Safety and efficacy of adzuki bean extract in subjects with moderate to high LDL-C: a randomized trial

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Safety and efficacy of adzuki bean extract in subjects with moderate to high LDL-C: a randomized trial.

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Introduction

Adzuki bean (*Vigna angularis*), a member of the Fabaceae family, has long been widely cultivated and consumed in traditional dishes throughout East Asia. This legume is rich in dietary fibers, proteins, minerals, vitamin A, and vitamin B9 [1]. Adzuki beans are regarded as an ethnopharmacologically well-known folk medicine in Korea, China, and Japan [2]. In Japan, the beans are often boiled and sweetened with sugar to produce adzuki (red) bean paste for use in traditional confectioneries. During the boiling process, several water-soluble bioactive compounds, such as polyphenols and fibres [3] are released from the beans. However, the water containing these compounds is commonly discarded after boiling. To exploit these underutilized materials, ‘adzuki bean extract’ has been developed from the released water obtained during adzuki bean paste production (Cosmo Foods Co., Ltd, Tokyo, Japan). The extract is purified and spray-dried to produce an adzuki bean extract powder that is rich in natural polyphenols, comprising approximately 59.3% of the powder (wt/wt). In our previous study, we partly determined that these polyphenols were chemically composed of approximately

38.2% (wt/wt) anthocyanidin glucosides and 61.8% proanthocyanidins (wt/wt) [4]. The main anthocyanidin glucosides in the product were found to be peonidin-3-rutinoside, followed by peonidin-3-(p-coumaroyl) glucoside, pelargonidin-3-O-glucoside, and malvidin-3-O-glucoside.

Recent interests have been focused on the biological activities of dietary polyphenols including cardiovascular protective, antioxidative, anti-inflammatory, anti-hypertensive, and anti-glycemic effects [5, 6]. Several studies have shown that adzuki bean-derived polyphenols can attenuate hypercholesterolemia and hyperglycemia as well as protect hepatic injury in animal models [7-9], although the effects of polyphenols on lipid metabolism are greater *in vitro* as compared to that *in vivo*.

In a previous study on testing the safety and efficacy of adzuki bean polyphenols, the data suggested that adzuki bean extract promotes lipid metabolism by disrupting lipid accumulation and adipocytokine production in animal and human adipocyte cultures. Furthermore, our research with animal models demonstrated that adzuki bean polyphenols can effectively inhibit hepatic lipid accumulation and reduce the plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (TG) with no detectable adverse effect [10]. These results raise the possibility that adzuki beans may be beneficial for the lipid metabolism in humans, including attenuation of hypercholesterolemia; however, there is a lack of research on the effects of dietary intake of purified adzuki bean extract in humans. Therefore, the present study aimed to determine the safety and efficacy of polyphenol-containing adzuki bean extract on the lipid metabolism in humans using a double-blind, 2-group parallel, placebo-controlled comparison study. The main outcomes measured were the percent changes in the plasma lipid composition, blood glucose, and blood pressure in comparison with baseline levels. The results of this study are expected to demonstrate the safety and potential phytotherapeutical applications of adzuki bean extract in humans.

Materials and methods

Extract and capsules preparation

The adzuki bean extract tested was supplied by Cosmo Foods (Adzukinomoto®, Cosmo Foods Co., Ltd., Japan). Briefly, the extracts were obtained from boiling water used for the production of adzuki bean paste. The water was purified, concentrated, sterilized, and freeze-dried. The total concentration of polyphenols in the adzuki bean extract was determined to be 59.3% according to the Folin–Ciocalteu method using (+)-catechin as a standard [11]. The absorbance was read at 750 nm using a spectrophotometer (1600-UV; Shimadzu, Kyoto, Japan). The results were expressed in milligram of

(+)-catechin equivalents per gram of bean extract powder. All the tests were performed in triplicate.

The adzuki bean extract powder was sent to a supplement manufacturer (Nakanihon Capsule Co., Ltd., Gifu, Japan) for encapsulation. Table 1 shows the nutrient composition of the adzuki bean polyphenol capsules and placebo capsules prepared in this trial. Each adzuki bean extract capsule weighed 338 mg, whereas the placebo capsule weighed 333 mg. Each adzuki bean extract capsule contained 71.2 mg adzuki bean extract with 42.2 mg polyphenols. The subjects were given a total of six test capsules (total of 427.0 mg adzuki bean extract with 253.2 mg polyphenols) or placebo capsules on the daily basis. The placebo capsules were prepared from inert ingredients and were identical to the active capsules in their appearance and odor.

A previous research reported that the no-observed-adverse-effect level for adzuki bean extract was a dose of 2,000 mg/kg body weight, based on both single and repeated-dose toxicity studies. Another report suggested that the intake of 200 mg per day adzuki bean extract product for 1 week did not result in any changes in body weight, blood pressure, or lipid profiles in healthy men and women aged >20 years (Unpublished data). Furthermore, the daily intake of 880 mg to 1,960 mg adzuki bean juice for 12 weeks did not result in any adverse effects in 33 healthy women of an average age 21.2 years [12].

Subjects selection

A total of 50 subjects aged 35–65 years with the LDL-C levels of 120–179 mg/dL were recruited from the volunteer databases registered at the Hokkaido Information University Center of Health Information Science through a screening test.

All the participants provided written informed consent before their enrollment. The exclusion criteria included individuals with hyperlipidemia, familial hypercholesterolemia, severe acute or chronic disease, surgery, unusually high and/or low blood pressure, severe anemia, pre or post menopause, allergic reaction to foods (particularly adzuki bean, pork, gelatin, and soybean), current use of any medication and supplement (particularly, eicosapentaenoic acid, docosahexaenoic acid, medium chain fatty acids, plant sterol, sesamin, turmeric, polyphenol, and dietary fiber), heavy smoker, alcohol abuse, pregnancy, concurrent or recent (within 30 days) intervention study participation, or any other medical reasons judged by the principal investigator.

The subjects participating in this study were randomly divided into two groups of 25 participants each: an adzuki bean extract group and a control group. The adzuki bean group received 6 capsules per day of 427 mg adzuki bean extract, which is equivalent to a daily dosage of 253.2 mg adzuki bean-derived polyphenols. The control group

received 6 placebo capsules per day containing no adzuki bean extract. The randomization sequence was created using a permuted-block randomization design stratified by age, gender, and LDL-C level, where the block size was a multiple of 2. Each subject was allocated by a third-party data center according to the random allocation sequence into a relevant group. The third-party data center concealed the allocation information, including the subjects' personal data, and kept them secure. This information was revealed only after the laboratory and analytical data were fixed, and the method of statistical analysis was finalized.

A CONSORT (consolidated standards of reporting trials diagram outlining the participant recruitment is depicted in Fig. 1. The recruited cohort comprised of middle-aged adults (mean age: 54.8 ± 7.3 years), and the majority of the participants were women (90%). A total of 50 participants were recruited: 25 were allocated to the adzuki bean extract group and 25 to the placebo group. Prior to the start of the experimental trial, one subject allocated to the placebo group dropped out for personal reasons. Finally, 49 subjects completed this trial (25 in the adzuki bean extract group and 24 in the placebo group).

Study design

The clinical study was based on a double-blind, placebo-controlled, parallel intervention study design. The timeline and schedule of events for this study is shown in Fig. 2. During the 8-week treatment period, the subjects were given either six test capsules or six placebo capsules on a daily basis after a washout period of 1 week during which the patients received placebo treatment.

The subjects were instructed to create four scheduled visits to the Hokkaido Information University Center of Health Information Science during screening and at weeks 0 (baseline), 4, and 8 of the treatment period. At each visit during the treatment period, body composition measurements were taken, including body weight (BW), body mass index (BMI), and body fat percentage (BFP). A doctor interviewed each subject and recorded their vital signs, specifically pulse rate, temperature, respiration rate, and blood pressure. During the study, the subjects were asked not to change their daily activities, including medications, exercise routines, and food consumption. The subjects were not allowed to take concomitant supplements and functional healthy foods that were believed to interfere with the interpretation of the study data. The primary outcomes measured were the LDL cholesterol levels. The secondary outcomes measured were the blood lipid profile, blood sugar levels, and markers of oxidative stress, including oxidized LDL (ox-LDL), thiobarbituric acid reactive substance (TBARS), adiponectine,

body composition (BW, BMI, and BFP), and blood pressure.

All the trial participants provided informed written consent prior to undergoing any of the tests and procedures needed to assess eligibility. The study protocol was approved by the Ethics Committee of Hokkaido Information University in conformity with the Helsinki Declaration (No.2015-29). This study was registered in UMIN (No. UMIN000021975).

Anthropometric and blood pressure measurements

Body weight (BW), height, blood pressure, and pulse rate were measured at each evaluation point. The BW was determined using the Body Composition Analyzer DC-320 (Tanita Corp., Tokyo, Japan) capable of calculating percent muscle and percent fat mass. BMI was calculated based on the BW and height recorded for each participant. The blood pressure and pulse rate were determined using the Automatic Blood Pressure Monitor HEM-7080IC (Omuron Colin Co., Ltd., Tokyo, Japan) with individuals in a seated position.

Blood collection and laboratory analyses

Venous blood samples were collected at weeks 0, 4, and 8 of the experimental period. The subjects were asked to fast for 12 h prior to the blood test, and blood collection was performed at the Hokkaido Information University Center of Health Information Science. Each blood sample was analyzed to determine the plasma lipid profile (i.e., total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and arteriosclerosis index); blood glucose status (i.e., fasting blood glucose level and HbA1c); complete blood count; white blood cell (WBC) count; red blood cell (RBC) count; hemoglobin content (Hb); hematocrit value (Ht); platelet count (Plt); liver function [i.e., aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)]; kidney function [i.e., blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UAC)]; and plasma protein [i.e., total plasma protein (TP), albumin (Alb), total bilirubin (T-Bil) and albumin-globulin ratio (A/G ratio)]. Oxidative stress markers (i.e., oxidized LDL and TBARS) and adiponectin were determined only at weeks 0 and 8 of the experimental period.

Hematological tests were conducted at the Sapporo Clinical Laboratory, Inc. (Sapporo, Japan). Ox-LDL and TBARS were measured using the ox-LDL ELISA Kit (Sekisui Medical Co., Ltd., Tokyo, Japan) and TBARS Assay Kit (Cayman Chemical, Michigan, USA). The TG, TC, HDL-C, and LDL-C were measured by the free glycerol method,

cholesterol oxidase method, selective inhibition method, and selective solubilization method, respectively. WBC, RBC, Hb, Ht, and Plt were measured by flow cytometry method, electrical resistivity measurement, sodium lauryl sulfate-hemoglobin method, and electrical resistivity measurements, respectively. The AST, ALT, γ-GTP, ALP, and LDH were measured by the Japan Society of Clinical Chemistry reference methods. BUN, Cr, and UAC were measured by the urease-GLDH method, enzyme assay, and uricase-peroxidase method, respectively. TP was determined by the Biuret method, Alb was measured by the BCG method, T-Bil was measured by the Vanadate oxidation method, and A/G ratio was calculated from measured total protein, measured albumin, and calculated globulin.

Statistical analysis

The sample size was statistically determined to obtain a power of 80% with an alpha error of 0.05. To demonstrate the postulated change in LDL-C at week 8 (0.50 reduction with a standard deviation of 0.50), a sample size of 40 (20 in the test group and 20 in the placebo group) was required. Assuming a 20% loss to follow-up, 50 subjects were included.

The mean and standard deviation of age and other parameters were calculated for each group. Two-way analysis of variance (ANOVA) was performed to assess the effect of diet and treatment period as well as interaction between diet and period on biochemical measures. Changes in subject value in each group were analyzed using Tukey's honestly significant difference (HSD) test. All statistical analyses were performed using SPSS Statistic20 (IBM, Armonk, NY, USA). $p < 0.05$ were considered to be significant.

Results

Baseline characteristics

Table 2 shows the baseline clinical and laboratory characteristics of the participants. No significant differences were noted at the baseline between the adzuki bean extract group and the placebo group for age, BW, BMI, BFP, serum lipid, glucose composition, or adiponectin. These results indicate the appropriate assignment of the subjects into the two groups.

Physical measurements

To determine the effects of adzuki bean extract supplementation on body composition, changes in BW, BFP, and BMI were evaluated. Measurements of the body composition were found to be stable across the trial period between the two groups. BW varied by

<0.30 kg and the derived fat mass values varied by <1.35% for all groups. None of the measurements showed a significant difference between the two groups, based on a Student's t-test, $p < 0.05$ (Data not shown).

Biochemical measurements

Serum lipid concentrations

Table 3 shows the changes in the biochemical measures for the two groups during the experimental period. The results of two-way ANOVA analysis revealed that (i) diet (adzuki bean extract supplementation) significantly affected the changes in HDL-C concentration ($p = 0.009$); (ii) period (weeks) affected the changes of both TC and HDL-C concentrations ($p = 0.007$ and $p = 0.022$, respectively); and (iii) no interaction between the diet and period was observed. The ΔHDL-C concentration (3.76 ± 7.79 mg/dl) was significantly increased in the adzuki bean extract group compared with that in the placebo group (−0.08 ± 6.03 mg/dl) at week 4. At 8 weeks, both the groups showed reduced ΔHDL-C concentrations compared with those at week 4, with the adzuki bean extract group showing a return to the baseline level (0.36 ± 5.36 mg/dl) and the placebo group showing a decrease to below the baseline level (−3.17 ± 7.79 mg/dl). There were no significant changes in LDL-C levels between the adzuki polyphenol group and placebo group after 4 and 8 weeks of capsule supplementation, as compared to baseline levels. Similarly, the TG and TC concentrations were largely unchanged following supplementation.

Given the possibility that adzuki bean extract retain HDL-C as opposed to the placebo, a subgroup analysis was performed to study the potential effects further. Participants were divided into subgroups on the basis of TG levels: the subgroups with low TG levels (< 72mg/dl) consisted of 12 participants in the placebo group and 10 participants in the adzuki bean extract group and the subgroups with high TG levels (≥72 mg/dl) consisted of 12 participants in the placebo group and 15 participants in the adzuki bean extract group. Fig. 3 shows the changes in the plasma HDL-C concentrations of subjects with low TG levels. Interestingly, there was a significant difference ($p = 0.011$) between the two groups with low TG levels when examining the changes in the plasma HDL-C concentrations after 8 weeks of supplementation. Participants in the placebo group showed a significant reduction in the ΔHDL-C concentration (−6.92 ± 1.68 mg/dl) as compared to those in the adzuki bean extract group (0.30 ± 1.98 mg/dl). Among the participants with high TG levels, no significant differences were observed in the HDL cholesterol levels between the adzuki bean extract and the placebo groups (results not shown).

Blood glucose status

The blood glucose status was determined by measuring the fasting blood glucose levels and HbA1c levels (Table 3). These measures were largely unchanged in both the adzuki bean extract group and the placebo group following supplementation, indicating that adzuki bean polyphenols do not affect glycometabolism.

Oxidative stress markers and adiponectin

The effects of adzuki bean extract on oxidative stress markers and adiponectin were evaluated in the adzuki bean and placebo groups throughout the study period. There were no significant differences between the groups (Table 3).

Adverse effects

No adverse effects related to peripheral blood analysis, liver function tests, and kidney function occurred in any of the participants in the two groups throughout the experimental period. These results confirmed the safety of adzuki bean extract intake at the levels received by participants in this study (6 capsules of 71.2 mg each per day; total daily intake = 427 mg) over a period of 8 weeks.

Discussion

Naturally derived bioactive compounds can have beneficial health effects with potential applications in the medicinal field. Therefore, the use of herbal medicine as a pharmacologic modality in the prevention and treatment of human diseases has received widespread attention. For centuries, adzuki bean has been an important ingredient in traditional herbal medicine in China and Korea owing to its beneficial effects on the diuretic functions and other diseases such as dropsy and beriberi [13].

In the current human trial, the tolerability and efficacy of adzuki bean extract capsules taken daily were investigated by randomized, double-blind, placebo-controlled, parallel intervention study design. The current study was designed to explore the tolerability of 8 weeks of supplementation with adzuki bean extract capsules in subjects with moderate-to-high LDL-C levels (120–179 mg/dl) as well as to examine the changes in the basic measures of body mass/composition and metabolic parameters. Subjects with moderate-to-high LDL-C levels were selected for this study due to their increased risk for developing heart diseases.

The participants in this study received a daily total of 427.0 mg adzuki bean extract containing 253.2 mg polyphenols, specifically anthocyanidins and proanthocyanidins.

The participants in the group receiving adzuki bean extract supplements showed significant increase in their HDL-C concentrations at week 4 as opposed to those in the placebo group, who showed a gradual decrease in their HDL-C levels over time. Subgroup analysis of the participants with low TG levels revealed that the intake of adzuki bean extract allowed patients to maintain their TG concentrations during the study period, while analysis of those in the placebo group showed a gradual increase in their TG concentrations over the same time period. To the best of our knowledge, this is the first human placebo-controlled trial to demonstrate both the safety of adzuki bean extract and its favorable effect on lipid metabolism, specifically on the maintenance of HDL-C levels. HDL-C has a direct effect on numerous cell types that influence the cardiovascular and metabolic health. It is known to decrease white adipose tissue mass, increase energy expenditure, and promote the production of adipose-derived cytokine adiponectin [14]. Despite the differences observed in the current study in terms of HDL-C levels, there were no significant differences in adiponectin levels following supplementation with adzuki bean extract supplements.

The results of this study partially support the findings of our previous animal experiments that indicated an association between polyphenol intake and a favorable lipid profile [10]. These effects are likely a result of the two main polyphenols in the adzuki bean extract: anthocyanins and proanthocyanidins. Anthocyanins, which are the main component of adzuki bean polyphenols, have been reported in previous studies to have beneficial effects on inflammatory disorders, lipid profile, platelet activation, and obesity-related disorders in human studies, although the literature remains controversial [15]. Proanthocyanidins (otherwise known as condensed tannins) are oligomers or polymers of flavan-3-ols (e.g., catechin and epicatechin) and have been linked to health benefits, such as hypolipidemic, anti-hypertensive, anti-inflammatory, and antioxidant effects [16].

Recently, Zhang et al. demonstrated that the plasma HDL-C levels significantly increased among hypercholesterolemic individuals after supplementation with purified anthocyanins (320 mg/day) for 24 weeks, accompanied by significant reductions in the LDL-C levels [17]. Furthermore, a 12-week study in which 120 dyslipidemic subjects were administered berry-derived anthocyanin supplements (320 mg/day) reported an increase in their HDL-C levels and a decrease in their LDL-C levels, with no changes in the TC or TG levels [18].

In addition, the administration of low molecular weight procyanidin rich extract from the French maritime pine bark (150 mg/day) for 5 weeks to stage-1-hypertension subjects was shown to significantly elevate the serum HDL-C levels with no changes in

the TC, LDL-C, or TG levels [19]. In contrast, another study reported that the TC, TG, HDL-C, and LDL-C levels were unaffected by the consumption of 150 mg/day of polyphenolic extract from grapes rich in procyanidins for 4 weeks among 24 healthy men considered to be heavy smokers [20]. Likewise, another study using a polyphenol-rich elderberry extract (500 mg/day) showed no significant changes in the markers of liver and kidney functions or in CVD risk, including the plasma HDL-C, LDL-C, and TG concentration, in healthy postmenopausal women after 12 weeks of supplementation [21]. There are several possible reasons for the contradictory results observed in these types of studies. One explanation is that the polyphenol family encompasses very diverse compounds with different levels of bioavailability, meaning that the results obtained for one polyphenol cannot be generalized to others. Contradictions may also arise due to the differences in the doses of polyphenols and foods used in each study, the duration of each intervention and the particular subjects used in each study.

Recent studies have shown that serum HDL-C concentrations are inversely related to the risk of developing cardiovascular disease. Therefore, maintaining and/or raising HDL-C concentration has been a viable and promising way to reduce the risk of cardiovascular mortality. However, increasing a patient's HDL-C levels has proven to be difficult using the existing medications, including niacin, due to the presence of various side-effects. Naturally derived functional ingredients have the potential to boost the HDL-C levels, and they are generally considered to be less toxic than synthetic drugs. Cross-over and randomized controlled trials in metabolic syndrome subjects with single phenolic compounds or specific foods, beverages, or extracts have indicated that polyphenol consumption can improve the lipid profiles by significantly reducing the LDL-C levels; however, only a few studies have shown significant effects on HDL-C concentrations [22]. Moreover, there is a general consensus that it is difficult to modulate the HDL-C levels using food ingredients. Therefore, the current findings could be important in that they present one of the few food ingredients available that modulate HDL-C levels in subjects with moderate-to-high LDL-C levels.

The results of our study combined with previous work have shown that oral administration of polyphenol containing adzuki bean extract is safe and well-tolerated in human subjects. A study by Nutr et al. indicated that adzuki bean-derived polyphenols can be tolerated by humans in large doses, even as high as 1960 mg/day, without any apparent toxicity [12], which is consistent with our findings that no adverse effects occurred in any of the participants who took adzuki bean extract. Specifically, we did not observe any adverse effects in terms of glucose metabolism, peripheral blood

analysis and liver function, or kidney function, which confirmed the safety of adzuki bean polyphenols. The study results also revealed a relationship between adzuki polyphenol supplementation and capability of maintaining HDL-C levels, indicating that adzuki bean extract may be a potential phytotherapeutic treatment for modulating lipid homeostasis without any negative side-effects. However, the interpretation of the results should be made cautiously, as the current study contained a small sample size and was conducted over a short duration.

Certain limitations of our study include a small sample size, consisting primarily of women, and the absence of a dose-response design. More research is needed to determine factors such as long-term safety, effective dosing range, optimal timing of administration, effects of dietary fat content, potential drug interactions, impact when co-administered with lipid-altering medications, and generalizability to other patient groups.

In conclusion, our study findings show that adzuki bean extract administered once a day can result in increased ΔHDL-C concentration. This finding suggests that adzuki bean polyphenols are a novel food ingredient capable of maintaining lipid homeostasis by modulating the HDL-C levels, without causing any adverse effects. This short-term study represents the first step in establishing the practicality, safety, and HDL-C-maintaining effects of adzuki bean extract in subjects with moderate-to-high LDL-C levels.

Authors contribution

K. Satoh, J. Nishihara, K. Ohba and M. Fukushima conceived and designed the research. K. Satoh and K. Sasaki performed experiments. R.Nagata and K.Satoh performed statistical analysis. T. Kitano-Okada, K. Han and N. Mikami drafted manuscript. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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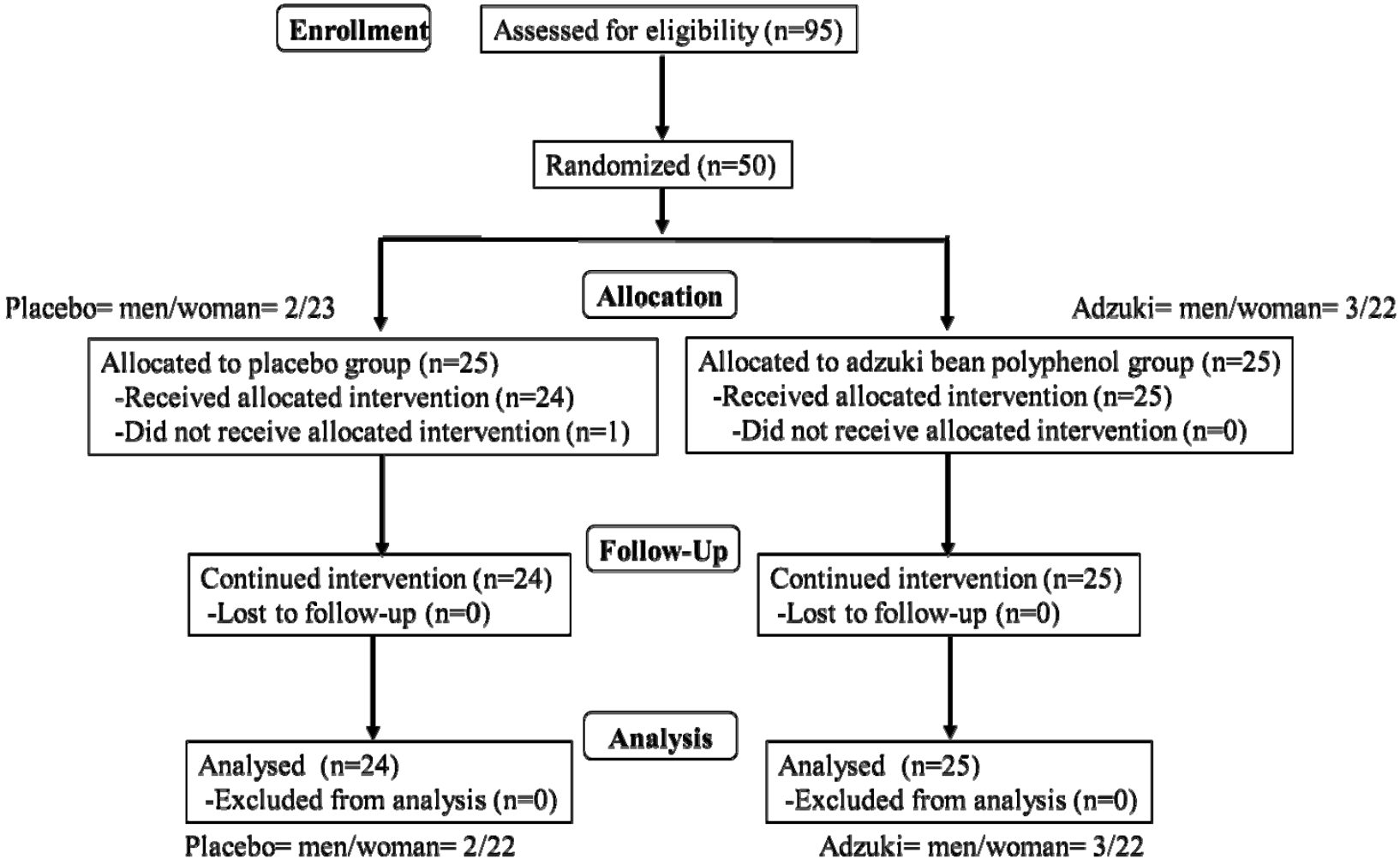

Figure 1 – CONSORT diagram to indicate participant flow and retention for the 8-week double-blind, placebo-controlled trial of adzuki bean extract supplementation (n = number of participants)

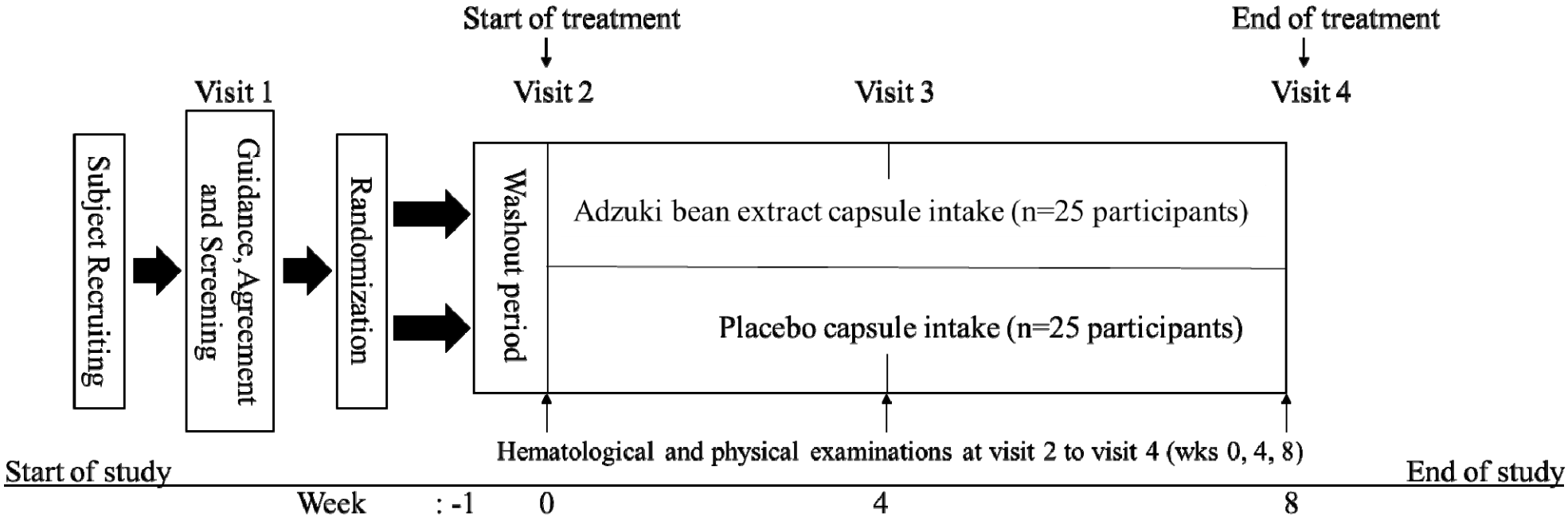

Fig.2 – Schedule of events for this study

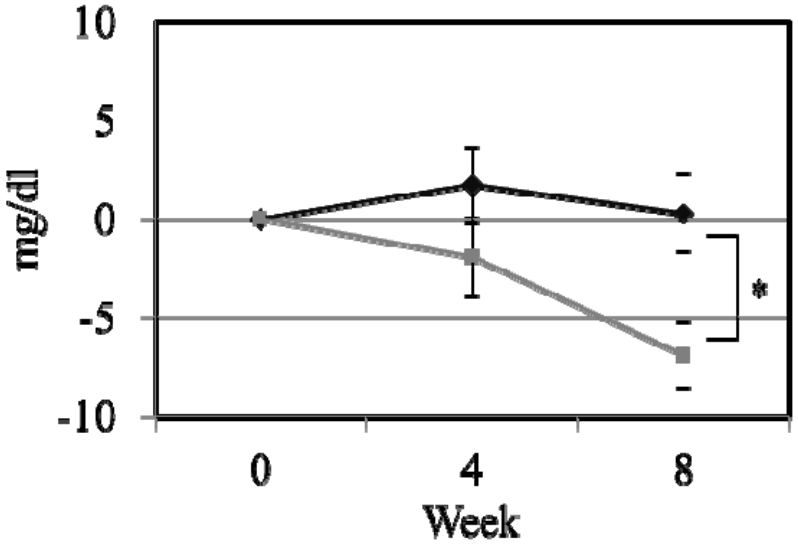

Fig. 3 – Changes in plasma HDL-C in subjects with low TG levels (<72mg/dl) compared to baseline. Gray bar: placebo group (n = 12), black bar: adzuki bean extract group (n = 10). Values are shown as mean ± standard deviation. *$p < 0.05$

Table 1 - Nutrient composition of each adzuki bean extract capsule (338 mg) and the placebo capsule (333 mg) used in this study. Participants received six capsules per day.

	Adzuki bean extract capsule	Placebo capsule
Calories (kcal)	1.03	1.29
Water (g)	0.01	0.01
Proteins (g)	0.06	0.05
Lipids (g)	0.01	0.01
Carbohydrates (g)	0.17	0.25
Ash (g)	0.01	0.01
Sodium (mg)	0.16	0.49
Adzuki bean extract (mg)	71.2	0.0
» Polyphenol (mg)	42.2	0.0

Analysis methods: Calories were caluculated by formula: proteins (g/100g) × 4 kcal/g + lipids (g/100g) + 9 kcal/g + carbohydrates (g/100g) × 4 kcal/g + fibre (g/100g) × 2 kcal/g; Water, atomospheric heat dring method; protein Kjeldahl method; lipid, acid digestion; carbohydrates were caluculated by formula: 100-(water + protein + lipid + fiber): ash, direct ashing method; sodium, atomic absorption analysis method. Polyphenol content was determined by Folin–Ciocalteu's method using (+)-catechin as a standard.

Table 2 – Baseline demographic, physical and biochemical measures for participants in the adzuki bean extract group and the placebo group.

	Adzuki bean extract capsule group	Placebo capsule group	*p* value
Subjects, n	25	24	-
Males, n (%)	3 (12.0%)	2 (8.3%)	1.000
Age, years	54.9 ± 7.5	52.3 ± 7.0	0.225
Physical Measures			
BW, kg	54.31 ± 7.00	54.09 ± 10.37	0.932
BMI, kg/m^2	21.62 ± 2.51	21.49 ± 3.15	0.871
BFP, %	28.73 ± 5.74	28.08 ± 6.23	0.706
Biochemical Measures			
TG, mg/dl	82.00 ± 34.06	73.21 ± 26.03	0.317
TC, mg/dl	235.88 ± 20.36	241.13 ± 24.12	0.414
HDL-C, mg/dl	71.20 ± 12.04	77.37 ± 17.07	0.149
LDL-C, mg/dl	155.0 ± 11.2	157.5±12.1	0.465
MDA-LDL	101.36 ± 16.18	96.33 ± 21.94	0.365
TBARS	4.03 ± 0.89	3.95 ± 0.76	0.727
Glucose, mg/dl	88.52 ± 6.20	86.96 ± 7.30	0.423
HbA1c, %	5.44 ± 0.30	5.36 ± 0.24	0.299
Adiponectin, µg/ml	12.51 ± 6.17	12.85 ± 5.65	0.841

BW, body weight; BMI, body mass index; BFP, body fat percentage; TG, triacylglycerol; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MDA-LDL, malonyldialdehyde low density lipoprotein; TBARS, thiobarbituric acid reactive substance; HbA1c, Hemoglobin A1c. Values shown are mean ±standard deviation. Changes in subject values were analysed using Student's t-test to compared the mean of adzuki polyphenol group and the placebo group at each test point.

Table 3 – Biochemical measures for participants in the adzuki bean extract group and the placebo group

		Changes in value at week 4	Changes in value at week 8	Two-way ANOVA (p)		
				Diet	Period	Interaction
Biochemical Measures						
LDL-C, mg/dl	Placebo	7.50 ± 17.09	-1.67 ± 15.69	0.486	0.078	0.388
	Adzuki polyphenol	6.92 ± 14.86	3.76 ± 20.33			
TG, mg/dl	Placebo	2.54 ± 19.75	5.50 ± 35.00	0.315	0.884	0.695
	Adzuki polyphenol	-0.84 ± 27.13	-2.20 ± 24.55			
TC, mg/dl	Placebo	11.42 ± 18.81	-1.08 ± 18.48	0.235	0.007	0.624
	Adzuki polyphenol	14.12 ± 16.75	5.40 ± 21.61			
HDL-C, mg/dl	Placebo	-0.08 ± 6.03	-3.17 ± 7.97	0.009	0.022	0.910
	Adzuki polyphenol	3.76 ± 7.79	0.36 ± 5.36			
Glucose, mg/dl	Placebo	-1.13 ± 4.32	-0.67 ± 4.85	0.434	0.193	0.418
	Adzuki polyphenol	-2.60 ± 4.82	-0.64 ± 4.23			
HbA1c, %	Placebo	-0.05 ± 0.08	-0.09 ± 0.11	0.568	0.176	0.765
	Adzuki polyphenol	-0.05 ± 0.11	-0.07 ± 0.14			