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Anti-obesity effects of highly polymeric proanthocyanidins from seed shells of Japanese horse chestnut (*Aesculus turbinata* Blume)

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ABSTRACT

Recently, we have shown that seed shells contain a large amount of highly polymeric proanthocyanidins having a series of heteropolyflavan-3-ols with doubly linked A-type linkages as well as single B-type bonds without gallic acid esterified to them. Here, we attempted to evaluate in vivo anti-obesity effects of the polymerized proanthocyanidins in mice. An oral starch or glucose tolerance test in mice revealed that the isolated two fractions of highly polymerized proanthocyanidins with the different degree of polymerization suppressed effectively the elevation of blood glucose from oral starch, but not from oral glucose, suggesting the preferential inhibition of the digestive enzymes of carbohydrates. Moreover, in vivo anti-obesity effects of the total fraction containing the proanthocyanidins as a drink were investigated in mice fed a high-fat diet. Their anti-obesity effects became more evident after 9 weeks as determined by the attenuation of the elevation in body weight, the mass of peritoneal adipose tissues, and the plasma levels of total cholesterol and leptin. Furthermore, the increased size of hepatocytes and the generation of steatosis with micro- and macrovesicles in liver were normalized by the dietary supplementation of the total proanthocyanidin fraction. The findings suggest the usefulness of highly polymeric proanthocyanidins from seed shells in the application to food as a dietary supplement with anti-obesity effects in vivo through the inhibition of digestive enzymes of carbohydrates and fats.

1. Introduction

Obesity is a well-known risk factor for a variety of life-style diseases, such as hyperlipidemia, hypertension, and diabetes (Kopelman, 2000). For the prevention of these life-style diseases, attempts are being made to develop nutraceuticals from different bioresources as well as the recommendation of the regular excise to promote energy expenditure. To suppress the abrupt rise in the blood glucose levels leading to the disorder of the pancreas and the generation of diabetes, extensive studies have been done to find effective materials to inhibit digestive enzymes including α-amylase and α-glycosidases. Moreover, the inhibition of pancreatic lipase by specific food factors is more promising for the prevention of obesity through the attenuation of the digestion and absorption of triacylglycerols (Hill et al., 1999).

The seeds of European horse chestnut (*Aesculus hippocastanum* L.), alternatively called marronnier, have been shown to contain a large amount of mixed triterpenoidal saponins called escins that have been used as herbal medicines with anti-inflammatory and anti-edematous activities (Yoshikawa et al., 1996). On the other hand, the seeds of the Japanese horse chestnut (*Aesculus turbinata* Blume) have been utilized as an emergency provision since ancient times. Nowadays, for the preparation of the edible seeds used as ingredients of traditional foods like rice balls and rice cake in Japan, the natural seeds are usually treated with wood ashes to remove bitter substances. Recently, our group has identified novel saponins including four types of deacetylescins and two types of desacylescins from edible seeds of the Japanese horse chestnut. These novel compounds were formed by the chemical transformation from four types of escins from the natural seeds during the food processing with wood ashes (Kimura et al., 2006). We have also reported that these saponins from both edible seeds and natural seeds have several biological activities to suppress the elevation of blood glucose levels in oral glucose tolerance test in mice (Kimura et al., 2006). Furthermore,

Abbreviations: TPA, total fraction of proanthocyanidins from seed shells of the Japanese horse chestnut; HF, high-fat diet; HDL, high-density lipoprotein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HE, hematoxylin and eosin; S.E.M., standard error of the mean.

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we have revealed that the novel saponins from edible seeds of the Japanese horse chestnut are effective in the suppression of the digestion and absorption of fats by inhibiting pancreatic lipase in vitro and they have in vivo anti-obesity effects in mice fed a high-fat diet (Kimura, Ogawa, Jisaka, Katsube, & Yokota, 2008).

Alternatively, we have been studying the biological activities of the ingredients from seed shells of the Japanese horse chestnut, which have been waste products for the preparation of edible seeds. Recently, we have reported that seed shells contain higher levels of polyphenolic antioxidants (Ogawa et al., 2008), as compared with typical foods such as cranberry, blueberry and almonds (Prior & Gu, 2005). The predominant polyphenolic compounds are highly polymeric proanthocyanidins having doubly linked A-type interflavan linkages as well as singly linked B-type bonds without gallic acid esterified to them (Fig. 1) (Ogawa et al., 2008). These proanthocyanins have been shown to serve as dose-dependent inhibitors in vitro for carbohydrate-digesting enzymes including pancreatic α-amylase and intestinal α-glycosidases (Ogawa et al., 2009) as well as pancreatic lipase (Kimura et al., 2009). Moreover, we have confirmed using oral fat tolerance test in mice that the highly polymerized proanthocyanidins can effectively suppress fat digestion (Kimura et al., 2009). In the present study, we attempted to conduct in vivo animal studies to determine the efficacy of highly polymeric proanthocyanidins from the Japanese horse chestnut to suppress the blood glucose levels using the oral starch tolerance test and their long-term anti-obesity effects in obese mice fed a high-fat diet with the drinks with them for several months.

2. Materials and methods

2.1. Materials

The seeds of the Japanese horse chestnut (*Aesculus turbinata* Blume) were collected from the forests of northern Hygo Prefecture in Japan and identified as described earlier (Kimura et al., 2006). Folin–Ciocalteu reagent was obtained from Sigma (St. Louis, MO). Glutestace R Kit was purchased from Sanwa Kagaku Kenkyusho (Nagoya, Japan). Diaion HP-20 and Chromatorex ODS 1024 T for column chromatography were supplied by Nippon Rensui (Tokyo, Japan) and Fuji Silysia (Kasugai, Japan), respectively. Sephadex LH-20 for column chromatography was purchased from GE Healthcare (Buckinghamshire, U.K.). All other reagents and chemicals were of analytical grade and purchased from Wako (Osaka, Japan), unless otherwise stated. Female ICR mice and standard MF chow were supplied by Shimizu Laboratory Supplies (Kyoto, Japan). HDF-60 pellets as a high-fat diet were purchased from Oriental Yeast (Tokyo, Japan).

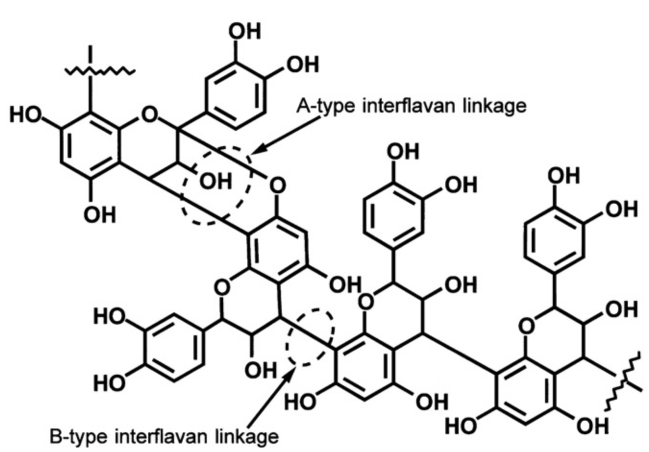

Fig. 1. Chemical structure of proanthocyanidins from seed shells of the Japanese horse chestnut.

2.2. Animals

Female ICR mice were individually housed in a polycarbonate cage at 23 °C on a 12 h/12 h light/dark cycle, and had free access to conventional MF chow and water *ad libitum* for one week before the experiments as described below. The present study was approved by the Institutional Animal Care and Use Committee of Shimane University. All applicable institutional and governmental regulations concerning the ethical use of animals were followed during our research.

2.3. Fractionation and isolation of proanthocyanidins

Proanthocyanidins were extracted, fractionated, and isolated according to our procedures as reported earlier (Ogawa et al., 2008). Briefly, seed shells of the Japanese horse chestnut (10 g as fresh weight) were grounded into powder and refluxed by boiling for 2 h in 200 mL of water. The extracts were fractionated by column chromatography on Diaion HP and subsequent Chromatorex ODS 1024T to recover the total fraction of proanthocyanidins from seed shells of the Japanese horse chestnut (TPA). The TPA fraction (100 mg) was applied to column chromatography on Sephadex LH-20 (100 mm × 10 mm i.d.) for the stepwise elution with 40 mL of ethanol, 40 mL of methanol, and 40 mL of 70% acetone to yield fractions of F1, F2, and F3, respectively. The eluates were collected every 2 mL and subjected to the determination of the amount of total polyphenols using (−)-epicatechin as a standard by the Folin–Ciocalteu method (Julkunen-Tiitto, 1985). The structural information regarding the isolated proanthocyanidins was confirmed by analyzing the isolated compounds using high-performance liquid chromatography, matrix-assisted laser-desorption ionization time-of-flight mass spectrometry and liquid chromatography electrospray-ionization mass spectrometry as described before (Ogawa et al., 2008).

2.4. Oral tolerance test of starch or glucose in mice

Female 6-week ICR mice were had free access to conventional MF chow and water for one week. The animals were randomly divided into different experimental groups (7 mice per group). After the mice were fasted for 16 h, the blood was withdrawn from the tail vein and subjected to the assay of blood level of glucose. The fractions, F2 and F3, at different doses were individually suspended in 100 μL of physiological solution to give 500, 1000, and 1500 mg/kg mouse with F2, and 250, 500, and 750 mg/kg mouse with F3. The resulting suspension was administered orally into the stomach of mice 10 min before a single oral injection of 250 μL of starch (2 g/kg mouse). In case of glucose tolerance test in mice, the fractions, F2 and F3, were individually suspended in 100 μL of physiological solution and used at doses of 1500 and 750 mg/kg mouse, respectively. After 10 min of the oral administration of the suspension of F2 or F3, a single oral injection of 250 μL of physiological solution containing glucose was performed to give 2 g glucose/kg mouse. Thereafter, the blood was withdrawn from the tail vein at 0.5, 1, and 2 h, and analyzed for the blood glucose levels using Glutestace R according to the manufacturer's instructions.

2.5. Anti-obesity evaluation of highly polymeric proanthocyanidins in mice fed a high-fat diet

Female 3-week-old ICR mice had free access to standard MF chow and water for 7 days for their adaptation. The experimental mice were divided into four groups after adaptation and fed the experimental diets as follows (5 mice per group). The high-fat diet (HF) group was fed the HDF-60 pellets (Oriental Yeast) containing 33% (w/w) lard oil, 2% soybean oil, 25.6% milk casein, 16% α-corn starch, 5.5% sucrose, 6.6% cellulose powder, 6% maltodextrin, 0.4% L-cysteine, 3.5% AIN-