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Full Length Research Paper

The beneficial effects of aged black garlic extract on obesity and hyperlipidemia in rats fed a high-fat diet

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The present study was conducted to evaluate the effectiveness of aged black garlic (ABG) extract in alleviating obesity and hyperlipidemia, and regulating antioxidant properties in rats fed high-fat diet. Six-week old male Sprague-Dawley rats were separately fed for 5 weeks with two types of diets; a normal diet (ND) or high-fat diet (HD). Then rats fed HD were randomly assigned into 5 groups and fed with one of the followings: a carrier (control), ABG extract (100, 250 and 500 mg/kg body weight) or simvastatin (1 mg/kg body weight) for another 5 weeks. The body and fat-pad weight, lipid parameter, antioxidant enzyme activity and lipid peroxidation (LPO) in serum and hepatic was investigated. ABG extract significantly lowered body and adipose tissue weight relative to the control. ABG extract significantly improved lipid profile by decreasing serum triglyceride and hepatic total cholesterol compared with the control. ABG extract-treated groups were also observed significant increases glutathione (GSH):oxidized GSH ratio in serum and hepatic compared with the control and near to the level of the normal. Consumption of ABG extract significantly decreased serum LPO level relative to the control. Based on these results, we suggest that the administration of ABG extract improves the body weight gain and dyslipidemia through the suppression of body fat and alteration in lipid profiles and antioxidant defense system.

Key words: Aged black garlic extract, antiobesity, hypolipidemic, antioxidant.

INTRODUCTION

Obesity has increased sharply and steadily in several decades (Karalis et al., 2009). Being obese or overweight is a significant public health problem in a number of countries (Nomura et al., 2008). The ongoing rise in obesity rates arise from the changes in lifestyle, such as the excess consumption of nutrients and declined physical activity (Armitage et al., 2008). Obesity can induce additional metabolic syndromes like fatty liver, dyphysical activity (Armitage et al., 2008). Obesity can induce additional metabolic syndromes like fatty liver, dyslipidemia, diabetes, hypertension and arteriosclerosis (Bray, 2000; Greenway and Smith, 2000). Excess intake of dietary fat is one of the most important factors contributed to the development of obesity and hyperlipidemia in human and rodents (McNamara, 2000; Woo et al., 2008). To prevent or control of overweight status, there are available many different approaches such as dietary control, exercise and medications. However, anti-obesity drugs have been reported on the adverse effects including headache, stomach-ache, vomiting and heart attack (Park et al., 2007). Therefore, there has been growing interests in exploiting the biological activities of medicinal plants because of their natural origin, cost effectiveness and lower side effects (Naik et al., 2003; Slanc et al., 2009). *Allium sativum*, commonly known as garlic, has been used worldwide not only as a food, spice or supplement but also as a traditional medicine since

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Abbreviations: ABG, aged black garlic; **GSH,** glutathione; **GSSG,** oxidized glutathione; **TBARS,** thiobarbituric acid reactive substances; **LPO,** lipid peroxidation; **SIM,** simvastatin.

ancient times (Rahman, 2007). Medicinal actions of garlic include lipid-lowering, antibacterial, antiviral, antifungal, antihypertensive, blood glucose lowering, antithrombotic, antioxidant and antiplatelet actions (Borrelli et al., 2007; Reinhart et al., 2009; El-Khayat et al., 2010). Although garlic is one of the popular food or herbal supplement in Korea and USA (Kim and Kwon, 2009; Blumenthal et al., 2006), some people express their discomfort due to strong odor and flavor of garlic. Thermal processes are commonly used in food manufacturing. One of the important objectives of thermal processes is to raise the sensory quality of foods, their palatability and to extend the range of colors, tastes, aromas and textures in food (Capuano and Fogliano, 2010).

Beside to these effects, heating processes leads to the formation of biological compounds that are not originally present in food. Recently, aged black garlic (ABG) is one of garlic-processed product available on the market in Korea (Choi et al., 2008; Lee et al., 2009). ABG is produced by ageing raw garlic through thermal process at high temperature and high humidity. During these processing, unstable and odorous compounds of raw garlic are converted to stable and odorless compounds S-allylcysteine (SAC) or decomposed to organosulfur compounds such as diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DATS), dithiins and ajoene (Amagase, 2006; Corzo-Martinez et al., 2007). It is also reported that ABG contained antioxidant activity and content of total polyphenol higher than fresh garlic (Choi et al., 2008; Shin et al., 2008). Yet, to date, only limited studies for beneficial effect of ABG is available.

Several studies have investigated that ABG exerted hypoglycemic and hypocholsterolemic effects in animal model of type 2 diabetes mellitus or *in vitro* antioxidant effect on human low density lipoprotein (LDL) (Yang, 2007; Lee et al., 2009; Seo et al., 2009). Therefore, little is known about the effect of ABG on regulation of body weight, level of lipid parameters and antioxidant activity *in vivo* animal model of obesity. For this reason, we investigated whether the effects of ABG on the accumulation of body fat, and the lipid levels and antioxidant activity in serum and liver of rats fed a high-fat diet.

MATERIALS AND METHODS

Chemicals and reagents

Simvastatin (SIM) was purchased from Sigma Chemical Company (St. Louis, USA). All other chemicals and reagent, unless noted otherwise, were obtained from Invitrogen (USA) or Sigma (USA). All the chemicals used were of the highest grade available.

Preparation of samples

Raw garlic was purchased from Uisung agricultural association (Gyeongsangbuk-do, Republic of Korea) in 2009. The unpeeled garlic packed in airtight containers, and was aged at 75°C and 50% humidity level for 14 days in constant temperature and humidity chamber. Aged black garlic were peeled off, mixed with 10 volumes of water, and blended. The samples were extracted with water for 3 h at 100°C and concentrated in a rotary evaporation at 50°C. The extracts were lyophilized to be used for the study.

3T3-L1 preadipocyte culture and differentiation

3T3-L1 preadipocytes were purchased from the Korean Cell Line Bank (KCLB). 3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum (CS) in 100 U/ml of penicillin and 100 mg/ml of streptomycin. Two days post confluence was reached (D0), differentiation was induced by changing the medium to DMEM supplemented with 10% fetal bovine serum (FBS) plus 0.5 mM/L isobutylmethylxanthine (IBMX), 1 mg/ml insulin and 1 mM/L dexamethazone (DEX). After two days (D2), the medium was changed to DMEM containing 10% FBS plus 1 mg/ml insulin. On day 4 (D4), the medium was replaced with DMEM containing 10% FBS and repeated with changes of medium every two days until day 8 (D8). The ABG extract was added to the media on D0, which is the day of the initiation of differentiation. All of cultures were incubated in humidified atmosphere of 5% CO_2 at 37°C.

Oil red O staining

Oil red O Staining and quantification of lipid droplet in 3T3-L1 adipocytes were carried out using adipogeneis assay kit (Cayman, USA). In brief, the cells were removed medium from the wells and then washed with PBS. The cells were added fixative to each well and incubated for 15 min at room temperature. The fixed cells were rinsed with wash solution twice for 5 min each and then dried completely. The well was added oil red O working solution and incubated for 20 min. The stained cells were washed with dH_2O 4 times and then added dye extraction solution to each well gently mixing for 30 min. The absorbance was measured at 510 nm by using microplate reader.

Experimental animals and design

Male Sprague-Dawley rats (5 weeks old) weighing 130 to 150 g were obtained from Shizuoka Laboratory Center Inc. (Hamamatsu, Japan) and acclimated for 1 week prior to experimental use. Sixty rats were randomly divided into 2 groups: normal (n = 10) and high-fat groups (n = 50). Normal group was fed the AIN-93G purified rodent diet while high-fat groups were fed hyperlipidemic diet containing 45% fat. The AIN-93 G purified diet contained 16% fat (soybean oil), 20% protein and 64% carbohydrates, as energy content. The high-fat diet contained 45% fat (soybean oil and lard), 20% protein and 35% carbohydrates, as energy content. The animals fed high-fat diets were then randomly assigned to five groups (n = 10/group): control, ABG extract-treated groups and SIM-administered group. The treated group was orally given the ABG extract with a multiple dose of 100, 250 and 500 mg/kg body weight daily and SIM with a dose of 1 mg/kg body weight daily for 5 weeks. The normal and control group received the vehicle at the same volume during the same period. For oral feeding of ABG extract or vehicles, all groups were consumed *ad libitum* water and AIN-93M diet. The animals were housed at 2 to 3 per plastic cage. They were kept in a temperature-controlled environment animal facility at 22 ± 2°C with a 12 h light/dark cycle. This experiment was approved by the Animal Experiment Committee of National Academy of Agricultural Science (NAAS) in Rural Development Administration (Republic of Korea). All animals were maintained and managed in accordance with NAAS animal use and care protocols.

Blood and tissue collection

After treatment period, animals were deprived of diets for at least 8 h. The rats were anesthetized by overexposure of CO_2 gas and exsanguinated through cardiac puncture. Blood samples were rapidly centrifuged at 3,000 rpm for 15 min at 4℃. The isolated serum was stored at -20℃ before analysis. Hepatic and adipose tissue were quickly removed, weighed, placed in ice-cold saline and stored at -20℃ for further studies.

Serum and hepatic lipids

Serum total cholesterol (TC) and triglyceride (TG) concentrations were determined using commercial kits (Asan, Korea) according to the instructions of the manufacturer. Total lipids from the liver were extracted using the procedure developed by Folch et al. (1957). The extracted samples were dried under nitrogen gas, resolved in 2-propanol containing 10% Triton X-100 (w:w). Hepatic TC and TG concentrations were analyzed with the same enzymatic kit used serum analyses.

Measurement of GSH:GSSG ratio

Reduced glutathione (GSH):oxidized gluthathione (GSSG) ratio in serum and liver were assayed using commercial kit (Calbiochem, USA). The analysis was done according to the manufacturer's instructions.

Assay of lipid peroxidation (LPO) level

Thiobarnituric acid-reactive substance (TBARS) concentrations in serum and liver were quantified spectrophotometrically. The levels of TBARS were estimated using commercial kit (Cayman Chemical, USA) according to the protocol of the manufacturer. Briefly, the 100 µl of serum was mixed with the same volume of sodium dodecyl sulfate (SDS) solution and the mixture was swirled to mix. The sample was mixed with thiobarbituric acid (TBA) reagent. The mixture was boiled at 95℃ for 60 min and then immediately cooled in the ice bath. After 10 min, the sample was centrifuged at 4,000 rpm for 10 min at 4℃. The absorbance of the supernatants was measured using a microplate reader (Molecular Devices, USA) at 535 nm. In liver, TBARS were assayed using 25 mg of hepatic tissue homogenized in 250 µl of RIPA buffer with protease inhibitor cocktail. The homogenates were centrifuged at 3,000 rpm for 10 min at 4℃. The measurement of TBARS level in hepatic tissue was evaluated using the same method of that in serum, described above. The TBARS levels were expressed as mM/mg protein.

Total protein determination

The protein content of the supernatant was determined using a commercial protein assay kit (Bio-Rad Protein Assay Kit, Bio-Rad Laboratories, Inc., CA, USA). Crystalline bovine serum albumin (BSA) was used for the standard curve. The concentration was measured using a spectrophotometer at 595 nm.

Statistical analysis

Data were expressed as means ± S.E. The differences between 2 groups were assessed by independent t-test. The results were also analyzed for statistically significant experimental differences using one-way analysis of variance (ANOVA) and post-hoc Duncan's multiple range tests. Differences were considered statistically significant at p-value < 0.05. All statistical analyses were performed using SPSS (version 12.0).

RESULTS

Inhibitive effect on ABG extract on 3T3-L1 adipocyte differentiation

Firstly, we investigated the effect of the ABG extracts to inhibit mature adipocyte differentiation *in vitro*. To assess the degree of differentiation, Oil red O staining was performed on day 8 (D8) of differentiation. The 3T3-L1 cells treated with AGE (Figure 1B, C, D) showed less color intensity as compared to the non-treated cells (Figure 1A). The Oil red O quantification showed the ABG extracts treatment significantly inhibited differentiation and decreased lipid cells (Figure 1E). At 10 µg/ml concentration, lipid accumulation was significant decreased to approximately 15% compared to the ABG extracts non-treated group.

Effect of ABG extract on weight of body and white adipose tissues

We verified whether the animals fed high-fat 45% diet were definitely induced obesity and hypercholesterolemia compared with rats fed normal diet for 5 weeks (Figure 2). The body weight, serum total cholesterol and adipose tissue weight of rats fed high-fat 45% diet for 5 weeks were increased significantly higher than those of rats fed the normal diet. After 5 weeks of administering vehicle, ABG extract or SIM, the body weights were significantly lower in the normal, ABG extract and SIM-treated groups than in the control group (Table 1). The paratesticular white adipose tissue (WAT) weights were significantly lower in the SIM-treated group than in the control group. The abdominal and perirenal WAT weights were shown significant decreases in the normal and ABG extract-treated groups compared to the control group. Therefore, total WAT weights were significantly shown decreases in the ABG extract and SIM-treated groups near to the level of the normal group. However, the food intakes were not significantly different among the groups.

Effect of ABG extract on lipid parameters of serum and hepatic tissue

The concentrations of serum and hepatic lipids are shown in Table 2. The serum TC concentration was not significantly different among the groups. A significant increase of serum TG concentration in the control group was observed relative to the normal group. However, AB G extract showed significant serum TG lowering effect compared to the control and SIM-treated group. The hepatic TC concentration in the control group was

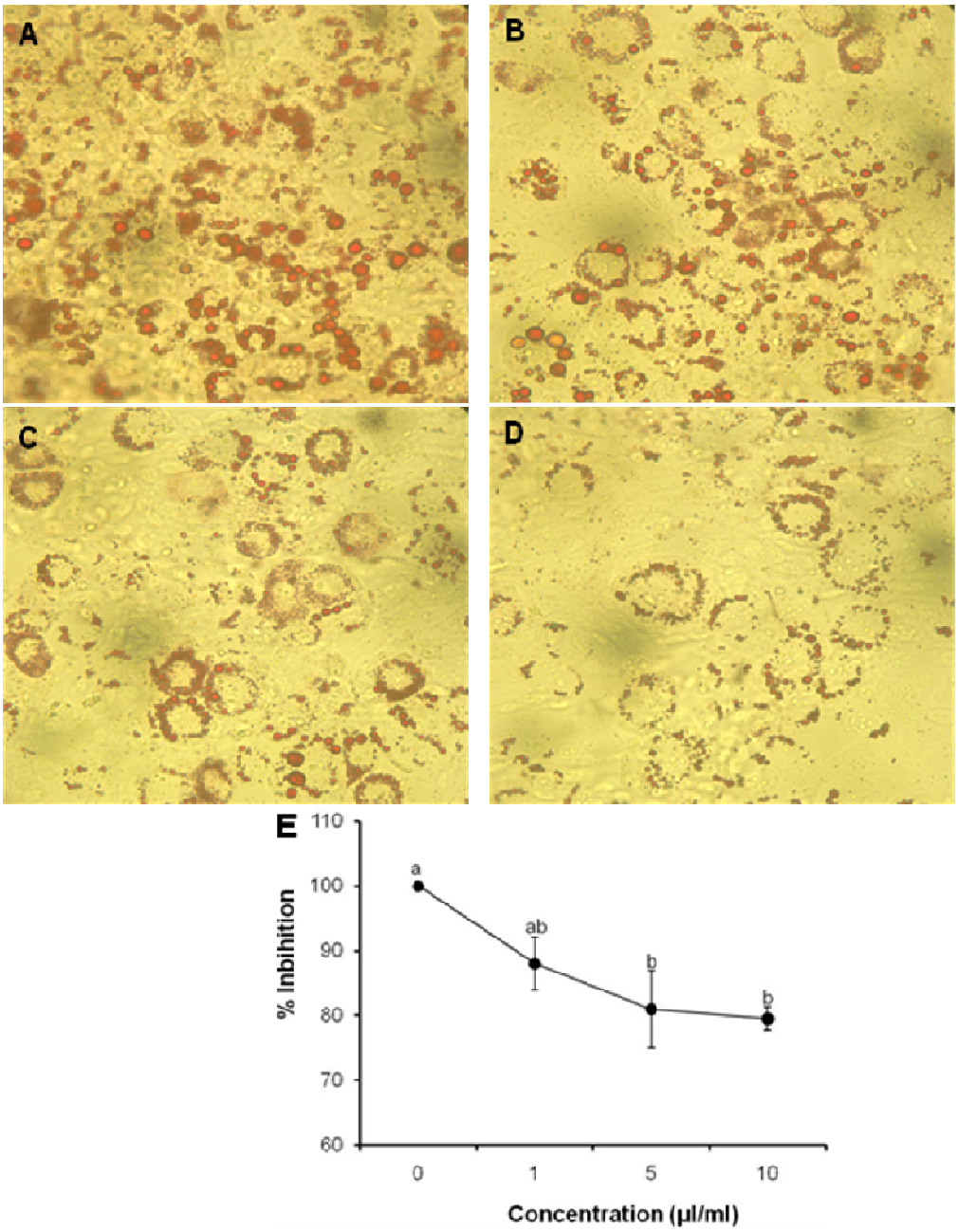

Figure 1. ABG extract inhibited preadipocyte differentiation in a dose-dependent manner. Fully differentiated 3T3-L1 cells both non-treated (A) and treated (B, 1 *µg*/ml; C, 5 *µg*/ml; D, 10 µg/ml) with ABG extract were stained with Oil red O staining solution. The absorbance at 510 nm from 3T3-L1 cells treated with different concentrations of ABG extract (E).

significantly increased higher than that in the normal group while ABG extract or SIM-treated groups were shown significant decreases near to the normal group. The hepatic TG concentration in the control group was also significantly higher than those of the normal group while the administration of ABG extract or SIM resulted in significant lower hepatic TG levels compared to that of the control group.

Effect of ABG extract on GSH:GSSG ratio in serum and hepatic tissue

The GSH:GSSG ratio in serum was significantly in the control group lower than the normal group (normal group, 124.85 ± 1.42; control group, 107.32 ± 3.15) while that of the ABG extract or SIM-treated groups (AGE100, 122.29 ± 2.64; AGE 250, 128.68 ± 1.49; AGE 500, 124.29 ± 3.47;

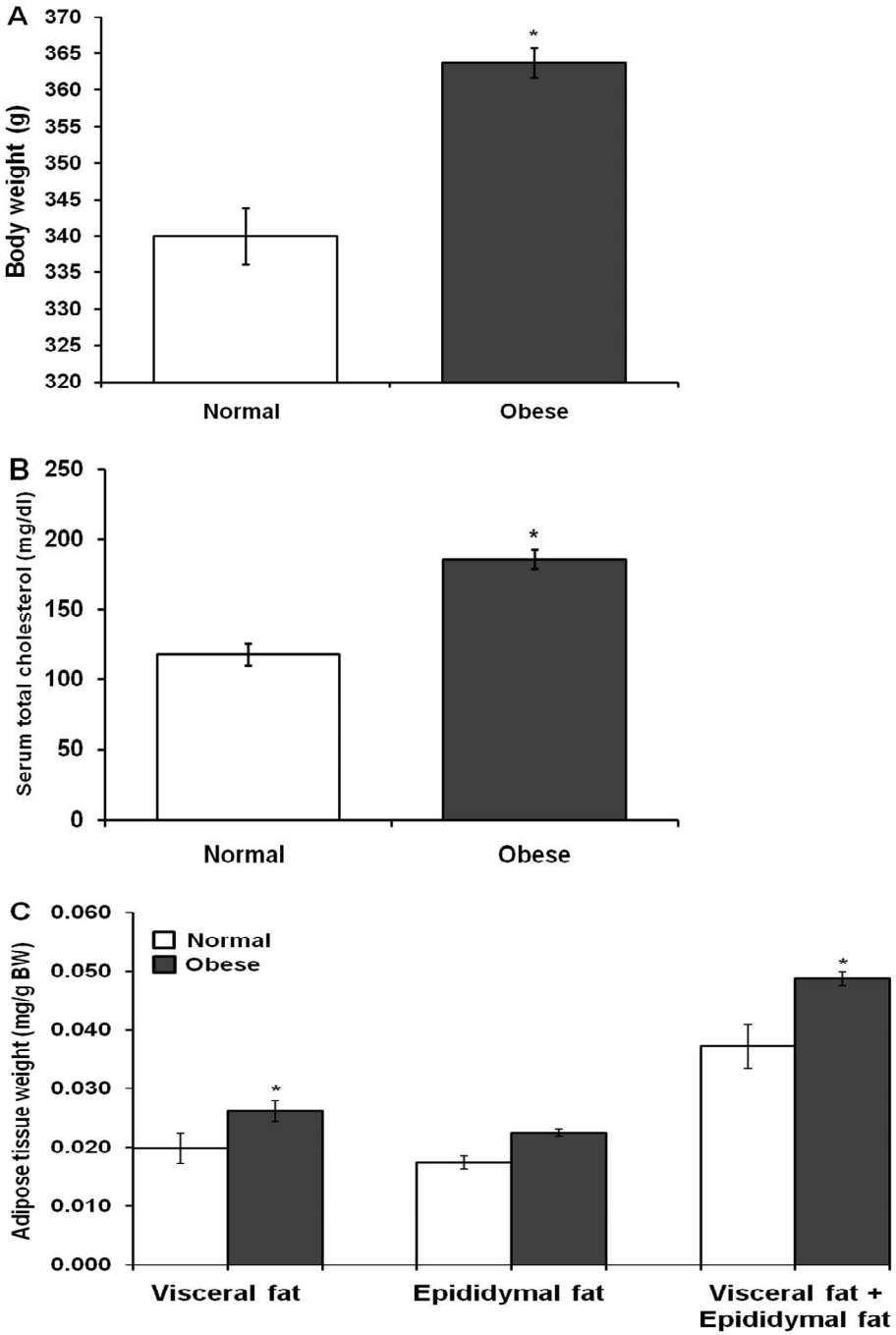

Figure 2. High-fat diet induced obesity and hypercholesterolemia in animals. The rats in each group were fed normal or high-fat 45% diet for 5 weeks (A). Serum total cholesterol level of normal and obese groups at 5-week were compared (B). Adipose tissue weight was measured in visceral and epididymal fats (C). Data are represented as mean ± S.E. for 10 rats per group. Normal, normal diet group; Obese, high-fat 45% diet group. *Significant at $P < 0.05$ compared to the Normal group.

SIM, 125.81 ± 3.28) were shown significant increases near to the normal group (Figure 3A). In liver, the GSH:GSSG ratio was significantly in the control group lower than the normal group (normal group, 221.52 ± 31.4; control group, 86.52 ± 3.43; Figure 3B). The supplementations of ABG extract or SIM significantly increased hepatic GSH:GSSG ratio compared to the control group. Especially, 250 and 500 mg/kg of ABG extract-administered groups were shown no significant differences compared to the normal group (AGE 250,

Table 1. Effects of ABG extract on body weight and white adipose tissue (WAT) weight.

Groups	Body weight (g)	WAT weight (g/100 bw)				Food intake(g/d)
		Paratesticular	Abdorminal	Perirenal	Total WAT	
Normal	515.8 ± 8.0^{b}	2.33 ± 0.10^{ab}	1.98 ± 0.09^{b}	3.65 ± 0.07^{b}	7.74 ± 0.13^{b}	30.1 ± 1.7^{ns}
Control	571.6 ±13.2^{a}	2.84 ± 0.17^{a}	2.52 ± 0.11^{a}	4.02 ± 0.08^{a}	9.25 ± 0.34^{a}	31.9 ± 1.0
AGE 100	532.6 ± 9.1^{b}	2.31 ± 0.08^{ab}	2.08 ± 0.17^{b}	3.48 ± 0.07^{b}	7.74 ± 0.38^{b}	32.0 ± 1.2
AGE 250	527.7 ± 15.6^{b}	2.39 ± 0.08^{ab}	2.07 ± 0.10^{b}	3.39 ± 0.76^{b}	7.79 ± 0.23^{b}	31.0 ± 0.5
AGE 500	524.5 ± 12.2^{b}	2.42 ± 0.10^{ab}	2.11 ± 0.11^{b}	3.36 ± 0.11^{b}	7.95 ± 0.25^{b}	28.8 ± 0.9
SIM	530.0 ± 11.4^{b}	2.24 ± 0.30^{b}	2.28 ± 0.15^{ab}	4.11 ± 0.22^{a}	7.96 ± 0.61^{b}	30.6 ± 0.2

Values are represented as mean ± S.E. for 10 rats per group. ^{a,b}Values with different letters are significantly different among columns at P-values < 0.05 by Duncan's multiple range test. ^{ns}Not significant.

Table 2. Effects of supplementation of ABG extract on concentrations of serum and hepatic lipid parameters.

Groups	Serum (mg/dl)		Hepatic (mg/100 mg)	
	TC	TG	TC	TG
Normal	128.5 ± 5.7^{ns}	150.0 ± 8.9^{d}	78.1 ± 3.8^{b}	148.4 ± 5.8^{b}
Control	119.3 ± 3.7	305.6 ± 9.4^{a}	118.9 ± 9.6^{a}	176.4 ± 9.4^{a}
AGE100	118.3 ± 3.3	186.4 ± 7.9^{c}	70.3 ± 5.4^{b}	154.0 ± 9.8^{b}
AGE250	128.5 ± 5.5	185.0 ± 8.5^{c}	63.5 ± 3.7^{b}	158.9 ± 9.0^{b}
AGE500	125.5 ± 6.7	147.1 ± 7.3^{d}	75.3 ± 3.3^{b}	158.7 ± 5.2^{b}
SIM	130.9 ± 8.3	244.3 ± 9.1^{b}	80.0 ± 7.4^{b}	140.3 ± 9.1^{b}

Values are represented as mean ± S.E. for 10 rats per group. ^{a,b,c,d}Values with different letters are significantly different among columns at P-values < 0.05 by Duncan's multiple range test. ^{ns}Not significant.

269.75 ± 22.47; AGE, 247.75 ± 20.62).

Effect of ABG extract on LPO in serum and hepatic tissue

The serum TBARS level of the control group (45.33 ± 1.37 mM/mg protein) was significantly higher than those of the normal group (21.08 ± 1.30 mM/mg protein, Figure 4A). However, the administration of ABG extract or SIM (AGE 100, 27.22 ± 5.29 mM/mg protein; AGE 250, 22.98 ± 2.38 mM/mg protein; AGE 500, 19.54 ± 1.12 mM/mg protein; SIM, 27.44 ± 1.52 mM/mg protein) significantly decreased serum TBARS levels compared with the control group. There were no significant differences in hepatic TBARS levels among the groups (Figure 4B).

DISCUSSION

Obesity has become a major public health problem worldwide. The concerns associated with obesity are related to numerous symptoms of metabolic syndrome, including hypertension, dyslipidemia, insulin resistance and glucose intolerance. A variety of approaches could be used to prevent and control obesity in human. Since perfect cure or prevention for obesity are yet to be found and most of anti-obesity medications could have side effects, there has been growing interests in investigating the discovery of new naturally occurring materials (Monteiro et al., 2008). Among these, extensive researches carried out on garlic have reported the biological effects, which include hypolipidemic (Ejaz et al., 2003), hypocholesteroliemic (Asdaq et al., 2009) and anti-obesity effect (Elkayam et al., 2003). Garlic contains a high content of organosulfur components and flavonoids. The major compounds in garlic that are known to contribute to the potent biological activity are sulfur-containing compounds. However, it has been known that allicin, the major biologically active component of garlic, is absent from garlic and garlic preparations because allicin is an unstable and transient compound with oxidant activity (Lawson et al., 1992; Lawson and Gardner, 2005). Therefore, allicin is nearly undetectable in blood circulation after garlic consumption due to decomposition into other organosulfur compounds. The ABG is a type of garlic-processed foods in the Korea, Thailand and Japan (Lee el al., 2011). It is manufactured by ageing whole bulbs of garlic at high temperature and high humidity.

During the ageing process, γ-glutamyl cysteine in intact garlic bulbs is converted to water-soluble SAC, the major unique organosulfur compound in ABG, which contributes

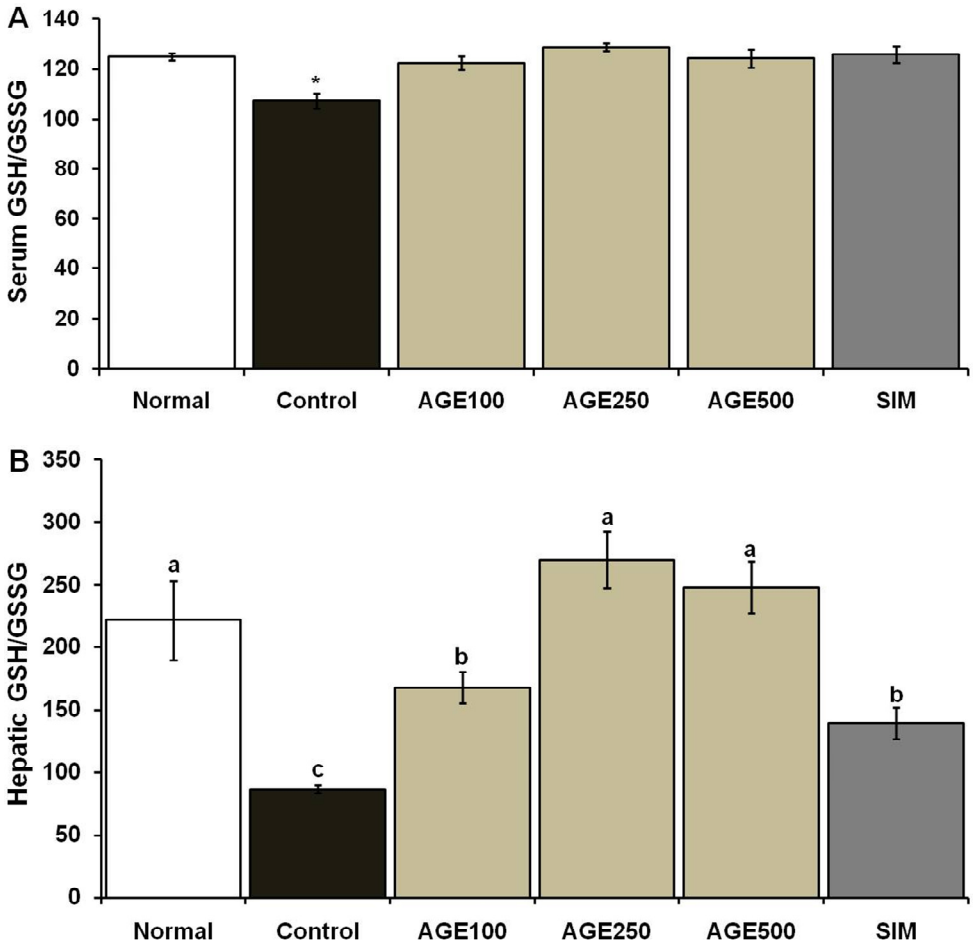

Figure 3. ABG extract recovered from reduction of the GSH:GSSG ratio by inducing high fat diet for 5 weeks. The GSH:GSSG ratio was measured in serum (A) and liver (B). Data are represented as mean ± S.E. for 10 rats per group. Normal, normal diet + orally vehicles; Control, high-fat 45% diet group + orally vehicles; AGE100, high-fat 45% diet + orally 100 mg/kg bw of ABG extract; AGE250, high-fat 45% diet + orally 250 mg/kg bw of ABG extract; AGE500, high-fat 45% diet + orally 500 mg/kg bw of ABG extract; SIM, high-fat 45% diet + orally 1 mg/kg bw of SIM. *Significant at $P < 0.05$ compared with among the groups. ^{abc}Values with different letters are significantly different among the groups at *P*-values<0.05 by Duncan's multiple range tests.

to the health benefits (Imai et al., 1994; Amagase, 2006). The phenolic compounds in ABG are also reported higher than fresh garlic. It was demonstrated that the total polyphenol content of ABG was approximately 10.0 mg/g while raw garlic contained 3.67 mg/g (Jang et al., 2008). In the present study, we tried to determine the possibility the effect of ABG extract for 5 weeks on antiobesity, control of lipid parameters and antioxidant activity in high-fat diet induced obese rats. We measured the inhibitive effect of ABG extract on mature adipocyte differentiation of 3T3-L1 preadipocyte. The results of the present study showed ABG extract inhibited 3T3-L1 preadipocyte differentiation and fat accumulation compared with the untreated cells. This is in agreement with Ambati et al. (2009) who reported that ajoene, a sulfur-containing compound of garlic, significantly decreased lipid accumulation in maturing 3T3-L1 preadipocyte. A significant decrease in final body weight was observed in the ABG extract and SIM-administered groups compared with the control group. In association with lower body weight, reduced WAT weights in the abdominal and perirenal were observed in the ABG extract-administered groups to the level of the normal group although the food intake was approximately the same for all groups. Previous studies reported that ABG consumption at the level of 5% of the diet did not influence body weight in db/db mice, type 2 diabetes mellitus animal model, during 7 weeks (Lee et al., 2009; Seo et al., 2009). This finding is shown the possibility that the body weight-lowering effect of ABG extract on high-fat diet induced obese male rats. We assume that these differences between the previous studies and ours are caused by dose of ABG, routes of

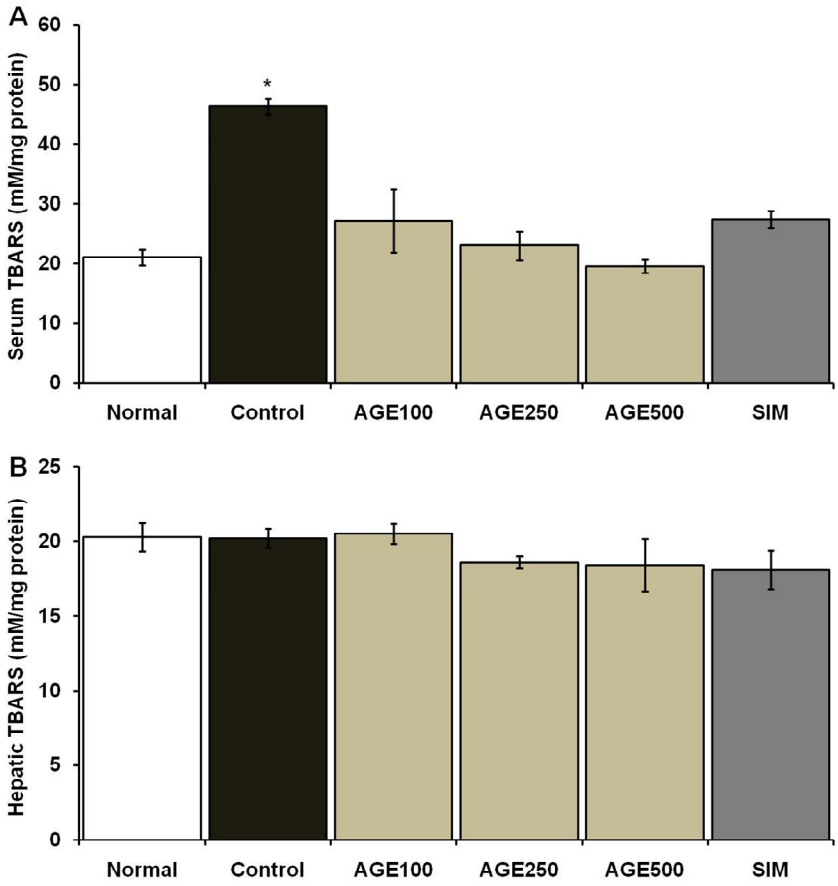

Figure 4. Effect of ABG extract on LPOs of rats. The LPO levels of the groups were compared in serum (A) and liver (B). Data are represented as mean ± S.E. for 10 rats per group. Normal, normal diet + orally vehicles; Control, high-fat 45% diet group + orally vehicles; AGE100, high-fat 45% diet + orally 100 mg/kg bw of ABG extract; AGE250, high-fat 45% diet + orally 250 mg/kg bw of ABG extract; AGE500, high-fat 45% diet + orally 500 mg/kg bw of ABG extract; SIM, high-fat 45% diet + orally 1 mg/kg bw of SIM.*Significant at $P < 0.05$ compared with among the groups.

administering ABG and animal model. We measured the effect of ABG extract on serum and hepatic lipid profile. In this study, consumption of ABG extract significantly decreased serum TG, hepatic TC and TG compared with the control group. However, Seo et al. (2009) reported that ABG significantly decreased not only serum TC concentration, but also serum TG concentration. Pourkabir et al. (2010) also reported that garlic powder supplementation at the level of 2.5 and 5% of the diet improved serum TG, TC, VLDL-C and LDL-C levels in hypercholesterolemic guinea pigs. Gorinstein et al. (2006) reported that the garlic samples of raw and boiled at 100°C for 20 min significantly hindered the cholesterol-induced increases in plasma TC and LDL-C but not plasma TG. In agreement with our research results, Lee et al. (2011) demonstrated that, by means of red garlic extract (light color than black garlic) supply, the TC level of serum did not show significant differences between obese group (control) and the groups fed red garlic extract while a significant decrease of TG level was observed in the groups fed red garlic extract compared to the control groups. These inconsistent results of re-searches could be attributable, in part, to methodological limitations of non-standardized extract used previous studies. Further study is needed to characterize the composition and standardize the ABG extract.

The superoxide anions including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in cells and tissues under increased oxidative stress induced by obesity and hyperlipidemia (Furukawa et al., 2004). The GSH is a central role to the cellular antioxidant defense system and acts as an essential cofactor for antioxidant enzymes such as glutathione peroxidase (GPx). GSH is consumed by the glutathione reductase (GR) to detoxify peroxides produced due to increased lipid peroxidation (Kumar et al., 2011).

Therefore, GSH:GSSG ratio is a good indicator of oxidative stress in cells and tissues. In this study, our results were shown that a significant decrease in the GSH:GSSG ratio was observed in the serum and hepatic tissue of the control group compared to the normal group, whereas the GSH:GSSG ratios in serum and hepatic tissue of the ABG extract-administered groups were comparable to the normal group and higher than the control group. This suggested that the balance between GSH and GSSG was maintained in the serum and liver when ABG extract was administered under oxidative stress status induced by high fat-diet. This is in agreement with earlier reports showing that ABG could be more effective in removing superoxide anions (Lee et al., 2009). The antioxidant action of ABG could be responsible for the higher content of the polyphenolic compounds and SAC. It was reported that the total polyphenol content of ABG was 3 times higher than that of raw garlic (Jang et al., 2008). The measurement of TBARS concentration is commonly used as an indicator of LPO process and indirectly of oxidative stress (Beltowski et al., 2000). We have observed a significant increase in the serum TBARS level of the control group, and a decrease that of the ABG extract-administrated groups to the normal group. Lee et al. (2009) investigated the effect on TBARS level in db/db mice, an animal model of type 2 diabetes mellitus, fed ABG-contained diet. They observed that ABG supplementation was effective in lowering hepatic TBARS compared to the control and garlic groups. These findings support our results that ABG triggers the decrease of LPO under oxidative stress status such as obesity, hyperlipidemia and diabetes mellitus.

In conclusion, our study showed evidences that the administration of ABG extract is capable of ameliorating weight control and lipid parameters, and restoring antioxidant balance in high-fat diet induced obesity. Based on the results obtained from present study, ABG extract may play a role in reducing body fat, lowering hyperlipidemia and protecting against oxidative stress in obese status. However, further studies are needed to elucidate the underlying mechanisms.

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