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EFFECT OF GARLIC ON HIGH FAT INDUCED OBESITY

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The present study was performed to examine the effects of garlic on obesity and blood lipid profiles in high-fat induced obesity mice model, and to elucidate the molecular mechanisms responsible for such effect. C57BL/6 mice were fed a standard diet (STD) or high-fat diet (HFD) for 5 weeks to induce obesity. Mice were then randomly divided into four groups with 10 mice per group, and fed experimental diet for 4 weeks; STD group, HFD group, HFD containing 2% or 4% garlic group (HFD + G2 or HFD + G4, respectively). Administration of garlic significantly reduced HFD-induced body weight, epididymal fat accumulation, hyperlipidemia and hypercholesterolemia. Consequently, the atherogenic indexes were reduced by 83% and 91%, respectively, in 2% and 4% garlic supplemented group. Liver steatosis induced by HFD was ameliorated by garlic supplementation. Furthermore, garlic affected the down regulation of expression patterns of epididymal adipose tissue genes such as peroxisome proliferator-activated receptor γ (PPARγ), acetyl CoA carboxylase (ACC), adipose specific fatty acid binding protein (aP2), and glycerol-3-phosphate dehydrogenase (GPDH). These results suggest that garlic may have a potential benefit in preventing obesity.

Keywords: Garlic – hyperlipidemia – fat accumulated genes – anti-obesity

INTRODUCTION

Obesity is one of the fastest growing major diseases in many areas of the world including Europe, United States and Asia. It is associated with increased risk of developing diabetics, hypertension and hyperlipidemia. Although the adipocyte has previously been viewed as a passive participant in the generation of obesity, this cell has recently been recognized as having a more active role in the regulation of energy homeostasis and body composition [8]. Thus, knowledge about the mechanisms that direct adipocyte differentiation and adipocyte specific gene expression is rapidly advancing.

Peroxisome proliferator-activated receptors (PPARs) and adipocyte specific fatty acid binding protein (aP2) are expressed in adipocytes, known as adipocyte differentiation markers [12, 21]. PPARγ has been implicated in mediating the expression of

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fat specific genes and in activating the program of adipocyte differentiation [21]. Furthermore, PPARγ plays an essential role as the transcription factors regulating expression of the aP2 [22].

Due to the side-effects associated with many of the anti-obesity drugs, more recent drug trials have focused on the herb medicines. Furthermore, it has been suggested that botanicals may, in the future supplement single-entity drugs in disease treatment and prevention [14, 18].

Garlic (*Allium sativum* L.) has attracted particular attention of modern medicine because of its widespread health use around the world. To date, many favorable experimental and clinical effects of garlic such as hypoglycemic, hypolipidemic, anti-atherosclerotic, anticoagulant, antihypertensive, anticancer, antioxidant, hepatoprotective and immuno modulation properties have been reported [1, 2, 7, 10]. Despite the positive evidence from few studies on the beneficial effect of garlic extract on hypolipidemic and diabetic properties, the effect on obesity, which is closely associated with diabetes and cardiovascular disease, has not yet been elucidated. Therefore, the present study was performed to examine the effects of garlic on obesity and blood lipid profiles in high-fat induced obesity mice model, and to elucidate the molecular mechanisms responsible for such effect.

MATERIALS AND METHODS

Preparation of garlic sample

Fresh garlic (*Allium sativum* L.), purchased from seosan (Chungnam, Korea) in July 2006, were identified by botanists in the herbarium of Hanseo University, Korea. The garlic bulbs were peeled, vacuum dried, and powdered.

Animals and diets

Four weeks old male C57BL/6 mice (Jungang Lab. Animal Inc. Seoul, Korea) were individually housed in cages and placed in a room with 12 : 12 h light-dark cycle and an ambient temperature of 24 ± 2 °C. Mice were fed commercial chow diet for 1 week after arrival for adaptation. For induction of obesity, mice were fed a commercial high fat diet (HFD, Feed Lab., Hanam, Korea) (% kcal; carbohydrate : protein : fat = = 20 : 20 : 60) for 5 weeks. Lard was used to induce obesity in HFD. They were then randomly assigned into three groups (10 mice per group): high fat diet (HFD) and high fat diet containing 2% or 4% garlic groups (HFD + G2 or HFD + G4, respectively). Garlic was substituted for a portion of HFD, and other ingredients were modified according to the composition of garlic to provide the same percentages of carbohydrate, protein, fat, fiber and mineral between the diet groups. Normal control group (STD) fed an AIN-93 based standard diet was also included in the experimental group. Food intake and body weights were recorded every three days during the 4

weeks of feeding period. Animals had free access to water and the care of the animals was consistent with the Korea National Institutes of Health guidelines on the care and use of laboratory animals.

Animal treatment and biochemical assays

All mice were fasted for 15 h prior to being sacrificed, and anesthetized using zoletil. Blood samples were taken from the abdominal aorta and were centrifuged for the determination of plasma biochemicals. Liver and epididymal fat tissue were dissected, and weighed. All samples were stored at –70 °C until analyzed. Plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, GOT and GPT concentrations were assayed using a commercial kit (Sigma-Aldrich, St. Louis, MO, USA).

Hepatic histology

The hepatic tissue samples were immediately collected from identical lobes of liver fixed in 10% formalin solution for 24 h. The hepatic tissue was subsequently dehydrated with a series of ethanol solution, from 75 to 100%, before being embedded in paraffin wax. Cross-sections of 4 μm in thickness were cut and stained with hematoxylin and eosin, and were examined by light microscopy (Olympus Optical Co., Tokyo, Japan).

Total RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from epididymal fat tissue using TRI reagent (Sigma-Aldrich, USA). According to the protocol, the reverse transcription (RT) was performed at 42 °C for 1 h, and heated at 95 °C for 5 min to inactivate the Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT) enzyme. The following primers were used: Peroxisome proliferation-activated receptor γ (PPARγ), forward (5'-AAAGACCC-AGCTCTACAACA-3') and reverse (5'-TCGTAGATGACAAATGGTGA-3'); acetyl CoA carboxylase (ACC), forward (5'-GGGCTACCTCTAATGGTCTT-3') and reverse (5'-CTACCTGATGGTAAATGGGA-3'); adipose specific fatty acid binding protein (aP2), forward (5'-CCTCGAAGGTTTACAAAATGTGTGA-3') and reverse (5'-AAACTCTTGTGGAAGTCACGCCTTT-3'); glycerol-3-phosphate dehydrogenase (GPDH), forward (5'-GAACTAAGGAGCAGCTCAAAGGTTC-3') and reverse (5'-CAGTTGACTGACTGAGCAAACATAG-3'). β-actin (forward:5'-AGGTATC-CTGACCCTGAAGTACCCC-3'; reverse:5'-GTTGCCAATAGTGATGACCTGG-CCG-3') was used as an internal standard.

The PPARγ amplification was performed by denaturing at 94 °C for 30 sec, annealing at 59 °C for 30 sec, and extension at 72 °C for 30 sec for 30 cycles and finally 72 °C for 5 min. The ACC amplification was performed by denaturing at 94 °C for 30 sec, annealing at 57 °C for 30 sec, and extension at 72 °C for 30 sec for 30 cycles and finally 72 °C for 5 min. The aP2 amplification was performed by denaturing at 94 °C for 30 sec, annealing at 60 °C for 30 sec, and extension at 72 °C for 30 sec for 30 cycles and finally 72 °C for 5 min. The GPDH amplification was performed by denaturing at 94 °C for 30 sec, annealing at 57 °C for 30 sec, and extension at 72 °C for 30 sec for 30 cycles and finally 72 °C for 5 min. The β-actin amplification was performed by denaturing at 94 °C for 30 sec, annealing at 63 °C for 30 sec, and extension at 72 °C for 30 sec for 25 cycles and finally 72 °C for 5 min. The PCR products were loaded onto 2% agarose gel and stained with ethidium bromide. The relative level of RT-PCR reaction products was measured with Forg gel image analysis system (Spot Densitometry Program, Core Bio, Seoul, Korea) under UV light.

Statistical analysis

Results were presented as mean ± SEM, and the data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Results were considered to be statistically significant at $P < 0.05$.

RESULTS

Effects on body weight, food intake, and food efficiency ratio (FER)

Table 1 shows the body weight gain and food intake of animals fed on the experimental diets. Consumption of HFD significantly increased food intake, body weight gain and FER ($P < 0.05$). The body weight gain of HFD was significantly increased by 3.4-fold compared with STD group ($P < 0.05$). Dietary garlic supplement significantly reduced the food intake in a dose-dependent manner in HFD fed mice. Food intakes of HFD + G2 and HFD + G4 groups were 88% and 80%, respectively, of the HFD control group. However, supplementation of garlic completely blocked the body weight gain and FER. Body weight gain and FER of HFD + G2 group were only 16% and 18%, respectively, of HFD group. Surprisingly, 4% garlic supplementation exhibited negative body weight gain and FER with only 20% reduction of food intake.

Effects on liver weight and epididymal fat mass

The relative weights of the liver and epididymal fat depots were significantly increased by HFD, and were significantly reduced by garlic supplementation. The

Table 1
Effects of garlic on food intake, body weight gain and food efficiency ratio in high fat diet fed mice

	Groups			
	STD	HFD	HFD + G2	HFD + G4
Food intake (g/day)	1.90±0.16^{d}	5.22±0.09^{a}	4.61±0.02^{b}	4.19±0.11^{c}
Initial body weight (g)	24.6±0.6^{b}	30.3±0.9^{a}	31.6±0.6^{a}	31.2±1.0^{a}
Final body weight (g)	27.2±0.4^{d}	39.3±1.2^{a}	33.0±0.6^{b}	30.0±1.2^{c}
Body weight gain (g/day)	2.6±0.4^{b}	8.9±1.2^{a}	1.4±0.6^{b}	–1.2±1.2^{c}
FER	1.4±0.2^{a}	1.7±0.2^{a}	0.3±0.1^{b}	–0.3±0.3^{b}

Data are mean ± SEM values ($n = 10$). STD, standard diet fed group; HFD, high fat diet fed group; HFD + G2, high fat diet supplemented with 2% garlic; HFD + G4, high fat diet supplemented with 4% garlic, FER = body weight gain/food intake. $^{a–d}$Different superscript letter means significantly different ($P < 0.05$) among groups.

Table 2
Effects of garlic on liver and epididymal fat tissue weights in high fat diet fed mice

Organ weight (mg/g body weight)	Groups			
	STD	HFD	HFD + G2	HFD + G4
Liver	41.3±1.3^{b}	47.2±1.0^{a}	39.5±2.1^{b}	37.0±3.7^{b}
Epididymal fat tissue	12.8±2.3^{c}	51.7±11.1^{a}	49.2±4.8^{a}	11.6±1.4^{b}

Data are mean ± SEM values ($n = 10$). STD, standard diet fed group; HFD, high fat diet fed group; HFD + G2, high fat diet supplemented with 2% garlic; HFD + G4, high fat diet supplemented with 4% garlic. $^{a–c}$Different superscript letter means significantly different ($P < 0.05$) among groups.

Table 3
Effects of garlic on plasma lipid concentration in high fat diet fed mice

	Groups			
	STD	HFD	HFD + G2	HFD + G4
Triglyceride (mg/dl)	90.6±15.9^{b}	213.6±14.8^{a}	118.7±21.4^{b}	102.4±16.5^{b}
Total cholesterol (mg/dl)	93.1±18.7^{c}	330.9±28.0^{a}	159.4±16.6^{b}	138.2±19.3^{b,c}
HDL cholesterol (mg/dl)	40.9±4.1^{c}	33.8±4.4^{a}	64±3.7^{a}	75.1±5.3^{b}
LDL cholesterol (mg/dl)	51.5±10.1^{b}	254.4±28.5^{a}	81±13.2^{b}	73.7±4.3^{b}
AI* (mg/dl)	1.3±0.4^{b}	8.8±0.8^{a}	1.5±0.2^{b}	0.8±0.2^{b}

Data are mean ± SEM values ($n = 10$). STD, standard diet fed group; HFD, high fat diet fed group; HFD + G2%, high fat diet supplemented with 2% garlic; HFD + G4%, high fat diet supplemented with 4% garlic. $^{a–c}$Different superscript letter means significantly different ($P < 0.05$) among groups. *Total cholesterol–HDL cholesterol/HDL cholesterol.

relative liver weights were reduced by 11% and 16%, respectively, in HFD + G2 and HFD + G4 group (Table 2). The effect of garlic on epididymal fat tissue was more dramatic exhibiting 78% reduction with 4% garlic supplementation ($P<0.05$).

Effects on blood biochemistry

HFD-induced hyperlipidemia was significantly improved by garlic supplementation (Table 3). The plasma TG, TC and LDL cholesterol concentrations were 45%, 52%, and 68% lower, respectively, in HFD + G2 group than HFD group ($P<0.05$), whereas the HDL cholesterol concentration was 1.9-fold higher in HFD + G2 group than in the HFD group ($P<0.05$). These effects of garlic were dose dependent. The plasma TG, TC, and LDL cholesterol concentrations of HDF + G4 group were 52%, 58%, and 71% lower, respectively, and the HDL cholesterol concentration was 2.2-fold higher in HFD + G4 group than HFD group. Consequently, the garlic supplementation in the HFD mice significantly reduced the atherogenic index (AI) by 83% and 91%, respectively, in HFD + G2 and HFD + G4 group.

The activities of liver function markers, such as plasma GOT and GPT concentration, were significantly elevated by HFD compared with the STD group (Fig. 1). Animals administered with garlic showed significant reduction in these marker enzyme activities to almost normal levels. The plasma GOT and GPT concentrations of HFD group were significantly increased by 2.3- and 1.6-fold, respectively, compared with those of STD group ($P<0.05$). Supplementation of 4% garlic in the HFD group significantly reduced the GOT and GPT levels by 73% and 41%, respectively.

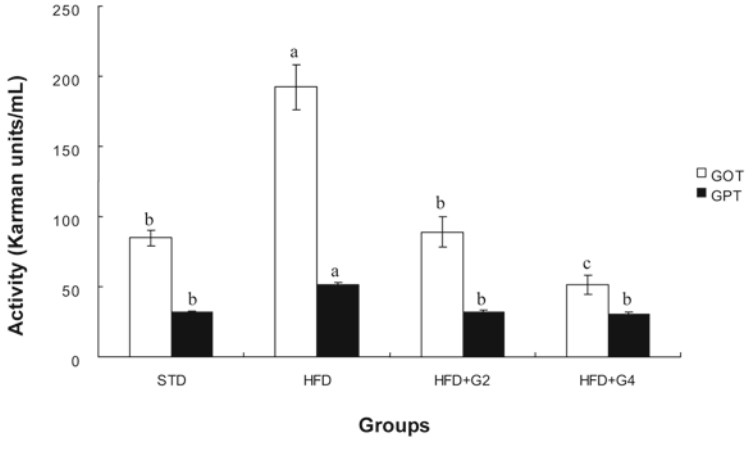

Fig. 1. Effects of garlic on activities of plasma GOT and GPT in high fat diet fed mice. Data are mean ± SEM values ($n = 10$). STD, standard diet fed group; HFD, high fat diet fed group; HFD + G2, high fat diet supplemented with 2% garlic; HFD + G4, high fat diet supplemented with 4% garlic. [a–c] Different superscript letters means significantly different ($P<0.05$) among groups

Effects on histological change of the liver

The HFD group showed the accumulation of hepatic lipid droplets (Fig. 2B), and severe steatosis compare to STD group which exhibited normal hepatic histology (Fig. 2A). The clear vacuoles would have contained lipid in the living cells, however the histological fixation caused it to be dissolved and hence only empty spaces remained. In the garlic supplemented groups, histological abnormalities were not present (Fig. 2C and D), and morphological liver condition was similar to that of the STD group.

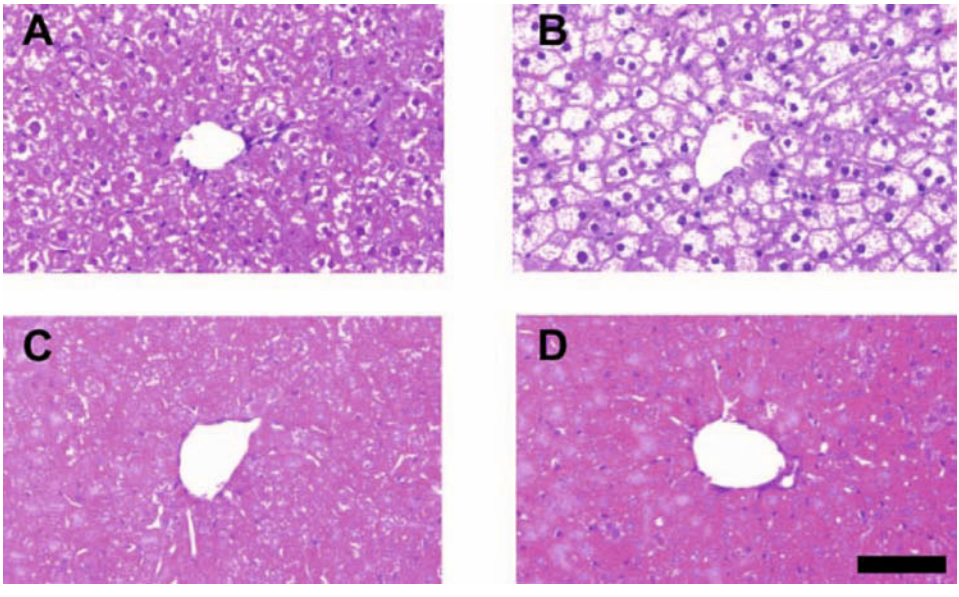

Fig. 2. Effects of garlic on hepatic tissue morphology in high fat diet fed mice. Fat accumulation, in the form of large fat droplets, is present in liver of mouse fed a high-fat diet (HFD, B) alone. Representative pictures of hematoxylin and eosin-stained sections of liver tissue from mouse fed a standard diet group (STD, A), high fat diet supplemented with 2% garlic (HFD + G2, C), and high fat diet supplemented with 4% garlic (HFD + G4, D) show few fat droplets. Scale bar represents 50 μm

Effects on gene expression in epididymal fat

To investigate whether garlic affects the expression of adipogenic genes, RT-PCR analysis was performed (Fig. 3A and B). The mRNA expressions of peroxisome proliferator-activated receptor γ (PPARγ), acetyl CoA carboxylase (ACC), aP2, and glycerol-3-phosphate dehydrogenase (GPDH) were significantly increased after high fat feeding as compared to standard diet fed animals. The expression levels of the PPARγ, ACC, aP2, and GPDH genes in HFD group were increased by 1.7-fold, 2.6-fold, 1.3-fold, and 1.4-fold, respectively, compared to those observed in STD group ($P<0.05$). In contrasts, dietary supplementation with garlic significantly reversed the

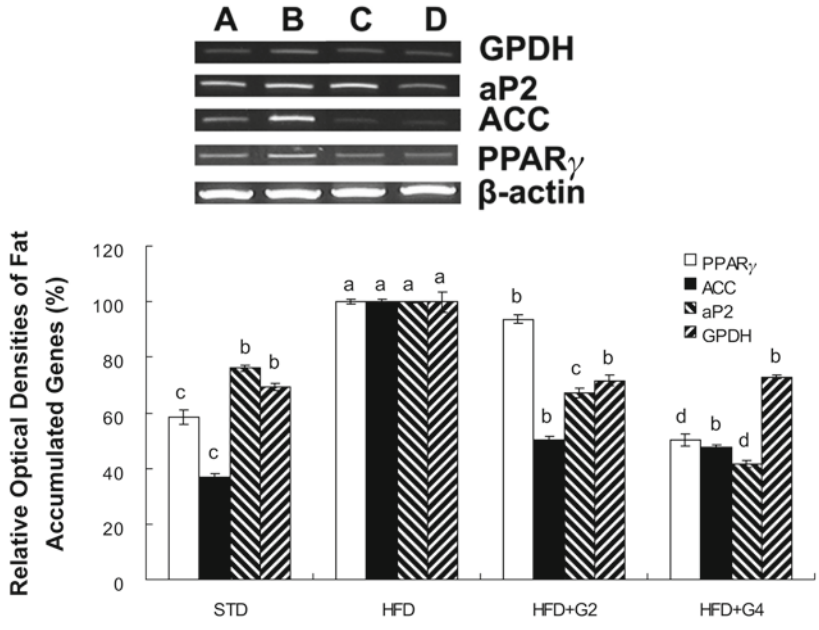

Fig. 3. Effect of garlic on gene expressions related to fat accumulation in epididymal fat tissues. Band density was quantified using a densitometer and normalized to that of HFD group. A: STD, standard diet fed group; B: HFD, high fat diet fed group; C: HFD + G2, high fat diet supplemented with 2% garlic; D: HFD + G4, high fat diet supplemented with 4% garlic. □, peroxisome proliferator-activated receptor γ (PPARγ); ■, acetyl CoA carboxylase (ACC); ▨, adipose specific fatty acid binding protein (aP2); ▧, glycerol-3-phosphate dehydrogenase (GPDH)

HFD-induced elevation of these genes implicated in adipogenesis. Significant down-regulation of the PPARγ (50% decrease), ACC (52% decrease), aP2 (58% decrease), and GPDH (27% decrease) genes were observed in 4% garlic supplemented group ($P<0.05$).

DISCUSSION

There are several reports showing that garlic has hypoglycemic, hypolipidemic, anti-coagulant, antihypertensive, anticancer and antioxidant properties [1, 2, 7, 10]. However, there have hitherto been no reports on the inhibitory effects on obesity induced by HFD. In this study, the anti-obesity effects of garlic were investigated through body weight, food intake, epididymal fat mass, plasma lipids profile, and the expression of fat accumulating genes, as well as the histological examination of the hepatic tissues in HFD-induced obese mice. The results of this study showed that garlic supplement significantly ameliorated HFD induced body weight gain, epididymal adiposity and dyslipidemia in mice. Additionally, garlic affected on improving

histological change of hepatic tissue through reducing plasma GOT and GTP levels, as well as plasma TG and TC levels. Serum GOT and GPT levels are the measure of liver function that increases in fatty livers and is associated with metabolic syndrome [9]. Furthermore, garlic supplement significantly decreased fat accumulating genes, such as PPARγ, ACC, aP2 and GPDH mRNA expression. PPARγ is the major adipogenic transcription factor, and induces glucose and fatty acid uptake by directly or indirectly enhancing the transcription of genes encoding proteins such as aP2 [19]. Consequently, activation of PPARγ could constitute an important part of the molecular mechanism behind the adipogenic effect of overfeeding. Vidal-Puig et al. [23] observed the 50% increases in $PPAR\gamma_2$ mRNA in normal mice exposed to a HFD. Margareto et al. [13] suggested that HFD induced adipocyte hypertrophy may be mediated by $PPAR\gamma_2$. They also observed elevated mRNA level of PPARγ with HFD was blunted in β_3-adrenergic agonist treated animals. A similar expression pattern was observed with aP2 mRNA expression. Our data suggest that the decrease in plasma TG and cholesterol levels by garlic supplement could be mediated by a diminution in the expression levels of lipogenic genes. In fact, garlic treatment decreased the expression levels of the crucial lipogenic enzymes. The major of enzyme in fat accumulation has been known acetyl CoA carboxylase (ACC) and glycerol-3-phosphate dehydrogenase (GPDH) [15]. ACC catalyzes the formation of malonyl CoA [4], which regulates fatty acid synthesis and, in an indirect manner, fatty acid oxidation inhibiting the action of carnitine palmitoyltransferase 1 enzyme [11]. GPDH is the enzyme linking glucose to lipid metabolism by catalyzing the synthesis of glycerol-3-phsophate. This reaction is the main source of glycerol-3-phosphate, the substrate for triglycerol synthesis in adipose tissue [16]. Our results showed a significant reduction in the expression levels of ACC and GPDH, supporting the potential role of garlic in the inhibition of lipogenesis, as well as in the indirect stimulation of fatty acid oxidation and, consequently, improvement of hyperlipidemia.

Triglycerides are synthesized in the liver, secreted into the bloodstream mainly as VLDL, and transported to the peripheral organs, including adipose tissues [5]. Duval et al. [6] reported that hepatic TG availability is altered by the balance between fatty acids synthesis and oxidation in the liver. Histological findings revealed macrovesicular steatosis in liver tissues of the HFD groups, and garlic supplementation to HFD reduced the level of steatosis.

In addition to its nutritional values, garlic has numerous applications in medicine. The beneficial effects of garlic on cardiovascular disorders are thought to be due to its inhibitory effect on angiotensin converting enzyme [20]. There are also many reports about the beneficial effects of garlic on diabetes and its related disorders [1, 2, 7, 10], and inhibition of atherosclerosis [19]. Augusti and Sheela [3] reported the s-allyl cysteine sulfoxide in garlic acts as an insulin secretagogue in diabetic rats. Orekhov and Grünwald [17] found that garlic indirectly affects atherosclerosis by reduction of hyperlipidemia, hypertension, and probably diabetes mellitus and prevents thrombus formation. However, potential biological activities against obesity from garlic have not yet been reported in the literature and remains to be elucidated.

CONCLUSIONS

In conclusion, the results of the present study indicate that garlic suppresses body weight, fat tissue mass, and plasma lipids levels, in part by down-regulating adipogenic transcription factor and other specific target genes. Since we used a HFD-induced obesity, we speculated that garlic might be another choice in the protection against visceral obesity, and it is highly possible to use garlic as one of anti-obesity functional foods.

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