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Research article

The involvement of ginseng berry extract in blood flow via regulation of blood coagulation in rats fed a high-fat diet

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ABSTRACT

Background: The present study investigated the effect of ginseng berry hot water extract (GBx) on blood flow via the regulation of lipid metabolites and blood coagulation in rats fed a high-fat diet (HFD).
Methods: Sixty rats were divided into five groups in descending order of body weight. Except for the control group, the other four groups were fed a HFD containing 45% kcal from fat for 11 wk without GBx. GBx groups were then additionally treated by gastric gavage with GBx dissolved in distilled water at 50 (GBx 50) mg/kg, 100 (GBx 100) mg/kg, or 150 (GBx 150) mg/kg body weight for 6 wk along with the HFD. To investigate the effects of GBx on rats fed a HFD, biochemical metabolite, blood coagulation assay, and histological analysis were performed.
Results: In the experiments to measure the serum levels of leptin and apolipoprotein B/A, GBx treatment attenuated the HFD-induced increases in these metabolites ($p < 0.05$). Adiponectin and apolipoprotein E levels in GBx-treated groups were significantly higher than the HFD group. Prothrombin time and activated partial thromboplastin time were increased in all GBx-treated groups. In the GBx-treated groups, the serum levels of thromboxane A_2 and serotonin were decreased and concentrations of serum fibrinogen degradation products were increased ($p < 0.05$). Moreover, histomorphometric dyslipidemia-related atherosclerotic changes were significantly improved by treatment with GBx.
Conclusion: These results suggest the possibility that GBx can ameliorate blood flow by decreasing intima-media thickness via the regulation of blood coagulation factors related to lipid metabolites in rats fed a HFD.

1. Introduction

Nowadays, foods that are palatable to most individuals are those high in fat, especially saturated fat. A high-fat diet (HFD) leads to the expansion of adipose tissue and abdominal obesity, which are associated with metabolic abnormalities including complex and chronic cardiovascular disease, liver disorders, and others [1,2]. In particular, cardiovascular disease is considered a serious public health issue globally and is among the top five causes of death in most countries [1,3]. Cardiovascular disease causes up to 17 million deaths every year and results from thrombosis, atherosclerosis, and myocardial infarction [4].

Hyperlipidemia is accompanied by platelet hyperactivity, hypercoagulability, and hypofibrinolysis, and plays a key role in the pathogenesis of cardiovascular diseases such as atherosclerosis [5,6]. The major pathological pathway involved in these disorders is the

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development of an occlusive thrombus in the arteries. Collapses of atherosclerotic plaques by rupture or erosion leads to prothrombotic conditions involving circulating platelets and procoagulant factors. The main thrombotic elements related to atherosclerotic plaques are tissue factor and collagen [7]. These result in platelet aggregation and activation to induce cardiovascular disease [8]. Because studies on the regulation of platelet hyperactivity have focused on approaches to prevent thrombosis, many therapies aimed at suppressing platelet function have been developed [8,9]. Moreover, for alternative treatments, numerous patients and healthcare specialists have sought oriental medicines or herbal remedies with potential effectiveness and without side effects such as gastrointestinal side effects, hemorrhage, and decreased platelets counts [1,8].

Among the natural product alternative treatments, ginseng and its related components have been widely used for thousands of years, either in food or herbal medicines, to treat various diseases. Ginseng has antiaging, antidiabetic, anticarcinogenic, and antifatigue functions through the promotion of DNA, RNA, and protein synthesis [10]. In addition, ginseng has been classified into 11 different species and contains approximately 200 ingredients, including ginsenosides, polysaccharides, polyacetylenes, fatty acids, mineral oils, peptides, and amino acids [11].

In particular, the bioactivity of ginseng results from the presence of ginsenosides in its roots [8]. Thus, until now, many studies have focused on the effect of the ginseng root [12]. However, these active ingredients are also distributed in other parts of the ginseng plant, such as the berries and leaves. Current studies have reported that the ginseng berry (GB) has higher ginsenoside content than that of the root and may exert more pharmacological effects on various diseases via its antihyperglycemic and antiobesity activities [6,13]. Moreover, the harvesting of GB is easier than that of the root because it can be harvested several times after the 3rd yr of growth, whereas the root is only harvested between the 4th and 6th yr of growth [14]. However, until now, GB has not received much attention [6].

Consumption of a HFD, which includes foods palatable to many individuals, induces cardiovascular disease through alterations in lipid metabolism and blood coagulation, for which treatments with potential effectiveness and without various side effects are needed. GB contains a high ginsenoside content, which might exert pharmacological effects on cardiovascular diseases. However, there have been no reports regarding GB. Therefore, the purpose of the present study was to investigate the possibility of GB as a putative agent to improve blood flow in rats fed a HFD.

2. Materials and methods

2.1. Animals and experimental diets

The animals used in this study were 5-wk-old male Sprague Dawley rats (140 ± 6.00 g) purchased from Central Lab. Animal Inc. (Seoul, Korea) and acclimatized for 1 wk. Each cage contained two rats. Rats were exposed to a 12-h light/dark cycle and maintained at a constant temperature of 22 ± 2°C and 55 ± 5% humidity. All animals were fed for 12 wk. The rats were selected randomly and assigned to five groups (12 rats/group). One group served as the control (CON) and was fed a normal diet (Zeigler Bros, Gardners, PA, USA). The other four groups were fed a HFD (D12451; Research Diets Inc., New Brunswick, NJ, USA) containing 45% kcal from fat for 11 wk without GB. Three of the HFD groups were additionally treated by gastric gavage with GB hot water extract (GBx) dissolved in distilled water at 50 (GBx 50) mg/kg, 100 (GBx 100) mg/kg, and 150 (GBx 150) mg/kg body weight for 6 wk (Fig. 1). This study was approved (EUIACUC 15-06) by the Eulji University Institutional Animal Care and Use Committee.

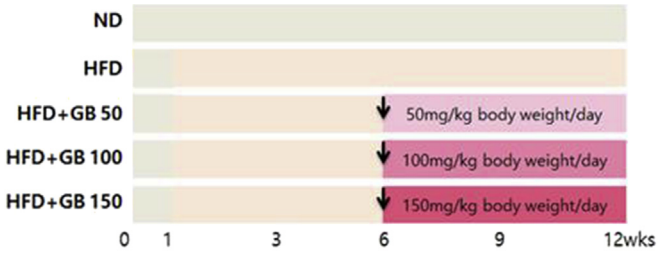

Fig. 1. Experimental study design. The rats were selected randomly and assigned to five groups. One group served as the control and was fed a normal diet (ND). The other four groups were fed a high-fat diet (HFD) containing 45% kcal from fat for 11 wk without ginseng berry (GB). Three of the HFD groups were additionally treated by gastric gavage with ginseng berry hot water extract (GBx) dissolved in distilled water at 50 (GBx 50) mg/kg, 100 (GBx 100) mg/kg, and 150 (GBx 150) mg/kg body weight for 6 wk.

2.2. Preparation of GBx

GB was extracted in three volumes of distilled water in a shaking incubator at 80°C for 10 h. The extract was filtered through a 60-mesh sieve filter and concentrated into a dry solid using a rotary vacuum evaporator (Daesung Machinery Co., Chuncheon, Korea) at 60°C, up to a 25% soluble solid content, depending on the GB ingredient. For freeze drying, extracts were frozen overnight at −40°C and lyophilized using a freeze dryer (PVTFA 10AT; Inshin Lab. Co. Ltd., Seoul, Korea) for 3 d. GB powder was obtained after drying for 12 h at 60°C, and the final dried extract was stored at −20°C until use. Constituents of the GBx were analyzed using HPLC. HPLC analysis of GB extract powder was done using an Agilent HPLC system (Agilent Technologies., Santa Clara, CA, USA) equipped with a binary pump and diode array detector. An aqueous solution of test materials (0.08 mg/mL) was injected at 10 μL. Separation was then done on a 5-μm Hector-M C18 column (4.6 mm × 250 mm; RS Tech Co., Daejeon, Korea). The mobile phase consisted of deionized water (A) and acetonitrile (B). The gradient was programmed as follows: 0–5 min: 20% B. 5–35 min: a linear gradient from 20% to 40% B; 35–45 min: 40% B. 45–45.1 min: a linear gradient from 40% to 20% B; 45.1–50 min: 20% B. The flow rate was 1.0 mL/min. The UV detector was set at 203 nm. Determination of major ginsenosides in the GB extract is shown in Fig. 2.

2.3. Biochemical metabolite assay

Leptin (R&D Systems, Minneapolis, MN, USA) and adiponectin (Shibayagi Co. Ltd., Gunma, Japan) were measured using a commercial enzyme-linked immunosorbent assay kit. Apolipoproteins (apo) A, B100, and E, thromboxane A_2, serotonin, and fibrinogen degradation products (FDP) were also measured using enzyme-linked immunosorbent assay kits (Elabscience Biotechnology Co. Ltd., Wuhan, China). After centrifugation, serum samples were mixed with substrate buffer and manganese solution and incubated for 2 h at 37°C, followed by further incubation with urea reagent at room temperature for another 30 min. Optical density was determined at 450 nm using a spectrophotometer.

2.4. Coagulation assays

Plasma for the coagulation assays was obtained by centrifugation (3,000 rpm, 10 min). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured within 2 h of sample collection using automated coagulation analyzers (Diagnostica Stago, Asnières, France) according to the manufacturer's instructions.

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