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Basic nutritional investigation

Lowering serum cholesterol level by feeding a 40% ethanol-eluted fraction from HP-20 resin treated with hot water extract of adzuki beans (*Vigna angularis*) to rats fed a high-fat cholesterol diet

Tomohiro Itoh, Ph.D.[a,*], and Yukio Furuichi, Ph.D.[b]

[a] *Department of Bio-active Substances Research, Gifu International institute of Biotechnology, Gifu, Japan*
[b] *Faculty of Home Economics, The Nagoya Women's University, Nagoya, Japan*

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Abstract

Objective: Hot water extract of adzuki beans (*Vigna angularis*) was subjected to HP-20 resin chromatography. The fraction eluted from the column using 40% ethanol (EtEx.40) was investigated by its effect on serum lipids in rats fed a high-fat cholesterol and/or cholesterol-free high-fat diet.
Methods: The rats were divided into 4 groups. Groups 1 and 2 were fed a high-fat cholesterol diet with or without 3.5% EtEx.40 for 2 wk. Group 3s and 4 were fed a high-fat cholesterol-free diet with or without 3.5% EtEx.40 for 2 wk.
Results: In the high-fat-cholesterol diet groups, there was no significant difference in food intake in the experimental diet group when compared with the control group. Serum total cholesterol level was significantly decreased in the rats fed the EtEx.40 diet, but there was no difference in fecal excretion of cholesterol and bile acid between the two dietary groups. Conversely, in the high-fat cholesterol-free diet groups, ingestion of EtEx.40 reduced serum triacylglycerol concentration.
Conclusion: Ingestion of EtEx.40 suppressed serum cholesterol level in rats fed the high-fat cholesterol and serum triacylglycerol level in rats fed the high-fat cholesterol-free diet. These mechanisms did not become clear in this experiment.

Keywords: Hot water extract of adzuki beans; Lipid metabolism; High-fat plus high-cholesterol diet; Serum cholesterol; Rats

Introduction

Recently metabolic syndrome, which is induced by several risk factors such as hyperlipidemia, high blood pressure, obesity, and diabetes, has increased worldwide. Therefore, it is important to decrease dyslipidemia and/or disturbances of carbohydrate metabolism that are the cause of these diseases.

Adzuki bean (*Vigna angularis*) is an ingredient known well in East Asia. In traditional Chinese medicine, adzuki beans familiar to *chi xiao dou* have been used habitually for many purposes including diuretics and antidotes and for symptoms of dropsy and beriberi. In Japan, adzuki beans are used mainly for the production of confectioneries such as *youkan*, *manjuu*, *amanatto*, etc. In the case of food processing, heat treatment of adzuki beans results in a large amount of hot water extracts (HWEs), which are usually discarded. Amarowicz et al. [1] reported the composition of phenolic compounds of adzuki beans. It is also well known that these polyphenols are extracted in the boiling process. To use this unused extract, we examined the effect of HWEs on several risk factors. We previously reported that the 40% ethanol-eluted fraction from HP-20 resin treated with HWE of adzuki (EtEx.40) is effective as antitumor treatment [2–5], antimetastatic treatment [6], suppression of postprandial blood glucose level [7], and melanogenesis [8]. Moreover, it has been reported that adzuki-resistant starch decreases serum cholesterol level [9–11]. The present study examined lipid metabolism improvement effects of EtEx.40 in rats fed a high-fat cholesterol and/or cholesterol-free high-fat diet.

* Corresponding author. Tel.: +81-583-71-4646; fax: +81-583-71-4412.
E-mail address: titoh@giib.or.jp (T. Itoh).

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Materials and methods

Preparation of test materials

Adzuki beans (2.5 kg) harvested in Tokachi, Hokkaido, Japan, were boiled and HWEs were obtained (15 L). We concentrated the HWE to 100 times by a plate heat exchanger (Izumi Food Machinery Co. Ltd., Hyogo, Japan). Thirty milliliters of concentrated HWEs were subjected to open-column chromatography on a Diaion HP-20 (Mitsubishi Chemical Co. ltd., Tokyo, Japan, column size: 5 $\varphi \times$ 300 mm, resin wet volume 400 g) and eluted stepwise with 5 L of distilled water and 40% ethanol. The respective fractions were evaporated to dryness.

Animal and diets

Five-week-old male Sprague-Dawley rats (Japan SLC, Ltd., Hamamatsu, Japan), weighing about 40–60 g, were housed individually in stainless-steal cages with screen bottoms. The animals were kept under controlled conditions with a 12-h light/12-h dark cycle (lighting from 0800 to 2000 h) at 22 ± 2°C. All rats were fed commercial MF pellets (Japan SLC, Ltd.) and water ad libitum for 1 wk to accustom them to the surroundings. After 1 wk, they were randomly divided into four groups of five animals each and were given the experiment diets ad libitum for 2 wk. The composition of the diets is listed in Table 1. In experiment 1, group 1 was fed a high-fat diet containing 0.5% cholesterol and 0.125% sodium cholate (HFC). Group 2 was fed a group 1 diet containing 3.5% EtEx.40 (HFC-E). In experiment 2, group 3 was fed a cholesterol-free high-fat diet (HF) and group 4 a group 3 diet containing 3.5% EtEx.40 (HF-E). EtEx.40 was added instead of sucrose.

Table 1
Composition of experimental diets

Component	High fat + cholesterol diets (weight%)		Cholesterol diets (weight%)	
	Control	EtEx.40	Control	EtEx.40
Casein*	20.0	20.0	20.0	20.0
Lard†	20.0	20.0	20.0	20.0
Mineral mixture (AIN-93)‡	3.5	3.5	3.5	3.5
Vitamin mixture (AIN-93)‡	1.0	1.0	1.0	1.0
α-Methionine‡	0.3	0.3	0.3	0.3
Choline bitartrate‡	0.2	0.2	0.2	0.2
Cholesterol‡	0.5	0.5	—	—
Sodium cholate‡	0.125	0.125	—	—
Corn starch§	15.0	15.0	15.0	15.0
Cellulose‖	5.0	5.0	5.0	5.0
EtEx.40	—	3.5	—	3.5
Sucrose¶	34.375	30.875	35.0	31.5

EtEx.40, 40% ethanol-eluted fraction from HP-20 resin treated with hot water extract of adzuki beans (*Vigna angularis*)

* Oriental Yeast Co., Tokyo, Japan.

† Yoneyama Reagent Industries Co., Osaka, Japan.

‡ Wako Pure Chemical Industries, Ltd., Osaka, Japan.

§ San-eisucrochemical Co. Ltd., Aichi, Japan.

‖ Toyo Roshi Kaisha Ltd., Tokyo, Japan.

¶ Abe Seito, Mie, Japan.

On the final day of the experiments, the mice were sacrificed by ethyl ether anesthesia, and the blood was immediately collected using a polyethylene tube with no heparin to measure total cholesterol (TC), triacylglycerol (TG), phospholipid (PL), non-esterified fatty acid (NEFA), and high-density lipoprotein cholesterol (HDL-C) levels. The blood was centrifuged at 1700 × *g* for 15 min to obtain serum. The liver was excised immediately. Feces were collected for the last 5 d of feeding, freeze-dried, and powdered. These samples were kept at −80°C until analysis. This study was approved by the Mie University animal use committee, and the animals were maintained according to the guidelines of Mie University for the care of laboratory animals.

Analytical procedures

The profiles of serum TC, TG, PL, NEFA, HDL-C and urinary glucose were measured with clinical analysis kits (Cholesterol CII, Triglyceride G, Phospholipid B-, and Nonesterified Fatty Acid C Tests, Wako Pure Chemical Industries, Ltd., Osaka, Japan; HDL-C2 Daiichi, Daiichi Pure Chemicals Co., Tokyo, Japan) and lipid contents in the liver, extracted by the method of Folch et al. [12], were measured in the same way. The cholesterol and bile acid contents of the feces were measured by the methods of Zak [13] and Sheltawy and Losowsky [14], respectively.

Statistical analysis

All data were expressed as mean ± standard error (SE), and subsequent inspection of the statistical significance of the difference of means was evaluated by Student's *t* test between two groups at a significance level of $P < 0.05$.

Results

The parameters of growth, serum lipid profiles (TC, TG, PL, NEFA, and HDL-C), hepatic lipid contents (liver TC, TG, and PL), and fecal neutral sterol and bile acid levels in rats fed the HFC diet are listed in Table 2. There were no differences in the body weights and food consumption of the HFC and HFC-E groups during the feeding period. For serum lipid profiles, TC was significantly lower in the HFC-E group than in the HFC group ($P < 0.01$). In addition, HDL-C/TC was significantly higher in the HFC-E group than in the HFC group ($P < 0.01$). In cntrast, hepatic lipid contents (TL, TC, TG, and PL) did not differ between the HFC and HFC-E groups. The amount of feces, which were collected on the last 5 d of feeding, had increased significantly in the HFC-E group compared with the HFC