J Ginseng Res 40 (2016) 160–168

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Black ginseng extract ameliorates hypercholesterolemia in rats

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in Revised form
29 June 2015
Accepted 6 July 2015
Available online 18 July 2015

Keywords:
black ginseng
hypercholesterolemia
lipogenesis
messenger RNA

ABSTRACT

Background: Ginseng (*Panax ginseng* Meyer) is a well-characterized medicinal herb listed in the classic oriental herbal dictionary as "Shin-nong-bon-cho-kyung." Ginseng has diverse pharmacologic and therapeutic properties. Black ginseng (BG, *Ginseng Radix nigra*) is produced by repeatedly steaming fresh ginseng nine times. Studies of BG have shown that prolonged heat treatment enhances the antioxidant activity with increased radical scavenging activity. Several recent studies have showed the effects of BG on increased lipid profiles in mice. In this study report the effects of water and ethanol extracts of BG on hypercholesterolemia in rats. To our knowledge, this is the first time such an effect has been reported.
Methods: Experiments were conducted on male Sprague Dawley rats fed with a high-cholesterol diet supplemented with the water and ethanol extracts of BG (200 mg/kg). Their blood cholesterol levels, serum white blood cell levels, and cholesterol-metabolizing marker genes messenger RNA (mRNA) expression were determined. Liver and adipose tissues were histologically analyzed.
Results: We found that BG extracts efficiently reduced the total serum cholesterol levels, low-density lipoprotein (LDL) levels with increased food efficiency ratio and increased number of neutrophil cells. It also attenuated the key genes responsible for lipogenesis, that is, acetyl-coenzyme A (CoA) acetyltransferase 2, 3-hydroxy-3-methyl-glutaryl-CoA reductase, and sterol regulatory element-binding protein 2, at the mRNA level inside liver cells. Furthermore, the BG extract also reduced the accumulation of fat in adipose tissues, and inhibited the neutral fat content in liver cells stained with hematoxylin and eosin and oil red O.
Conclusion: Administration of BG extracts to Sprague Dawley rats fed with high-cholesterol diet ameliorated hypercholesterolemia, which was mediated via modulation of cholesterol-metabolizing marker genes. This data throw a light on BG's cardioprotective effects.

1. Introduction

Hypercholesterolemia is a serious problem faced by many countries. Hypercholesterolemia is a major concern for health professionals, because it is one of the primary risk factors for the development and growth of cardiovascular diseases such as atherosclerosis and its complications, acute infarction of the myocardium or hypertension, accumulation of low-density

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p1226-8453 e2093-4947/$ – see front matter
http://dx.doi.org/10.1016/j.jgr.2015.07.003

lipoproteins (LDLs), stroke, and cerebral infarctions [1,2]. Diet-related chronic diseases such as type 2 diabetes, cardiovascular diseases, several types of cancer, and increased morbidity and mortality can be attributed to obesity and overweight. In general, obesity occurs due to an imbalance between energy intake and energy output. An imbalance in this ratio results in accumulation of fat inside adipose tissues and elevated LDLs inside blood and other fat-accumulation organs [3]. Although allopathic drugs provide a quick relief and show rapid effects for mitigating cardiovascular anomalies, their serious side effects cannot be ignored. Therefore, increasing attention is paid to utilizing natural products for alleviation of these chronic diseases.

Ginseng and ginsenosides have been studied for a long time for their therapeutic effects on body vitality, the immune system, various types of cancer, inflammatory anomalies, nervous system disorders, reproductive system functioning, and cardiovascular diseases. Studies in the past have demonstrated ginseng's potent suppressing effects on *in vitro* pancreatic lipase activity as well as its ability to reduce obesity and plasma lipid profile in rodents fed with high-fat diet [4,5]. Here our compound of interest is black ginseng (BG). BG is prepared from raw ginseng by steaming it nine times at a high temperature for approximately 3 h up to the point where it becomes black. At this point, the BG possesses better qualities than red ginseng, including better anti-inflammatory, antimetastatic, and antioxidant effects [6]. This pharmacologically active agent has been studied for many aspects previously, however, its effects on hypercholesterolemia have not yet been studied in detail. We used both water and ethanol extracts of BG in this study. Because the ethanol extract has already been identified for its therapeutic effects, we aimed to investigate the effects of the water extract. Herein, we report for the first time the amelioration of hypercholesterolemia in high-cholesterol-fed rats by the water and ethanol extracts of BG. Our results show that the water and ethanol extracts of BG effectively lowered the total serum levels of cholesterol. It also increased the food efficiency ratio (FER) as well as the differential white blood cell (WBC) count. The key gene markers for fat metabolism such as acetyl-coenzyme A (CoA) acetyltransferase 2 (ACAT2), sterol regulatory element-binding protein 2 (SERBP2), and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAr) were also reduced by the BG extract at the messenger RNA (mRNA) levels. Moreover, the histopathological images also show reduction in fat accumulation in liver and adipose tissues. Therefore, in a nutshell, BG appears to be a promising antihypercholesterolemic agent.

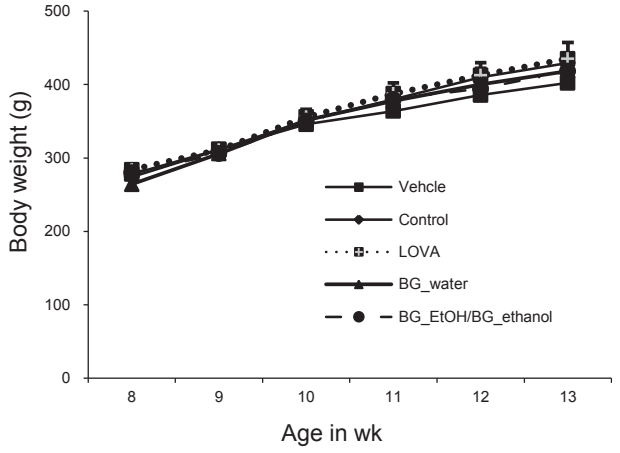

Fig. 1. Effects of black ginseng (BG) supplementation on body weight. Experimental rats were fed with normal chow rat diet, high-cholesterol diet, high-cholesterol diet supplemented with lovastatin (LOVA), high-cholesterol diet supplemented with water extract of black ginseng (200 mg/kg, BG_water), and ethanol extract of black ginseng (200 mg/kg, BG_ethanol) for 4 wk. Weight gain after 4 wk is expressed in grams. Values are expressed as mean ± standard deviation ($n = 4$). EtOH, ethanol.

Table 1
Food efficiency ratio

Group	Food intakes (g/d)	Body weight gain (g/d)	Food efficiency ratio (%)
Vehicle	19.95	4.44 ± 0.15	22.23 ± 0.75
Control	23.51	5.55 ± 0.34	23.62 ± 1.43
Lovastatin (20 mg/kg)	23.58	5.40 ± 0.56	22.89 ± 2.38
BG_water (200 mg/kg)	23.38	5.51 ± 0.72	23.59 ± 3.07
BG_EtOH (200 mg/kg)	22.47	4.94 ± 0.24	21.98 ± 1.05

Sprague Dawley rats were supplemented with a high-fat diet and black ginseng (BG) extracts for 4 wk and after completion of the experimental period, the food efficiency ratio (FER) was calculated as follows: FER = (food intake/body weight gain) × 100.
EtOH, ethanol.

2. Materials and methods

2.1. Sample preparation

Black ginseng (BG) was prepared according to the procedures described in previous reports, but with some minor modifications [7,8]. In brief, the BG sample was ground in a cutting mill to pass through a 50-mesh sieve to obtain a fine powder and then extracted in 10-times volume of distilled water or 50% ethanol (V/W) at 80°C for 8 h in a water bath. It was then extracted again in seven-times volume of distilled water at 80°C for 8 h, which was repeated once more (third-time extraction). The total extract solution was filtered through a filter paper (medium fast: CHM F1001, CHMLAB GROUP, Barcelona, Spain). The filtrate solution was then concentrated in a low-vacuum evaporator at 60°C, and the water extract (water content 34.45%) and ethanol extract (35.55%) were prepared as the test samples.

2.2. Animals and experimental diets

Male Sprague Dawley rats, 8-wk-old, were obtained from Central Lab Animal Inc. (Seoul, Korea) and housed in standard conditions with free access to chow and water. All animals were acclimated for 1 wk before use. All *in vivo* experiments were conducted in accordance with internationally accepted guidelines in a specific pathogen-free facility, and the protocols were approved by

Table 2
Mean liver and kidney weights

Group	Liver (mean ± SEM)	Kidney (mean ± SEM)
Vehicle	11.75 ± 0.48	2.80 ± 0.11
Control	22.06 ± 0.49	2.96 ± 0.12
Lovastatin (20 mg/kg)	22.07 ± 0.78	2.87 ± 0.04
BG_water (200 mg/kg)	20.40 ± 1.44	2.66 ± 0.11
BG_EtOH (200 mg/kg)	19.25 ± 0.36 *	2.50 ± 0.07 *

The values presented were obtained after the experimental 13-wk period. The dry weight of the liver and kidney is significantly (* $p < 0.01$) reduced by treatment with the ethanol (EtOH) extract of black ginseng (BG). Values are presented as mean ± standard deviation ($n = 4$).
SEM, standard error of the mean.