blood glucose. Herein, we evaluated the effects of proanthocyanidins derived from acacia bark on fasting and postprandial blood glucose levels in healthy Japanese adults with fasting blood glucose (FBG) levels between 110–125 mg/dL.

Methods: Subjects were randomly allocated into 2 groups (n = 33 per group) and consumed 6 tablets/day of either tablets of acacia bark extract containing proanthocyanidins (Acacia group) or placebo for 12 weeks. Evaluation points were at the screening (Scr), and after 4-week, 8-week, and 12-week intervention (4w, 8w and 12w, respectively). The primary outcome was FBG level at 12 w, whereas the secondary outcomes were FBG level at 4w

and 8w, the FBG changes from Scr to each-week intervention, the percentage of subjects with FBG levels below 110 mg/dL after 12 w, the measured value and the change value from Scr to each-week intervention of HbA1c, the measured value and the change value from Scr to each-week intervention of postprandial blood glucose levels. Additionally, we performed a safety assessment.



Results: A total of 33 subjects (18 men, 15 women) from each group were analyzed. The Acacia group had significantly lower FBG levels at 8w and 12w than the placebo group (P = 0.030 and P = 0.014, respectively). The percentage of subjects with FBG <110 mg/dL at 12w was marginally higher in the Acacia group than in the placebo group (P = 0.079). HbA1c at 12w was significantly lowered in the Acacia group compared to the placebo group (P = 0.015). No medically problematic changes were observed due to the continued ingestion of the test food.

Conclusions: Proanthocyanidins derived from acacia bark were confirmed to have FBG-lowering effects.

Trial registration: UMIN000039414

Foundation: Acacia-No-Ki Co.,Ltd.

Keywords: Acacia bark extract, Proanthocyanidins, Fasting blood glucose level, HbA1c

INTRODUCTION

The National Health and Nutrition Examination Survey conducted by Japan's Ministry of Health, Labor, and Welfare classifies diabetic individuals as those who are strongly suspected of having diabetes, and pre-diabetic individuals as those who cannot deny the possibility of having diabetes [1]. Those who are strongly suspected of having diabetes meet either of the following conditions: 1) diagnosed with diabetes mellitus by a medical institution or on health checkup; 2) described by a physician as being borderline, suspected of having diabetes mellitus, about to develop diabetes mellitus, or has high blood glucose levels; or 3) is undergoing treatment or regular hospital visits and has a hemoglobin A1c (HbA1c; NGSP) level of 6.5% or higher. On the other hand, those who cannot deny the possibility of having diabetes have an HbA1c (NGSP) level of 6.0% or higher but less than 6.5% and are not strongly suspected of having diabetes.

The 2019 National Health and Nutrition Survey in Japan [1] reported that, overall, 14.6% (19.7% of men and 10.8% of women) of Japanese adults are strongly suspected of having diabetes, whereas 12.7% (12.4% for men and 12.9% for women) cannot deny the possibility of having diabetes. Based on the population of Japan at the time of the survey (November 2019), approximately 11.7 million people are strongly suspected of having diabetes, while 10.7 million cannot deny the possibility of having diabetes. In total, approximately 22.4 million Japanese people have some forms of diabetes risk.

The Japan Diabetes Society [2] classifies patients according to the results of fasting blood glucose level (FBG) or 2-hour blood glucose level on 75 g oral glucose tolerance test (75g OGTT) of venous blood collection as follows: normal type (FBG < 110 mg/dL or 2-hour 75g OGTT < 140 mg/dL), borderline type (FBG of 110–125 mg/dL or 2-hour 75g OGTT of 140–199 mg/dL), and diabetic type (FBG ≥ 126 mg/dL or 2-hour 75g OGTT ≥ 200 mg/dL). Approximately less than 1% of patients worsen from the normal to diabetic type each year. Worsening from borderline to diabetic type is more common, causing a high frequency of atherosclerotic complications [2]. Moreover, diabetes mellitus cannot be cured once it develops. If left untreated, it can lead to complications such as retinopathy, nephropathy, and neuropathy, which may eventually lead to blindness and the need for dialysis treatment. In addition, diabetes is known to promote the onset and progression of cardiovascular diseases such as stroke and ischemic heart disease [2]. Therefore, guidance lifestyle improvement is the main treatment for borderline type patients, and blood glucose levels should be monitored periodically to determine the effect of treatment [3]. Aside from blood glucose levels, HbA1c is also used as an important biomarker.

The acacia bark extract is a hot water extract obtained from the bark of *Acacia mearnsii* De Wild. This extract is a mixture of compounds consisting of single, dimeric, trimeric, and further condensed polymers with a basic structure of flavan-3-ol such as gallocatechin, and robinetinidol [4-5]; approximately 80% of its compounds is related to polyphenols [6-7].

A previous study in which mice were fed a high-fat diet (high-fat group) and a high-fat diet containing 2.5% or 5.0% acacia bark extract (the acacia bark extract group) for 7 weeks demonstrated significantly lower FBG levels in the acacia bark extract group than

in the high-fat group [8]. The study suggested that the mRNA expression changes by the acacia bark extract may lower FBG levels [8].

Additionally, the acacia bark extract moderated the increase in postprandial blood glucose levels. In healthy Japanese adults with high fasting blood glucose levels or those prone to high blood glucose levels (FBG of 110–125 mg/dL or 2-hour 75g OGTT of 140–199 mg/dL), taking 250 mg of the acacia bark extract (containing 163 mg proanthocyanidins derived from acacia bark) before eating cooked rice significantly suppressed their glucose absorption and moderated an increase in postprandial blood glucose levels [9].

Based on these previous studies, the acacia bark extract is expected to improve glucose metabolism and inhibit postprandial blood glucose elevation in humans. Therefore, this study aimed to clarify the effect of proanthocyanidins in acacia bark extract on the reducing blood glucose levels in healthy Japanese adults with FBG levels from 110–125 mg/dL. We set the daily intake of the proanthocyanidins derived from acacia bark at 245 mg to examine the effect of the acacia bark extract on glucose metabolism. This intake is 1.5 times higher than that reported by Takeda et al. [9], who confirmed the inhibitory effect of the acacia bark extract on elevated blood glucose levels and is the dose that was confirmed as a safe intake in other previous studies using proanthocyanidins derived from acacia bark [10]. However, the efficacy of proanthocyanidins derived from acacia bark on glucose metabolism at an intake of 245 mg/day remains unclear. Hence, we also verified the inhibitory effect of the acacia bark extract on elevated postprandial blood glucose levels at high doses, and we assessed its safety during 12 weeks of intake.

METHODS

Study design: This was a randomized, double-blind, placebo-controlled parallel-group comparison study, and the allocation followed a 1:1 ratio. This study was approved by the Ethics Committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan; Approval ID: 2001-2001-AK01-01-TC; January 24, 2020) and registered at the University Hospital Medical Information Network Clinical Trial Registry (Registry no. UMIN000039414). This study was conducted in accordance with the principles of the Declaration of Helsinki (2013) and the ethical guidelines for medical and health research involving human subjects in Japan, with complete consideration of medical ethics.

Subjects: This study included healthy Japanese adults with relatively high levels of blood glucose or those who are anxious about or suffering from their blood glucose. The exclusion criteria were defined as follows: (a) a medical history of current treatment for malignancy, heart failure, or myocardial infarction; (b) use of a pacemaker or an implantable cardioverter defibrillator; (c) current treatment for cardiac arrhythmia, hepatic, renal, or cerebrovascular disease, rheumatism, diabetes mellitus, hyperlipidemia, hypertension, or other chronic diseases; (d) daily consumption of “foods for specified health uses,” “foods with function claims,” or other functional foods or beverages; (e) regular use of medications, including herbal medicines or supplements; (f) allergic reaction to medications or products used in

the study; (g) pregnancy, lactation, or planning to become pregnant; (h) enrollment in other clinical trials within the previous three months before agreeing to participate in this trial or planning to participate in another trial during this trial; and (i) ineligibility to participate in the study based on the evaluation of the principal physician. Subjects were recruited online via a website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The study protocols were comprehensively explained to all subjects at the office of ORTHOMEDICO Inc., and all subjects provided informed consent before their participation in the study. No subject was part of the sponsors or funding companies. The study was conducted at Medical Corporation Seishinkai, Takara Clinic.

Determination of sample size: To date, no studies have been conducting regarding the effect of proanthocyanidins derived from acacia bark on the FBG of humans for a period of 12 weeks. Thus, the sample size was calculated while assuming a large difference in the FBG between the Acacia and placebo groups at 12 weeks. As suggested by Cohen [11], the sample size was calculated with an assumed effect size (d) of 0.80, significance level (α) of 0.05, and statistical power (1−β) of 0.80, leading to 52 subjects per group (26 subjects in each group). To maximize the statistical power, the target number of patients was set at 60 (30 subjects in each group), resulting in a statistical power of 0.86. In addition, the number of patients was set at 66 (33 in each group) to allow for dropouts and noncompliance with the protocol during the study period.

Selection, randomization, and blinding: Of the 347 subjects who signed the informed consent forms, 66 eligible subjects were considered appropriate for the purposes of this study and were thus selected by the physician. Inclusion criteria were defined as follows: (a) eligible to participate in the study based on the evaluation of the principal physician, and (b) FBG of 110–125 mg/dL at screening (Scr; before test food consumption). Test foods were provided to the contract research organization by the sponsor. After the test foods were confirmed to be indistinguishable and after entering and verifying the data at Scr, an individual in charge of shipping (a member of the contract research organization), provided the code of the test foods to the allocation controller who was not directly involved in the studies. The allocation controller then assigned subjects to either the Acacia group or the placebo group (n=33 per group) according to the computer-generated randomization list with stratified random allocation adjustment factors (i.e., sex, age, and FBG levels at Scr). Then, the allocation controller coded the name of test foods of the allocation list and provided this list only to the person in charge of shipping, who sent the test foods to each subject according to the list. The sponsors, principal investigator, sub-investigators, entire contract research organization staff (i.e., director of the trial, director of trial conduction, individual in charge of monitoring, director and staff of statistical analysis, and individual in charge of shipping), medical institution staff, institutional review board members, contract laboratory, and others who were related to this study. The allocation controller locked the allocation table until the key opening day.

Intervention: The components of the test foods in each tablet are shown in Table 1. All subjects were given 6 tablets a day of either acacia bark extract or placebo. Six tablets of the acacia bark extract food contained 245 mg of proanthocyanidins derived from acacia bark, and placebo tablets were free from proanthocyanidins. In addition, both test foods did not contain any other effective polyphenols for glucose metabolism. The ethics committee declared that both tablets were indistinguishable in appearance.

(a) Glucose tolerance loading test

The glucose tolerance loading food was designated as Sato-No-Gohan (200 g; retort cooked rice) (Sato Foods Industries Co., Ltd., Niigata, Japan), and subjects took 6 tablets of either acacia bark extract or placebo with water without chewing 5 minutes before glucose tolerance loading.

(b) Intervention periods

The subjects took 6 tablets of either acacia bark extract or placebo before meals with water without chewing.

Table 1. Nutritional composition of test food

		Acacia bark extract tablets	Placebo tablets
Calorie	kcal	7.182	7.2
Protein	g	0.054	0.0432
Fat	g	0.0522	0.0522
Carbohydrate	g	1.6236	1.6398
Salt equivalent	g	0.001179	0.000522
Proanthocyanidins derived from acacia bark	mg	245	0

The content per 6 tablets (1800 mg) is shown.

Outcomes: The schedule of this study is shown in Table 2. Postprandial blood glucose was assessed via a glucose tolerance loading test before test food consumption (0w). Assessment of the FBG was conducted at Scr and at 4 weeks, 8 weeks, and 12 weeks following initial intake (4w, 8w, and 12w, respectively). HbA1c (NGSP) was assessed at Scr and 12w. Safety assessments were conducted at Scr, 0w, 4w, 8w, and 12w.

(***1) Primary outcome: The measured value of FBG level at 12w:*** Approximately 13 mL of venous blood was collected from the subjects, and the analysis was entrusted to LSI Medience Corporation (Tokyo, Japan), and the results were measured according to conventional methods.

(2) Secondary outcomes:

(***a) The percentage of subjects with FBG <110 mg/dL after 12 weeks:*** We calculated the percentage of subjects with FBG <110 mg/dL, which is defined as a

normal range in Japan [2], 12 weeks after the intervention.

(b) FBG: Aside from the primary outcome (FBG at Scr, 4w, and 8w), the amount of changes of FBG at Scr, 4w, 8w, and 12w were also evaluated. As with the primary outcome, analysis was entrusted to LSI Medience Corporation, and the results were measured according to conventional methods.

(c) HbA1c (NGSP): Approximately 13 mL of venous blood was collected from the subjects, and the analysis was entrusted to LSI Medience Corporation (Tokyo, Japan). Results were measured according to conventional methods.

(d) Blood glucose level in glucose tolerance loading test: The blood glucose values at each measurement time were evaluated in terms of incremental area under the curve (IAUC) and maximum blood concentration (Cmax) before consuming test food and 120 min after consuming the loading diet. Venous blood was collected from subjects at before consuming test food and 30, 60, 90, and 120 min after consuming the loading diet.

(3) Safety evaluation: Safety evaluation was accomplished via physical examination, urinalysis, and blood analysis.

Physical examination items included the subjects' weight, body mass index, body fat percentage, systolic and diastolic blood pressures, and pulse rate. Height was measured to calculate the body mass index.

In urinalysis, urine samples were collected to evaluate the levels of protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood. The collected samples were entrusted to LSI Medience Corporation, and the results were measured according to conventional methods.

Hematological tests were conducted to assess the following: leukocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and white blood cell differential count (i.e., neutrophil, lymphocyte, monocyte, eosinophil, and basophil count). For biochemical tests, we evaluated the following parameters: aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase, calcium, serum amylase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glycoalbumin, serum iron, sodium, potassium, chloride, inorganic phosphorus, glucose, and nonspecific immunoglobulin E. Nonspecific immunoglobulin E was only measured at Scr. The level of each parameter was measured by LSI Medience Corporation.

All subjects were asked to complete a medical questionnaire to understand their health conditions at each examination. Subjects were also asked to keep a daily record of the consumption of the test food, health conditions, use of medications, and lifestyle.

Table 2. Schedule of enrollment, intervention, and assessments

	Screening				Intervention Period			
	Examination	Screening (Scr)	Enrollment	Allocation	Start intake (0w) / Glucose tolerance loading test	4 weeks after the start of the test food consumption (4w)	8 weeks after the start of the test food consumption (8w)	12 weeks after the start of the test food consumption (12w)
ENROLLMENT:								
Eligibility screen	●		●					
Informed consent	●							
Other procedures	●							
Allocation				●				
INTERVENTIONS:								
Acacia group					←	—	—	→
Placebo group					←	—	—	→
ASSESSMENTS:								
Postprandial blood glucose					●			
Fasting blood glucose		●			●			●
Physical examination	●	●			●	●	●	●
Urinalysis		●			●	●	●	●
Blood test		●			●	●	●	●
Medical questionnaire		●			●	●	●	●
Daily record					←	—	—	→

Closed circles (●) display the execution timing of each item.

Statistical analysis: All statistical analyses in this study were two-sided, and significance level was set at 5%, with no adjustment for multiple comparisons. Data analyses were performed using Windows SPSS version 23.0 (IBM Japan, Ltd., Tokyo, Japan).

The subjects' background data were demographically aggregated according to the enrolled and analyzed subjects. Age, height, and nonspecific immunoglobulin E were compared between both groups using Student's *t* test.

The primary and secondary outcomes (except for the percentage of subject with FBG <110 mg/dL after 12 weeks) were presented as mean ± standard deviation.

The values at Scr and blood glucose level before glucose tolerance loading food were considered as baseline. The baseline, IAUC and Cmax of glucose tolerance loading tests, and the change in HbA1c (NGSP) were compared between groups using Student's *t* test.

The measured values of the FBG and HbA1c (NGSP) after the intervention, and blood glucose level after glucose tolerance loading at each time were analyzed using post-hoc comparisons with a linear mixed model, with the baseline values utilized as covariates and using time, group, group-time interaction, and subject as factors. The changes in and blood glucose level after glucose tolerance loading at each time were analyzed using post-hoc comparisons of the linear mixed model, with time, groups, group-time interaction, and subjects as factors.

To compare the percentage of subjects, logistic regression analysis with the factors of group, sex, age, and blood glucose level at Scr was performed to compare the percentage of the number of subjects evaluate the odds ratio (OR), 95% confidence interval, and significance probabilities are presented.

Physical examination (except for subjects' height) and blood test were compared between groups using Student's *t* test. The urinalysis data was assigned codes, wherein "1" and "0" defined as within and outside the normal range, respectively. For the safety assessment items, the principal investigator evaluated and checked the data case-by-case to confirm that there were no medical problems associated with the consumption of the test food.

When calculating the difference between groups, the estimated marginal mean and their 95% confidence intervals were obtained. In the case of missing values, they were not supplemented in statistical analysis.

RESULTS

Analysis set: The study flowchart is shown in Figure 1. This study was conducted from February 10, 2020, to June 28, 2020, and the subjects were recruited from February 10, 2020, to October 19, 2020. In the placebo group, one subject dropped out because they did not come to the clinic since the 8-week intervention. However, the relevant data were treated as missing values and analyzed. All subjects completed this study and were included in the validity and safety analyses on an intention-to-treat dataset. Included in the final analysis were 33 subjects (18 men and 15 women) each in the Acacia group and placebo group.

Tables 3-1 and 3-2 show the subjects' background information. There were no significant differences in the background factors between both groups.

Fasting blood glucose level: Figure 2 and Table 4 show the FBG levels. At 8w, Acacia group had significantly lower FBG levels than the placebo group (Acacia group: 99.6 ± 14.9 mg/dL, placebo group: 119.4 ± 29.3 mg/dL; $P = 0.030$). This trend was seen at 12w as well (Acacia group: 100.4 ± 12.8 mg/dL, placebo group: 114.3 ± 26.6 mg/dL; $P = 0.014$).

The changes in FBG from Scr to 8w in the Acacia group were significantly lower than those observed in the placebo group (Acacia group: −14.4 ± 14.4 mg/dL, placebo group: −2.3 ± 30.1 mg/dL; $P = 0.027$). The FBG changes from Scr to 12w in the Acacia group were significantly lower than that in the placebo group

(Acacia group, −14.0 ± 12.1 mg/dL; placebo group, −0.1 ± 27.3 mg/dL; *P* = 0.013).

The percentage of subjects with fasting blood glucose levels below 110 mg/dL: Table 5 shows the percentage of subjects with FBG <110 mg/dL. Although no significant difference was observed, 24 subjects in the Acacia group and 17 in the placebo group who met the criteria, with the Acacia group tending to outnumber the placebo group (*P* = 0.079).

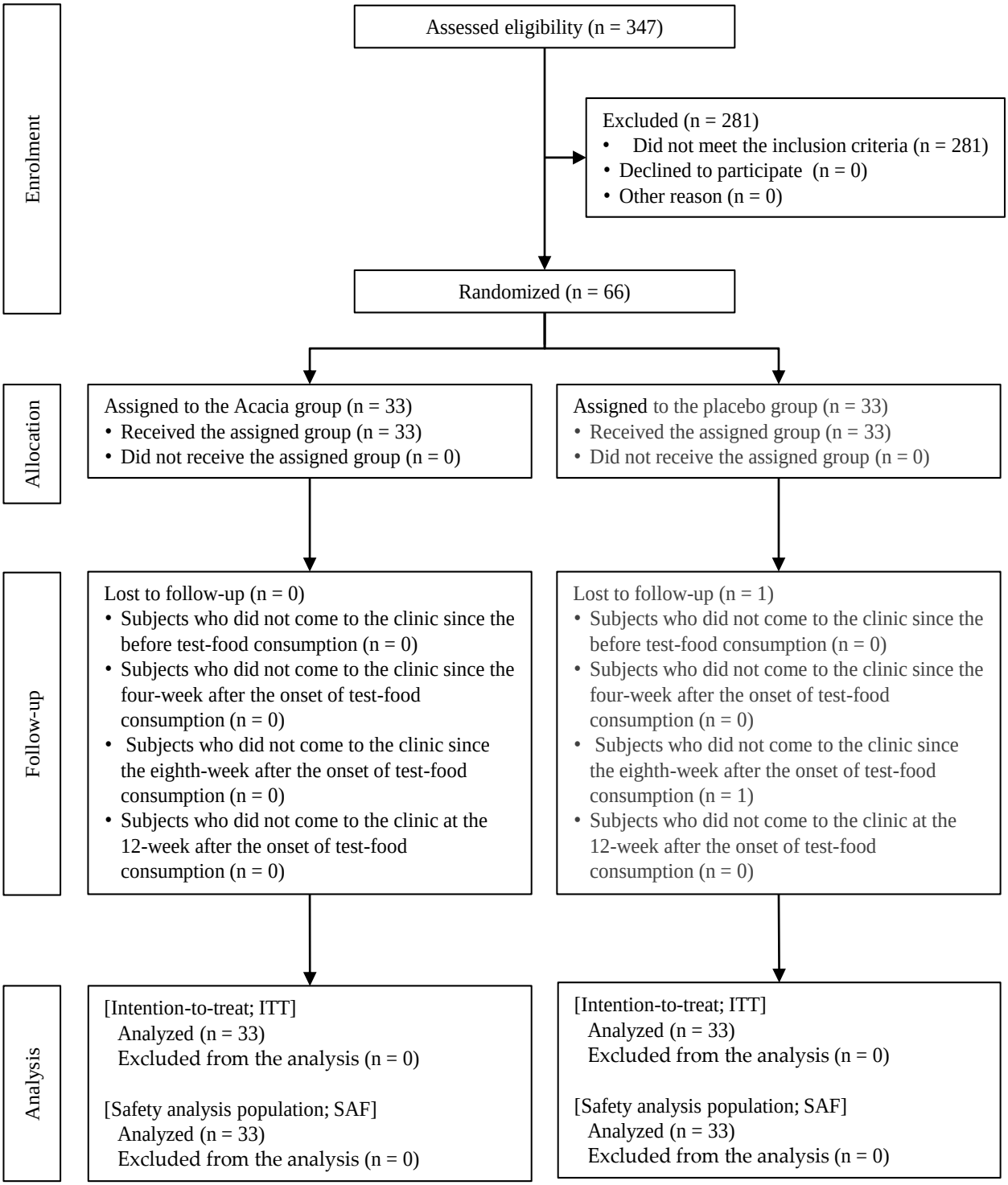


Figure 1. Flowchart of subjects in this study

Table 3-1. Subjects' background information (Gender, Age)

Item	Acacia group (n = 33)		Placebo group (n = 33)		*P* value
	Men	Women	Men	Women	
Gender	18 (54.5%)	15 (45.5%)	18 (54.5%)	15 (45.5%)	1.000
Age (years)					
30–39	1 (3.0%)	2 (6.1%)	4 (12.1%)	2 (6.1%)	-
40–49	7 (21.2%)	4 (12.1%)	4 (12.1%)	3 (9.1%)	-
50–59	5 (15.2%)	5 (15.2%)	8 (24.2%)	7 (21.2%)	-
60–69	5 (15.2%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	-
70–79	0 (0.0%)	2 (6.1%)	0 (0.0%)	1 (3.0%)	-

The data are presented as the number of subjects and as a percentage of each group.

Table 3-2. Subjects' background information (Age, Height, Nonspecific IgE)

	Unit	Acacia group (n = 33)		Placebo group (n = 33)		*P* value
		Mean	SD	Mean	SD	
Age	years	50.8	11.3	49.8	9.6	0.682
Height	cm	165.6	8.1	166	7.4	0.862
Nonspecific IgE	IU/mL	211.3	451.5	141.8	201.1	0.422
BMI	kg/m^2	24.6	4.7	24.1	3.7	0.584
FBG	mg/dL	114.4	3.7	114.1	3.9	0.771
HbA1c (NGSP)	%	5.6	0.5	5.9	1.1	0.199

The data are presented as the number of subjects, or the mean and standard deviation (SD).

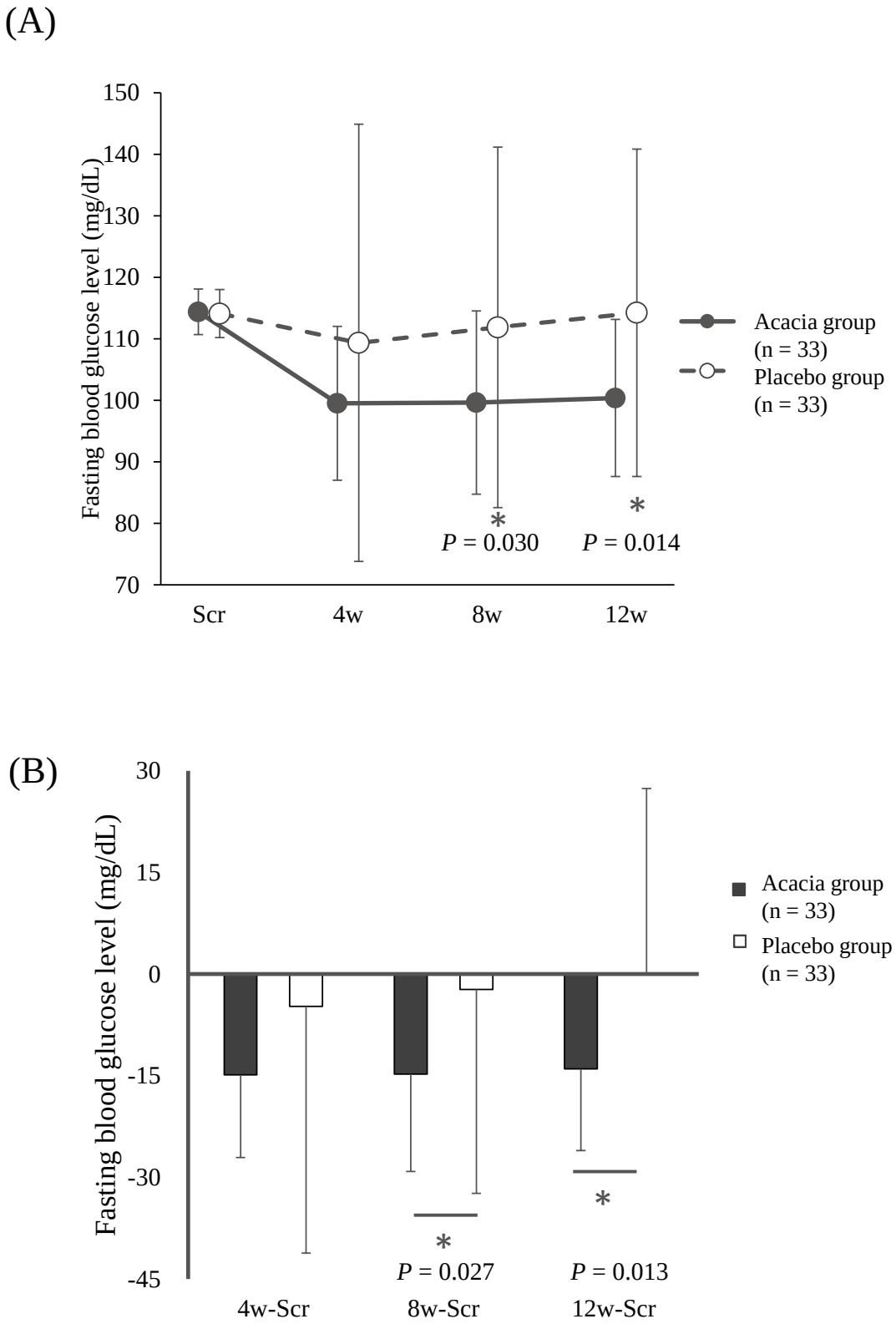


Figure 2. Fasting blood glucose level

(A) Measured values; (B) Change values

In the placebo group, one subject dropped out because they did not come to the clinic since the 8-week intervention. However, the relevant data were treated as missing values and analyzed.

Table 4. Intergroup differences and their 95% confidence intervals in fasting blood glucose level

	Intergroup differences	95% CI
Measured values		
4w	−9.84	−20.92 to 1.24
8w	−12.35	−23.45 to −1.24
12w	−13.96	−25.07 to −2.86
Changes values		
4w−Scr	−10.12	−21.28 to 1.04
8w−Scr	−12.62	−23.81 to −1.43
12w−Scr	−14.24	−25.43 to −3.05

The data are presented as estimated marginal mean and 95% confidence interval of EMM (95% CI−, 95% CI+).

Table 5. The ratio of the number of subjects with FBG <110 mg/dL after 12 weeks

	Decision criterion	
	Acacia group (n = 33)	Placebo group (n = 30)
Number of subjects who achieved the criteria	24	17
(percentage)	(72.2%)	(53.1%)
Odds ratio against placebo group	2.654	
[95% confidence interval]	[0.892, 7.896]	
***P* value**	0.079	

The data are presented as the number of subjects, percentage of subjects, and the odds ratio (OR) and its 95% confidence interval.

HbA1c (NGSP): Figure 3 and Table 6 show the HbA1c (NGSP). At 12w, the Acacia group had significantly lower HbA1c (NGSP) values than the placebo group (Acacia group: 5.6% ± 0.4%, placebo group: 6.0% ± 1.2%; $P = 0.015$). The changes in HbAlc (NGSP) from Scr to 12w in the Acacia group were significantly lower than those observed in the placebo group (Acacia group: 0.0% ± 0.2%, placebo group: 0.1 ± 0.2%; $P = 0.012$).

Glucose level after glucose tolerance loading at each time: There were no significant differences between the groups in the glucose level after glucose tolerance loading at each time (Data not shown).

Safety assessment: Tables 7-1 and 7-3 illustrate the safety assessment results. Under the conditions of this study, no side effects or adverse events were observed. Although significant differences in several test items were observed between the groups, we assumed that these differences were not clinically meaningful because the measured values of the relevant items were within the reference values. Furthermore, these changes did not induce nor facilitate any medical emergencies or abnormalities. Therefore, no medically problematic changes were observed due to the continued ingestion of the test food.

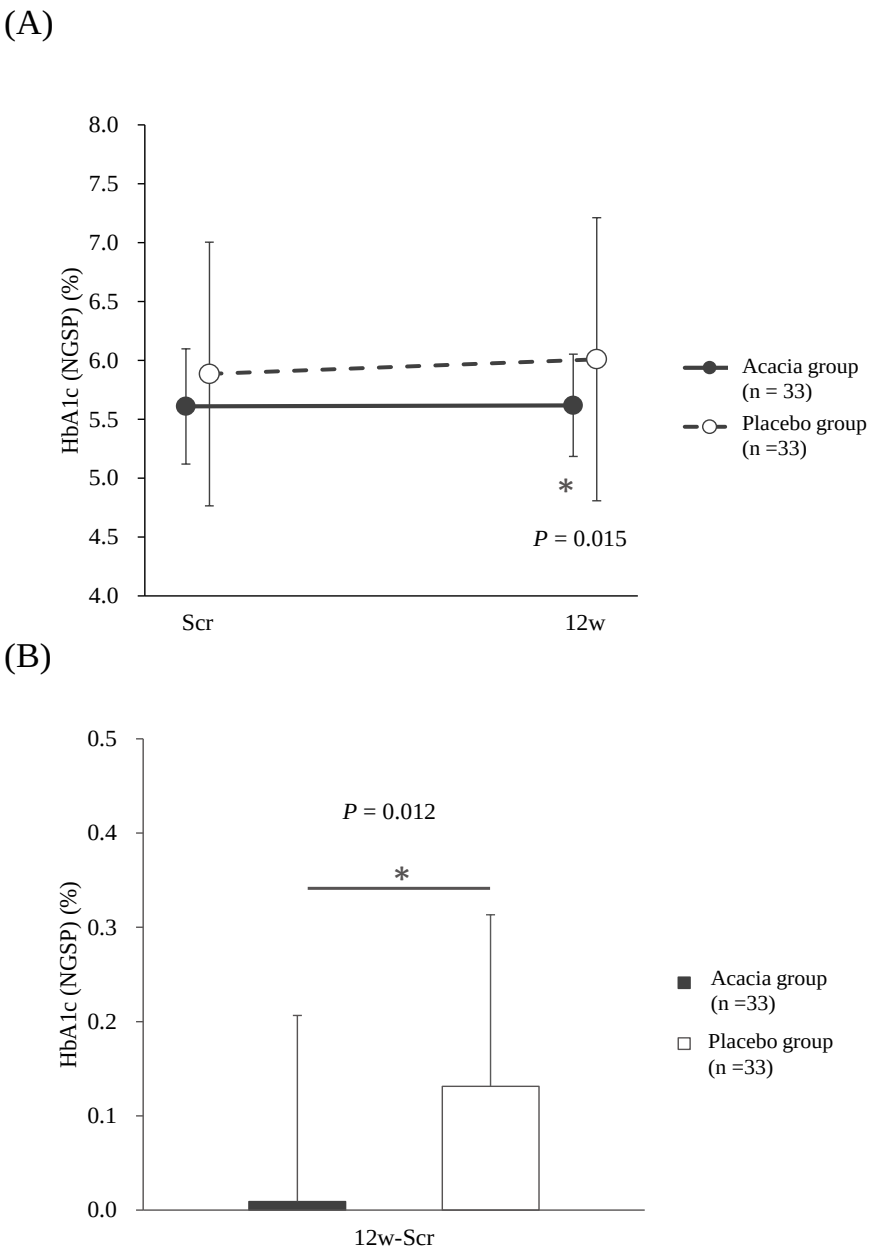


Figure 3. HbA1c (NGSP)
(A) Measured values; (B) Change values

Table 6. Intergroup differences and their 95% confidence intervals in HbA1c (NGSP)

	Intergroup differences	95% CI
12w	−0.1	−0.2 to 0.0
12w−Scr	−0.1	−0.2 to 0.0

The data are presented as estimated marginal mean and 95% confidence interval of EMM (95% CI−, 95% CI+).

Table 7-1. The results of the safety evaluation (physical examination)

	Unit		Scr		0w		4w		8w		12w		*P* value				
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	0w	4w	8w	12w
Body weight	kg	Acacia group (n = 33)	67.4	14.1	67.6	14.0	67.1	14.0	67.3	13.8	66.7	14.1	0.777	0.764	0.860	0.872	0.990
		Placebo group (n = 33)	66.5	13.9	66.6	13.8	66.5	13.7	66.7	14.3	66.5	14.4					
BMI	kg/m^2	Acacia group (n = 33)	24.6	4.7	24.7	4.7	24.5	4.7	24.6	4.6	24.4	4.7	0.584	0.586	0.683	0.682	0.833
		Placebo group (n = 33)	24.1	3.7	24.1	3.7	24.1	3.7	24.1	3.8	24.0	3.8					
Body fat percentage	%	Acacia group (n = 33)	26.5	9.5	26.5	9.4	26.4	9.8	27.0	9.5	27.0	10.4	0.778	0.802	0.879	0.601	0.707
		Placebo group (n = 33)	25.9	6.3	26.0	6.3	26.1	6.1	25.6	6.6	25.9	7.0					
Systolic blood pressure	mmHg	Acacia group (n = 33)	125.6	14.2	125.7	14.0	125.9	13.4	125.2	15.3	127.8	12.9	0.687	0.567	0.274	0.756	0.585
		Placebo group (n = 33)	124.1	18.8	123.6	14.3	121.8	13.5	124.8	17.3	126.5	16.6					
Diastolic blood pressure	mmHg	Acacia group (n = 33)	79.8	10.2	80.1	10.6	81.2	10.7	79.6	11.8	81.0	10.8	0.683	0.653	0.594	0.639	0.864
		Placebo group (n = 33)	80.9	12.9	81.4	10.8	79.6	11.2	81.3	14.6	81.9	13.0					
Pulse rate	bpm	Acacia group (n = 33)	75.4	11.6	76.2	11.0	76.7	8.9	75.6	8.9	76.2	10.5	0.064	0.409	0.372	0.965	0.858
		Placebo group (n = 33)	80.5	14.0	78.5	11.3	79.2	12.7	75.1	10.4	76.3	10.7					
Body temperature	°C	Acacia group (n = 33)	34.0	0.7	33.8	0.7	33.8	0.7	33.9	0.8	33.2	0.9	0.976	0.617	0.169	0.013*	0.525
		Placebo group (n = 33)	34.0	0.7	33.7	0.8	33.5	0.8	33.4	1.0	33.3	0.9					

The data are presented as the number of subjects, or the mean and standard deviation (SD).

*: $P < 0.05$ vs. the placebo group.

Table 7-2. The results of the safety evaluation (urinalysis)

	Reference range		Scr		4w		8w		12w		*P* value			
			Within the reference range	Outside the reference range	Within the reference range	Outside the reference range	Within the reference range	Outside the reference range	Within the reference range	Outside the reference range	Scr	4w	8w	12w
Protein	−	Acacia group (n = 33)	29	4	30	3	31	2	28	5	0.733	0.708	0.672	0.537
		Placebo group (n = 33)	27	6	28	5	29	3	25	7				
Glucose	−	Acacia group (n = 33)	32	1	33	0	33	0	33	0	1.000	0.492	0.238	0.238
		Placebo group (n = 33)	31	2	31	2	30	2	30	2				
Urobilinogen	±	Acacia group (n = 33)	33	0	33	0	33	0	33	0	N.A.	N.A.	N.A.	N.A.
		Placebo group (n = 33)	33	0	33	0	32	0	32	0				
Bilirubin	−	Acacia group (n = 33)	33	0	33	0	33	0	33	0	N.A.	N.A.	N.A.	N.A.
		Placebo group (n = 33)	33	0	33	0	32	0	32	0				
pH	5.0–7.5	Acacia group (n = 33)	32	1	33	0	33	0	33	0	1.000	N.A.	0.492	0.492
		Placebo group (n = 33)	32	0	33	0	31	1	31	1				
Occult blood	−	Acacia group (n = 33)	32	1	32	1	33	0	30	3	0.197	1.000	0.238	1.000
		Placebo group (n = 33)	28	5	31	2	30	2	30	2				
Ketone bodies	−	Acacia group (n = 33)	33	0	33	0	31	2	33	0	1.000	N.A.	0.492	0.492
		Placebo group (n = 33)	32	1	33	0	32	0	31	1				

N.A.: Not Available.

Table 7-3. The results of the safety evaluation (blood test)

	Reference range	Unit		Scr		4w		8w		12w		*P* value			
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	4w	8w	12w
Leukocyte count	3300–9000	/μL	Acacia group (n = 33)	5233.3	1516.1	5315.2	1600.6	5245.5	1345.4	5212.1	1389.4	0.684	0.603	0.636	0.889
			Placebo group (n = 33)	5375.8	1334.2	5497.0	1447.1	5059.4	1501.4	5240.6	1481.0				
Erythrocyte count	Men: 430–570 Women: 380–500	$\times 10^4$/μL	Acacia group (n = 33)	455.3	40.2	460.1	40.3	461.0	39.6	463.2	39.5	0.970	0.609	0.371	0.631
			Placebo group (n = 33)	455.7	54.2	454.3	52.6	451.4	50.0	458.3	48.8				
Hemoglobin	Men: 13.5–17.5 Women: 11.5–15.0	g/dL	Acacia group (n = 33)	14.2	1.4	14.3	1.5	14.2	1.5	14.4	1.5	0.994	0.488	0.303	0.400
			Placebo group (n = 33)	14.2	1.9	14.0	1.8	13.9	1.7	14.1	1.7				
Hematocrit Value	Men: 39.7–52.4 Women: 34.8–45.0	%	Acacia group (n = 33)	43.4	3.7	44.8	4.2	44.8	4.0	45.4	4.2	0.959	0.480	0.211	0.339
			Placebo group (n = 33)	43.4	4.8	44.1	4.7	43.6	4.7	44.5	4.4				
Platelet count	14.0–34.0	$\times 10^4$/μL	Acacia group (n = 33)	25.5	5.0	25.5	5.4	25.7	5.1	25.7	4.8	0.352	0.329	0.742	0.533
			Placebo group (n = 33)	26.7	4.5	26.7	4.9	26.2	4.9	26.6	4.8				
MCV	85–102	fL	Acacia group (n = 33)	95.5	5.6	97.5	6.2	97.3	5.3	98.1	6.0	0.962	0.773	0.600	0.396
			Placebo group (n = 33)	95.5	4.5	97.2	4.3	96.8	4.0	97.1	4.2				
MCH	28.0–34.0	pg	Acacia group (n = 33)	31.2	2.2	31.0	2.2	30.9	2.1	31.0	2.1	0.877	0.594	0.587	0.395
			Placebo group (n = 33)	31.1	1.8	30.8	1.5	30.7	1.5	30.7	1.5				
MCHC	30.2–35.1	%	Acacia group (n = 33)	32.7	1.0	31.8	1.1	31.7	1.1	31.6	1.0	0.724	0.635	0.919	0.794
			Placebo group (n = 33)	32.6	1.1	31.7	1.0	31.8	1.0	31.6	1.0				
Percentage of neutrophils	40.0–75.0	%	Acacia group (n = 33)	57.7	7.5	57.7	7.8	58.7	9.2	59.1	8.1	0.287	0.855	0.049*	0.081
			Placebo group (n = 33)	55.5	8.5	57.4	8.5	54.5	7.6	55.4	8.4				
Percentage of lymphocytes	18.0–49.0	%	Acacia group (n = 33)	33.9	7.0	33.7	7.6	32.9	8.5	32.5	7.3	0.544	0.919	0.155	0.207

	Reference range	Unit		Scr		4w		8w		12w		*P* value			
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	4w	8w	12w
			Placebo group (n = 33)	35.0	7.6	33.5	7.6	35.7	7.0	35.1	7.6				
Percentage of monocytes	2.0–10.0	%	Acacia group (n = 33)	5.1	1.2	5.1	1.3	5.0	1.4	4.8	1.2	0.073	0.231	0.043*	0.003**
			Placebo group (n = 33)	5.8	1.5	5.5	1.6	5.8	1.4	6.0	1.4				
Percentages of eosinophils	0.0–8.0	%	Acacia group (n = 33)	2.7	1.9	2.8	1.7	2.6	1.7	2.8	2.0	0.343	0.616	0.134	0.753
			Placebo group (n = 33)	3.2	2.2	3.1	1.7	3.3	1.8	3.0	1.7				
Percentages of basophils	0.0–2.0	%	Acacia group (n = 33)	0.6	0.3	0.6	0.3	0.7	0.5	0.7	0.3	0.400	0.177	0.300	0.259
			Placebo group (n = 33)	0.5	0.3	0.5	0.2	0.6	0.3	0.6	0.3				
Neutrophils	-	/μL	Acacia group (n = 33)	3066.9	1163.3	3095.9	1125.7	3131.4	1080.4	3127.0	1081.6	0.734	0.779	0.224	0.527
			Placebo group (n = 33)	2980.5	862.3	3167.4	1007.6	2795.7	1032.7	2941.3	1072.5				
Lymphocytes	-	/μL	Acacia group (n = 33)	1730.3	498.2	1775.5	628.5	1689.1	530.8	1661.7	492.3	0.247	0.661	0.522	0.305
			Placebo group (n = 33)	1887.0	603.3	1834.6	602.8	1781.1	533.6	1806.2	526.2				
Monocytes	-	/μL	Acacia group (n = 33)	265.5	88.3	269.8	107.1	257.9	78.4	250.9	86.3	0.056	0.216	0.234	0.014*
			Placebo group (n = 33)	308.4	103.0	297.5	93.1	286.3	84.0	308.5	93.9				
Eosinophils	-	/μL	Acacia group (n = 33)	140.2	98.2	143.6	88.3	133.4	87.7	140.7	88.7	0.241	0.317	0.202	0.554
			Placebo group (n = 33)	171.2	126.4	170.1	106.0	165.9	105.1	154.9	101.6				
Basophils	-	/μL	Acacia group (n = 33)	30.5	12.6	30.3	12.8	34.0	19.1	32.1	13.2	0.674	0.453	0.354	0.290
			Placebo group (n = 33)	29.0	16.5	27.5	12.4	30.6	13.5	28.2	14.4				
AST (GOT)	10–40	U/L	Acacia group (n = 33)	23.2	12.5	25.1	9.8	25.4	9.9	24.8	8.1	0.921	0.559	0.941	0.568
			Placebo group (n = 33)	23.5	10.1	23.7	8.8	25.4	11.0	26.4	10.9				

	Reference range	Unit		Scr		4w		8w		12w		*P* value			
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	4w	8w	12w
ALT (GPT)	5–45	U/L	Acacia group (n = 33)	25.8	26.8	26.0	19.6	27.4	23.5	24.1	14.5	0.507	0.501	0.318	0.087
			Placebo group (n = 33)	29.8	25.9	30.1	25.6	33.9	30.0	35.0	32.5				
γ-GT (γ-GTP)	Men: ≤80 Women: ≤30	U/L	Acacia group (n = 33)	40.3	40.9	44.0	45.5	41.4	35.0	41.0	42.0	0.630	0.909	0.574	0.376
			Placebo group (n = 33)	44.9	35.0	45.1	36.3	47.3	39.7	50.0	40.8				
ALP	100–325	U/L	Acacia group (n = 33)	194.0	52.6	198.6	52.0	193.5	55.1	190.7	51.0	0.266	0.435	0.567	0.279
			Placebo group (n = 33)	209.6	66.2	209.5	61.7	201.5	58.6	205.8	55.6				
LD (LDH)	120–240	U/L	Acacia group (n = 33)	182.4	26.8	187.8	28.2	187.5	26.8	191.6	28.5	0.060	0.292	0.479	0.448
			Placebo group (n = 33)	196.7	38.7	195.7	33.5	192.0	31.1	196.5	29.4				
LAP	Men: 45–81 Women: 37–61	U/L	Acacia group (n = 33)	51.9	8.8	53.0	9.2	53.0	9.2	52.5	8.9	0.056	0.318	0.411	0.124
			Placebo group (n = 33)	57.1	14.0	55.7	11.7	55.3	11.0	56.8	13.5				
Total bilirubin	0.2–1.2	mg/dL	Acacia group (n = 33)	0.73	0.23	0.83	0.21	0.82	0.19	0.81	0.21	0.879	0.189	0.320	0.667
			Placebo group (n = 33)	0.74	0.25	0.75	0.26	0.77	0.25	0.79	0.28				
Direct bilirubin	0.0–0.2	mg/dL	Acacia group (n = 33)	0.08	0.05	0.11	0.04	0.10	0.04	0.11	0.04	0.623	0.028*	0.032*	0.106
			Placebo group (n = 33)	0.07	0.06	0.08	0.05	0.08	0.05	0.09	0.06				
Indirect bilirubin	0.2–1.0	mg/dL	Acacia group (n = 33)	0.65	0.20	0.72	0.19	0.72	0.17	0.69	0.20	0.775	0.331	0.541	0.922
			Placebo group (n = 33)	0.67	0.22	0.67	0.23	0.69	0.22	0.69	0.25				
Cholinesterase (ChE)	Men: 234–493 Women: 200–452	U/L	Acacia group (n = 33)	342.4	71.8	346.4	65.5	347.8	66.8	347.2	61.8	0.992	0.784	0.790	0.934
			Placebo group (n = 33)	342.2	70.3	341.7	72.8	340.8	73.3	346.2	72.1				
Total protein	6.7–8.3	g/dL	Acacia group (n = 33)	7.1	0.4	7.2	0.4	7.1	0.4	7.1	0.3	0.617	0.597	0.364	0.762

	Reference range	Unit		Scr		4w		8w		12w		*P* value			
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	4w	8w	12w
			Placebo group (n = 33)	7.2	0.5	7.1	0.4	7.0	0.5	7.1	0.5				
Urea nitrogen	8.0–20.0	mg/dL	Acacia group (n = 33)	13.8	3.8	12.8	2.9	12.7	3.1	13.6	3.1	0.658	0.024*	0.074	0.567
			Placebo group (n = 33)	14.2	3.2	14.6	3.6	14.2	2.3	14.2	2.4				
Creatinine	Men: 0.61–1.04 Women: 0.47–0.79	mg/dL	Acacia group (n = 33)	0.75	0.13	0.74	0.13	0.73	0.13	0.72	0.13	0.270	0.949	0.682	0.950
			Placebo group (n = 33)	0.79	0.13	0.74	0.15	0.73	0.12	0.73	0.13				
Uric acid	Men: 3.8–7.0 Women: 2.5–7.0	mg/dL	Acacia group (n = 33)	5.8	1.6	5.7	1.6	5.8	1.5	5.8	1.7	0.348	0.318	0.181	0.281
			Placebo group (n = 33)	5.4	1.3	5.4	1.3	5.2	1.2	5.4	1.1				
CK	Men: 60–270 Women: 40–150	U/L	Acacia group (n = 33)	118.3	60.5	116.6	72.6	124.9	80.9	118.8	73.1	0.308	0.708	0.794	0.343
			Placebo group (n = 33)	141.9	152.1	125.3	87.9	119.9	66.8	141.8	134.4				
Sodium	137–147	mEq/L	Acacia group (n = 33)	140.3	1.7	140.3	1.4	140.1	1.6	140.7	1.8	0.588	0.635	0.392	0.235
			Placebo group (n = 33)	140.5	2.0	140.1	2.0	140.5	2.1	140.2	1.8				
Potassium	3.5–5.0	mEq/L	Acacia group (n = 33)	3.9	0.3	3.9	0.3	3.9	0.3	3.9	0.3	0.516	0.097	0.076	0.242
			Placebo group (n = 33)	3.9	0.3	4.0	0.3	4.0	0.3	4.0	0.3				
Chloride	98–108	mEq/L	Acacia group (n = 33)	101.6	2.0	100.6	2.0	100.5	1.8	101.0	2.2	0.285	0.953	0.201	0.878
			Placebo group (n = 33)	101.1	2.1	100.6	2.2	101.1	2.5	100.8	1.7				
Calcium	8.4–10.4	mg/dL	Acacia group (n = 33)	9.2	0.4	9.3	0.3	9.3	0.3	9.3	0.3	0.149	0.872	0.783	0.942
			Placebo group (n = 33)	9.3	0.3	9.3	0.3	9.3	0.3	9.4	0.3				
Inorganic phosphorus	2.5–4.5	mg/dL	Acacia group (n = 33)	3.4	0.5	3.4	0.6	3.3	0.5	3.3	0.5	0.423	0.504	0.439	0.211
			Placebo group (n = 33)	3.5	0.6	3.5	0.6	3.4	0.5	3.5	0.4				

	Reference range	Unit		Scr		4w		8w		12w		*P* value			
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Scr	4w	8w	12w
Serum iron	Men: 50–200 Women: 40–180	μg/dL	Acacia group (n = 33)	108.8	40.8	114.8	47.9	109.8	32.6	114.9	45.0	0.711	0.400	0.635	0.559
			Placebo group (n = 33)	112.6	45.2	106.2	41.3	114.6	34.1	109.0	33.5				
Serum amylase	40–122	U/L	Acacia group (n = 33)	76.2	32.3	75.5	30.9	74.1	21.7	73.9	20.2	0.201	0.513	0.553	0.739
			Placebo group (n = 33)	67.3	17.4	71.0	23.6	70.4	21.7	72.0	22.1				
Total cholesterol	120–219	mg/dL	Acacia group (n = 33)	217.5	35.6	219.1	26.1	218.9	33.5	217.3	26.8	0.691	0.407	0.880	0.534
			Placebo group (n = 33)	220.7	30.0	225.7	36.5	217.4	29.2	221.9	32.0				
HDL cholesterol	Men: 40–85 Women: 40–95	mg/dL	Acacia group (n = 33)	65.2	16.0	68.2	20.2	67.4	20.0	69.8	19.4	0.817	0.898	0.933	0.677
			Placebo group (n = 33)	66.3	18.1	68.8	20.7	66.9	18.5	71.7	20.3				
LDL cholesterol	65–139	mg/dL	Acacia group (n = 33)	128.3	34.6	126.7	32.1	125.4	33.9	122.8	31.4	0.857	0.459	0.583	0.290
			Placebo group (n = 33)	129.8	29.7	132.6	33.0	129.6	29.8	131.2	30.5				
Triglyceride	30–149	mg/dL	Acacia group (n = 33)	126.3	94.3	136.5	95.9	153.2	158.3	169.8	273.2	0.746	0.820	0.458	0.137
			Placebo group (n = 33)	138.3	100.4	145.0	144.1	124.7	72.5	113.3	78.3				
Glycoalbumin	12.3–16.5	%	Acacia group (n = 33)	14.8	1.8	14.3	1.8	14.0	1.5	14.0	1.8	0.263	0.240	0.074	0.123
			Placebo group (n = 33)	15.5	3.0	15.1	3.4	15.2	3.8	15.0	3.6				
Insulin	1.7–10.4	μU/mL	Acacia group (n = 33)	8.3	9.2	5.9	3.5	6.2	4.5	6.9	5.7	0.706	0.198	0.553	0.363
			Placebo group (n = 33)	9.1	9.1	7.8	7.9	7.0	5.3	5.8	3.6				

The data are presented as the number of subjects, or the mean and standard deviation (SD).

*: $P < 0.05$; $P < 0.01$ vs. the placebo group.

DISCUSSION

Insulin is involved in glucose metabolism and is secreted from the pancreas during elevations in postprandial blood glucose level. By doing so, it promotes glucose uptake in the target organs and maintains a constant blood glucose level in the body. However, lowered insulin sensitivity in the target organ, also known as insulin resistance, is likely to cause diabetes. Insulin resistance is caused by lifestyle disorders such as overeating, lack of exercise, and obesity. Insulin resistance reduces the uptake of glucose in the muscle and adipose tissue. This leads to unsuppressed gluconeogenesis in the liver, thus making it difficult to lower blood glucose levels. Thus, more insulin is needed to return blood glucose levels to normal. Continuously decreased insulin secretion and deficiency of glucose utilization in the body results in the development of type II diabetes. Therefore, maintaining a normal blood glucose level on a daily basis is considered to be important from the perspective of reducing the risk of developing diabetes.

A number of polyphenol-rich foods have been reported to be beneficial for glucose metabolism [12]. The test food in this study contained extract from the bark of *Acacia mearnsii* De Wild., which has a high polyphenol content with confirmed efficacy in the glucose metabolism of humans [9] and mice [8]. Proanthocyanidins are also reportedly effective for glucose metabolism in several substrates [13]. Since acacia polyphenols also contain proanthocyanidins, an effect on glucose metabolism is expected as well. However, the efficacy of proanthocyanidins from the acacia bark extract on FBG levels in humans has not been confirmed yet. The present study examined the effects of proanthocyanidins derived from acacia bark on reducing FBG levels, with the intake of proanthocyanidins derived from acacia bark set at 245 mg per day.

The primary outcome of the study, FBG levels at 12w, was significantly lower in the Acacia group than in the placebo group. The secondary outcomes, measured values of FBG levels at 8w and changes in FBG levels at 8w and 12w, were significantly lower in the Acacia group than in the placebo group. Moreover, the measured values and changes in HbA1c (NGSP) at 12w were significantly lower in the acacia group than in the placebo group. HbA1C is used to measure blood glucose control over the past 3 months [14–16]. Thus, the significant change in HbA1c (NGSP) at 12w was consistent with the result of the significant change in FBG level in the Acacia group at 8w. These results demonstrate that long-term intake of proanthocyanidins derived from acacia bark for 12 weeks reduced FBG.

The Japan Diabetes Society [2] divides blood glucose level status into the normal type, borderline type, and diabetic type, with the borderline type being more prone to complications of atherosclerosis than the normal type [2]. In a cohort study with a mean observational period of 6.4 years [17], the incidence of diabetes in people with FBG levels of 100–109 mg/dL and HbA1c of 5.6–5.9% was 4.9% in men and 2.4% in women. In contrast, the incidence of diabetes in people with FBG levels of 110–125 mg/dL and HbA1c of 5.6–5.9% is 14.3% in men and 19.0% in women. Thus, there is a large difference in the incidence of diabetes among people with different blood glucose levels. In this study, the mean FBG level in the acacia group decreased significantly from 114.4 to 100.4 mg/dL compared to the placebo group, and the HbA1c was also significantly suppressed compared to the placebo group (Acacia group: from 5.6% to 5.6%, placebo group: from 5.9% to 6.0%). Our results have been shown a clinically meaningful change.

The reduction of FBG levels in mice due to the intake of acacia bark extract was confirmed in a study

by Ikarashi et al [8]. Recently, Kashiwada et al. [18] identified proanthocyanidins as the components of acacia bark extract associated with blood glucose lowering [18]. In that study, the acacia bark extract was fractionated into two: one containing proanthocyanidins and the other containing mainly carbohydrates, and mice were used to determine which fraction showed FBG-lowering effects. As the result, the proanthocyanidins in the acacia bark extract were identified as the functional components involved in lowering FBG levels. Moreover, that study also suggested several mechanisms by which proanthocyanidins lower FBG levels. First, the proanthocyanidins ameliorated insulin resistance in white adipocytes by enhancing adiponectin production, which potentiates insulin action and reduces TNF-α. Second, the proanthocyanidins also suppressed glycogenesis and fatty acid synthesis in the liver by decreasing the mRNA expression of fatty acid synthase (FAS) and lipoprotein lipase (LPL). Respectively, these encode fatty acid synthase, a rate-limiting enzyme for fatty acid synthesis, and lipoprotein lipase, which is associated with fat uptake into the liver. Third, proanthocyanidins significantly upregulated the expression of peroxisome proliferator-activated receptor δ (PPARδ), Uncoupling protein 3 (UCP3; deconjugated protein), and Acyl-CoA (ACO) mRNA in skeletal muscle, indicating that intake of proanthocyanidins suppressed the decrease in energy production. Fourth, proanthocyanidins activated AMP-activated protein kinase (AMPK), suggesting that proanthocyanidins suppressed AMPK-mediated glycogenesis and fatty acid synthesis. Fifth, proanthocyanidins inhibit dipeptidyl peptidase (DPP)-4 action. DPP-4 is one of the enzymes that inactivate incretin, a hormone with postprandial hypoglycemic effects. DPP-4 inhibitors are known to be in high demand in clinical practice for the treatment of diabetes because of their lower risk of hypoglycemia compared to sulfonylureas and glinides.

Collectively, our data demonstrating reduction of FBG levels suggested that proanthocyanidins play a role in improving insulin resistance, inhibiting glycogenesis and fatty acid synthesis in the liver, increasing energy expenditure in skeletal muscle, suppressing glycogenesis and fatty acid synthesis via AMPK activation, and promotion of insulin secretion via inhibiting DPP-4 activation. In addition, a Cochrane meta-analysis [19] which reviewed the effects of insulin and blood glucose-lowering drugs on patients with diabetes and chronic kidney disease, reported that DPP-4 inhibitors have the potential to lower HbA1c. Furthermore, in a mouse study reported by Kashiwada, et al [18], HbA1c levels were significantly lower in the proanthocyanidins group compared to controls, and no significant difference in HbA1c levels was identified between the carbohydrates group and controls. Therefore, the DPP-4 inhibitory effect of proanthocyanidins might be involved in the significant suppression of HbA1c increase observed in our study.

One limitation of this study is that the subjects were Japanese adult men and women with baseline FBG levels of 110–125 mg/dL. Hence, further studies are needed to verify the effect of proanthocyanidins in those with FBG levels >125 mg/dL. The other limitation was that we did not account for the effect of the meal on the day before the test. Additionally, proanthocyanidins were also found to have strong inhibitory activity against α-amylase and lipase [20]. In healthy Japanese men and women with high FBG levels or those prone to high blood glucose levels (FBG of 110–125 mg/dL or 2-hour 75g OGTT of 140–199 mg/dL), consuming of 250 mg of acacia bark extract (163 mg as proanthocyanidins) significantly reduced the IAUC from before to 60 mins after glucose loading compared to the placebo group [9]. Because of these

findings, we also expected our study's test food containing proanthocyanidins derived from acacia bark to inhibit the increase in postprandial blood glucose level, but no significant difference was found in the postprandial blood glucose levels. It has been reported that approximately 30% of those with FBG levels of 110–125 mg/dL had normal results on 2-hour 75g OGTT [21]. Thus, it is possible that many of the subjects in our study had a low postprandial blood glucose level. Moreover, the contents of the previous day's dinner may be involved in the second meal effect, which affects postprandial blood glucose levels at breakfast [22]. In this study, fasting for at least 6 hours before the test was done, but the diet was not unified. By standardizing the contents of the dinner on the day before the postprandial blood glucose test, it may be possible to verify the efficacy of the test on postprandial blood glucose as well as the reduction of FBG in patients with high FBG.

CONCLUSIONS

This study was conducted on healthy Japanese adult men and women with relatively high blood glucose levels and who are worried about their blood glucose levels (FBG levels of 110–125 mg/dL [borderline levels] at screening). We examined the effect of 12 weeks of proanthocyanidins intake (245 mg/day) on the reduction of FBG levels and the safety of long-term intake. The measured values and change in FBG levels at 8w and 12w, as well as the measured values and change in HbA1c (NGSP) levels at 12w were significantly lower in the Acacia group than in the placebo group. Furthermore, it was confirmed that there were no safety problems under the conditions of this study. Consequently, these results indicate that long-term consumption of foods containing proanthocyanidins derived from acacia bark can reduce borderline FBG levels to normal levels, maintain healthy FBG levels, and maintain HbA1c levels. Foods containing proanthocyanidins derived from acacia bark are expected to be suitable for those with borderline FBG levels, those who concerned about their blood glucose levels and HbA1c levels.

List Of Abbreviations: AMPK: AMP-activated protein kinase, ANCOVA: analysis of covariance, Cmax: maximum blood concentration, DPP: dipeptidyl peptidase, FBG: fasting blood glucose, GLUTs: glucose transporters, HbA1c: hemoglobin A1c, IAUC: incremental area under the curve, OGTT: oral glucose tolerance test, OR: odds ratio, TNF: tumor necrosis factor, FAS: fatty acid synthase, LPL: lipoprotein lipase, PPARδ: peroxisome proliferator-activated receptor δ, UCP3: Uncoupling protein 3, ACO: Acyl-CoA.

Competing Interests: The sponsor of this study, Acacia-No-Ki Co., Ltd., entrusted ORTHOMEDICO Inc. with conducting the study. S.O. is a member of Acacia-No-Ki Co., Ltd., and A.B. and T.H. are employees of ORTHOMEDICO Inc. T.T. (MD), the principal investigator of this study, is a staff of Medical Corporation Seishinkai, Takara Clinic, and he monitored all the conditions of the subjects.

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