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Bioavailability of polyphenols from peanut skin extract associated with plasma lipid lowering function

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ABSTRACT

Peanut skin is a rich source of polyphenols including procyanidins and is shown to have hypolipidemic properties. This study investigated the bioavailability of peanut skin polyphenols using a rat model. First, the bioavailability of peanut skin polyphenols in rat plasma was evaluated. Our results showed procyanidin A2 levels in plasma peaked within 30 min of ingestion. The results of a second study show that peanut skin extract supplemented in addition to oil gavage resulted in significant decrease in plasma triglyceride and VLDL within 5 h. In the third study, rats were given a Western type diet for 5 weeks with peanut skin extract at a dose of 150 and 300 mg/kg body weight. The main effects observed were lowering of total blood lipid and reduction of the plasma fatty acids profile. Our results suggest that procyanidin A may impart a key role of hypolipidemic effect seen in peanut skin polyphenols.

1. Introduction

Dyslipidemias are abnormal amounts of lipid and/or lipoprotein in the blood (Peterson & McBride, 2012). It is one of the major risk factors for cardiovascular diseases in diabetes mellitus, obese and overweight individuals (Mooradian, Haas, Wehmeier, & Wong, 2008; Weiss et al., 2004). It has been reported that 88% of U.S. adults have abnormalities of all three standard lipid parameters (LDL-C, HDL-C, TG) and are in need of therapy with lifestyle modification in an effort to relieve insulin resistance (Toth, Potter, & Ming, 2012). Similarly, approximately one-third of American children are overweight or obese and rates of pediatric dyslipedimia in the United States are rising (Kennedy, Jellerson, Snow, & Zacchetti, 2013; Weiss et al., 2004).

In recent times, research has been focused on understanding the influence of diet on health and well-being (Serra et al., 2010). Foods enhanced with bioactive components, such as polyphenols, are attracting growing interest (Franck, 2006; Grassi et al., 2008; Schroeter et al., 2010; Shoji et al., 2006). Natural polyphenols range from simple molecules such as phenolic acids to highly polymerized compounds such as tannins and proanthocyanidins (Yang et al., 2010). Proanthocyanidins comprises of the most abundant subclasses of phenolic compounds in our diet (Gu et al., 2004). The most common subgroup of proanthocyanidins are the procyanidins, which are oligomers of (epi)catechin units and their galloyl derivatives (Appeldoorn, Vincken, Gruppen, & Hollman, 2009). Procyanidins consists of two sub-types, namely A-type and B-type. Procyanidin B-type is common in most food sources, while A-type is additionally present in cranberries, peanuts, plums and spices like cinnamon (Gu et al., 2004).

We have recently demonstrated that peanut skin extract prevents hepatic steatosis and showed hypolipidemic effect in rats on a Western type diet (Bansode, Randolph, Hurley, & Ahmedna, 2012). In the present study the bioavailability of peanut skin procyanidins and its derivatives in rat plasma is evaluated. This study also investigated the effect of peanut skin extract on the lipid and free fatty acid profile of rats on a Western type diet.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol, chloroform, isoflurane, tetrahydrofuran, Folin–Ciocalteu reagent, gallic acid, sodium chloride, sodium hydroxide, potassium hydroxide, acetonitrile, ammonium fluoride,

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ammonium actetate,formic acid, β-glucoronidase, catechin, epicatechin were purchased from Sigma–Aldrich (St. Louis, MO). *trans*-Resveratrol, was purchased from Cayman Chemicals (Ann Arbor, MI). Procyanidin A2 and Procyanidin B1 were from Indofine Chemical Company (Hillsborough, NJ). Isooctane was purchased from Thermo Fisher Scientific Inc., (Waltham, MA). Heptadecanoic acid and fatty acid methyl ester was from Nu-Check Prep, Inc (Elysian, MN). All reagents were greater than or equal to 99% purity unless stated otherwise.

2.2. Extraction of polyphenols from peanut skin

Polyphenol-rich extract was prepared from peanut skin as described previously by Bansode et al. (2012). Mildly roasted peanut skins were generously gifted by American Peanut Shellers Association (Albany, GA). Peanut skins were suspended in distilled water and boiled for 30 min as described by Shimizu-Ibuka et al. (2009). The residue was decanted and freeze dried. The lyophilized water-soluble fraction was further treated with 80% ethanol at 5 °C for 24 h and subsequently centrifuged at 10,000 rpm for 10 min. The supernatant was separated and concentrated by using a rotary evaporator *in vacuo* at 40 °C. The above-mentioned extraction and purification process were completed under dim light to minimize light-induced degradation/oxidation of phenolics, which are generally light sensitive. The final concentrate of peanut skin extract (PSE) was stored at −30 °C until further use.

2.3. Determination of total phelonics assay

Total phenolics in the peanut skin extract were evaluated by the Folin–Ciocalteu method as described by Yu, Ahmedna, and Goktepe (2005). Gallic acid was used as the standard and the phenolic concentrations in the PSE samples were calculated as gallic acid equivalent (mg GAE ml^{-1}). Samples were analyzed in triplicate.

2.4. Animal study and experimental design

2.4.1. Polyphenols bioavailability in rats receiving peanut skin extract gavage

Male Wistar rats, approximately 6 weeks old, were used in this study. Each animal was individually housed and acclimated in laboratory conditions (18–23 °C, humidity 55–60%, 12 h light/dark cycles) for at least 1 week before the study. All rats were fed with a standard Purina 5001 chow diet (Purina Mills, Inc.) and given free access to water. After one week of acclimation, the rats were randomly divided into two groups ($n = 6$ per group). Food was withheld from the rats for 15 h prior to the experiments. Peanut skin polyphenols were dissolved in water at the concentration of 25 mg/ml and administered intragastrically by direct stomach intubation to six rats at a dose of 250 mg/kg of body weight. In a control group, six rats were administered 2 ml of saline solution. Blood (70–100 μl) was withdrawn from the intravenous catheter at 0, 10, 20, 30, 45, 60, 90, 150, 240, 300 and 360 min post administration.

2.4.2. Gastrointestinal absorption of lipids in rats receiving vegetable oil gavage with and without peanut skin extract

The effect of peanut skin polyphenols on intestinal absorption of lipids after an acute lipid load was assessed in rats. Male wistar rats 8 weeks of age were individually housed at 22 °C with 12 h light/dark cycle and were fed with standard chow diet *ad libitum*. The animals were given a standard Purina chow diet and water *ad libitum* for a week. Food was withheld from the rats for 15 h prior to the experiment. On the experimental day, the rats were fed an oral gavage of peanut-skin extract (0, 100, 200, 300 and 400 mg/kg body weight) in 1 ml vegetable oil emulsion containing 0.1% lecithin as emulsifier or an oral gavage with vehicle (saline). The used procyanidin dose is one-fifth of the no-observed-adverse-effect level (NOAEL) described for grape seed procyanidin extract (GSPE), tested in male rats (Yamakoshi, Saito, Kataoka, & Kikuchi, 2002). Five hours after treatment, blood samples (200 μl) were collected by retro-orbital bleeding into heparinized tubes. Retro-orbital bleeding was conducted while the animals were under the influence of deep anesthesia attained using Isoflurane. Plasma was obtained by centrifugation and analyzed for lipids, glucose and liver enzymes (ALT and AST).

2.4.3. Evaluation of blood lipids in rats fed with a Western-type diet

Male wistar rats were randomly divided into three groups ($n = 6$ per group). The rat groups were fed with one of the following diets: AIN93G based Western-type diet (WD) containing 30% of fat from lard, 30% from butterfat, 30% from Crisco (hydrogenated vegetable oil), and for EFA, 7% from soybean oil and 3% from corn oil. Cholesterol content was 0.15% and the approximate energy from fat was 39.9%, and 44.0% of energy from carbohydrate. WD with a gavage of peanut skin extract (PSE) at a low-dose of 150 mg/kg body weight/day (WD + PSE150) and WD with a high-dose of 300 mg/kg body weight/day (WD + PSE300). Animals on WD received gavage of distilled water. Animals on WD + PSE150 and WD + PSE300 received gavage of peanut skin extract at the mentioned dosages five times a week for 5 weeks. Body weights of the animals and food intake were measured twice a week. The experiment was terminated after 5 weeks. Plasma samples were collected for further lipid profile and free fatty acid estimation. All animal-experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of North Carolina A&T State University, Greensboro, North Carolina.

2.5. Collection of plasma samples

Rats were fasted overnight (approximately 15 h) in order to measure plasma biochemical parameters. Blood samples were collected by retro-orbital bleeding by putting animals under deep anesthesia using isoflurane. Plasma was separated from blood by centrifugation at $3000\times g$ for 10 min and immediately analyzed for blood profile. Plasma aliquots were frozen immediately for free fatty acids analysis.

2.6. Determination of plasma profile

Plasma was analyzed for lipid profile including total cholesterol (TC), triglyceride (TG), high-density-lipoprotein cholesterol (HDL), low-density-lipoprotein (LDL), and very-low-density-lipoprotein (VLDL) as well as glucose (GLU), alanine aminotransferase (ALT), aspartate amino- transferase (AST) using an Abaxis Piccolo express chemistry analyzer (Abaxis, CA).

2.7. Determination of plasma free fatty acids analysis by GC–MS

For free fatty acids (FFA) analysis, lipids were extracted from plasma with chloroform–methanol (2:1, v/v). The extract was washed with a saline solution to remove proteins. The chloroform layer containing the lipids was transferred into an amber vial. The chloroform phase was concentrated under a stream of nitrogen and redissolved in 3 ml of chloroform–methanol. The free fatty acids extract was dried under N_2 and resolubilized in 1 ml tetrahydrofuran at ambient temperature and 1 ml methanolic 1 M KOH and vortexed briefly. One ml BF_3-Methanol (14%, w/w) was added and mixed thoroughly. The solution was subsequently heated for 15 min in a 100 °C water bath and cooled, then mixed with 0.5 ml of saturated NaCl. Fatty acid methyl esters (FAME) prepared