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Anti-obesity and anti-inflammatory effects of high hydrostatic pressure extracts of ginseng in high-fat diet induced obese rats

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ABSTRACT

Obesity is associated with a state of chronic, low-grade inflammation. High hydrostatic pressure, a non-thermal food processing technique, increases extraction efficiency without destroying heat-sensitive bioactive constituents. This study investigated effects of high hydrostatic pressure extract of ginseng (PEG) and hot water extract of ginseng (WEG) on obesity and inflammation in rats fed a high-fat diet. The contents of total phenolics, saponins and acidic polysaccharides of PEG were higher than those of WEG. PEG reduced the body weight and white adipose tissue mass. PEG increased faecal triacylglycerol, whereas WEG did not. PEG reduced mRNA levels of adipogenic genes such as PPARγ and aP2. The mRNA levels of pro-inflammatory genes such as TNF-α, IL-6 and MCP-1 were down-regulated by the PEG, whereas WEG did not. These results suggest PEG may have more beneficial effects on obesity and inflammation than WEG, partially mediated by increase of faecal triacylglycerol and regulation of gene expression.

1. Introduction

Obesity is characterized by excessive body weight and abnormal body fat accumulation (Hamilton, Hamilton, & Zderic, 2007). It has become a worldwide health epidemic due to its rising prevalence and strong association with a number of metabolic disorders such as hyperlipidaemia, hypertension, and insulin resistance (Lee, Kim, Kim, & Kim, 2011b). Accumulating evidence suggests that obesity causes chronic low-grade inflammation, which contributes to systemic metabolic dysfunction that is related to obesity-linked disorders (Ouchi, Parker, Lugus, & Walsh, 2011). Accordingly, in the field of food science, studies on obesity have focused on searching for food components that have potential to suppress body fat accumulation.

Ginseng has been widely used as a medicinal plant in many countries for a long time. Its therapeutic effects on metabolic disease such as hypercholesterolaemia (Lee et al., 2013b), hyperglycemia (Sen, Querques, & Chakrabarti, 2013), obesity (Lee,

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Park, & Yoon, 2013a) and immune function (Attele, Wu, & Yuan, 1999) have been reported. Biologically active constituents found in ginseng are ginsenosides (saponins), polyacetylenes, polyphenolic compounds, and acidic polysaccharides such as uronic acid (Lee et al., 2011a). Among them, ginsenosides have been attributed to major pharmacological actions of ginseng (Xu et al., 2013). More than forty ginsenosides have been isolated up to date, and diversity of the ginsenoside composition contributes to different efficacies of ginseng (Karu, Reifen, & Kerem, 2007). There is a wide variation in the ginsenoside content of ginseng according to species, growing environment, geographical conditions and picking times (Qi, Wang, & Yuan, 2011). Moreover, it has been reported that ginsenoside composition is affected by cooking or processing methods (Qi et al., 2011), which means ginseng processing technique may be very important to bring the maximum effect of the ginseng function.

High hydrostatic pressure (HHP) is a non-thermal food processing technique that has been used as an alternative to high-heat processing in the food industry (Lopes, Valente Mesquita, Chiaradia, Fernandes, & Fernandes, 2010). An important application of the HHP technique is to extract bioactive constituents from plants or herbal materials at a lower temperature between –20 and 60 °C than other thermal extraction methods (Lee et al., 2011a). Since the HHP extraction uses low temperature, increased extraction efficiency without destroying heat-sensitive bioactive constituents can be expected (Oey, Lille, Van Loey, & Hendrickx, 2008). In a previous study, Lee et al. (2011a) reported that HHP used for ginseng extraction improved extraction efficiencies of functional materials such as ginsenosides and its metabolites through cell structure modification, which could influence bioefficacy of the ginseng.

Korean red ginseng (*Panax ginseng*) is reported to reduce body weight and white adipose tissue (WAT) mass by inhibiting angiogenesis (Lee et al., 2013b). Reduction in the body weight by ginsenoside Rg_3 supplementation is accompanied by increased AMP-activated protein kinase (AMPK) signaling pathway and reduced peroxisome proliferator-activated receptor (PPAR)-γ gene expression (Hwang et al., 2009). In addition, decreased inflammatory gene expression by orally administrated ginsenoside Rh_1 in diet-induced obese mice is also reported (Gu, Kim, & Kim, 2013). Therefore, this study was designed to investigate the effects of high hydrostatic pressure extract of ginseng (PEG) and hot water extract of ginseng (WEG) on obesity and inflammation using high-fat (HF) diet induced obese rats. Lipid profiles in serum, liver and faeces were analysed. In addition, mRNA expression of genes related to adipogenesis and inflammation were evaluated in WAT.

2. Materials and methods

2.1. Preparation of PEG and WEG

PEG and WEG were kindly supplied by the Korea Food Research Institute (Songnam, Gyeonggi, Korea). Six-year-old Panax ginseng root from Gimpo-Paju Ginseng Agricultural Cooperative (Gimpo, Gyeonggi, Korea) was used for preparation of PEG and WEG. For preparation of PEG, ginseng root suspension was poured into plastic bags with 25 mL of each enzyme (Thermamyl 120 L, Celluclast 1.5 L and Viscozyme L) and transferred to a programmable high-pressure treatment apparatus (TFS-10 L, Innoway Co., Bucheon, Korea), set at pressure of 100 MPa for 24 h at 50 °C. After incubation, the extract was heated at 100 °C for 10 min to inactivate the enzyme. After cooling, the extract was centrifuged at 11,000 × *g* for 10 min, and the supernatant was filtered using Whatman No. 4 filter paper. The filtrate was freeze-dried and used as PEG. In order to prepare the WEG, ginseng root suspension was placed into a round-bottom flask fitted with a cooling condenser, and extraction was performed at 100 °C for 3 h. The extract was followed by centrifugation at 11,000 × *g* for 10 min, and the supernatant was filtered using Whatman No. 4 filter paper. The filtrate was freeze-dried and used as WEG.

2.2. PEG and WEG analysis

2.2.1. Total phenolic, total flavonoid and acidic polysaccharides

Total phenolic content was determined using a method modified from Singleton and Rossi (1965) and the Folin–Ciocalteu reagent (Korean Food Standards Codex, 2011). Ginseng extract (0.1 ml) was added to 3.5 ml of distilled water, and 0.5 ml of 1N Folin–Ciocalteu reagent was added. And then 1 ml of 20% sodium carbonate was added and shaken thoroughly. After being kept for 2 h in the dark room at room temperature, the absorbance was measured at 710 nm in a spectrophotometer (V-550, Jasco, Tokyo, Japan) using catechin as a standard. The concentration of the total phenolic compounds was expressed as mg of catechin equivalents per 1 g of dry weight.

Total flavonoids were determined using a method modified from AOAC (1990). To 1 ml of ginseng extract, 10 ml of diethylene glycol and 1 ml of 1 M NaOH were added, and mixed thoroughly. The mixed solution was incubated 1 h at 37 °C and the absorbance was measured at 420 nm in a spectrophotometer (V-550, Jasco) using rutin as a standard.

Acidic polysaccharides were determined using a method modified from Bitter and Muir (1962). One gram of dried ginseng extract was dissolved into 10 ml of distilled water and heated in the water bath for 10 min and then cooled to room temperature. After that, 1 ml of solution was taken and 0.2 ml of 0.125% carbazole was added and mixed thoroughly, and then incubated for 30 min at room temperature. The absorbance was measured at 530 nm in a spectrophotometer (V-550, Jasco) using galacturonic acid as a standard. The measured total polyphenol, total flavonoids and acidic polysaccharide levels are shown in Table 1.

2.2.2. Total saponin and crude saponin

Since ginseng saponins are the main bioactives of ginseng, analyses of saponins from the PEG and WEG were additionally carried out. Total saponin content of each extract was determined using the method described by Hiai, Oura, and Nakajima (1976). Briefly, 100 μL of ginseng extract were mixed with vanillin (8% w/v, 0.3 mL) and sulphuric acid (75% (w/v), 4 mL). The mixture was then incubated at 60 °C for 10 min. After cooling, the absorbance was measured at 545 nm, and ginsenoside Re (Wako Chem. Co., Osaka, Japan) was used as a reference standard. To determine the crude saponin content,