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Anti-obesity effects of black ginseng extract in high fat diet-fed mice

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Black ginseng is produced by a repeated steaming process. The aim of this study was to investigate the anti-obesity effects of black ginseng ethanol extract (BG-EE) in high fat (HF) diet-fed mice. Two groups were fed either a normal control (NC) diet or a HF diet (45% kcal fat). The other three groups were given a HF diet supplemented with 1% BG-EE, 3% BG-EE, and 5% BG-EE for 12 wk. The anti-obesity effects of the BG-EE supplement on body weight, the development of fat mass, and lipid mechanisms were assessed in obese mice. HF-induced hyperlipidemia, fat accumulation in the liver, and white adipose tissues were reduced after BG-EE supplementation. Total fecal weight and the amount of fecal fat excretion also were increased after BG-EE supplementation. These results suggest that BG-EE may be useful to ameliorate HF-induced obesity through the strong inhibition of fat digestion.

Keywords: *Panax ginseng*, Black ginseng, High fat diet, Obesity, Hyperlipidemia

INTRODUCTION

Obesity and being overweight are also associated with diet-related chronic diseases, including type 2 diabetes, cardiovascular disease, and several types of cancer, leading to a substantial increase in morbidity, and mortality [1,2]. In general, it is accepted that obesity results from an imbalance between energy intake and expenditure, and is characterized by increased fat accumulation in adipose tissue and elevated lipid concentrations in the blood [3]. The mechanism of high fat (HF) diet-induced obesity is still unclear, but long-term exposure to a HF diet can increase body weight and adiposity in human and animals [4]. Only two drugs, orlistat and sibutramine, have been approved for long-term use in significantly obese patients by the U.S. Food and Drug Administration. Due to their adverse effects including gastro-intestinal discomforts, flatulence, and diarrhea, the potential of natural products to treat obesity is under exploration [5].

Ginseng and ginsenosides have been demonstrated to exert therapeutic effects on vitality, immune function, cancer, and cardiovascular diseases, and to improve cognitive, physical, and sexual performance [6]. Moreover, many studies have suggested that dietary ginseng saponins may have beneficial effect on obesity and hyperlipidemia as well as reducing total serum cholesterol level [7,8]. It has also been reported that ginseng has an effect on obesity and lipid metabolism. Previous studies report that ginseng extract and isolated ginsenosides exhibited strong inhibitory effects on pancreatic lipase *in vitro* and were shown to reduce plasma lipid levels and obesity when administered to rodents fed a HF diet [9,10].

Black ginseng (BG) is developed from raw *Panax ginseng* by steaming nine times at 98°C for 3 h and nine

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times drying, at which point it becomes black in color. BG possesses better biological activity than red ginseng, inducing anti-stress, anti-cancer, anti-inflammatory, and free radical scavenging pharmacological effects [11,12]. Although BG is pharmacologically effective, the antiobesity effect of BE has not been examined yet.

In the present study, to examine the effects of BG-ethanol extract (EE) on HF diet-induced obesity, we used the obesity model, inducing it by feeding a HF diet containing 45% kcal of fat for 12 wk. BG-EE was supplemented at the dose of 1%, 3%, and 5%, based on the previous studies about toxic safety of BG [13] and antiobesity effect of red ginseng [14].

MATERIALS AND METHODS

Black ginseng ethanol extraction

BG was obtained from Daedeok Bio Corporation Research Institute (Daejeon, Korea). To prepare the extract, ginseng was crushed into a powder and ultrasonicated 3 times in 10 volumes of 80% ethanol at 50°C for 1 h, then was filtered and lyophilized.

Experiment diets

Forty male C57BL/6J mice 6 wk of age were obtained from Dahan BioLink (Eumseong, Korea). They were individually housed in stainless steel cages in a room maintained at 22±2°C with 50% to 55% relative humidity and 12 h of light/dark cycle (light on at 08:00). The animals were fed a pelletized chow diet for 1 wk. They were then randomly divided into 5 dietary groups (n=8). Two groups were fed either a normal control (NC) diet or a HF diet (45% kcal fat). The other three groups were given a HF diet supplemented with 1% BG-EE, 3% BG-EE, and 5% BG-EE. The composition of the experimental diet was based on the AIN-93 semisynthetic diet (Table 1). The mice were allowed free access to food and water during the 12-week experimental period. Food consumption and weight gain were measured daily and weekly, respectively. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Chungnam National University.

Collection of serum, organs, and feces

At the end of the experiments, all animals were induced to fast for 12 h. All mice were anesthetized by carbon dioxide. Blood was collected using a polyethylene tube with no heparin and centrifuged at 1,000 ×g for 15 min at 4°C to obtain the serum and stored at -70°C until analysis. Selected organs, the liver, kidneys, spleen, brain, heart, testes, and white adipose tissue such as retroperitoneal, subcutaneous, and epididymal fat pads were weighed. Feces were collected during the final 3 d using metabolic cages, and dried feces were used for fecal lipid analysis.

Analytical procedures

The concentrations of total cholesterol (TC), triglycer-

Table 1. Composition of the experimental diets

Contents (g/kg)	NC	HF	BG-EE		
			1%	3%	5%
Casein	200	200	200	200	200
Corn starch	457	260	250	230	210
Sucrose	200	200	200	200	200
Soybean oil	43	25	25	25	25
Lard	0	215	215	215	215
Cellulose	50	50	50	50	50
Choline bitatrate	2	2	2	2	2
L-cysteine	3	3	3	3	3
Mineral mix	35	35	35	35	35
Vitamin mix	10	10	10	10	10
BG-EE	-	-	10	30	50
Total grams (g)	1,000	1,000	1,000	1,000	1,000
Calories from fat (%)	10	45	45	45	45

Mineral and vitamin mixtures (AIN-93) were purchased from Samtako (Osan, Korea).
NC, normal control; HF, high fat diet (45% kcal fat); BG-EE, black ginseng ethanol extract.

ide (TG), and high density lipoprotein cholesterol (HDL-C) in serum were determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea). Hepatic and dried fecal lipid extractions were measured according to the method of Folch et al. [15]. Briefly, hepatic and dried fecal lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2. Measurement of malondialdehyde (MDA) contents of the liver was performed using the thiobarbituric acid reactive substances assay [16].

Histology

Liver tissues were preserved in a 10% buffered formaldehyde solution. They were processed into paraffin blocks, sectioned at a nominal 5 μm, mounted on glass microscope slides and stained with hematoxyline and eosin.

Statistical analysis

All the results were expressed as mean±SD. Data were analyzed by one-way ANOVA followed by Duncan's test for multiple comparisons. A difference of $p<0.05$ was regarded as being statistically significant. Analysis was performed using IBM SPSS ver. 20.0 (IBM, Armonk, NY, USA).

RESULTS

Body weight, food intake, and organ weights

The change in body weight and food intake during the experimental period is shown in Table 2 and Fig. 1. There was no significant difference in the initial body weight among the groups. However, consuming a HF diet for 12 wk caused a 17% significant increase in body weight compared with the NC group. The mice fed a HF diet supplemented with 1%, 3%, and 5% BG-EE had 14%, 12%, and 18% lower body weights, respectively, compared with mice fed the HF diet. All of the diets containing BG-EE significantly reduced the weight gain. The food consumption and food efficiency ratio in the HF diet group was significantly higher than those of the NC group. No significant differences were observed in the weights of liver or, testicles in relation to body weight among groups (Table 3). However, heart, brain, and kidney weights were significantly lower in the HF diet group than in the NC group.

Adipose tissue

The white adipose tissue weights of the groups are shown in Fig. 2. The total adipose tissue weights were significantly increased by 153.1% in the HF diet group compared to the NC diet group. Interestingly, the subcu-

Table 2. Effect of BG-EE supplementation on body weight gain, food intake, and food efficacy ratio in mice fed a HF diet

Group	Initial weight (g)	Final weight (g)	Food intake (g/d)	FER[1)](%)
NC	24.66±0.24	32.92±1.51^{b}	2.78±0.08^{b}	41.05±8.96
HF	23.45±0.28	39.60±1.16^{a}	3.11±0.10^{a}	69.35±9.62
1% BG-EE	23.25±0.40	33.92±1.74^{b}	2.68±0.08^{bc}	50.34±8.54
3% BG-EE	22.83±0.25	34.50±1.28^{b}	2.50±0.06^{c}	54.23±9.93
5% BG-EE	23.66±0.25	32.50±1.10^{b}	2.63±0.07^{bc}	47.10±9.53

Experimental mice were fed NC, HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8).
Different superscripts in the same row indicate significant differences between the groups ($p<0.05$).
NC, normal control; HF, high fat; BG-EE, black ginseng ethanol extract; FER, food efficiency ratio.
[1)]FER=(food intake/body weight gain)×100.

Table 3. Effect of BG-EE supplementation on organ weights fed a HF diet

g/100 g BW	NC	HF	1% BG-EE	3% BG-EE	5% BG-EE
Liver	2.95±0.04	3.23±0.15	3.16±0.17	3.19±0.15	3.23±0.14
Spleen	0.29±0.06^{a}	0.21±0.00^{ab}	0.18±0.04^{b}	0.20±0.11^{b}	0.21±0.00^{ab}
Heart	0.39±0.17^{a}	0.32±0.01^{b}	0.36±0.02^{ab}	0.39±0.02^{a}	0.37±0.01^{ab}
Brain	0.96±0.06^{a}	0.78±0.03^{b}	0.89±0.04^{ab}	0.90±0.03^{ab}	0.91±0.05^{ab}
Kidney	1.04±0.09^{a}	0.86±0.02^{b}	0.88±0.05^{ab}	0.89±0.04^{ab}	0.96±0.31^{ab}
Testicles	0.56±0.04	0.52±0.03	0.58±0.04	0.52±0.05	0.60±0.02

Experimental mice were fed NC, HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8).
Different superscripts in the same row indicate significant differences between the groups ($p<0.05$).
NC, normal control; HF, high fat; BG-EE, black ginseng ethanol extract; BW, body weight.

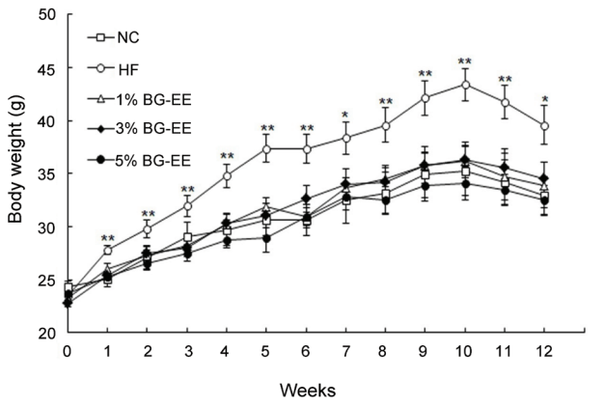

Fig. 1. Effects of black ginseng ethanol extract (BG-EE) on body weight in high fat (HF) diet-fed mice. Experimental mice were fed normal control (NC), HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8). *p<0.05, **p<0.01 vs. control group.

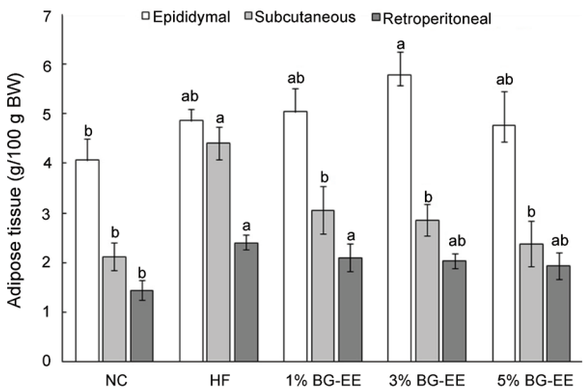

Fig. 2. Effects of black ginseng ethanol extract (BG-EE) on white adipose tissue weight in mice fed with 45% kcal fat diet for 12 wk. Values are expressed as mean±SD (n=8). Different superscripts in the same bar chat indicate significant differences between the groups (p<0.05). NC, normal control; HF, high fat diet.

Table 4. Serum biochemistry of mice fed experimental diets

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/ dL)
NC	173.44±8.97	82.79±1.65^{ab}	89.50±5.23^{c}
HF	197.74±8.17	95.82±8.28^{a}	94.99±3.67^{bc}
1% BG-EE	168.15±13.07	72.76±5.37^{b}	104.26±6.04^{ab}
3% BG-EE	179.48±14.97	82.08±3.16^{ab}	109.12±2.08^{a}
5% BG-EE	181.53±10.11	81.54±4.76^{ab}	109.84±2.52^{a}

Experimental mice were fed NC, HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8). Different superscripts in the same row indicate significant differences between the groups (p<0.05).
TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; NC, normal control; HF, high fat; BG-EE, black ginseng ethanol extract.

taneous fat and retroperitoneal fat mass in the HF group were 208% and 167% greater than in the NC group, respectively. However, BG-EE supplementation with a HF diet significantly decreased subcutaneous and retroperitoneal fat mass compared to the HF diet group. Particularly, subcutaneous and retroperitoneal fat were significantly decreased by 46% and 20% in the HF plus 5% BG-EE group compared to the HF group, respectively.

Serum lipid profiles

Table 4 shows the data of serum lipid profiles containing TC, TG, and HDL-C of the groups. There was no significant difference in the serum TC levels among groups. The HF group had elevated serum TG levels, by 16%, compared to the NC group. The 1% BG-EE group showed a significantly lower serum TG level, by 24%, compared to the HF group. There was no significant difference between the NC and HF group concerning HDL-C levels, however, 3% BG-EE and 5% BG-EE group significantly increased serum HDL-C levels.

Table 5. Effects of BG-EE supplementation on fecal weight, fecal lipid in HF diet-fed mice

Group	Fecal weight (g/d)	Fecal lipid (mg/g)
NC	0.17±0.01^{a}	39.27±1.09^{d}
HF	0.11± 0.02^{b}	85.27±1.20^{a}
1% BG-EE	0.15±0.00^{a}	57.07±2.12^{c}
3% BG-EE	0.17±0.01^{a}	64.43±0.76^{b}
5% BG-EE	0.18±0.00^{a}	66.00±1.97^{b}

Experimental mice were fed NC, HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8). Different superscripts in the same row indicate significant differences between the groups (p<0.05).
NC, normal control; HF, high fat; BG-EE, black ginseng ethanol extract.

Fecal weights and fecal fat

As shown in Table 5, fecal weights of the HF group showed the lowest value. The HF plus BG-EE group had significantly increased fecal weights, ranging from 136% to 164%, compared to the HF diet. The mice fed the HF diet showed a marked increase in fecal fat compared with the NC group, but BG-EE supplementation in the HF

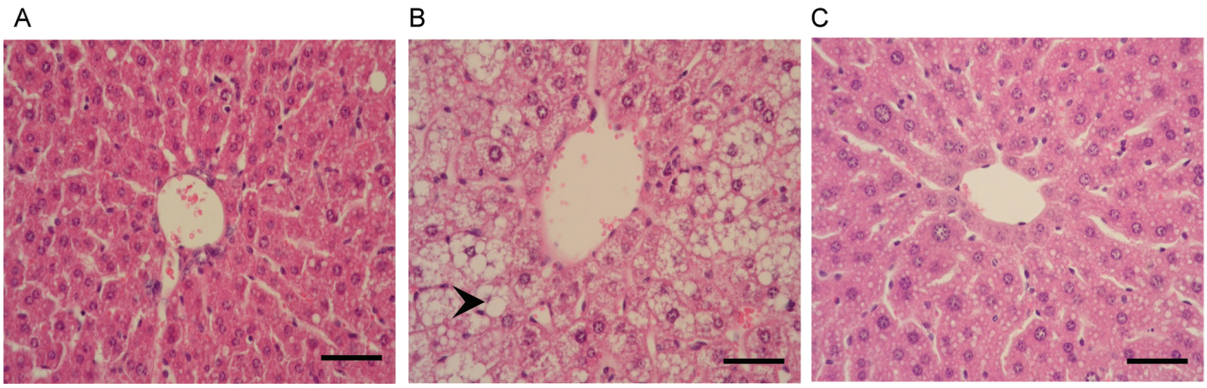

Fig. 3. Hematoxylin and eosin-stained photomicrographs showing the liver (×200). (A) normal control, (B) high fat group, (C) 5% black ginseng ethanol extract group. Bar=50 μm. Fat accumulation, indicated by the arrowhead, in the form of large fat droplet is present in liver of mice fed a high fat diet.

Table 6. Effects of BG-EE supplementation on lipid contents and lipid peroxidation of liver in HF diet-fed mice

Group	Lipid contents (mg/g)	MDA (nmole/mg protein)
NC	129.22±18.54^{b}	2.89±0.40^{ab}
HF	189.62±15.07^{a}	3.27±0.31^{a}
1% BG-EE	139.99±17.41^{ab}	2.49±0.19^{b}
3% BG-EE	149.95±12.16^{ab}	2.89±0.13^{ab}
5% BG-EE	148.88±13.02^{ab}	2.26 ± 0.09^{b}

Experimental mice were fed NC, HF, and HF supplemented with 1%, 3%, or 5% BG-EE for 12 wk. Values are expressed as mean±SD (n=8). Different superscripts in the same row indicate significant differences between the groups (p<0.05).
NC, normal control; HF, high fat; BG-EE, black ginseng ethanol extract; MDA, malondialdehyde level.

diet showed a markedly significant decrease in fecal fat.

Liver lipid contents and peroxidation

As shown in Table 6, liver lipid accumulation and lipid peroxidation were reduced in mice fed a HF diet supplemented with BG-EE. HF diet ingestion caused the liver to accumulate a higher lipid content. The liver lipid level was 1.47 fold higher than that of the NC group. However, BG-EE supplementation inhibited the accumulation of hepatic lipid caused by a HF diet but not significantly. The amount of MDA in the HF diet group was 3.27±0.40 nmole/mg protein, which was 113% higher than that in ND diet group. 1% and 5% BG-EE supplementation significantly reduced MDA levels by 24% and 31%, respectively. The 3% BG-EE group restored the elevated liver MDA level to a level similar to the control group.

Morphological comparison in hepatocytes

Histopathological analyses indicated that the livers of the mice fed a HF diet developed hepaticsteatosis (Fig. 3) but, the 5% BG-EE group showed small fat droplets.

DISCUSSION

Crude extracts of ginseng have also been traditionally used as remedies for lifestyle-related diseases such as arteriosclerosis, diabetes mellitus, hyperlipidemia, hypertension, and obesity [17-19]. This present study was designed to examine the anti-obesity effects of BG-EE on mice fed with a 45% kcal HF diet. It has previously been shown that a HF diet is a good strategy for inducing obesity [20]. The HF diet thus leads to an increase in body weight, adipose tissue weight, and hyperlipidemia in animals.

The present study determined that BG-EE could improve HF diet-induced obesity, through an examination of whole body weight and adipose tissues, serum cholesterol and TG, lipid peroxidation and lipid contents of the liver, and histopathological assay. Body weight and adipose tissue mass were higher in the HF diet group than in the NC diet group. The present study showed that BG-EE supplementation in a HF diet for 12 wk reduced body weight and adipose tissue mass in all treated groups. Reportedly, Korean red ginseng possesses appetite suppressive properties [21]. Although research has identified several active constituents in these substances possessing appetite suppressive capabilities (e.g., glycosides, saponin, and flavonoids), the ways in which they work to suppress appetite are unclear.

Several studies have shown that ginseng extract and crude saponin reduced body weight and adipose tissue and elevation of plasma lipid levels in mice fed a HF diet [21-23]. It has also been reported that ginsenoside

Rg3 and Rh2, rich ginsenosides in BG, induced the inhibition of adipocyte differentiation in 3T3-L1 cells [24,25]. Obesity is a cause of dyslipidemia characterized by increased TG and decreased HDL-C concentration [26]. It is well known that dietary fat is not absorbed from the intestine unless it has been subjected to the action of pancreatic lipase during the digestion process. Therefore, inhibition of the hydrolysis of dietary fat may decrease intestinal absorption of fat, leading to a reduction in obesity and hyperlipidemia. In our study, we found that serum TG level was significantly decreased in the HF plus 1% BG-EE group. This observation suggests that BG-EE may delay the absorption of dietary fat via the inhibition of pancreatic lipase. The HF diet containing 3% and 5% BG-EE significantly increased HDL-C levels compared with unsupplemented HF diet group. The present results were supported by the report that administration of *Panax ginseng* extract in humans for 8 wk decreased cholesterol levels while increasing HDL level [27]. Long-term ingestion of a HF diet leads to dyslipidemia, increased liver mass, and hepatic steatosis [28,29]. Mice fed a HF diet in this study showed increased liver weight and high lipid content, elevated lipid peroxidation, and developed hepatic steatosis. However, BG-EE treatment decreased the liver lipids and lipid peroxidation which may have an anti-obesity effect through the suppression of dyslipidemia and hepatic steatosis in obese mice. Mean fecal fat values increased in a dose-dependent manner in the BG-EE groups. Sidhu and Oakenfull [30] reported that ingestion of foods containing saponins would increase fecal bile acids and could lower plasma cholesterol concentrations in hypercholesterolemic subjects.

The application of pancreatic lipase inhibitor has been examined as a treatment for diet-induced obesity in humans. It has been clinically reported that a pancreatic lipase inhibitor, orlistat, prevented obesity and hyperlipidemia through the increment of fat excretion into feces and the inhibition of pancreatic lipase [31].

From the results of this study on mice fed a HF diet, it is possible to consider that BG-EE supplementation influences weight control, suppression of hepatosteatosis, amelioration of the serum lipid profile, and improvement of fat-binding capacity. Therefore, BG-EE appears to exert an anti-obesity effect through fat digestion inhibition.

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